

**PLANT RESPONSE TO RICE-ROOT NEMATODE,
Hirschmanniella gracilis (de Man, 1880) Luc and Goodey, 1963
IN CONSORTIUM WITH MICROBIAL INOCULANTS**

Thesis submitted in part fulfilment of the requirements for the award of the degree of
DOCTOR OF PHILOSOPHY (AGRICULTURE) in PLANT NEMATOTOLOGY
to the Tamil Nadu Agricultural University, Coimbatore.

BY
BYOMAKESH DASH
ID.No.99-811-001

**DEPARTMENT OF NEMATOTOLOGY
CENTRE FOR PLANT PROTECTION STUDIES
TAMIL NADU AGRICULTURAL UNIVERSITY
COIMBATORE – 641 003**

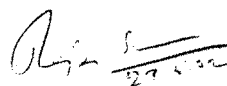
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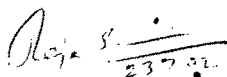


[Dr.(Tmt.) RAJESWARI SUNDARA BABU]

Chairperson

APPROVED BY

Chairperson :



[Dr.(Tmt.) RAJESWARI SUNDARA BABU]

Members :



[Dr.S.RAMAKRISHNAN]



[Dr.B.THAYUMANAVAN]

[Dr.S.SURESH]

External Examiner :



[Dr.P.PARVATHA REDDY]

Ex-Director,

**Indian Institute of Horticultural Research,
Bangalore-560 089.**

Place : Coimbatore

Date : 23-07-2002

ABSTRACT

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PLANT RESPONSE TO RICE-ROOT NEMATODE, *Hirschmanniella gracilis* (de Man, 1880) Luc and Goodey, 1963 IN CONSORTIUM WITH MICROBIAL INOCULANTS

BY

BYOMAKESH DASH

**Degree : DOCTOR OF PHILOSOPHY (AGRICULTURE)
IN PLANT NEMATOLOGY**

**Chairperson : Dr. (Tmt.) RAJESWARI SUNDARA BABU,
Professor,
Department of Nematology,
Tamil Nadu Agricultural University,
Coimbatore-641 003.**

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In search of resistance to the rice-root nematode, *Hirschmanniella gracilis*, a total of ninety-one short, medium and long duration rice varieties was screened. Apparently none of the variety had absolute resistance against the nematode. Most of the varieties evaluated were categorised as susceptible, evident from the proposed rating index. A few among the tested cultivars viz., TKM 5, TKM 7, TKM 8 and TKM 9 (short duration); ASD 8, MDU 4, PTB 12, PTB 15, PTB 23 and PY 1 (medium duration) and CR 1009 (long duration) were moderately resistant.

By virtue of their capabilities, microbial inoculants either individually or in combinations with *Glomus intraradices* or *Pseudomonas fluorescens* enhanced plant growth parameters at seedling and panicle initiation stage, yield components at maturity stage, grain and straw yield at harvest and reduced root and rhizosphere soil nematode population at all stages of observation in *H. gracilis* infected short and long duration rice cvs. IR 50 and ADT 44, respectively both in glasshouse and field conditions. The relative

efficiencies of bioagents in general followed a consistence trend with *P. fluorescens* combinations being more effective though variable by the influence of cultivars, nematode population densities, crop stage and experimental situations. Inverse co-relationship existed between plant performance with nematode population in root and soil. The treatments comprising of *P. fluorescens* combined inoculations with *G. intraradices* and *Azospirillum lipoferum* which had enhanced overall plant growth and yield components ultimately resulted in maximum grain and straw yield of both short and long duration *H. gracilis* infected rice cultivars. Upon treatments with microbial inoculants, flowering was advanced and typical disease symptoms on roots were suppressed.

Microbial inoculants were able to improve the physiological functions by reducing leaf temperature and transpiration rate and increasing photosynthetic active radiation and diffusive resistance of both short and long duration rice cultivars during panicle initiation stage infected with *H. gracilis* at one nematode per g inoculum level. *P. fluorescens* combined treatments were more efficient. Combined treatments of *P. fluorescens* with *G. intraradices* and *A. lipoferum* were equally efficient and most effective in limiting the water loss due to nematode infection.

Treatments of *H. gracilis* infected rice cvs. IR 50 and ADT 44 with microbial inoculants resulted in quantitative increase of biochemical components of roots such as total free amino acids, total sugars, chlorophyll 'a', chlorophyll 'b' and total chlorophyll against decrease in untreated plants. Further, enhancement in concentrations of reducing sugars and total and ortho dihydroxy phenols in roots were achieved in supplement to increase with *H. gracilis* infection. Soluble protein contents of roots in microbial inoculant treated plants failed to reach the level of untreated *H. gracilis* infected plants. Activities of peroxidase and catalase enzymes were increased over and above the

enhancement by nematode infection. All the biochemical attributes followed the similar trend at panicle initiation stage of rice cvs. IR 50 and ADT 44 with *P. fluorescens* combinations being more effective in response to the infection of *H. gracilis* at an inoculum level of one nematode per g soil. However, rate of increase of the studied biochemical attributes varied between the varieties and among the evaluated microorganisms. Combined inoculations of *P. fluorescens* with *G. intraradices* and *A. lipoferum* were most efficient and equally effective in increasing the quantities of biochemical components and activities of oxidative enzymes in roots of *H. gracilis* infected rice cvs. IR 50 and ADT 44 at panicle initiation stage under glasshouse conditions.

Both macro- and micronutritional status of shoot and root of *H. gracilis* infected rice cvs. IR 50 and ADT 44 were enhanced at panicle initiation stage following the application of microbial inoculants. Combined treatments were more effective. *P. fluorescens* combined inoculations with *G. intraradices* and *A. lipoferum* were most effective in enhancing the macronutrients (nitrogen and potassium) and micronutrients (iron, copper, zinc and manganese), except for shoot and root phosphorus contents as an exception, which were enhanced to a maximum in dual inoculation of *G. intraradices* with *Bacillus megaterium*.

Serial microtomed sections of *H. gracilis* infected rice roots revealed that the nematode feed on cortical tissues and caused damage to the root parenchyma cell walls by forming cavities and necrosis.

**Dedicated
to
most respected heavenly abode teacher
and esteem nematologist
Dr. Narendra Nath Padhi**

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Byomakesh Dash

[BYOMAKESH DASH]

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INTRODUCTION

CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) *per se* remained as the main focus of Indian Agriculture, evident from ancient religious texts and folklores since prehistoric era. This annual grass is regarded as the precious gift of God in the subcontinent. Originated in orient, rice has become the most important primary staple food for two third of world's population and is referred to as "the staff" of human race. The cereal, belongs to family Poaceae, has its importance in supplying half of the daily caloric intake. Out of 201.56 million tonnes of total national food grain production in 2000 – 01, rice has contributed 86.30 million tonnes, covering an area of 44.79 million hectares (Anon., 2001). India ranks second in rice grown area and production. Notwithstanding, the benefits of green revolution, India is still *in arriere* with a meagre average yield of 1.927 tonnes per hectare (Siddiqui, 2000). This yield has to be increased by at least 20 to 30 per cent to remain self-sufficient in near future. The productivity is however unenviable as there exists a *quantum sufficit* of yield gap between farmers' and experimental fields. This illumines the pathway for tactical farming (Swaminathan, 1999). Several factors contribute to this achievable yield loss. Loss due to biotic and abiotic stresses, and their sub-optimal management is one of the prime causes. There is an urgent necessity, to curtail the loss through economically viable ecotechnologies.

Plant parasitic nematodes are one of the economic constraints in rice production. There is hardly any agroclimatic zone where, the crop is free from a complicity of phytonematodes, which prevail mainly as poly-specific communities. According to earlier surveys, out of 346 million acres of world's rice growing area, 263 million acres were infected with the damaging levels of plant parasitic nematodes (Hollis and Keoboornrueng, 1984). About 144 different species of stylet bearing nematodes are

associated with rice culture (Gerber and Smart, 1987). But, only a few are preponderant, present continuously in rice fields and are economically important. Nematodes attacking rice have diverse parasitic habits, but they cause mechanical damage and/or malfunctions of physiological processes involved in plant development, resulting in poor growth and yield loss (Bridge *et al.*, 1990). Worldwide, annual yield loss of rice due to nematode infections was estimated at ten per cent, which may extend up to 25 per cent in underdeveloped Asian countries (Sasser and Freckman, 1987; Jairajpuri and Baqri, 1991). Although rice is important as the world crop, and phytonematodes are omnipresent and potential pathogens of rice, still there is a critical shortage of basic information and their accuracy (Mai, 1985; Ichinohe, 1988). In India, fifty-one species of phytoparasitic nematodes were recorded from rice fields (Gunathilagaraj and Kumar, 1997).

A number of species of *Hirschmanniella* Luc and Goodey, 1963 (Tylenchida : Pratylenchidae) present in the rice rhizosphere are collectively known as rice-root nematodes. Of the 29 valid species, a few are recorded as migratory ecto-endoparasites of rice (Lin and Chiu, 1971; Bridge *et al.*, 1990). More than one species of this genus is generally prevalent in all rice growing countries and infecting about 200 million acres i.e., 58 per cent of world's rice fields (Hollis and Keoboornueng, 1984). The nematodes are mainly subterranean in nature and are generally confined to low land and deep-water rice. In fields, the above ground symptoms are non-specific that include mortality of seedlings, stunted growth and retardation of growth rate, reduction in number of leaves, leaf area and leaf chlorosis, depressed and delayed tillering and delayed flowering. Intercellular migration of the nematode in the cortex degenerates the normal functioning of roots. Roots invaded by *Hirschmanniella* spp. generally have small more or less spindle shaped lesions, and turn yellowish brown, which darken later and rot. Yield loss by the rice-root nematodes are influenced by soil fertility, cropping pattern, seasonal conditions, crop status and initial population levels. The disease severity and yield loss

are further compounded by ability of the nematode of over wintering in stubble and fallow soil even in extreme temperatures and prolonged flooding in quiescent stage, having a wide range of weed and alternate crop hosts and involvement in disease complex with *Rhizoctonia solani*, *Corticium rolfsii*, *Xanthomonas oryzae* and *Tylenchorhynchus annulatus*. Assessing the degree of damage caused by the nematode, the European Community made a legislation recently under "European Community Plant Health Legislation" to include the nematode as new pest risk in order to control its import and spread (Bartlett, 2000). The nematode genus prevail in all rice growing areas of Indian sub-continent and are endemic in multicropped rice areas having the potential to cause yield loss to a tune of forty per cent (Prasad *et al.*, 1987).

Among the species of *Hirschmanniella* pathogenic to rice, *H. gracilis* (de Man, 1880) Luc and Goodey, 1963 is one of the important species and distinguishably different from closely related species *H. oryzae* having longer stylet length of 20 to 24 μm (Dey and Baqri, 1985). The species is predominant in temperate regions of Europe and North America but still prevalent in warmer countries. In India, *H. gracilis* was found to be associated with crop cultures of various rice growing states and in some areas as a key pest of rice (Khera and Chaturvedi, 1970; Mathur and Prasad, 1971; Baqri *et al.*, 1983).

Realizing the economic impacts of the rice-root nematode, control strategies were adopted in the later half of twentieth century to suppress them below damaging threshold level. Paradigms of spectacular results were obtained in chemical control methods both as overall main field broadcasting and more specific limited scale prophylactic application to seeds, seedlings and nursery beds. Concerns about the adverse effects of agrochemicals lead to alternative nematode management strategies. Various cultural management practices were experimented for the purpose and many of them were found to be neither

economically viable nor commercially acceptable. Other possible approaches like crop rotation and shifting of planting dates failed to satisfy the ultimate goal of the rice growers. A fair number of high yielding rice cultivars were evaluated and majority of them were observed as good hosts for the nematode. Development of resistant cultivars or transgenics is a distant dream because of its non-specific and sympatric association with rice crops.

Antagonistic microorganisms are the recent and welcome additions to the list of nematode management programmes. The biocontrol agents were proved to have promising potentiality for restricting nematode multiplication either by direct or indirect involvement (Jatala, 1986; Sayre, 1986; Sikora, 1992; Khan, 1998). Ever since the development and commercialization of microbial inoculants as biofertilizers or biopesticides, notable endeavours were made to utilise their efficacies as the alternative to chemical nematicides in conjugation with improvement of plant health and yield. Imperceptible report on management of rice-root nematode with these bioenhancers as supplement to the agrochemicals is in record. It is felt that the research efforts in this direction thus far have been empirical and random.

To mitigate the challenges faced to manage the rice-root nematode, *H. gracilis*, in subsistence agricultural systems, studies on the evaluation of performances of microbial inoculants either as individual suppressant or their combinations are considered worth investigating in totality of the prevailing socio-economic and ecological considerations.

Considering the above problems and prospects, the present investigations were carried out both in glasshouse and field conditions to elucidate information on the following objectives:

1. Screening of popular high yielding rice varieties for their reaction to the rice-root nematode, *H. gracilis*.
2. Management of the rice-root nematode, *H. gracilis* with microbial inoculants in popular high yielding short and long duration varieties both in glasshouse and field conditions.
3. Physiological responses of short and long duration popular high yielding rice varieties treated with microbial inoculants against the infection of the rice-root nematode, *H. gracilis*.
4. Biochemical changes of short and long duration popular high yielding rice varieties to the infection of the rice-root nematode, *H. gracilis* in consortium with microbial inoculants.
5. Nutritional (macro- and micronutrients) status of the rice-root nematode, *H. gracilis* infected popular high yielding short and long duration rice varieties upon treatment with microbial inoculants.
6. Histopathological changes of rice roots to the infection of the rice-root nematode, *H. gracilis*.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Over the years, a wide array of rice cultivars screened and management strategies evaluated with an aim to suppress the plant parasitic nematode population below injury level, with a varied degree of success. Literature pertaining to resistance response of rice to the rice-root nematode, *Hirschmanniella* spp.; suppression of phytonematodes with microbial inoculants; suppression of the rice-root nematode, *Hirschmanniella* spp. with bioagents; biochemical, nutritional and physiological responses of vesicular arbuscular mycorrhizal fungi, *Pseudomonas fluorescens*, and combination of microbial inoculants treated plants; responses of rice plants to phytonematode infections; plant responses to plant parasitic nematodes in consortium with microbial inoculants and histopathological changes of rice roots in response to the infection of *Hirschmanniella* spp. are reviewed in this chapter.

2.1 Resistance response of rice to the rice-root nematode, *Hirschmanniella* spp.

Variety Gendjah Ratji contained only 3,740 *Radopholus oryzae* (= *H. oryzae* Van Breda de Haan, 1902) per plant, while, Tjina contained 15,300, although both received the same amount of inoculum indicating the differences in varietal response to the nematode (Van der Vetch and Bergman, 1952).

The plants of fine grained rice varieties were found to escape the attack of the rice-root nematode, when grown with coarse grained varieties (Birat, 1965). Selections from crosses Taichung Native 1 x T 141, Peta x DgWg and Taichung Native 1 x CO 29 appeared resistant to the rice-root nematode (Anon., 1970).

Out of 270 varieties tested in field, 6 including Suwon, were less preferred by *H. oryzae* (less than 10 nematodes in 10 g root) and 3 including Ilizin had more than 1,000 nematodes per 10 g root (Park *et al.*, 1970).

Among the varieties and lines evaluated against *H. mucronata* Das, 1960 though no immunity was found, CR 44-32, CR 52-10, N 136, Taichung 65, Tinpakhia and W 113, and a few selections from the cross Taichung Native 1 and TKM 6 were observed to be less susceptible (Israel and Rao, 1971).

Rice varieties Annapurna, Aswathy, Bharati, Jaya, Jyothi, Mashoori, Sabari and Triveni were found to be infected with *H. oryzae* naturally under field conditions in Kerala (Venkitesan *et al.*, 1979).

Ten varieties of rice *viz.*, Annapurna, Bharati, CO 25, IR 8, Jaya, Jyothi, PTB 12, Rohini, Sabari and Triveni were screened for resistance to *Rhizoctonia solani* and *H. oryzae* under the field conditions and were found susceptible to *H. oryzae*. High incidence of the nematode encouraged severe *Rhizoctonia* infection (Gokulapalan and Nair, 1983).

Out of the rice varieties IR 20, IR 50, Paiyur 1, TKM 6 and TKM 9 evaluated against *H. oryzae*, only TKM 9, the deep rooted rice cultivar was found to be resistant to the nematode at all growth stages (Ramakrishnan *et al.*, 1984).

Infection of *H. oryzae* was investigated over a period of 3 years in 23 provinces and cities of China. No variety was found immune to the nematode, but differences in varietal response existed among the varieties under field conditions (Yin and Li, 1984).

Rice varieties Kao Paung Klang, Kao Paung, Kao Tah Jue, Kao Yaun and Kao Klang Pee with Rf values between 1.54 to 1.98 after 60 days of transplanting were classified as resistant to *Hirschmanniella* spp. (Arayarungsarit *et al.*, 1986).

Rice varieties and breeding lines, viz., Annapurna, CR 44-140-2-1051, CR 130-203, CR 141-6058-1-35, CR 142-3-2, CR 294-548, CR 320, RP 1155-128-1, MTU 8 and PTB 27 were found to be resistant or tolerant to the rice-root nematode (Rao *et al.*, 1986).

Higher population build-up of *H. oryzae* was observed in Pak Basmati and Basmati 370, while Basmati 385 supported lowest population among 7 basmati rice cultivars evaluated (Randhawa *et al.*, 1991; 1992). Ten rice varieties were identified as resistant to *H. oryzae* (Gao, 1991).

All the eleven rice cultivars viz., PR 103, PR 106, PR 108, PR 109, Jaya, PAU 1126, PAU 1628, PUSA 44, PUSA 429, HKR 120 and HKR 126 were found susceptible to *H. oryzae*, while PR 108 and PAU 1126 having the greatest nematode build-up and PR 103 the least (Singh *et al.*, 1992).

Of the 18 wild rice species tested for their reaction to *H. mucronata*, *Porteresia coarctata* showed the highest degree of resistance, while *Oryza collina* and *O. nivara* were moderately resistant. Though, resistance in *P. coarctata* could not be attributed to any particular genome, some degree of resistance was attributed to CC and AA genomes (Panigrahi and Mishra, 1995).

Six cultivars belonging to Japonica (Sakha 101, Sakha 102, Giza 176 and Giza 177), Indica-Japonica (Giza 178) and Indica (Yamini) were evaluated against *H. oryzae* infection, and the rice varieties belonging to Japonica group were more susceptible than the other two groups (Youssef, 1998; 1999).

Rice cultivars belonging to *Oryza sativa* and *O. glaberrima* parents and their progenies (5 varieties) were found susceptible to *H. oryzae* (Coyne *et al.*, 1999).

2.2 Suppression of phytonematodes with microbial inoculants

2.2.1 *Azolla*

Azolla is a free-floating water fern and fixes atmospheric nitrogen in association with nitrogen fixing blue green alga *Anabaena azollae* (Moore, 1969). The agronomic potential of *Azolla* is quite significant particularly for rice crop and is widely used as a biofertilizer for increasing rice yields (Kannaiyan, 1992; Kannaiyan *et al.*, 1982).

Maximum plant growth of okra and tomato and minimum root knot index were obtained by the application of dry *Azolla* at 3 per cent soil weight basis to the plants infected with *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (okra) and *M. incognita* (Kofoid and White, 1919) Chitwood, 1949 (tomato) (Patel *et al.*, 1985; Thakar *et al.*, 1988).

Introduction of dry *Azolla* at 1 to 3 per cent w/w to soil reduced infection of *M. incognita* in *Hibiscus esculentus* cv. Pusa Sawani (Thakar *et al.*, 1987).

Dry *Azolla pinnata* at 3 per cent w/w exhibited enhanced growth and yield of okra and reduced the number of adult female, egg masses and eggs per egg mass and soil population of *M. incognita* in a pot experiment (Ramakrishnan *et al.*, 1996).

Soil amendment with dry and green *Azolla* enhanced aubergine plant growth and suppressed root galling of *M. javanica*. Better response in reducing galls and improving plant growth were observed with dry *Azolla* as compared to its extract (Maleck *et al.*, 1996).

2.2.2 *Azospirillum*

Azospirillum, a nitrogen fixing bacterium had been successfully tested for its effect on rice growth and yield (Roger *et al.*, 1993). The beneficial effect of *Azospirillum* can be attributed to a combination of increased associative biological N₂ fixation in the

rhizosphere, production of plant growth regulating substances that favour rice growth, nutrient utilization and increased nutrient availability through solubilization of immobilized nutrients (Kannaiyan, 1999).

A. brasilense at 1.5 per cent w/w was effective in reducing adult females, eggmasses, eggs per eggmass and soil nematode population of *M. incognita* infecting okra (Ramakrishnan *et al.*, 1996).

A. lipoferum reduced infection level of *Heterodera avenae* Wollenweber, 1924 in wheat under pot culture, and found effective among all the rhizosphere bacteria tested. Results indicated ammonia but not siderophores or indole acetic acid (IAA) had a role in control of nematode infection as the bacterium possessed high ammonia excretion ability and negligible IAA production (Bansal *et al.*, 1999).

In field microplots, application of *A. lipoferum* controlled *M. incognita* infection in mung bean (*Vigna radiata*) through reduction of number of galls, eggmasses / root system and J2/kg soil. Of the eight bacterial and fungal bioagents tested, *A. lipoferum* improved the plant growth and biomass of nematode inoculated plants and synergised root nodulation (Khan and Kaunsar, 2000).

2.2.3 Blue green algae

The blue green algal culture, is a composite inoculum consisting of genera *Nostoc*, *Anabaena*, *Calothrix*, *Tolypothrix*, *Plectonema*, *Gloeocapsa*, *Oscillatoria*, *Cylindrospermum*, *Aulosira* and *Scytonema*. These N₂ fixing diazotrophic cyanobacterial cultures have better adaptability in wetland rice (Kannaiyan, 1985). These are efficient, ideal biological system under tropical situation for better non-symbiotic nitrogen fixation and are renewable biofertilizers. Rice cropping system provides favourable aquatic

environment, in which algae contribute about thirty kg N/ha (Tilak, 1993a; Kannaiyan, 1999).

'Dried algal catch', an organic waste significantly reduced the root galling of *M. incognita* and increased fruit yield of aubergine and okra (Kaul and Chhabra, 1993).

Exudates and aqueous extracts (5 per cent) of two species of cyanobacteria, *Aulosira fertilissima* and *Nostoc muscorum* and a green algae, *Chlorella vulgaris* suppressed the hatching of *M. tritricoryzae* Gaur, Saha and Khan, 1993 in *in vitro* condition (Gaur, 1995).

The algal preparation 'Bioalgeen S 90 plus 2' at its recommended dose reduced the cyst population of *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975 and increased potato tuber yields of cultivars Quarta, Sieglinde and Selma in the nematode infected fields (Lung, 1996).

Three species of blue green algae (*Anabaena oryzae*, *Nostoc calcicola* and *Spirulina* sp.) in combination reduced the number of galls and eggmasses of *M. incognita* and increased plant growth and number of nodules in cowpea cv. Baladi (Youssef and Ali, 1998).

In a pot experiment, the blue green algae, *Microcoleus vaginatus* (2.4×10^6 endospores per ml) increased tomato plant growth and yield, and decreased *M. incognita* population and gall formation. Optimum dose was observed to be 20 ml of endospore suspension per plant (Khan and SoDeuk, 1999).

Blue green algal (*N. muscorum*) based biofertilizer at 10 kg/ha reduced *Tylenchorhynchus mashoodi* Siddiqui and Basir, 1959 population in wheat under wheat-rice cropping system by 6.8 per cent (Gaur *et al.*, 2000).

Hatching of *M. triticoryzae* J2 (collected from rice field) was suppressed in culture filtrates and standard aqueous extracts of the blue green algae, *Aulosira fertilissima*, but hatching resumed when the blue green algae was replaced with distilled water. Low concentrations of the aqueous extract stimulated hatching, while high concentrations were nematostatic (Chandel *et al.*, 2001).

2.2.4 Phosphobacteria

Phosphorus solubilising heterotrophic microorganisms include various bacterial, fungal, actinomycetes forms, which convert insoluble and fixed inorganic phosphate into simple soluble forms. These microorganisms have consistently shown their efficiency to solubilize chemically-fixed phosphorus and rock phosphate from different sources. In India, field trials conducted in different soil types, revealed the increase of rice yields by the inoculation of phosphobacterium, *Bacillus megaterium* var. *phosphaticum* (Tilak, 1993b).

Inoculation of *B. megaterium* ($2.5 - 3.9 \times 10^6$ c.f.u./ml) reduced the population densities of both *M. chitwoodi* Golden, O'Bannon, Santo, and Finley, 1980 and *Pratylenchus neglectus* Rensch, 1924 up to 50 per cent in potato both in glasshouse and field microplots (Al Rehiyani *et al.*, 1999).

Among the resident microflora of established apple orchard soil, *B. megaterium* and *Burkholderia capacia* dominated the bacterial communities and were responsible for the suppression of lesion nematode population infecting 'Gala' apple seedlings (Mazzola, 1999).

2.2.5 VAM fungus (*Glomus intraradices* Schenck and Smith)

Frank (1885) coined the term 'Mycorrhiza' to describe the symbiotic association of plant roots and fungi. Mycorrhiza literally means fungus root and by far, the most

common mycorrhizal association is the vesicular arbuscular type, which produces structures (vesicles and arbuscles) in the cortex region of the root. The vesicular arbuscular mycorrhizal association is found in most of the plant families growing in arctic, temperate and tropical regions. They are categorised as non-septate zygomycetous fungi, belonging to genera *Aucalospora*, *Entrophosphora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora* (Bagyaraj, 1995).

It is well documented, that VAM fungi improve growth of plants that are important in agriculture. VAM fungi provide greater absorptive surface as compared to root hair and help in absorption of relatively immobile ions in soil, such as phosphorus, copper and zinc and water in plants (Bagyaraj, 1992). In addition, VAM fungi are shown to increase host tolerance or resistance of crop plants against nematode infection (Jain and Hassan, 1998).

Inoculation of *G. intraradices* increased the tolerance of susceptible cotton cultivars to *M. incognita* and increased yield of cotton cultivars in field; but not in glasshouse (Smith *et al.*, 1984). VAM fungus *G. intraradices* influenced penetration, development and reproduction of *M. incognita* in susceptible cotton cv. Stoneville 213, resulting in reduction of nematode infection. Mycorrhizal fungus increased host tolerance to the nematode under field conditions (Smith *et al.*, 1986a; 1986b).

Roots of rough lemon (*Citrus limon*) seedlings colonized with *G. intraradices* improved growth and 'P' nutrition, but had no effect on *Radopholus similis* Cobb, 1893 population (Smith and Kaplan, 1988). *G. intraradices* inoculated mycorrhizal cantaloupe plants had not affected gall formation by *M. incognita* as well as number of eggs per eggmass (Herald *et al.*, 1989).

Micropropagated BA-29 quince rootstocks achieved higher values of all plant growth parameters in low 'P' soil through the inoculation of *G. intraradices*, even in the presence of *P. vulnus* Allen and Jensen, 1951. Enhanced root mass production accounted for increase in final nematode population in mycorrhizal plants (Calvet *et al.*, 1995).

Fresh top weight and stem diameter of *G. intraradices* inoculated Santa Lucia 64 cherry rootstocks were significantly higher than *P. vulnus* inoculated or uninoculated rootstocks under microplot conditions but had not affected nematode population (Pinochet *et al.*, 1995b).

Two soyabean cultivars, Bragg (nematode intolerant) and Wright (moderately tolerant) showed differential response to combined inoculation of VAM fungus, *G. intraradices* and the soyabean cyst nematode (SCN), *H. glycines* Ichinohe, 1952 under glasshouse conditions in soils with low (35 micro g/g) and high (70 micro g/g) 'P' content. Cultivar Wright grew better than Bragg in soil with high 'P', indicating a greater response to 'P' and VAM, and was less damaged by SCN because of more efficient root system (Price *et al.*, 1995).

Inoculation with *G. intraradices* and *G. mosseae* increased growth of micropropagated OHF-333 pear rootstock and was more effective than 'P' fertilization under field microplot conditions. Root colonization by mycorrhiza suppressed *P. vulnus* build up in host roots. Early inoculation with mycorrhiza favoured pear rootstock growth and conferred protection against the nematode by inhibiting its reproduction and by enhancing plant nutrition (Lspez *et al.*, 1997).

Inoculation of *G. intraradices* in the micropropagated Grand Naine banana (Musa AAA) showed significant improvement in plant growth but mycorrhizal plants were more galled with *M. javanica* (Pinochet *et al.*, 1997).

Early mycorrhizal inoculation with *G. mosseae* and *G. intraradices* on Myrobalan 29 C (*Prunus cerasifera* x *P. munsoniana*) plum rootstock, infected with *P. vulnus* indicated that only *G. intraradices* increased tolerance of rootstock to the damage of nematode by stimulating plant nutrition and vegetative growth (Pinochet *et al.*, 1998).

Among the three species of arbuscular mycorrhizal fungi, *G. aggregatum*, *G. intraradices* and *G. mosseae*, the degree of suppression of *M. incognita* infecting white clover (*Trifolium repens*) varied with the species of mycorrhizal fungus, but was not related to the degree to which they colonized roots or their effectiveness in enhancing host growth (Habte *et al.*, 1999).

Of the four VAM fungi, *G. fasciculatum* recorded the highest plant growth rates of *Crossandra undulaefolia* and least *M. incognita* damage in pot experiments, followed by *G. mosseae*, *G. intraradices* and *Aucalospora laevis* (Nagesh *et al.*, 1999).

Life cycles of both the arbuscular mycorrhizal fungus (AMF), *G. intraradices* and the burrowing nematode, *R. similis* were completed in the presence of each other under dioxenic culture condition and a transformed carrot root as host. The AMF protected the root against the nematode by suppressing male and female nematode population by almost 50 per cent. There was no correlation between the nematode population densities and either the AMF internal root colonization, external hyphal development or spore production (Elsen *et al.*, 2001).

The infectivity of *G. intraradices* against *P. brachyurus* Godfrey, 1929 and *M. javanica* was inhibited by the natural chemical compounds like cinnamaldehyde, salicylaldehyde, thymol, carvacrol, panisaldehyde and benzaldehyde in saturated atmospheres (Calvet *et al.*, 2001).

A mixture inoculum of *Gigaspora rosea*, *Glomus mosseae* and *G. intraradices* exhibited significant increase of resistance in grapevine rootstocks and cultivars and suppressed pathogens including nematodes. However, the degree of responsiveness varied, depending upon the plant genotype and pathogen. Untreated plants were clearly stunted and showed foliar symptoms of undetermined nutrient deficiencies in the presence of pathogens (Linderman and Davis, 2001).

2.2.6 *Pseudomonas fluorescens* Migula

Fluorescent pseudomonads are the major bacterial groups capable of surviving and colonizing in rhizosphere of all field crops. They are known as 'Plant Growth Promoting Rhizobacteria' (PGPR) as they promote plant growth by secreting auxins, gibberellins and cytokinins (Vidyasekharan, 1998).

Fluorescent pseudomonad species has emerged as the largest group and potentially the most promising group of biocontrol agent for suppression of plant parasitic nematodes (Oostendrop and Sikora, 1989). They induce resistance in plants against ingressed nematode pests (Sikora, 1988) and systemic resistance against nematodes (Oostendrop and Sikora, 1990; Sikora, 1992; Sikora and Hoffman Hergarten, 1992). Among the fluorescent pseudomonads, *P. fluorescens* is the most experimented species and in recent years it was found to be highly effective biocontrol agent for suppression of a number of plant parasitic nematode species.

Khan *et al.* (1976) were first to report the bacterium, *P. fluorescens* in the alimentary canal of a female plant parasitic nematode, *M. incognita*.

Significant reduction in root knot nematode galling was observed, when *P. fluorescens* was applied around the roots of infected plants (Jowaraski *et al.*, 1986). *P. fluorescens* was found to be effective in suppressing the populations of *H. avenae*,

H. cajani Koshy, 1967, *H. zae* Koshy, Swarup and Sethi, 1971 and *M. incognita* under *in vitro* conditions (Gokte and Swarup, 1988).

Eight isolates of *P. fluorescens* from sugarbeet rhizosphere were found effective against *H. schachtii* Schmidt, 1871. Seed treatment with these isolates reduced nematode penetration up to 75 per cent under field condition. The antagonistic activity of *P. fluorescens* was attributed to bacterial alteration of root exudates that influenced nematode hatch, attraction and penetration behaviours. In addition, the mechanism is related to the ability of the bacterium to bind itself to the root surface lectins, thereby interacting with normal host recognition of the nematode (Oostendrop and Sikora, 1989; 1990).

Application of the specific rhizobacterium *P. fluorescens* (Strain T 58) as seed dressing in either liquid or solid state caused up to 42 per cent reduction in root penetration of *H. schachtii* (Hoffman Hergarten and Sikora, 1992).

Application of *P. fluorescens* as seed treatment reduced *H. cajani* population in both roots of cowpea and rhizosphere soil by 80 and 69 per cent, respectively under pot culture (Sujatha, 1995).

Three strains (Pf 1, Pf 2 and Pf 3) of *P. fluorescens* as seedling root dip significantly increased plant growth of tomato infected with *M. incognita* and reduced the levels of infection. Degree of nematode suppression was found related to the bacterial concentration and their root colonizing ability (Shanthi and Sivakumar, 1995).

P. fluorescens (Strain F 113) was proposed as a potential biocontrol inoculant for the protection of potato crops against the potato cyst nematode since *G. rostochiensis* was reduced by threefold following their incubation in the presence of *P. fluorescens* on artificial media (Elsherif and Grossmann, 1996).

Grapevine seedlings root dip with *P. fluorescens* was found effective in reducing *M. incognita* and caused 40 per cent reduction of root galls under glasshouse conditions (Mani, 1996).

None of the thirty isolates of *P. fluorescens* from the rhizoplane of black pepper gave absolute protection to the plant against *M. incognita* infection. However, five isolates reduced the nematode damage and another three promoted the growth of seedlings, though they had no effect on nematode suppression (Eapen *et al.*, 1997).

Exposure of *G. rostochiensis* cyst to *P. fluorescens* (Strain F 113) that produces 2,4-diacetylphloroglucinol under *in vitro* conditions or in soil microcosms, almost doubled the ability to hatch and reduce juvenile mobility and infectivity on potato (Cronin *et al.*, 1997). Transposon mutagenesis strain F 113 of *P. fluorescens* had the biocontrol ability against *G. rostochiensis* and was mediated by 2,4-diacetylphloroglucinol (PHL) or lytic enzyme production. Purified PHL, lytic enzymes and chitinolytic or phloroglucinol producing bacterial inoculants including *P. fluorescens* F 113 negatively influenced hatching of nematode eggs and decreased subsequent viability of juveniles both under *in vitro* and soil conditions (Dunne *et al.*, 1998).

The internal population of endophytic bacterial strains including 89B-61 of *P. fluorescens* increased, when introduced in the roots of cotton and cucumber in the presence of *M. incognita*. The endophytic bacterial strains increased in roots and suppressed the nematodes under greenhouse conditions (Hallmann *et al.*, 1998).

A strain of *P. fluorescens* significantly reduced *R. similis* invasion by 50 per cent but had no effect on live or sonicated entomopathogenic nematode, *Steinernema carpocapsae* Weiser, 1955 (Aalten and Gowen, 1998). Out of three strains of *P. fluorescens*, only one strain significantly reduced the number of *R. similis* after initial

invasion. All the strains exhibited an *in vitro* repellent effect towards the nematodes *R. similis* and *Meloidogyne* spp., with later nematode being more affected. Chemical compounds of isolates might have affected nematode infectivity or strains elicited induced systemic resistance in plants (Aalten *et al.*, 1998).

Commercial formulation of *P. fluorescens* as seed treatment at 5, 10 and 15 g/kg seed significantly increased the shoot length of tomato against the infection of *M. incognita*. Significant reduction of number of galls and egg masses per plant was observed in the two higher doses (Verma *et al.*, 1998).

Standard concentration (S), S/10 and S/100 of culture filtrate of *P. fluorescens* reduced the emergence of *H. avenae* juveniles, but S/1000 was found to have stimulatory effect. Standard concentration of filtrate showed 100 per cent mortality within 24 hours (Kamra and Dhawan, 1998).

Application of talc based formulation of *P. fluorescens* (2.5×10^8 c.f.u. per g) at 10 and 20 kg ha⁻¹ reduced *Globedera* spp. Skarbilovich, 1959 parasitization in potato roots by 47.7 and 62.6 per cent, respectively (Mani *et al.*, 1998).

Among the antagonists viz., *Trichoderma viride*, *T. harzianum*, *Paecilomyces lilacinus*, *P. fluorescens* and *Bacillus subtilis*, soil application of culture filtrate of *B. subtilis* caused maximum reduction in cyst and juvenile population of *H. cajani* infecting blackgram (*Vigna mungo*), followed by *P. fluorescens* and *P. lilacinus* (Latha and Sivakumar, 1998).

Glasshouse and microplot application of 2.5×10^8 c.f.u. per g TNAU strain Pf 1 talc based formulation of *P. fluorescens* at 1, 2, or 4 g per grapevine significantly reduced the severity of infection by *M. incognita* (race 3) and enhanced root colonization of the rhizobacterium. Colonization was observed in the roots produced during second season

(Shanthi *et al.*, 1998). In the long run, carbofuran maintained superiority in controlling the nematode. Field application of the bacterium (commercial formulation) at 20 or 40 g/tree affected the multiplication of *Tylenchulus semipenetrans* Cobb, 1913 in sweet orange (*Citrus sinensis*) and lemon (*Citris limon*). Suppression of the nematode was enhanced with the application of carbofuran @ 2 g a.i./tree. Disease symptoms appeared in *P. fluorescens* treated trees after 4 months, but thereafter no further deterioration was observed in the trees up to 10 months (Shanthi *et al.*, 1999).

Commercial products formulated with microbial inoculants including *P. fluorescens*, when applied through soil against *T. semipenetrans* and *Paratrichodorus lobatus* (Colbran, 1965) Siddiqui, 1974 on *Citrus* spp. (oranges and lemons) exhibited poor performance both in pot and field experiments. Nutri-life 3/20 (a dry formulation of *P. fluorescens*) at the recommended dose inhibited growth of citrus and caused leaf chlorosis (Walker and Morey, 1999).

In soil microcosm tests, inoculation of pea seeds with *P. fluorescens* strain F 113 lac ZY or F 113 G 22 increased the number of plant parasitic nematodes in the rhizosphere. This suggested that nematicidal compounds released by the germinating seeds were metabolised by microbial inoculants before they affect nematode population in spermosphere or rhizosphere of pea. In contrast, inoculation of wheat seeds with the same strain resulted in significantly lower nematode population (Brimecombe *et al.*, 1999).

In bioassays and greenhouse tests, both *B. thuringiensis* and *P. fluorescens* showed nematicidal activity against hatched juveniles and adults of *M. incognita* infecting tomato. The mortality levels of *M. incognita* increased with the higher concentration of bacterial cells (5×10^8 c.f.u/ml). Generally, the percentage of decrease of gall formation and root-gall index were maximum when the antagonistic bacteria were

introduced prior to nematode as compared to simultaneous introduction of both the nematode and bacteria (Hanna *et al.*, 1999).

In a tomato field infected with *M. incognita* and *Fusarium oxysporum* f. sp. *lycopersici*, greatest enhancement of plant growth and yield of tomato was observed in *P. fluorescens* treatment among the treatments viz., *P. lilacinus*, *Gliocladium verens*, *P. fluorescens* PRS 9, *Bacillus polymyxa* and combination of aldicarb and thiram tried. Hatching of larvae, gall formation, eggmass production, fecundity and soil population of *M. incognita* and wilting index of disease complex were decreased by the application of all the above biocontrol agents, except *B. polymyxa* (Khan and Akram, 2000).

Among three strains of *P. fluorescens* (Pf 1, Pf 2 and Pf TNAU 1), maximum enhancement of shoot length and weight of *Cajanus cajan* was observed in Pf 1 treatment against *H. cajani* infection with highest reduction of cysts in soil and root system. Principal factor of nematode suppression contributed to the enhanced root colonizing ability of Pf 1 strain (2.5×10^8 c.f.u/g root) than other two strains (Sujatha *et al.*, 2000).

Application of *P. fluorescens* in tomato nursery at 20 g/m² (formulated dose) proved better than carbofuran individually or in combination with *P. fluorescens*, against *M. incognita* in respect to seed germination, reduction of nematode population and formation of root galls. In field, all the treatments were effective in minimizing root gall index and enhancing the yield (Khan, 2000).

Mortality of *M. incognita* juveniles was observed to be similar both in unheated and heated culture filtrates of *P. fluorescens*, suggestive of its non-proteins in nature. However, mortality increased with increase in concentration (Sirohi *et al.*, 2000).

Improvement of plant growth of tomato, and reduction in galling and nematode population of *M. incognita* was the result of application of *P. fluorescens* GRP 3 sixty

days after treatment under glasshouse condition. Combination of *P. fluorescens* GRP 3 and organic manure was the best treatment for the management of *M. incognita* on tomato among the treatments of two strains of *P. fluorescens* (GRP 3 and PRS 9), organic manure and inorganic fertilizers (urea, diammonium phosphate, muriate of potash and monocalcium phosphate) either individually or in combinations (Siddiqui *et al.*, 2001).

Criconemella xenoplax (Raski, 1962) Luc and Raski, 1984 was suppressed by the introduction of *P. fluorescens* BG 33R, isolated from peach orchard soil. But five mutants derived from the strain were incapable of ovicidal activity of the nematode and production of siderophore and protease. The results indicated the involvement of specific genes of the bacterium in the production of egg-killing factor (Wechter *et al.*, 2001).

Seed bacterization of tomato with *P. fluorescens* (Pf 1) improved germination per cent and reduced *Rotylenchulus reniformis* Linford and Oliveira, 1940 penetration and multiplication. However, maximum reduction of the nematode was obtained through seed inoculation with the bacterium followed by soil drench (Niknam and Dhawan, 2001).

Application of *P. fluorescens* or *B. subtilis* (cultured on nutrient broth) increased the growth and yield of chickpea and reduced the infection of *M. incognita* by minimizing the number of galls/root system, eggmass production and soil population (Khan *et al.*, 2001).

When the bioagents *P. fluorescens* and *Trichoderma viride* were applied separately in soil (3 kg/ha) and seed (20 g/kg) of chickpea in a field trial, significant reduction in gall formation by *M. incognita* and disease intensity of *Fusarium oxysporum* f. sp. *ciceri* was observed resulting in increase in plant growth parameters and yield (Devi and Pandey, 2001).

Under *in vitro* conditions, mobility of *R. reniformis* was reduced significantly 48 hours after exposure to culture filtrate of *P. fluorescens* and immobility of the nematode increased with increase in concentration of culture filtrate and exposure time. In pot experiment with cotton cv. MCU 5, significant reduction in nematode penetration was observed in roots treated with *P. fluorescens* (Jayakumar *et al.*, 2001).

In a pot experiment, *P. fluorescens* was more effective in the management of *M. incognita* in turmeric. Application of *P. fluorescens* at 10 g/kg of turmeric rhizome performed best by improving plant growth characters and decreasing *M. incognita* population and gall indices than *T. viride* (4 g/kg) and neem cake (2 per cent w/w) (Srinivasan *et al.*, 2001a; 2001b).

Among the bioagents (*P. fluorescens*, AMF, *T. viride* at 3 per cent w/w); plant products, botanical pesticides, organic amendments, hot water treatment, green leaves and chemicals (check), tried, *P. fluorescens*, AMF and hot water treatment (55°C for 5 minutes) when applied to the rhizome, significantly improved the plant growth parameters and yield of Kacholam, and reduced the mixed soil nematode population of root-knot and burrowing nematodes in the ratio of 2:1 (Nisha *et al.*, 2001).

2.2.7 Combination of microbial inoculants

Combined application of VAM fungus, *Glomus fasciculatum* (nursery bed treatment 100 g/m²), seed treatment (200 g/kg seeds), seedling root dip (1 kg/ha seedling) and main field application (1 kg/ha) of *Azospirillum* and phosphobacterium recorded maximum yield and low nematode multiplication rate of *R. reniformis* in ragi and *Pratylenchus zaei* Graham, 1951 in maize. The VAM fungus was able to prevent entry of the nematode in the presence of biofertilizers and offset the damage caused by the nematodes (Rajeswari Sundara Babu *et al.*, 1996).

The replacement of inorganic fertilizers with biofertilizers (mixture of *Azospirillum*, phosphobacterium and VAM fungus) enhanced growth of papaya cv. CO 6 and reduced *Meloidogyne* sp. population in root and soil (Rani and Sathiamoorthi, 1997).

In pot culture experiment combined inoculation of phosphobacterium (*B. megaterium* var. *phosphaticum*) and VAM fungus (*G. mosseae*) recorded the highest yield of blackgram and the lowest gall index and nematode population of *M. incognita* than individual inoculation. Application of phosphobacterium to mycorrhizal plants, nullified the damage caused by the nematode and increased spore and mycorrhizal colonization. There was direct relationship between 'P' uptake and extent of root colonization of VAM fungus (Sankaranarayanan and Rajeswari Sundara Babu, 1997).

Application of *G. fasciculatum* (1 spore per g) at 100 g per m² in furrows along with maize seeds and seed treatment with *Azospirillum* and phosphobacterium (1 x 10⁸ cells per g of carrier material) at 200 g per 8 kg seed per acre, obtained lowest multiplication rate of *P. zeae* and the highest cob yield of maize (Rajeswari Sundara Babu *et al.*, 1998).

Combined application of *Azospirillum* and phosphobacterium at 50 g per vine and in combination with neem cake at 200 g per vine to a four year old Muscat-Hamberg vineyard, reduced infection of *M. incognita* and recorded higher fruit yield than individual treatments (Sivakumar and Vadivelu, 1998).

G. mosseae, *P. lilacinus* and *P. fluorescens* when used alone or in combination suppressed wilt disease complex of pigeonpea caused by *H. cajani* and *Fusarium udum*, and increased plant growth and nodulation in pot experiments. Reduction in nematode multiplication was the most important factor in the suppression of wilt disease complex (Siddiqui *et al.*, 1998).

A commercial mixture of microorganisms (mixture of nitrogen fixing bacteria, humus producing bacteria, moulds and algae) in a liquid suspension marketed as RET-FLO PX 357 when applied to banana plantations (cv. Dwarf Cavendish) at its recommended dose decreased *Helicotylenchus* sp. Steiner, 1945 number, improved pseudocoelom circumference, number of fingers per hand, and advanced flowering time (Farnandez Falscn *et al.*, 1998).

The reproduction factor of *R. reniformis* on okra was significantly decreased by combined applications of *Azospirillum*, VAM fungi and *Azotobacter* in different combinations, than individual applications. There was also a compatible interaction between *Azospirillum*, VAM fungi and low dose of carbofuran, as indicated by significant improvement of plant growth characters and lower nematode population (Sharma and Pankaj, 2000).

Six new commercial Egyptian biofertilizers containing a mixture of nitrogen fixing bacteria, nitrogen fixing *A. brasilense*, phosphate dissolving bacteria and a nitrogen fixing blue green algae (*Cyanobacterium*) applied at 3 different doses (base lower, recommended and higher) reduced the population of *M. incognita* and improved the growth of sunflower cv. Giza 101 (Ismail and Hasabo, 2000).

VAM (*G. fasciculatum* soil application at 15 g/kg soil) *P. fluorescens* (seed treatment at 10 g/kg seed) and FYM at 15 g/kg soil as soil application on okra against *M. incognita* either individually or in combinations were evaluated. Maximum reduction in nematode galling, eggmass formation and final soil population was observed in carbosulfan (seed treatment at 3 per cent a.i. w/w) treatment followed by combination of all the three nematode managing components (Verma, 2001).

When added individually *G. mosseae*, a symbiont at 4 g was better in improving plant growth of chickpea (*Cicer arietinum*) and reducing galling and population of *M. javanica* than the rhizobacteria viz., *P. fluorescens*, *A. chroococcum* and *A. brasilense* and symbiont *Rhizobium* sp. *P. fluorescens* treatment was the next best individual treatment and was similar to *Rhizobium* sp. Combined treatment of *P. fluorescens* with *G. mosseae* was better at improving plant growth, reducing galling and nematode multiplication than any other combined treatment (Siddiqui and Irshad Mahmood, 2001).

2.3 Suppression of the rice-root nematode, *Hirschmanniella* spp. with bioagents

2.3.1 Nematode trapping fungi

A soil conditioner containing spores (3 per cent w/w) of the nematode trapping fungi (*Arthrobotrys* spp.) decreased the numbers of *Hirschmanniella* spp. and increased free living nematode population, which led to improvement of number of tillers, number of grains per ear, grain and straw yield (Yakoo, 1971).

2.3.2 Obligate parasite

Spore encumbrance of *Pasteuria* sp. was observed on *H. oryzae* collected from rice field of Assam under natural conditions (Dhawan and Kamra, 1995).

2.3.3 Plant growth promoting rhizobacteria

Six strains of *P. fluorescens* isolated from rice rhizospheres and applied as seed treatment at 10 g/kg seed (talc base containing $2-3 \times 10^8$ c.f.u/g) caused 70 per cent reduction of *H. oryzae* in cv. ADT 38 under glasshouse conditions. Seed treatment with *P. fluorescens* along with shrimp waste (1 per cent w/w) and neem cake (1 ton per ha) decreased *H. oryzae* population by 51 per cent in roots and soil population by 37 per cent under field conditions. The treatments either individually or in combinations encouraged natural fungal flora and the microbial activities in soil. The major fungi isolated were

species of *Aspergillus* and *Trichoderma* which were found to be antagonistic to the nematode (Swarnakumari, 1996).

Applications of *P. fluorescens* Pf 1 (commercial formulation of TNAU) as seed treatment (10 and 20 g per kg seed) and nursery soil application (3 g per m²) either separately or in combinations reduced *H. gracilis* population in cvs. CO 43, ADT 36 and ADT 38 both in nursery and main field. However, nursery treatment was not effective as seed treatments (Ramakrishnan and Sivakumar, 1999; Ramakrishnan *et al.*, 1998a; 1998b).

Among eleven strains of *P. fluorescens* isolated from rice rhizospheres, strain Pf TN 1 caused maximum (64 per cent) reduction of *H. oryzae* population and increased plant biomass under field condition. All the eleven strains suppressed the nematode population when applied as seed treatment at 25 ml inoculum suspension (10⁸ c.f.u./ml) per kg seed of cv. CO 38. Effectiveness of the same strain was attributed to intensive root colonizing ability. Various dilutions and standard concentration of culture filtrate of *P. fluorescens* (Pf TN 1) exhibited toxic effect on *H. oryzae* population *in vitro*. The percentage of mortality was increased with enhanced concentration and exposure time. Toxic metabolites produced during the microbial decomposition of broth was responsible for mortality of the nematode (Sreenivasan *et al.*, 2000a; 2000b).

2.3.4 Predaceous mites

Under *in vitro* conditions two nematode feeding mites, *Hypoaspis calcuttaensis* and *Tyrophagus putrescentiae* were observed to be predaceous to both *M. incognita* and *H. oryzae* (Bilgrami, 1997).

2.3.5 Predatory nematodes

Biotic and abiotic factors *viz.*, prey density, temperature, periods of incubations of prey, starvation of predator, agar concentration and thickness and distance of predator's inoculation influenced the suppression of prey nematodes (*H. oryzae* and *M. incognita*) by the predatory nematodes (*Laimydorus baldus* and *Discolaimus major*) under *in vitro* conditions. Differences in suppressiveness were attributed to inert behaviour of predatory nematodes, their preference for a particular species of prey, chemical composition, concentration, quality and quantity of prey attractants (Parvez and Bilgrami, 2000).

In vitro study revealed feeding, attraction and aggregation behaviour of predatory nematodes, *Mesodorylaimus bastiani* and *Aquatides thornei* on *H. oryzae* were influenced by concentrations, thickness and pH of agar. There were differences among the predators with respect to time taken by the predator to feed upon prey, number of feeding sites formed by the predator, duration of feeding and post feeding aggregation. All the parameters were interdependent which were related to feeding behaviour of the predator (Parvez *et al.*, 2000).

2.3.6 Sulphur solubilising rhizobacteria

Survey carried out in Senegal revealed the presence of soluble sulphides due to higher number of sulphur solubilising bacterial cells which effectively excluded *H. oryzae* population in the rhizosphere of mangrove paddies (Fortuner, 1976). The toxicity assays of soluble sulphides produced by sulphur reducing bacteria had a shock effect on *H. oryzae*, *H. spinicaudata* Schuurmans Stekhoven, 1944 and *T. mashoodi* due to sudden contact, but immediate mortality per cent of nematodes was less. However, lethal concentrations of hydrogen sulphide was reached in 3 days, as there was gradual increase in soluble sulphides (particularly hydrogen sulphide) concentrations produced by

the bacteria. At higher concentrations of soluble sulphides all the nematodes had similar sensitivity (Fortuner and Jacq, 1976).

In microplot experiment, the *H. oryzae* population was decreased by the application of sulphur solubilising bacterium (*Desulfotribrio* spp). Mortality rates were directly correlated with population levels of the bacterium and the concentrations of soluble sulphides. The activity of the bacterium was enhanced by flooding the soil, application of mineral sulphur and organic matter resulting in further decrease in nematode population. Bacterium was found to be harmless and increased yield (Jacq and Fortuner, 1979).

2.4 Biochemical, nutritional and physiological responses of VAM fungi inoculated plants

Maize plants inoculated with *G. fasciculatum* were resistant to seedling blight fungus, *Fusarium moniliforme* which was attributed to the increase of total phenol content in mycorrhizal plants (Chhabra *et al.*, 1992).

Increase in chitinase activity in leek (*Allium porrum*) root extracts was observed, when the roots were colonized with *G. versiforme* or *G. intraradices*. In *G. fasciculatum* colonized root extracts of onion (*A. cepa*) four additional bands with chitinase activity was found in 2 dimensional PAGE tests (Dumas Gaudot *et al.*, 1992).

Cotton seeds treated with *G. mosseae*, *G. versiforme* or *Sclerocystis sinuosa* improved plant growth, increased yield and reduced the incidence and disease index of *Verticillium* wilt. In roots and leaves of VAM fungi inoculated plants some new soluble proteins were detected. More than ten types of new proteins were identified as pathogenesis related proteins (PRs) (Liu *et al.*, 1995).

VAM species *Acaulospora morrowae*, *Gigaspora margarita*, *Glomus deserticola*, *G. fasciculatum* and *Scutellospora calospora* when inoculated on *Ziziphus mauritiana* under greenhouse conditions in sterilized soil resulted in the increase of biomass production, nutrient uptake, amino acids, net photosynthesis, total chlorophyll, protein, carotenoid, sugar and starch concentrations. Inoculation also increased stomatal resistance, thereby reducing the rate of transpiration (Mathur and Vyas, 1995a; 1996b; 2000).

Inoculation of vesicular arbuscular mycorrhizae, increased activities of nitrogen metabolism and soluble protein concentrations in leaves and roots of *Ziziphus nummularia*. Enhanced activities of enzymes of importance for nitrogen metabolism and plant defence mechanisms including peroxidase, protein, phenolic and catechin contents of *Z. xylopyrus* were observed after 180 days of VAM fungal inoculation. Increased peroxidase and polyphenol (catechol oxidase) activities of cluster bean was reported in response to VAM fungi application (Mathur and Vyas, 1995b; 1996a; 1997).

Maize genotypes inoculated with *G. fasciculatum* showed increase in net photosynthesis, stomatal conductance and growth, more particularly in the unimproved genotypes (Aguilera Gsmez *et al.*, 1998).

The concentration of total amino acids in root tissues of *G. caledonium* inoculated eucalyptus seedlings increased by 31 per cent than uninoculated plants (Chen *et al.*, 1998).

Colonization of maize roots by *G. fasciculatum* increased vegetative growth, total chlorophyll content and uptake of nutrients ('N', 'P', 'K', 'Ca' and 'Mg'). VAM fungus density of 200 spores per plant was observed optimum for root colonization (Sitaramaiah *et al.*, 1998).

Micropropagated banana plantlets inoculated with *Acaulospora scrobiculata*, *G. clarum* or *G. etunicatum* had increased plant growth and higher rates of photosynthesis and transpiration (Yano Melo *et al.*, 1999).

Inoculation of maize cv. Pioneer 3905 (hybrid) with *G. aggregatum*, *G. etunicatum*, *G. mosseae* and *G. versiforme* resulted in enhancement of shoot and root mass, chlorophyll, soluble protein, total and reducing sugars, carbon, nitrogen concentrations and glutamine synthetase activity. However, the effectiveness of VAM fungi depended upon the species of *Glomus* inoculated (Boucher *et al.*, 1999).

Significant enhancement of 'N', 'P', total chlorophyll, chlorophyll 'a', chlorophyll a:b (ratio) and total sugars was detected in roots of nodulated bean (*Phaseolus vulgaris*) vars. Ceca and Montalban, when inoculated with *G. spurum* strain IES-3 and *G. mosseae* strain IES-8 in low 'P' content soil (Izaguirre Mayoral *et al.*, 2000).

Cotton plants grown from *G. intraradices* treated seeds had lower transpiration rate and improved plant stand. Plant phenology, soil water profile, leaf chlorophyll and leaf blade nutrients were also improved (Makus, 2000).

Growth, photosynthetic pigments, total soluble protein, total free amino acids and nutrient contents were increased in *G. intraradices* no. LAP 8 inoculated sorghum (*Sorghum bicolor*) plants (Abdel Fattah and Mohamedin, 2000).

Increase in the activities of catalase and ascorbate peroxidase were achieved in *Nicotiana tabacum* treated with *G. mosseae*. This indicated the first reaction of the host root cells due to invasion of VAM fungus as a defence response (Blilou *et al.*, 2000).

Mycorrhizal formation by *G. mosseae* in the bark of *Populus beijingensis* infected with *Dothiorella gregaria* (cause canker disease) was positively correlated with relative

turgidity, activities of peroxidase, polyphenol oxidase, availability of phosphorus and total phenol content of the bark, but negatively related to total soluble sugars. Induced peroxidase and polyphenol oxidase activities resulted due to the increase of phenolics (Tang *et al.*, 2000).

Acid phosphatase, alkaline phosphatase, superoxide dismutase and chitinase activities and proteins content in roots of *Pennisetum pedicellatum* plants were increased in the beginning, but declined later as post-treatment effects of *G. mosseae*, *G. aggregatum* and *G. margarita* (Ramesh *et al.*, 2000).

Inoculation of *A. scrobiculata* increased total chlorophyll content and all the plant growth parameters of gerbera (*Gerbera jamesonii*) plants, whereas *G. mosseae* increased plant height, fresh and dry weights of leaves and enhanced 'P', 'K', 'Cu' and 'Zn' content in leaves and 'Ca', 'Cu' and 'Zn' in the roots. *A. scrobiculata* increased only 'P' content in leaves and 'N' content in roots (Petraza Santos *et al.*, 2001).

Colonization of *Pisum sativum* L. (cv. Frisson) roots with *G. mosseae* leads to the induction of four acidic symbiosis-related chitinase (SR-chi) isoforms. The results strongly supported the argument that SR-chi isoform is of plant origin (Slezacek *et al.*, 2001).

2.5 Biochemical, nutritional and physiological responses of *Pseudomonas fluorescens* inoculated plants

Inoculation of *P. fluorescens* to 2 days old soyabean (cv. Hudson) seedlings increased leaf area and photosynthetic rate per pot, but had not affected photosynthetic rate per unit leaf area (Shabaev *et al.*, 1992).

The chlorophyll loss of *Poa pratensis* (Kentucky bluegrass) due to the infection of *Sclerotinia homeocarpa* and *Bipolaris sorokiniana* was prevented by the inoculation of four strains of *P. fluorescens* (Hodges *et al.*, 1994).

Increase in protein content in the intracellular washing fluids (IWF) of leaves of bean (*Phaseolus vulgaris*) infected with *Pseudomonas syringe* pv. *Phaseolicola* was observed, when plants were grown from seeds inoculated with *P. fluorescens*. Certain phenolic compounds, proteins were accumulated in the leaf extracts of protected plants (Alstrvm, 1995).

Wheat (cv. Krasnoyarskaya-83) seedling had decreased rate of transpiration and higher organic nitrogen content when treated with *P. fluorescens*. There was no competition between bacterium and plants for 'N' sources (Pechurkin *et al.*, 1997).

Application of *P. fluorescens* WCS 417r suppressed *Fusarium* wilt of tomato in commercial farming. Bacterial application led to accumulation of pathogenesis related (PR) proteins in plants. This finding ascribed to systemic induced resistance of plants by *P. fluorescens* (Duiff *et al.*, 1998).

Analysis of root exudates revealed enhanced total amount of amino acids in spinach roots, which was colonized by *P. fluorescens*. The relative increase in total amino acids in low salinity soil was more compared to soil with high salinity (Futamata *et al.*, 1998).

Application of *P. fluorescens* (Pf 1) increased chitinase activity and phenolic content in leaves of rice to a maximum after 3 and 4 days of inoculation, respectively. Groundnut plants infected with late leafspot and rust diseases showed increase in phenolic content one day after *P. fluorescens* treatment, and increase in chitinase activity

after four days. There was also accumulation of a 23 KDa thaumatin like protein (TLP) and a 30 KDa Glucanase (Meena *et al.*, 1999; 2000).

In a calcareous soil, lime-tolerance ability of one year old vine of *Vitis vinifera* cv. Pinot blanc grafted on Kober 5BB hybrid was enhanced by the inoculation of *P. fluorescens*. This was attributed to increase of chlorophyll and improvement of 'P', 'K' and 'Fe' mineral nutrition of vines (Bavaresco *et al.*, 2000).

Nitrogen fixation in rhizosphere of winter wheat was improved by inoculation of culture of *P. fluorescens*. The bacterial inoculant also enhanced the uptake of 'N', 'P', 'K', 'Ca', 'Mg', 'Fe', 'Zn', 'Mn' and 'Cu' in the crop (Shabaev and Smolin, 2000).

Application of *P. fluorescens* strains Pf 1 and Fp 7 as bacterial suspension or talc based formulation through seed, root, soil and foliar either individually or in combination (seed + root + soil + foliar) promoted plant growth and yield of rice (cv. IR 50) against the infection of sheath blight fungus (*Rhizoctonia solani*). Increase in activities of chitinase and peroxidase enzymes, led to speculation of induction of systemic resistance of plants against *R. solani* (Nandakumar *et al.*, 2001).

Three fold increase of peroxidase activity was noticed in rice (cv. TN 1) after seed and foliage inoculation of *P. fluorescens* Pf 1 and sharp increase in lignification was reported against the challenge inoculation of *Xanthomonas oryzae* pv. *oryzae* (Vidyasekharan *et al.*, 2001).

2.6 Biochemical, nutritional and physiological response of plants inoculated with combined microbial inoculants

Chlorophyll concentration, photosynthetic rate, nitrate reductase and glutamine synthetase activities and grain yield of wheat (cv. HD 2428) were higher in plants which received combined inoculation of *A. brasilense* and *G. fasciculatum* than individual

inoculation. Under stress, leaf water content, total chlorophyll concentration and nitrate reductase activity were increased and leaf ion leakage was decreased with dual inoculation to wheat (Panwar, 1991; 1993).

Dual inoculation of seed with *P. fluorescens* and *G. mosseae* increased yield and 'N', 'P', 'K', 'Ca', 'Mg', 'Fe', 'Zn', 'Cu', 'Mn' and 'Co' contents of soyabean plant whereas *P. fluorescens* individual inoculation increased only 'K', 'Zn' and 'Mn' concentrations of above ground plant parts (Smolin and Shabaev, 1992).

Combined inoculation of VAM fungi (*G. fasciculatum*, *G. margarita* or *Aucalospora laevis*) with *A. brasilense* enhanced 'N' and 'P' uptake and micronutrients like 'Fe', 'Cu', 'Zn' and 'Mn' of coffee seedlings in nursery beds (Kumari and Balasubramanian, 1993).

Dual inoculation of *P. fluorescens* 20 and *G. mosseae* stimulated nodulation, nitrogen fixation, photosynthesis, nitrogenase activity of nodules and enhanced the amount of 'biological' nitrogen in soyabean. Enhancement of photosynthesis was due to increase in leaf area, whereas plant growth promoting substances increased both nodulation and photosynthetic rate (Shabaev *et al.*, 1996).

Siderophore producing bacterium, *P. fluorescens* when combinedly inoculated with *G. mosseae* reduced chlorosis symptoms of grapevine grafted on susceptible lime rootstocks and improved ferrous iron and chlorophyll concentrations of leaves in calcareous soil (Bavaresco and Fogher, 1996a; 1996b).

Combined application of biocontrol agents viz., *A. lipoferum*, *Azotobacter chroococcum* and *P. fluorescens* to seeds at the ratio of 1:1:1 reduced damping off and root-rot diseases of pea (cv. Little Marvel) and improved nodulation, growth parameters, protein percentage in nodules, seeds and pod yield (Beshir and El Ghany, 1997).

Highest grain yield per plant and protein per cent of plants were observed in maize (cv. Single Cross 10) in response to combined inoculation of *G. macrocarpum* with biofertilizer (HALEX, containing the N₂-fixing bacteria *Azotobacter*, *Azospirillum* and *Klebsiella*) (Radwan, 1998).

Dual inoculation of *Glomus* sp. and *P. fluorescens* increased mineral fertilizer efficiency, grain yield, total and soluble protein and nutrient contents of maize plants as compared to individual inoculations (Attia, 1999).

Six arbuscular mycorrhizal fungi (*G. margarita*, *G. deserticola*, *G. fasciculatum*, *G. monosporum*, *G. mosseae* and *G. versiforme*) inoculated individually, in various factorial combinations with or without *Azospirillum* improved 'N', 'P', 'K' concentrations in 30 days old seedlings of *Tectona grandis* and *Eucalyptus tereticornis*. Dual inoculation of *G. versiforme* or *G. margarita* in conjugation with *Azospirillum* was observed to be the best (Sugavanam *et al.*, 1998; 2000).

Biofertilizers (*Azospirillum*, phosphobacterium) and VAM (*Glomus* sp.) either individually or in combinations improved plant growth and nutrient content of black pepper (Kandiannan *et al.*, 2000).

A synergistic interaction between *G. mosseae* and *B. megaterium* was observed with regard to growth and phosphorus uptake of red clover (*Trifolium pratense*) grown in river sand, but similar result was not obtained when plants were grown in field soil (Qin *et al.*, 2000).

2.7 Biochemical, nutritional and physiological responses of rice plants to phytonematode infections

Many genera of plant parasitic nematodes are associated with rice, but few among them are potential pathogens. Though, they have diverse parasitic habits, all cause

mechanical damage and/or malfunctions of the physiological processes involved in plant development, resulting in alteration of biochemical constituents, nutritional status and vital physiological processes which ultimately leads to poor growth and yield loss (Bridge *et al.*, 1990). On the other hand, plants inflict some resistance through modification of the same as defense mechanisms to parasitic nematodes.

2.7.1 Biochemical response

Among 170 rice varieties tested against *M. graminicola* Golden and Birchfield, 1965, the varieties which were observed to be resistant had definite structure and chemical characteristics of the roots. Resistant varieties had low content of starch, nitrogen, protein and high content of lignin, aspartic acid and alanine (Anon., 1973).

Reducing sugars were found decreased in roots of cv. Pankaj (Peta x Tonkai rotan) at 10 and 30 days after inoculation of *Hoplolaimus indicus* Sher, 1963 but levels of reducing and non-reducing sugars were more than control at 20 days after inoculation. Increase in amino nitrogen and total phenols were observed in all the 3 periods of observations (Ramana and Rao, 1976).

Leaf chlorophyll (total and 'a', 'b' fractions) was reduced by 30-40 per cent in *M. graminicola* infected rice plants, which resulted in leaf chlorosis (Mohanty and Rao, 1978).

There was no detectable difference in total chlorophyll contents of foliage of cv. Jaya among 0, 10, 100, 1000 and 10000 inoculum levels of *H. oryzae* after 37 days of inoculation. Only slight fluctuations of total sugars, reducing sugars, phenols and amino acid levels in shoots and total sugars in roots were observed in plants, receiving 10 and 100 nematode inoculum. But, at higher inoculum levels there were distinct reductions in

total and reducing sugars, phenols and amino acids both in shoot and root in pot experiments (Jayaprakash *et al.*, 1979; 1981).

Reduction in chlorophyll was observed in plants separately infected with three migratory endoparasites viz., *H. mucronata*, *Hoplolaimus indicus* and *Pratylenchus indicus* Das, 1960. Reduction in total and reducing sugars both in shoot and root was observed in *H. indicus* infected plants, whereas these were increased in plants infected with both *H. mucronata* and *P. indicus*. All the three nematode species increased soluble amino acid content, whereas total phenols were increased in plants in response to *H. mucronata* and *P. indicus* (Prasad *et al.*, 1982).

Increase in total phenols and decrease in non-reducing and reducing sugars were observed in cv. IR 50 infected with *H. mucronata*. Reduction in sugar content might be due to lower rate of metabolism of roots following nematode injury, contributing the degradation in the physiological functioning of roots (Mohapatra *et al.*, 1982).

The amount of total phenolic substances was increased about two times in *Hirschmanniella caudacrena* Sher, 1968 infected roots than in healthy roots at 3 and 7 days after inoculation, but the difference among them was reduced at 14 days after inoculation. Catalase activity was two times more in nematode infected roots than the healthy ones only at 3 days after inoculation, thereafter the difference in enzyme activity among them was reduced (Hollis and Keoboonrueng, 1984).

Inoculation of *Heterodera oryzae* Rao and Jayaprakash, 1978 juveniles on cv. Sattari and *M. graminicola* on cv. IR 8 resulted in reduction of leaf chlorophyll and reducing sugars in their shoots and roots after 45 days. Total sugars, protein, IAA, cytokinin and thiamine were reduced in cv. Sattari inoculated with *H. oryzae*, whereas phenols were reduced in *M. graminicola* infected cv. IR 8. There was decrease in soluble

amino acids, abscisic acid (ABA), ribonucleic acids (RNA) and starch observed both in shoots and roots of nematode infected plants of cv. Sattari, on the other hand soluble amino acids, total sugars, protein, deoxyribo-nucleic acid (DNA) and RNA found increased in *M. graminicola* infected variety. These disorders in infected varieties induced typical symptoms of leaf chlorosis, wilting and stunting of plants (Rao *et al.*, 1986; 1988).

Among the varieties MW 10, IR 36, Udaya (resistant), Annapurna and Parijat (susceptible), the chlorophyll 'a' and 'b' fractions were increased in Udaya when infected with 100 B infective juveniles of *M. graminicola*, while there was decrease in chlorophyll fractions in cv. Annapurna (Swain and Prasad, 1988).

Total chlorophyll content of cv. Satia was decreased in response to *Helicotylenchus multicyctus* (Cobb, 1893) Golden, 1956 infection in 15, 25 & 35 days old seedlings, whereas the same was increased in healthy plants. The root oxidising activity was reduced in both infected and healthy plants, but the reduction was more pronounced in uninfected plants which decreased as disease progressed (Dutta *et al.*, 1990).

The amino acids alanine, aspartic acid, serine and methionine were on the increase in cvs. Annada and Udaya roots in response to the inoculation of *M. graminicola* and the increase was more in Udaya than in Annada. In the susceptible cultivar Parijat there was specific reductions in the amino acids (Swain and Prasad, 1991).

Chlorogenic acid levels increased in resistant varieties after inoculation of *Ditylenchus angustus* (Butler, 1913) Filipjev, 1936. A newly formed compound, the phytoalexin Sakuranetin was isolated in resistant cultivar Rayada 16-06. There was a

correlative evidence that chlorogenic acid and phytoalexin (phenolics) had a functional role in the resistance of rice to *D. angustus* (Plowright *et al.*, 1996).

Biochemical changes due to *M. graminicola* infection in *Annapurna* (susceptible) and Manika resistant cultivars recorded increase in the amount of amino acids and total sugars in both the varieties. However, amount of increase of total sugars in resistant variety was more in comparison to the susceptible one. Phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) enzyme activities were more in resistant plants (Mohanty *et al.*, 1997).

Higher initial inoculation (1000 and 2500) of *D. angustus* decreased chlorophyll content and increased total and reducing sugars and total free amino acids contents of cv. Reiho seedlings. In the initial stages of infection, amount of increase of sugars and amino acids were more than the later stages (Ali *et al.*, 1997).

Cellular modifications of rice and tomato roots were observed as defence response to dorylaimids. Ultrastructural observations and biochemical analysis indicated callus-like production, an osmotic imbalance in protein and starch metabolism together with phenolic deposits and increase of lytic processes of roots. These indicated a strong hypersensitive reaction of roots to nematode infection (Leone *et al.*, 1999). Changes in chlorophyll content and relative biochemicals were observed in plants in response to *H. gracilis* infection (Wu, 1999).

Decrease in relative leaf chlorophyll content (RLCC) with increase in cyst density of *Heterodera sacchari* Luc and Merny, 1963 was observed in cv. IDSA 6 under upland conditions both in sandy and clay loam soils. However, RLCC was comparatively more in plants grown in sandy soils (Coyne and Plowright, 2000).

2.7.2 Nutritional response

Nitrogen content of plants was significantly reduced after infection of *H. oryzae*, while in *H. spinicaudata* infected plants it remained unchanged. It appeared that *H. oryzae* and to some extent, *H. spinicaudata* were responsible for the limitation of non-symbiotic nitrogen fixation in submerged rice soil (Rinaudo and Germani, 1981).

Infection of cv. Sattari with *H. oryzicola* and cv. IR 8 with *M. graminicola* (200 infective juveniles) resulted in nutritional disorders within the plants after 45 days of inoculation. Both the nematodes reduced 'N', 'P' and 'Fe' in shoot and root. *M. graminicola* caused a reduction of 'K', 'Mn' and 'Mg' in shoots, but these nutrients were enhanced in roots. There was an increase in 'Ca' and 'Na' levels in both shoot and root of *H. oryzicola* infected plants. Infection of both the nematodes caused chlorosis of foliage limiting the uptake of nitrogen and phosphorus (Rao *et al.*, 1986; 1988).

Total nitrogen in shoots was reduced both at maximum tillering and flowering stages of cv. UPL Ri5 in response to *M. graminicola* infection. Root nitrogen content was found decreased both at flowering and maturity stages and its concentrations were positively correlated with initial inoculum level and the number of juveniles recovered from the roots (Prot *et al.*, 1994).

2.7.3 Physiological response

Reduced photosynthetic rate was observed that in *M. graminicola* infected susceptible varieties (Annapurna and Parijat) than that of resistant varieties (Annada and IR 36). In contrast, a significant increase in photosynthetic rate was observed in the resistant cultivar Udaya (Swain and Prasad, 1989).

In *H. multicinctus* inoculated cv. Satia there was a decrease in photosynthetic rate, but the percentage of reductions gradually decreased as disease progressed (Dutta *et al.*, 1990).

In a glasshouse experiment, significant reductions of net photosynthetic rates on leaf area basis were observed from seventh to forty-second days after inoculation of *D. angustus* (Ali *et al.*, 1997).

In cv. IDSA 6 (*Oryza sativa*) with a mean population density of 39.4 *H. sacchari* eggs per 200 ml soil, it was observed at 81 days after sowing a decrease in physiological parameters like stomatal conductance, leaf water potential and relative water content, resulting in near total yield loss, only leaf chlorophyll was enhanced in diseased plants. In a pot experiment, *H. sacchari* infected plants of cv. CG 14 (*O. glaberrima*) when exposed to five days of imposed draught showed reduction in stomatal conductance and leaf water potential in comparison to only nematode infection, although the variety was drought resistant (Audebert *et al.*, 2000).

2.8 Biochemical, nutritional and physiological responses of plants to phytonematodes in consortium with microbial inoculants

The beneficial effects of *G. etunicatum* on peach infected with *M. incognita* was observed in the form of increased plant growth, which was evident by improvement of foliar 'P', 'Cu' and 'Zn' status of plants. However, the concentrations of these nutrients were more in VAM treated healthy plants (Strobel *et al.*, 1982).

Mycorrhizal inoculation of *G. fasciculatum* was found to reduce *M. incognita* infection in tomato and enhanced concentrations of 'P', 'K', 'Ca', total and reducing sugars, and amino acids *viz.*, glutamic acid, aspartic acid, phenylalanine and serine both

in shoot and root. It was hypothesized that these chemicals had possible roles in the suppression of the development of root-knot nematode (Suresh and Bagyaraj, 1984).

The interaction of *G. fasciculatum* and *R. similis* resulted in higher contents of 'N', 'P', 'K', 'Ca' and 'Mg', reducing and total sugars, phenols and total free amino acids in the roots of banana cv. Dwarf Cavendish (Umesh *et al.*, 1988).

Post-infectional detrimental effects of *M. incognita* were nullified by VAM fungi (*Glomus* spp. and *Gigaspora* spp.) inoculation in Egyptian clover (*Trifolium alexandrinum*) grown in pots. There was an increase in total 'N' and 'P' contents in shoots in response to inoculation of VAM fungi, but were decreased when *M. incognita* was inoculated either with or following the VAM fungi inoculations (Taha and Abdel Kadar, 1990).

Inoculation of *G. fasciculatum* increased lignin and phenol content of tomato (cv. Pusa Ruby) roots infected with *M. javanica* (Singh *et al.*, 1991).

Inoculation of *Glomus* spp. significantly enhanced total 'N' and 'P' contents of common bean (*Phaseolus vulgaris* L.) infected with *M. incognita* in pots, when inoculated 30 days prior to nematode infection. However, 'P' content was decreased when VAM fungi were inoculated along with *M. incognita* (Osman *et al.*, 1991).

Root colonization of *G. mosseae* was not affected by the presence of *P. vulnus* on apple rootstock (FMLA-26). The analysis for nutrients revealed that there was no nutrient deficiency in foliage, although low levels of 'S', 'Mg', 'Mn' and 'Zn' were detected in *P. vulnus* inoculated plants, whereas mycorrhizal plants had the highest levels of 'N', 'P', 'K', 'Na' and 'Fe' (Pinochet *et al.*, 1993).

Nemared peach rootstocks inoculated with *G. intraradices* and *P. vulnus* recorded low levels of 'Ca', 'Mn' and 'Fe' compared to mycorrhizal plants which had the highest value of 'N', 'P', 'Fe' and 'Zn' and increased absorption of 'Ca' and 'Mn'. Copper was found to be the only deficient element in Santa Lucia 64 cherry rootstocks, although low levels of 'Na', 'Mg', 'Mn' and 'Zn' were detected in *P. vulnus* inoculated plants. *G. mosseae* inoculated plants had the highest values of 'Cu' and 'Al'. It was concluded that early mycorrhizal inoculation of rootstock increased plant growth even in the presence of *P. vulnus* (Pinochet *et al.*, 1995a; 1995b).

Low levels of 'Al', 'Fe', 'Mn' and 'Zn' were estimated in non-mycorrhizal, *P. vulnus* infected micropropagated BA 29 quince rootstocks, but *G. intraradices* inoculated plants recorded the highest foliar levels of 'N', 'Ca', 'Mg', 'Mn', 'Cu' and 'Zn'. Mycorrhizal plants infected with *P. vulnus* maintained normal to high levels of 'Mn', 'Cu' and 'Zn' (Calvet *et al.*, 1995).

Seed treatment of talc based formulation of *P. fluorescens* strain Pf RS 1 at 10 g per kg seed enhanced total and ortho dihydroxy phenol contents and chitinase enzyme activity of *H. oryzae* infected rice seedlings (cv. CO 38) after 10 days of inoculation. *P. fluorescens* either individually or in combination with shrimp waste (1 per cent w/w) and neem cake (1 ton per ha) increased ammoniacal and nitrate nitrogen status of *H. oryzae* infected rice plants under field conditions (Swarnakumari, 1996).

Nitrogen, phosphorus and potassium contents of tomato plants were significantly higher prior to simultaneous application of VAM fungus (*G. fasciculatum*) with the nematode, *M. incognita*, than that of nematode alone inoculation (Mishra, 1996; Mishra and Shukla, 1997).

Inoculation of *G. intraradices* and/or *G. mosseae* increased foliar levels of 'N', 'P' and 'Zn' of *P. vulnus* infected micropropagated OHF 333 pear rootstocks, however 'N' and 'P' were deficient in *P. vulnus* infected plants. Early inoculation with mycorrhiza either individually or in combination with nematode were observed to favour rootstock growth and conferred protection against the nematode by inhibiting nematode reproduction and by enhancing plant nutrition (Lspez *et al.*, 1997). Earlier inoculation of *G. intraradices* improved growth and nutrition of micropropagated Grand Naine banana (Musa AAA) infected with *M. javanica* (Pinochet *et al.*, 1997). Application of *G. mosseae* to micropropagated banana plants infected with *M. incognita* exhibited higher levels of 'N', 'P', 'K', 'Ca' and 'Mg' contents compared with non-mycorrhizal plants. Mycorrhizal plants suppressed reproduction and galling of the nematode during early stages of plant development (Jaizme Vega *et al.*, 1997).

Inoculation of *G. fasciculatum* enhanced total phenols, total sugars, total free amino acids and acid phosphatase activity of cowpea (*Vigna unguiculata*) inoculated with *H. cajani*. Biochemical constituents and enzyme (acid phosphatase) activity were maximum in mycorrhizal plants, while they were minimum in diseased plants (Nageswari, 1997).

Activities of oxidative enzymes were increased by the treatments of three strains of *P. fluorescens* isolated from rhizosphere of banana, maize and tomato combinedly infected with *R. similis* and *Meloidogyne* spp. Goeldi, 1892 (Aalten *et al.*, 1998).

A commercial preparation RET-FLO PX 357 (mixture of nitrogen fixing bacteria, humus producing bacteria, moulds and algae) improved 'P', 'Ca' and 'Fe' levels in roots of banana (cv. Dwarf Cavendish) infected with *Helicotylenchus* sp. under field conditions. Sodium concentrations and stomatal conductance of roots were decreased, leading to

improvement of pseudocoelom circumference, number of fingers per hand, and advanced flowering time (Farnandez Falscn *et al.*, 1998).

Leaf chlorophyll and NPK contents of greengram (*Vigna radiata*) plants were enhanced by the application of either *G. fasciculatum* or *Rhizobium phaseoli* (strain M 14) with or without *M. incognita* inoculation (Ray and Dalei, 1998).

Total nitrogen and phosphorus contents of *G. fasciculatum* or *G. mosseae* inoculated and *M. incognita* infected blackgram (*Vigna mungo*) roots were enhanced either individually or in combination with *Rhizobium* spp. (Sankaranarayanan and Rajeswari Sundara Babu, 1998).

Pigeonpea plants inoculated with *H. cajani* and *Fusarium udum* recorded higher phosphorus content when treated with *G. mosseae*, while *P. fluorescens* treatment enhanced P content of only in *F. udum* infected plants (Siddiqui *et al.*, 1998).

Suppression of *R. similis* in banana roots by the application of endomycorrhiza (*G. margarita*, *G. occultum* and *Entrophosphora colombiana*) was correlated with increase in concentrations of nutrients in the foliage, particularly 'Ca' and 'Mg'. It was observed to be the compensatory mechanism to overcome nematode damage (Lugo Urribarrm *et al.*, 2000).

2.9 Histopathological changes of rice roots in response to the infection of rice-root nematode, *Hischmanniella* spp.

All stages of *H. oryzae* caused disruption of cell walls and formation of large cells or cavities in rice roots (Keoboonrueng, 1972).

After penetration, *H. oryzae* positioned near the vascular bundle and formed big cavities in the cortex. The nematode was observed to feed on cortical cells, seldom reaching the central vascular, region and stele (Mathur and Prasad, 1972a; 1972b).

Feeding of *H. caudacrena* on rice roots revealed that nematode made a tunnel almost perpendicular to the rice root surface, however, depth of penetration varied among individual nematodes. During feeding, the nematode usually became oriented parallel to the long axis of the root. In some instances, the nematode moved transversely across the cortex and damaged root parenchyma cells of the cortex. Single nematode feeding in roots had not always disrupted adjacent cells, but when several nematodes were associated with an infection a cavity was always formed that included the nematodes. In some instances, rounded large cells were developed (Hollis and Keoboonrueng, 1984).

Naturally or artificially inoculated rice roots with a Chinese population of *H. oryzae* revealed necrosis along burrowed channels in parenchyma and the nematode was found surrounded by broken and necrotic cells (Vovlas *et al.*, 1996).

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The experimental details and methods adopted to study resistance response of popular high yielding rice cultivars and the growth, yield, physiological, biochemical and nutritional responses of plant to the rice-root nematode, *Hirschmanniella gracilis* in association with microbial inoculants (*Azolla*, *Azospirillum*, blue green algae, phosphobacterium, *Glomus intraradices* and *Pseudomonas fluorescens*) either individually or in combination with *G. intraradices* or *P. fluorescens*, and the reaction of the nematode and histopathological changes of rice roots to the infection of *H. gracilis* are described in this chapter.

3.1 Monoculture of rice-root nematode, *H. gracilis*

The rice-root nematode, *H. gracilis* isolated from rice roots and soil, was multiplied in glasshouse as a monoculture on susceptible rice cultivar, IR 50 and used for all glasshouse investigations.

3.1.1 Extraction of *H. gracilis*

Nematodes required for maintaining monoculture were obtained from samples of rice root and soil collected from the infected fields of Wetland and Paddy Breeding Station attached to Tamil Nadu Agricultural University, Coimbatore. Collected soil samples were processed by Cobb's wet sieving and decantation technique (Cobb, 1918) for isolation, followed by modified Baerman funnel method (Baerman, 1917; Schindler, 1961) for recovery of nematodes. The infected roots were thoroughly washed and cut into small pieces of 8 to 10 mm length (Rao and Nandakumar, 1973). Root pieces were macerated for 15 seconds by using a warring blender (Follis, 1943; Stermerding, 1964). The nematodes from macerated roots were recovered by modified Baerman funnel

Plate 1. The rice-root nematode, *Hirschmanniella gracilis*

- A. Female**
- B. Anterior part**
- C. Female tail**
- D. Vulva**
- E. Male tail**

Plate 1

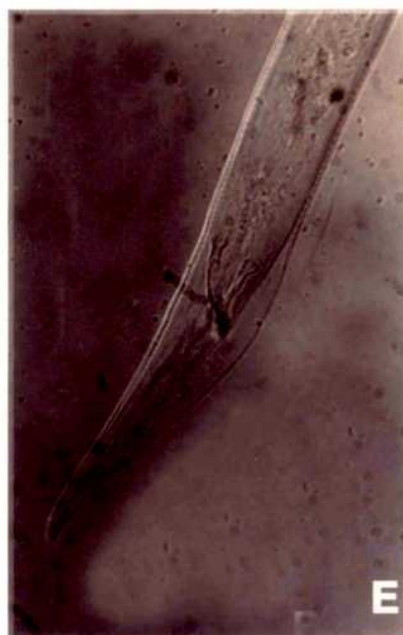
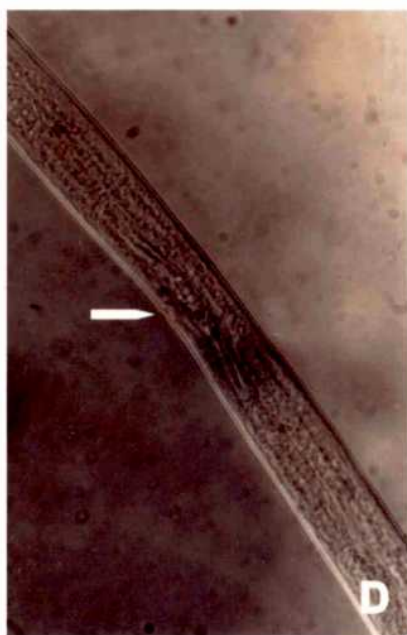
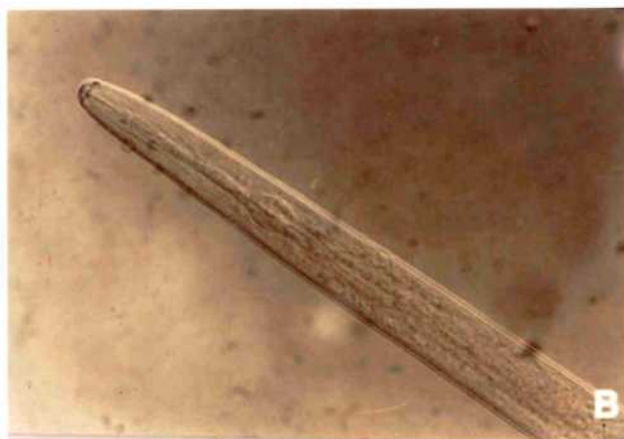
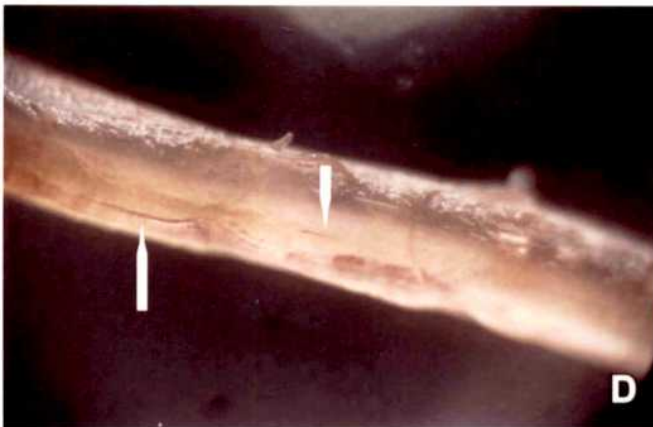
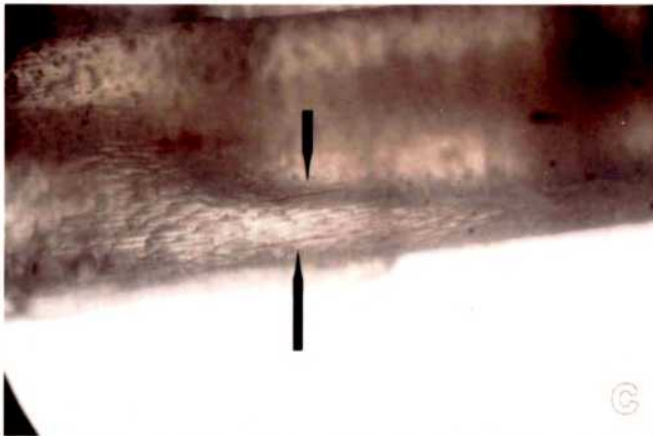
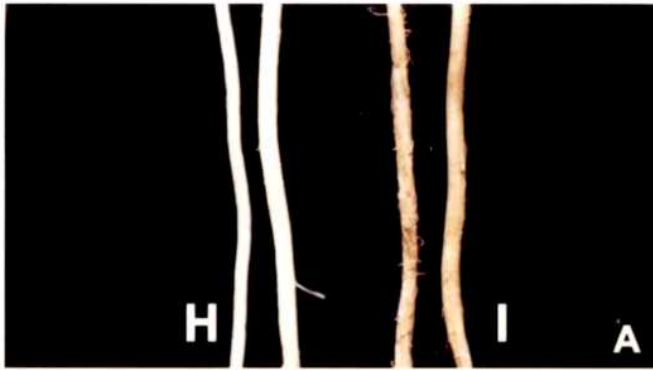


Plate 2. Pathogenicity of the rice-root nematode, *H. gracilis* on rice

- A. Healthy (H) and infected (I) rootlets**
- B. Necrotic lesions on rootlet**
- C & D. Nematodes inside the infected roots**
- E. Healthy (H) and infected (I) root
 systems**

Plate 2



method Nematode population was enumerated after 24 hours with the use of plastic square counting dish under stereozoom microscope (Hooper, 1986)

3.1.2 Surface sterilization of seeds and nematode, and sterilization of soil

Pre-soaked healthy paddy seeds (cv IR 50) were given hot water treatment at 52 – 55°C for 15 minutes (Todd and Atkins, 1959) The treated seeds were surface sterilized by shaking for one minute with 1 per cent liquid detergent, followed by shaking in 0.1 per cent mercuric chloride for 20 minutes, and immediately washed with sterile distilled water (Street and Henshaw, 1966)

The extracted nematodes were surface sterilized by rinsing with sterile distilled water then with 0.1 per cent streptomycin sulphate for fifteen minutes, and finally with sterile distilled water (Mountain, 1955)

Wetland clay soil (clay 43.2%, silt 8.9% and sand 45.6%) was filled in 5 kg capacity earthen pots and were steam sterilized in an autoclave at 20 lb inch⁻² (121°C) for 30 minutes

3.1.3 Maintenance of monoculture

In the pots with sterilized soil, calculated amount of NPK was added Paddy seeds after surface sterilization were sown at the rate of fifteen seeds per pot in five equidistant hills After 7 days, seedlings were thinned to five per pot Incubation of nematodes was done at room temperature for 2 days prior to inoculation Incubated nematodes were inoculated fifteen days after sowing Nematodes were introduced to the rhizosphere through punctured holes by a pipette after thorough agitation of suspension at the rate of 5000 nematodes per pot The pots were regularly irrigated with tap water passed through 350 mesh sieve The nematodes thus multiplied were used as starter inoculum

3.2 Rice varieties

Rice plants of a number of varieties were raised as hosts to *H. gracilis* during the course of investigation. Breeder seeds of these varieties were collected from Paddy Breeding Station, TNAU, Coimbatore.

3.2.1 Cultivars screened for their reaction to *H. gracilis*

A total of ninety-one popular high yielding rice varieties were screened for their reaction to *H. gracilis* in glasshouse (Table 1)

Table 1. High yielding rice varieties screened for their reaction to *H. gracilis*

Sl. No.	Duration	Number of varieties screened	Varieties
1	Short (< 120 days)	27	ADT 7, ASD 3, Basmati 370, CO 9, CO 13, CO 20, CO 21, CO 28, CO 29, CO 31, CO 35, CO 41, IR 32, IR 36, IR 46, IR 48, IR 50, IR 56, IR 58, IR 60, MDU 1, TKM 2, TKM 3, TKM 5, TKM 7, TKM 8, TKM 9
2	Medium (120-150 days)	60	ADT 1, ADT 2, ADT 5, ADT 8, ADT 10, ADT 11, ADT 12, ADT 21, ADT 22, ADT 25, ADT 39, ASD 1, ASD 7, ASD 8, ASD 16, ASD 18, ASD 19, Bhavani, CO 1, CO 2, CO 3, CO 4, CO 6, CO 8, CO 12, CO 16, CO 25, CO 30, CO 38, CO 42, CO 43, CO 44, CO 45, CO 46, IR 20, IR 34, IR 44, IR 54, IR 62, MDU 2, MDU 4, Ponni, PTB 12, PTB 15, PTB 16, PTB 18, PTB 20, PTB 23, PTB 33, PUSA 33, PUSA 167, PUSA 169, PUSA 205, PY 1, PY 2, TKM 1, TKM 6, TKM 10, TKM 11, White Ponni
3	Long (> 150 days)	4	ADT 40, ADT 44, CO 40, CR 1009

3.2.2 Cultivars used as hosts for assessment of microbial inoculants

Two high yielding popular rice varieties susceptible to *H. gracilis*, viz, IR 50 (short duration) and ADT 44 (long duration) were grown as hosts both in glasshouse and field to evaluate the relative performance of microbial inoculants, and their combinations against the infection of *H. gracilis*. Various responses viz, physiological, biochemical and nutritional status of these cultivars were also analysed.

3.3 Microbial inoculants

Azolla, *Azospirillum*, blue green algae, phosphobacterium, VAM fungus (*G. intraradices*) and *P. fluorescens* were evaluated for their effectiveness in the suppression of *H. gracilis* and improving plant growth either individually or in combination with *G. intraradices* or *P. fluorescens*.

Azolla (*A. microphylla*) was obtained from culture raised in Wetland, commercial formulations of *Azospirillum* (*A. lipoferum* AZ 204), phosphobacterium (*Bacillus megaterium* var *phosphaticum* PB 1) and VAM fungus (*G. intraradices*) were collected from Department of Microbiology, blue green algae (heterogenous mixture of genera *Aulosira*, *Tolypothrix*, *Scytonema*, *Nostoc*, *Anabaena* and *Plectonema*) was obtained from *Azolla* Laboratory, and commercial formulation of *P. fluorescens* Pf 1 was collected from Department of Plant Pathology, TNAU, Coimbatore. The microbial inoculants were treated as per the guideline prescribed in Crop Production Guide, 1999 (Anon, 1999).

3.3.1 *Azolla*

A. microphylla was applied to main field at the rate of 250 kg ha⁻¹ after 5 days of transplanting. It was allowed to be raised as a dual crop along with rice and trampled during subsequent hand weedings.

3.3.2 *Azospirillum*

A. lipoferum AZ 204 M culture containing 1×10^8 cells g^{-1} of lignite based carrier on dry weight basis was used as an inoculum. It was used as seed treatment, seedling root dip, and broadcast in the main field. For seed treatment, 0.6 kg ha^{-1} of the inoculum was uniformly mixed with 1250 ml of rice gruel and made into a slurry. The gruel mixed seeds were thus treated with *A. lipoferum*. Treated seeds were shade-dried for 30 minutes over a polythene sheet and then soaked overnight in sufficient quantity of water. Excess bacterial suspension was poured over the designated nursery beds. *A. lipoferum* treated seedlings were subjected to bare root dip for 30 minutes just before transplanting. Root portions of seedlings were dipped in bacterial suspension of 1.0 kg ha^{-1} in 40 litres of water. Excess bacterial suspension was poured over the prescribed net plots in main field. Two kg of the commercial formulation of the bacterium was mixed uniformly with finely powdered 25 kg farm yard manure for one hectare and broadcasted over the prescribed plots in the main field before transplanting.

3.3.3 Blue green algae

Dried and powdered, soil based composite starter culture of blue green algae flakes was uniformly broadcasted over the ear-marked plots in main field at the rate of 10 kg ha^{-1} after 10 days of planting. A thin film of water was maintained over the plots after treatment.

3.3.4 *Phosphobacterium*

Lignite based carrier containing 3 to 4×10^8 cells g^{-1} of *B. megaterium* on dry weight basis was uniformly broadcasted over the prescribed plots in main field at the rate of 2 kg ha^{-1} along with equal amount of sand before transplanting.

3.3.5 VAM fungus

G. intraradices cultured on maize roots having 85 to 88 per cent colonization with vermiculite as carrier was used as inoculant. One hundred gram of dry commercial formulation contained about 220 to 240 chlamydospores. VAM fungus was broadcasted at the rate of 50 g m⁻² over the ear-marked nursery beds before sowing. The inoculant was thoroughly mixed with equal quantity of sand before application.

3.3.6 *Pseudomonas fluorescens*

Talc based formulation of *P. fluorescens* containing 2.5×10^8 c.f.u. g⁻¹ on dry weight of carrier was used for seed treatment. The seeds were soaked overnight in bacterial suspension containing 10 g formulation in one litre of water per kg of seed. Excess bacterial suspension was decanted and applied uniformly over the respective nursery beds. The treated seeds were allowed to sprout for twenty-four hours before sowing.

3.4 Screening of popular high yielding rice varieties for their reaction to the rice-root nematode, *H. gracilis*

A pot culture experiment was conducted in glasshouse to study the varietal reaction of high yielding popular rice cultivars against *H. gracilis*.

3.4.1 Methodology

A total of ninety-one high yielding rice (27 short, 60 medium and 4 long duration) varieties was screened (Table 1). The seeds were sown in one kg capacity pots containing steam sterilized wetland clay soil. Three sterilized seeds per pot were sown under aseptic conditions. Variety IR 50 was used as susceptible check. After seven days of sowing, the seedlings were thinned to one per pot. Each pot was inoculated with sterilized nematodes at 1000 per pot after fifteen days of sowing. Screened rice varieties were uprooted at their maximum tillering stages viz., at 45 days for short, 60 days for medium and 90 days after

sowing for long duration. Each variety was replicated five times. The experiment was arranged in completely randomised block design.

3.5 Studies on management of the rice-root nematode, *H. gracilis* with microbial inoculants

Two pot culture and three field experiments were carried out to study the effect of microbial inoculants either individually or in combinations with *G. intraradices* or *P. fluorescens* on the growth and yield of rice cvs IR 50 and ADT 44 infected with *H. gracilis*, and suppression of nematode population in root, and rhizosphere of plants.

3.5.1 Pot culture experiments

Pot culture experiments were conducted on cvs IR 50 and ADT 44 simultaneously in the Nematology glasshouse with same treatments.

Earthen pots of five kg capacity were filled with wetland clay soil. Designated seeds were treated with *A. lipoferum*, *P. fluorescens* and their combination. *G. intraradices* and carbofuran 3G were applied to respective pots prior to sowing. Treated and untreated seeds were sown in the respective pots at the rate of fifteen per pot at five equidistant hills (three per hill). Freshly extracted nematodes were incubated at room temperature for two days. Inoculation of nematodes were conducted just after sowing of seeds, at the rate of 5000 per pot. The required nematode suspension was evenly poured over the pots. A thin layer of freshly autoclaved clay soil was spread on top after inoculation. The seedlings were thinned to ten (two per hill) after seven days of sowing.

After 30, and 35 days of sowing, the seedlings of cv IR 50 and cv ADT 44 respectively were uprooted. Five average sized seedlings were selected from each pot. Seedlings grown from *A. lipoferum* treated seeds were root dipped in the same bacterial

suspension for 30 minutes sufficient enough for dipping of root portion. Excess bacterial suspension was poured over the respective pots. The selected seedlings were planted in the respective hills (one per hill). Before planting, *A. lipoferum*, *B. megaterium* and carbofuran 3G were applied to the specific pots. *A. microphylla* and blue green algae were treated in the designated pots respectively after 5 and 10 days after planting.

Necessary agronomic practices were followed during the period of study. The experiments were terminated during panicle initiation stage i.e. 75 and 120 days after sowing for cv. IR 50 and cv. ADT 44 respectively.

The experiments consisted of following eighteen treatments, replicated three times, and arranged in a completely randomised block design.

- T₁ - *Azolla* (*A. microphylla*) pot application (625 mg pot⁻¹)
- T₂ - *Azospirillum* (*A. lipoferum*) [seed treatment (1.5 mg pot⁻¹ seed) + seedling root dip (2.5 mg pot⁻¹ seedling) + pot application (5.0 mg pot⁻¹)]
- T₃ - Blue green algae pot application (25 mg pot⁻¹)
- T₄ - Phosphobacterium (*B. megaterium*) pot application (5.0 mg pot⁻¹)
- T₅ - VAM fungus (*G. intraradices*) pot application (1.25 g pot⁻¹)
- T₆ - *Azolla* (*A. microphylla*) pot application (625 mg pot⁻¹) + VAM fungus (*G. intraradices*) pot application (1.25 g pot⁻¹)
- T₇ - *Azospirillum* (*A. lipoferum*) [seed treatment (1.5 mg pot⁻¹ seed) + seedling root dip (2.5 mg pot⁻¹ seedling) + pot application (5.0 mg pot⁻¹)] + VAM fungus (*G. intraradices*) pot application (1.25 g pot⁻¹)
- T₈ - Blue green algae pot application (25 mg pot⁻¹) + VAM fungus (*G. intraradices*) pot application (1.25 g pot⁻¹)
- T₉ - Phosphobacterium (*B. megaterium*) pot application (5.0 mg pot⁻¹) + VAM fungus (*G. intraradices*) pot application (1.25 g pot⁻¹)
- T₁₀ - *P. fluorescens* seed treatment (10 mg g⁻¹ seed)

- T₁₁ - *Azolla* (*A. microphylla*) pot application (625 mg pot⁻¹) + *P. fluorescens* seed treatment (10 mg g⁻¹ seed)
- T₁₂ - *Azospirillum* (*A. lipoferum*) [seed treatment (1.5 mg pot⁻¹ seed) + seedling root dip (2.5 mg pot⁻¹ seedling) + pot application (5.0 mg pot⁻¹)] + *P. fluorescens* seed treatment (10 mg g⁻¹ seed)
- T₁₃ - Blue green algae pot application (25 mg pot⁻¹) + *P. fluorescens* seed treatment (10 mg g⁻¹ seed)
- T₁₄ - Phosphobacterium (*B. megaterium*) pot application 5.0 mg pot⁻¹) + *P. fluorescens* seed treatment (10 mg g⁻¹ seed)
- T₁₅ - VAM fungus (*G. intraradices*) pot application (1.25 g pot⁻¹) + *P. fluorescens* seed treatment (10 mg g⁻¹ seed)
- T₁₆ - Carbofuran 3G pot application (109.4 mg pot⁻¹ at the time of sowing and 40.6 mg pot⁻¹ at the time of planting)
- T₁₇ - Untreated control
- T₁₈ - Uninoculated control

3.5.1.1 Observations recorded

Four average sized plants were selected from each pot. Two plants were used as samples for biometric observations and other two for nematode population estimation in root and the mean was worked out. The samples were cleaned with running water before taking observations. Population of nematodes in root and soil were estimated after randomly selecting one g root and 200 g soil samples from each pot by quartering method. Following observations were recorded during the course of study.

3.5.1.1.1 Biometric observations of rice plants

Plant growth parameters such as shoot and root length (cm), total leaf area (cm²), root volume (cc), fresh and dry shoot and root weights (g) and number of tillers per plant were recorded by adopting standard procedures (Gomez, 1972).

Total leaf area (cm²) – Total leaf area was calculated as per the method suggested by Palanyswami and Gomez (1974)

$$LA = L \times W \times K \times n$$

where,

- LA = Total leaf area
- L = Length of the third leaf from top of the middle tiller
- W = Maximum width of the same leaf blade
- K = A constant factor (0.75)
- n = Total number of leaves of the plant sample

Root volume (cc) – Root volume of the samples was measured by water displacement method. The total root system of the sample was submerged in a completely filled 500 ml beaker kept inside a pan. The dispersed water was collected in the pan measured by means of a measuring cylinder and root volume was estimated.

3.5.1.1.2 Estimation of nematode population

Nematode population per g root and per 200 g soil were enumerated.

3.5.2 Field experiments

Three field experiments were conducted in Wetland, TNAU, Coimbatore. Two experiments were carried out on cv IR 50 during summer and *kharif* season, 2001 and one experiment on cv ADT 44 during summer, 2001.

3.5.2.1 Field location

In summer the experiments were conducted in 'E' Block (Field no 3) on cv IR 50 (February-June) and cv ADT 44 (February-July) and during *kharif* in 'J' Block (Field no 2) on cv IR 50 (June-October). The nursery beds were raised for all the three experiments in 'F' Block (Field no 12).

Table 2. Monthly average weather data during the period of field experiments (summer and *kharif*, 2001)

Month (2001)	Temperature (°C)		Relative humidity (%)		Total rainfall (mm)	Number of rainy days	Sunshine (h d ⁻¹)	Evaporation (mm d ⁻¹)	Wind velocity at 8 feet (km h ⁻¹)	Solar radiation (Cal cm ⁻² d ⁻¹)
	Max.	Min.	0722 h	1422 h						
February	33.5	20.0	87	37	--	--	82	5.5	4.0	425.5
March	35.3	22.1	80	35	--	--	88	6.8	4.9	412.2
April	34.7	23.6	87	42	96.0	5	70	5.6	4.0	380.9
May	35.0	23.5	82	44	6.5	1	86	6.8	8.5	427.5
June	31.2	22.1	78	52	52.6	5	49	6.9	13.0	367.6
July	31.2	22.7	80	54	19.9	3	56	6.8	11.0	371.9
August	31.4	22.1	85	53	22.8	6	57	6.0	8.7	354.7
September	32.7	22.3	89	50	96.6	10	68	4.9	5.0	371.0
October	31.0	22.1	90	61	286.7	6	53	4.3	3.6	335.1

3.5.2.2 Soil properties

The soil of the experimental fields were moderately well drained, low to medium in depth, deep clay loam, classified taxonomically as Typic Haplustalf with low to medium in available 'N', medium in available 'P' and neutral to high in available 'K'

3.5.2.3 Weather and climate

The monthly mean weather data during the period of study are furnished in Table 2

3.5.2.4 Treatments and design

A total of seventeen treatments were imposed for each experiment. Each treatment was replicated three times. Experiments were arranged in randomised block design. The treatments imposed for the study were based on the prescribed guideline given in the Crop Production Guide, 1999 (Anon, 1999) are as follows

- T₁ - *Azolla* (*A. microphylla*) main field application (250 kg ha⁻¹)
- T₂ - *Azospirillum* (*A. lipoferum*) [seed treatment (0.6 kg ha⁻¹) + seedling root dip (1 kg ha⁻¹) + main field application (2 kg ha⁻¹)]
- T₃ - Blue green algae main field application (10 kg ha⁻¹)
- T₄ - *Phosphobacterium* (*B. megaterium*) main field application (2 kg ha⁻¹)
- T₅ - VAM fungus (*G. intraradices*) nursery bed application (50 g m⁻²)
- T₆ - *Azolla* (*A. microphylla*) main field application (250 kg ha⁻¹) + VAM fungus (*G. intraradices*) nursery bed application (50 g m⁻²)
- T₇ - *Azospirillum* (*A. lipoferum*) [seed treatment (0.6 kg ha⁻¹) + seedling root dip (1 kg ha⁻¹) + main field application (2 kg ha⁻¹)] + VAM fungus (*G. intraradices*) nursery bed application (50 g m⁻²)
- T₈ - Blue green algae main field application (10 kg ha⁻¹) + VAM fungus (*G. intraradices*) nursery bed application (50 g m⁻²)
- T₉ - *Phosphobacterium* (*B. megaterium*) main field application (2 kg ha⁻¹) + VAM fungus (*G. intraradices*) nursery bed application (50 g m⁻²)

- T₁₀ - *P. fluorescens* seed treatment (10 g kg⁻¹ seed)
- T₁₁ - *Azolla* (*A. microphylla*) main field application (250 kg ha⁻¹) + *P. fluorescens* seed treatment (10 g kg⁻¹ seed)
- T₁₂ - *Azospirillum* (*A. lipoferum*) [seed treatment (0.6 kg ha⁻¹) + seedling root dip (1 kg ha⁻¹) + main field application (2 kg ha⁻¹)] + *P. fluorescens* seed treatment (10 g kg⁻¹ seed)
- T₁₃ - Blue green algae main field application (10 kg ha⁻¹) + *P. fluorescens* seed treatment (10 g kg⁻¹ seed)
- T₁₄ - Phosphobacterium (*B. megaterium*) main field application (2 kg ha⁻¹) + *P. fluorescens* seed treatment (10 g kg⁻¹ seed)
- T₁₅ - VAM fungus (*G. intraradices*) nursery bed application (50 g m⁻²) + *P. fluorescens* seed treatment (10 g kg⁻¹ seed)
- T₁₆ - Carbofuran 3G [(nursery bed application 3.5 kg ha⁻¹) + main field application (16.25 kg ha⁻¹)]
- T₁₇ - Untreated control

Ten and 20 g of *A. lipoferum* was used for seed treatment per kg of seed of cv IR 50 and cv ADT 44 respectively

3.5.2.5.1 Nursery management

Nursery beds of 3 x 1 m² were laid out for each treatment. The nursery area was flooded two days prior to ploughing and water was allowed to soak. The soil was kept under shallow submergence. The area was ploughed and brought to puddle condition. Bunds were raised around the demarcated nursery plots and channels were made for providing irrigation. Dry and well pulverised farm yard manure was spread uniformly at the rate of 1000 kg ha⁻¹. *G. intraradices* and carbofuran 3G were treated in the ear-marked beds.

Seed rates of 60 kg ha⁻¹ (cv IR 50) and 30 kg ha⁻¹ (cv ADT 44) were adopted. *A. lipoferum* and *P. fluorescens* seed treatments were carried out on pre-soaked seeds. The sprouted seeds were sown uniformly with a film of water on the beds.

The nursery beds were fertilized with 40 kg ha⁻¹ di-ammonium phosphate after 20 days of sowing. Irrigation was provided regularly to keep the seedlings in a thin layer of water after germination.

A total of eight treatments were imposed. Each bed was equally divided into three parts representing each replication. The nursery beds were laid out in randomised block design.

Treatments

- T₁ - *Azospirillum* (*A. lipoferum*) seed treatment (10 g kg⁻¹ of seed for cv IR 50 or 20 g kg⁻¹ of seed for cv ADT 44)
- T₂ - VAM fungus (*G. intraradices*) nursery bed application (50 g m⁻²)
- T₃ - *P. fluorescens* seed treatment (10 g kg⁻¹ seed)
- T₄ - *Azospirillum* (*A. lipoferum*) seed treatment (10 g kg⁻¹ for cv IR 50 or 20 g kg⁻¹ seed for cv ADT 44) + VAM fungus (*G. intraradices*) nursery bed application (50 g m⁻²)
- T₅ - *Azospirillum* (*A. lipoferum*) seed treatment (10 g kg⁻¹ for cv IR 50 or 20 g kg⁻¹ seed for cv ADT 44) + *P. fluorescens* seed treatment (10 g kg⁻¹ seed)
- T₆ - VAM fungus (*G. intraradices*) nursery bed application (50 g m⁻²) + *P. fluorescens* seed treatment (10 g kg⁻¹ seed)
- T₇ - Carbofuran 3G nursery bed application (4 735 g m⁻²)
- T₈ - Untreated control

Observations

After 30 days of sowing, twenty seedlings were uprooted randomly avoiding boundary seedlings from each replication and washed carefully in water. Ten samples were assessed for plant growth performance and other ten processed for estimation of nematode population in root. The mean was worked out for each treatment. Soil samples

Figure 1. Management of the rice-root nematode, *H. gracilis* with microbial inoculants on cvs. IR 50 and ADT 44 (Main field layout during Summer, 2001)

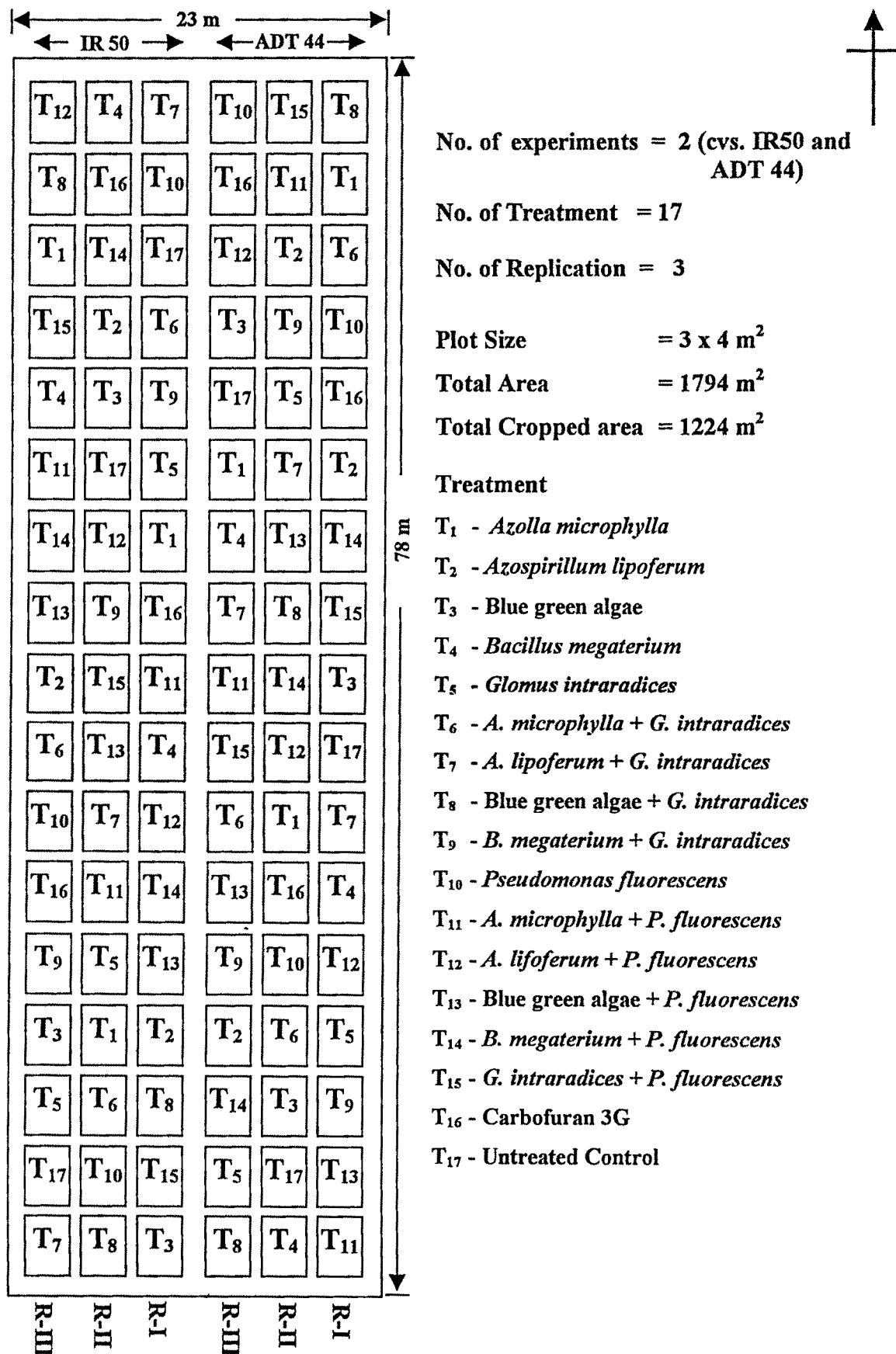
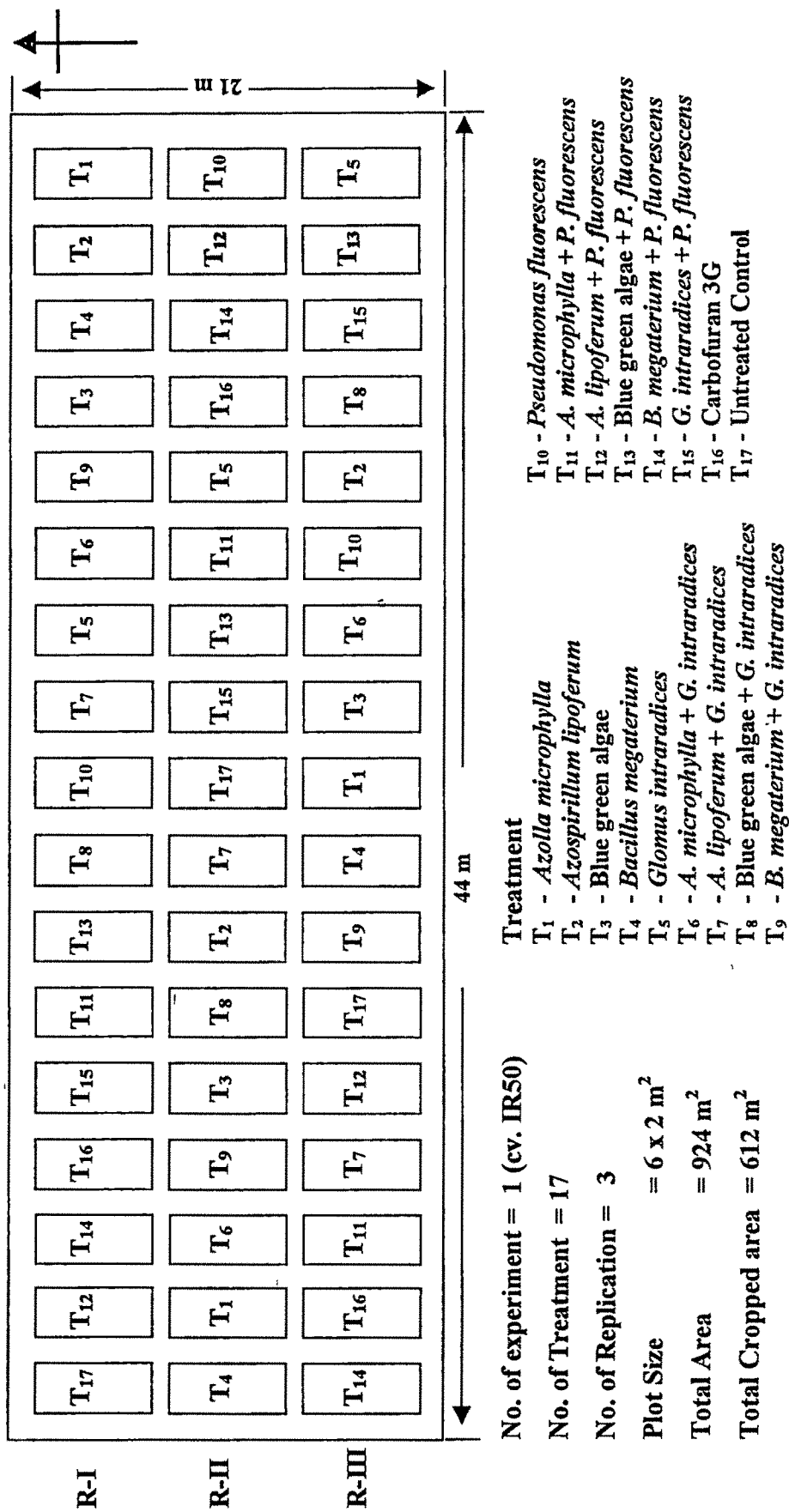


Figure 2. Management of the rice-root nematode, *H. gracilis* with microbial inoculants on cv. IR 50
(Main field layout during *Kharif*, 2001)



were collected randomly from each replicated area and made to 200 g by quartering method. Soil samples were processed for enumeration of nematode population.

3.5.2.5.2 Main field preparation

The selected main fields were thoroughly ploughed with mould board plough and after final ploughing and levelling, plots were laid out of net size 4 x 3 m² during summer (Figure 1) and 6 x 2 m² during *kharif* season (Figure 2). Plots were demarcated with raised bunds. Channels of 0.5 m width were provided among the plots and 1 m around the plots representing irrigation channels.

Fertilization prior to planting

Grounded dry farm yard manure was broadcasted at the rate of 12.5 tonnes ha⁻¹. Nitrogen, phosphorus and potassium fertilizers were applied in the form of urea (46% N), single super phosphate (16% P₂O₅) and muriate of potash (60% K₂O) respectively. In total, for cv IR 50 120 38 38 and for cv ADT 44 150 50 50 NPK kg ha⁻¹ were applied. Before transplanting at 30 kg 'N', 38 kg 'P' and 19 kg 'K' per ha for cv IR 50 and at 25 kg 'N', 50 kg 'P' and 25 kg 'K' per ha for cv ADT 44 were applied as basal dose.

Treatments

Respective net plots were treated with *A. lipoferum*, *B. megaterium* and carbofuran 3G by uniform broadcasting.

Thirty and 35 days old seedlings of cv IR 50 and cv ADT 44 respectively were pulled out of nursery for transplanting. Seedlings of *A. lipoferum* treated seeds were subjected to bare root dip for 30 minutes just before planting.

Planting was carried out in main fields with 2 cm of standing water over the plots

Following specifications were adhered to during planting

Specification	cv. IR 50	cv. ADT 44
Number of seedling (hill ⁻¹)	3	2
Depth of planting (cm)	3	3
Spacing (cm ²)	12.5 x 10	20 x 15
Number of hills (m ⁻²)	80	33

After transplanting recommended agronomic practices were followed

Fertilizer application

Rest of the nitrogen and potassium fertilizers was applied in subsequent split doses as follows

Variety	Days after sowing	Nitrogen (kg ha ⁻¹)	Potassium (kg ha ⁻¹)
IR 50	35	30	-
	50	30	19
	70	30	-
ADT 44	40	25	-
	55	25	-
	70	25	-
	95	25	25
	115	25	-

Treatments

A. microphylla was broadcasted over the designated plots to be raised as dual crop after 5 days of planting. Blue green algae was broadcasted after 10 days of planting. Standing water was maintained at the time of imposition of both the treatments.

Irrigation

Up to seven days of planting 2 cm of water level was maintained. After this establishment stage, cyclic 5 cm submergence of water was continued throughout the crop period. Irrigations were provided immediately before disappearance of water from field. Irrigation was stopped 10 days prior to harvest.

Gap filling

Casualty replacement was carried out on seventh (cv IR 50) and tenth (cv ADT 44) day of planting.

Weeding

Hand weeding was carried out twice (40 and 80 days after planting) in cv IR 50 and thrice (40, 80 and 120 days after planting) in cv ADT 44.

Harvesting and thrashing

Plot wise harvesting of cv IR 50 and cv ADT 44 was performed at 110, and 155 days after sowing and was carried out when more than 80 per cent of the panicles turned straw colour. Harvested grains and straw were sun dried separately to remove excess moisture.

3.5.2.5.3 Observations recorded

Biometric observations

Plant growth parameters were recorded at panicle initiation stage (75 days after sowing for cv IR 50 and 120 days for cv ADT 44). Five hills from each plot were randomly selected and tagged, avoiding boundary plants. The plant growth characters were recorded after uprooting and cleaning.

The leaf area index (LAI) per hill was calculated using the following formula (Williams, 1946)

$$\text{LAI} = \frac{\text{Total leaf area per hill}}{\text{Area of the land occupied by the hill}}$$

Yield components

The yield attributes were recorded at maturity stage, 105 days and 150 days after sowing for cv IR 50 and cv ADT 44 respectively. The selected and tagged hills (5 per plot) were subjected to various observations

- i Number of panicles (hill^{-1})
- ii Panicle length (cm) – five panicles randomly selected from middle tillers and their lengths were measured
- iii Number of filled grains (panicle^{-1}) – Filled grains were counted after discarding chaffy grains from five panicles of middle tillers
- iv Thousand grain weight (g) – Randomly selecting 1000 grains from middle tillers, their weights were recorded

Yield

The thrashed grains and straw bundles were air dried. Grain and straw yield (t ha^{-1}) were recorded at 14 per cent moisture level

Nematode population estimation

Nematode population in root and rhizosphere soil were recorded at panicle initiation stage and at harvest

3.6 Physiological, biochemical and nutritional responses of cvs. IR 50 and ADT 44 to the infection of rice-root nematode, *H. gracilis* in consortium with microbial inoculants

Plants from the two pot culture experiments (cvs IR 50 and ADT 44) conducted at Nematology glasshouse were subjected to various physiological, biochemical and nutritional responses of *H. gracilis* infected rice crops

3.6.1 Observations recorded

All the physiological, biochemical and nutritional parameters were studied during panicle initiation stage (75 days after sowing for cv IR 50 and 120 days for cv ADT 44)

3.6.1.1 Physiological parameters

Vital gas exchange parameters such as leaf temperature ($^{\circ}\text{C}$), transpiration rate ($\mu\text{g cm}^{-2} \text{sec}^{-1}$), photosynthetic active radiation ($\mu\text{E m}^{-2} \text{sec}^{-1}$) and diffusive resistance (sec cm^{-1}) were measured with the steady state porometer (LICOR 1600, Model LI 301 COR Inc, USA make) Observations were recorded between 10 00 to 11 00 A M under glasshouse temperature of 36°C The third leaves of the middle tiller of plants were fed to the hum set of the instrument after null adjustment The observations were recorded directly by shifting the head switch, from one point to the other (Sen *et al*, 1989)

3.6.1.2 Biochemical attributes

Quantitative estimation of biochemical components such as total free amino acids, soluble proteins, total and reducing sugars, total and ortho dihydroxy (OD) phenols and chlorophyll 'a', chlorophyll 'b' and total chlorophyll were carried out following the standard procedures Absorbance was measured using Hitachi 200-20 spectrophotometer as and when required

3.6.1.2.1 Total free amino acids

The amount of total free amino acids present in root samples were estimated, following the method described by Lee and Takahashi (1966)

Reagents

Citrate buffer of 0.5 M (pH 5.5) – Buffer was prepared by dissolving 1.44 g citric acid and 5.34 g sodium citrate in 100 ml water

Ninhydrin-citrate-glycerol reagent – It was prepared by mixing 25 ml of ninhydrin solution (one g ninhydrin in 100 ml of citrate buffer, pH 5.5) with 60 ml glycerol in 10 ml 0.5 M citrate buffer (pH 5.5). The final pH of the mixture was adjusted to 5.5.

Standard solution

One hundred mg leucine was dissolved in 100 ml of distilled water. From this stock solution 10 ml was pipetted out and diluted to 100 ml for preparing working standards. One ml of working standard solution contained 100 µg leucine.

Procedure

One gram of root sample from each replication was added to ten ml of 80 per cent ethanol for extraction and macerated using pestle and mortar. Root extract of 0.1 ml was evaporated and the residue dissolved in 1 ml of distilled water. To this, 4 ml of ninhydrin-citrate-glycerol reagent was added and thoroughly mixed. The tubes were heated for 12 minutes in a boiling water bath. Then the tubes were cooled to room temperature. The purple colour developed after 15 minutes was read at 570 nm against a reagent blank using spectrophotometer within one hour of development of colour.

From the standard graph of various concentrations of leucine and their respective absorbance values, the amount of total free amino acids present in the samples were estimated. Total free amino acids in the samples were expressed as percentage equivalent of leucine.

3.6.1.2.2 Soluble proteins

Estimation of soluble proteins was carried out following the procedure of Lowry *et al* (1951)

Reagents

Phosphate buffer (pH 7.0) – Sixty-one ml of 0.2 M dibasic sodium phosphate was mixed with 39 ml 0.2 M monobasic sodium phosphate and the solution was diluted to a total volume of 200 ml

Alkaline-copper reagent – Fifty ml of 2 per cent sodium carbonate in 0.1 N sodium hydroxide (solution A) was mixed with one ml 0.5 per cent copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in 1 per cent potassium sodium tartarate (solution B)

Folin-Ciocalteu reagent – A mixture consisting of 100 g sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$), 25 g sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$), 700 ml distilled water, 50 ml of 85 per cent phosphoric acid, and 100 ml of concentrated hydrochloric acid was refluxed for 10 hours in a 1.5 litre flask. To this mixture 150 g lithium sulphate, 50 ml water and two drops of bromine water was added. The mixture was boiled for 15 minutes without condenser to remove excess bromine and then cooled. Later on, it was diluted to 1 litre followed by filtration. The reagent (0.5 ml) was titrated against 1 N sodium hydroxide using phenolphthalein as indicator. The acidity of the solution was calculated and diluted to 1N in acid, for use in protein estimation.

Standard solution

Bovine serum albumin (Fraction V) 50 mg was mixed in distilled water and volume was made up to 50 ml, served as stock solution. The stock solution was diluted to 50 ml in distilled water for preparation of working standard solution. One ml of this working standard solution contained 200 μg of soluble protein.

Procedure

One hundred mg of sample was macerated in pestle and mortar with 10 ml of phosphate buffer (pH 7.0) for extraction, followed by centrifugation at 3000 rpm for 10 minutes. From this 0.5 ml supernatant was taken and 5 ml of alkaline-copper reagent was added. The solutions were mixed thoroughly and allowed to stand for 10 minutes. Then, 0.5 ml Folin-Ciocalteu reagent was added and incubated at room temperature in dark for 30 minutes. Blue colour was developed indicating the presence of soluble protein. The absorbances were read at 660 nm against the reagents blank using a spectrophotometer.

From the standard graph drawn by plotting absorbance values against series of concentrations, the quantity of soluble protein present in the samples was calculated.

3.6.1.2.3 Total sugars

The amount of total sugars present in the root samples was estimated by Anthrone method (Hedge and Horreiter, 1962).

Reagent

Anthrone reagent – Two hundred mg of anthrone was dissolved in 100 ml of ice cold 95 per cent sulphuric acid.

Standard solution

One hundred mg of glucose was dissolved in 100 ml of distilled water to prepare stock solution. From stock solution, 10 ml was pipetted out and diluted to 100 ml with distilled water to make working standard. One ml of this working standard solution contained 100 µg of glucose.

Procedure

Powdered dry root sample of 100 mg was homogenised in pestle and mortar with three ml of 70 per cent aqueous ethyl alcohol for extraction. From this extract, 0.5 ml was pipetted out and kept in boiling water bath for complete evaporation of the solvent. One ml distilled water was added to dissolve the sugars and to it freshly prepared 4 ml of Anthrone reagent was added. Heated for eight minutes in a boiling water bath and then, cooled rapidly. Green to dark green colour developed was read at 630 nm using a spectrophotometer against the reagent blank.

By plotting a series of concentration of glucose and their absorbance values, a standard graph was prepared and from it amount of total sugars present in the samples were enumerated. Total sugars present in the samples were expressed as per cent equivalent of glucose.

3.6.1.2.4 Reducing sugars

The amount of reducing sugars present in the root samples was estimated by Nelson-Somogyi method (Somogyi, 1952).

Reagents

Alkaline-copper-tartrate reagent – Anhydrous sodium carbonate (2.5 g), sodium bicarbonate (2 g), potassium sodium tartrate (2.5 g) and anhydrous sodium sulphate were dissolved in 80 ml water and volume was made up to 100 ml (solution A). Copper sulphate (15 g) was dissolved in a small volume of distilled water. To it, one drop of sulphuric acid was added and volume was made up to 100 ml (solution B). Just before use solution A (96 ml) and solution B (4 ml) were thoroughly mixed.

Arsenomolybdate reagent – Ammonium molybdate (2.5 g) was dissolved in 45 ml distilled water. To it, 2.5 ml of sulphuric acid was added and thoroughly mixed. Then,

0.3 g disodium hydrogen arsenate dissolved in 25 ml water. The solution was thoroughly mixed and was incubated at 37°C for about 36 hours.

Standard solution

Standard solution was prepared similar to total sugars.

Procedure

The root samples collected from different treatments were subjected to extraction. Root sample of 100 mg was homogenised with pestle and mortar. Eighty per cent hot ethanol was added twice to the sample, 5 ml each time for extraction of sugars. Each time centrifugation was done at 10,000 rpm for 20 minutes and the supernatant was collected. The volume was made up to 10 ml with distilled water to dissolve the sugars. From this suspension, 0.5 ml was pipetted out and the volume was made to 2 ml with distilled water. To this, 1 ml alkaline-copper-tartrate reagent was added and kept in the boiling water bath for 10 minutes. Then the tubes were cooled and 1 ml of Arsenomolybdate reagent was added. The absorbance was read at 620 nm by spectrophotometer against the reagent blank.

By taking a series of glucose concentrations and their absorbance values a standard graph was drawn and from it amount of reducing sugars present in the samples were calculated. The quantity of reducing sugars present in the samples was expressed as per cent equivalent of glucose.

3.6.1.2.5 Total phenols

The amount of total phenol content of root samples was estimated as per the procedure prescribed by Mallick and Singh (1980).

Standard solution

Stock solution was prepared by dissolving 100 mg of catechol in 100 ml of distilled water. Working standard was prepared by diluting 10 ml of the stock standard solution to 100 ml. One ml of this working standard solution contained 100 µg of catechol.

Procedure

Five ml of 80 per cent ethanol was added to 100 mg of root sample for extraction using pestle and mortar, and macerated. The homogenate was centrifuged at 10,000 rpm for 20 minutes and repeated 5 times for full extraction. After centrifugation, 0.5 ml of pooled supernatant was taken and evaporated to dryness. Then 5 ml of distilled water was added to dissolve phenol. Folin-Ciocalteu reagent of 0.5 ml was added to it. After three minutes, 2 ml of 20 per cent sodium carbonate solution was added and mixed thoroughly. The tubes were kept in boiling water bath for one minute. The intensity of blue colour developed was read at 650 nm by spectrophotometer against the reagent blank.

A standard curve was prepared by taking different concentrations of catechol against their absorbance values. From this curve, total phenols present in the samples were estimated. The quantity of total phenol content of samples was expressed as per cent equivalent of catechol.

3.6.1.2.6 Ortho dihydroxy (OD) phenols

Ortho dihydroxy phenol present in the samples were quantified by using Arnow's reagent (Arnow, 1937). The method for estimation was followed as per the procedure of Johnson and Schaal (1952).

Reagent

Arnow's reagent – Ten g each of sodium nitrate and sodium molybdate added to 100 ml distilled water and stored in brown bottle

Standard solution

Standard solution was prepared as in total phenols

Procedure

Five ml of 80 per cent ethanol was added to 100 mg of root sample in pestle and mortar and macerated. The solution was repeatedly centrifuged at 10,000 rpm for 20 minutes and the supernatants were pooled. To this ethanolic extract of 3 ml, one ml of 0.5 N hydrochloric acid and one ml of Arnow's reagent were added. The blue colour developed was read at 515 nm by spectrophotometer against the reagent blank.

A standard graph was drawn by plotting different concentrations of catechol against their absorbance values. From this standard graph, ortho dihydroxy phenols present in the samples were estimated. The ortho dihydroxy phenol content of samples was expressed as per cent equivalent of catechol.

3.6.1.2.7 Chlorophylls

Chlorophyll 'a', chlorophyll 'b' and total chlorophyll present in the leaves of the plants were estimated as per the procedure described by Witham *et al* (1971). The third leaves from middle tillers of plant samples were plucked and used as representative samples for estimation of chlorophylls.

Finely cut one g leaf samples were grinded in a pestle and mortar to a fine pulp with the addition of twenty ml 80 per cent acetone. The extract was centrifuged at 3000 rpm for 30 minutes. The supernatant was added to 100 ml volumetric flask. The residue

was added with 20 ml more of 80 per cent acetone and centrifuged again. The supernatant was added to the same flask. The process was repeated until the residue became colourless. Then the pestle and mortar were washed thoroughly with 80 per cent acetone and the washings were collected to the volumetric flask. The volume was made up to 100 ml with 80 per cent acetone. The absorbance of the made up solutions were read at 645 and 663 nm against solvent blank.

The amount of chlorophyll present in the extract of leaf tissue were calculated according to the following equations

$$\text{mg chlorophyll 'a'/g leaf tissue} = 12.7 (D_{663}) - 2.69 (D_{645}) \times \frac{V}{1000 \times W}$$

$$\text{mg chlorophyll 'b'/g leaf tissue} = 22.9 (D_{645}) - 4.68 (D_{663}) \times \frac{V}{1000 \times W}$$

$$\text{mg total chlorophyll/g leaf tissue} = 20.2 (D_{645}) + 8.02 (D_{663}) \times \frac{V}{1000 \times W}$$

where,

D = absorbance at specific wave lengths

V = final volume of 80 per cent acetone and chlorophyll extract

W = Fresh weight of leaf tissue extracted

3.6.2 Enzyme activity

Enzymes are associated with lignification and disease resistance mechanism in nematode infected plants. Activities of important enzymes such as peroxidase and catalase were studied during the course of investigation to ascertain the degree of resistance provided by microbial inoculant treated plants.

3.6.2.1 Peroxidase

Reagent

Pyrogallol solution 0.05 M – 630 mg pyrogallol in 100 ml of 0.01 M phosphate buffer (pH 6.5)

Procedure

One g of fresh root tissue sample was dissolved in three ml of 0.1 M phosphate buffer, pH 7.0. The sample was macerated with pre-cooled pestle and mortar. The homogenate was centrifuged at 18,000 rpm at 5°C for fifteen minutes using ultra centrifuge. The supernatant was used as enzyme source. From the enzyme extract 0.05 ml was pipetted out. Three ml 0.05 M pyrogallol solution was added to it. To the above suspension 0.5 ml of 1 per cent hydrogen peroxide was added in the test cuvette. The cuvette was read by spectrophotometer at 430 nm against the reagent blank. The change in absorbance for every thirty seconds upto three minutes were recorded by spectrophotometer. The average was worked and the peroxidase (Donor Hydrogen peroxide oxidoreductase EC 1.11.1.7) activity of the sample was estimated as enzyme units hr^{-1} (Reddy *et al*, 1985).

3.6.2.2 Catalase

Catalase (Hydrogen peroxide Hydrogen peroxide oxidoreductase EC 1.11.1.6) was estimated indirectly by estimating the amount of hydrogen peroxide changed per minute in the root samples after arresting the catalase enzyme activity. The content of hydrogen peroxide amount is inversely proportional to catalase activity.

Reagent

Phosphate buffer (pH 7.8) – Monobasic sodium phosphate 0.2 M (8.5 ml) was mixed with 91.5 ml 0.2 M solution of dibasic sodium phosphate and the volume was diluted to a total of 200 ml.

Procedure

Five hundred mg of root sample from each treatment was taken in pestle and mortar and to it, 10 ml of phosphate buffer, pH 7.8 was added and macerated. Then centrifuged at 2000 rpm for 10 minutes to obtain the enzyme extract. Five glass beakers of 100 ml capacity were numbered from 0 to 4. To the numbered beakers one ml of enzyme extract was added per beaker, followed by five ml 1.5 per cent sodium perfluoroborate and 1.5 ml phosphate buffer, pH 7.8 were added. To the control beaker (number 0), ten ml of 2N sulphuric acid was added to arrest catalase activity, followed by five ml 1.5 per cent sodium perfluoroborate, 1.5 ml phosphate buffer, pH 7.8 and one ml of enzyme extract respectively. Immediately, the control beaker was titrated against 0.05 per cent potassium permanganate in burette till the appearance of pink colour persisted for a few seconds and taken as end point. The other four numbered beakers were added with ten ml of 2 N sulphuric acid at 1, 2, 3 and 4 minutes according to their number after the addition of sulphuric acid to the control beaker. Immediately followed by, titration against 0.05 per cent potassium permanganate. Difference between the readings of burette indicated the amount of hydrogen peroxide present in the samples. The average is worked out to estimate the quantitative change (μg) of hydrogen peroxide per g per minute and thus catalase activity of samples were determined (Okuda *et al*, 1991).

3.6.3.1 Nutritional status

Nutritional status of host plants is an important factor in deciding the course of plant-nematode interaction. Nutrient contents of plant samples were studied during the course of investigation.

3.6.3.1.1 Macronutrients

Macronutrients such as nitrogen, phosphorus and potassium contents of shoot and root samples of cvs IR 50 and ADT 44 were estimated following the standard laboratory procedures.

3.6.3.1.1.1 Total Nitrogen

Nitrogen content of shoot and root were estimated by Microkjeldahl method (Jackson, 1962)

Procedure

In a conical flask, one g sample was taken. A quantity of 10 ml diacid mixture (sulphuric acid and perchloric acid in the ratio of 5:2) was added to convert organic nitrogen to inorganic and flask's mouth is covered with funnel. Digestion was performed over a sand bath at a temperature range of 290 – 295°C till a clear solution was obtained. The volume of the digested material was made up to 250 ml. Ten ml of the extract was put in a distillation flask. Twenty five ml 2 per cent boric acid was pipetted out to a beaker and two drops of double indicator (bromocresol green methyl red) was added and kept at the delivery end of the distillation set. The extracted plant material was distilled with 40 per cent sodium hydroxide and distillate (liberated ammonia) was collected in 2 per cent boric acid. Completion of distillation was tested with a moistened red litmus paper and absence of blue colour indicated that all ammonia had been distilled. The contents in beaker was titrated against 0.1 N sulphuric acid. The end point was determined by the change of colour from blue to green. From the titrate value, the quantity of nitrogen content in the sample was estimated, taking into consideration the moisture content of samples and expressed in percentage.

3.6.3.1.1.2 Phosphorus

Reagent

Barton's reagent – Ammonium molybdate (25 g) was dissolved in 400 ml of distilled water and then mixed with a solution containing 1.25 g of ammonium meta vanadate dissolved in 300 ml concentrated nitric acid and volume of the mixture was made up to one litre.

Standard solution

Stock solution was prepared by dissolving 0.22 g potassium hydrogen phosphate (dried at 40°C) in 400 ml of distilled water. Then, 25 ml of 1N sulphuric acid was added and the volume was made up to 1000 ml to obtain 50 ppm of phosphorus.

Preparation of triple acid extract

Phosphorus content in plant samples was estimated by calorimetric method (Snell and Snell, 1937). Triple acid extract was prepared by transferring two g of plant sample into a 100 ml conical flask. The triple acid mixture (nitric, sulphuric and perchloric acids in the ratio of 9:2:1 on volume basis) of 12 ml was added and the mouth of the flask was covered with a funnel. The contents of the flask were digested over a sand bath till a clear suspension was obtained, then filtered through filter paper (Whatman No 41) and the filtrate was collected in a 250 ml volumetric flask. The conical flask was washed with small additions of hot water and the washings were also filtered. The residue on the filter paper was also washed with hot water. The volumetric flask was cooled under tap water and volume of the triple acid extract was made up to 250 ml with cold distilled water.

Procedure

Five ml of triple acid extract was pipetted out into a 25 ml volumetric flask and Barton's reagent of 5 ml was added and shaken. The volume was made up to 25 ml and shaken thoroughly for obtaining homogenous solution. Thirty minutes was allowed for development of colour. The yellow colour developed was read at 470 nm by using a spectrophotometer.

The standard curve was prepared by plotting absorbance values against concentrations of phosphorus. From this standard graph, the phosphorus content in the samples was estimated and expressed as percentage of weight of the samples.

3.6.3.1.1.3 Potassium

Standard solution

Stock solution was prepared by dissolving 19g potassium chloride in one litre of distilled water to give 1000 ppm of potassium. One hundred ml of the stock solution was diluted to one litre to give 100 ppm potassium solution to be served as working standard. From this, various concentrations of potassium solutions were prepared ranging from 10 to 100 ppm.

Preparation of triple acid extract

The triple acid extract was prepared as in case of phosphorus estimation.

Procedure

To five ml of triple acid extract in a 25 ml volumetric flask, five ml of ammonium hydroxide was added and the volume was made up to 25 ml with distilled water. The content was transferred to an injection vial and fed to the flame photometer (Mediflame 127) after adjusting the flame photometer to read zero with distilled water and standardising with 100 ppm of potassium solution to read 100 of galvanometer readings (Jackson, 1962).

A standard curve was prepared by plotting flame photometer readings against different concentrations of potassium solution. By referring the standard curve, the potassium content of samples was estimated and expressed in percentage.

3.6.3.1.2 Micronutrients

Contents of divalent and trivalent micronutrients viz., iron, copper, zinc and manganese of shoot and root of rice cvs IR 50 and ADT 44 were estimated at panicle initiation stage.

Preparation of triple acid extract

Two g of plant sample was added to 12 ml of the triple acid mixture (nitric, sulphuric and perchloric acids in the ratio 9 2 1 on volume basis) in a conical flask and was covered with a funnel. The contents were digested over a sand bath till a clear solution was obtained. Then, the solution was filtered through filter paper (Whatman No 10) and filtrate was collected. The suspension was cooled under tap water and volume was made up to 250 ml with distilled water.

Procedure

The triple acid extract of plant sample was fed to the cuvette of the atomic absorption spectrophotometer (Tetra 20 BQ, Simens, USA make). The concentrations of the micronutrients in the suspension was read at various absorbance viz, 250, 325, 215 and 280 nm for iron, copper, zinc and manganese respectively and the contents of the micronutrients were estimated on dry weight basis (Lindsay and Norvell, 1978).

3.7 Histological study for cellular responses of rice root to the ingress rice-root nematode, *H. gracilis*

Rice plants infected with *H. gracilis* were dug out of the pots maintained for monoculture washed gently and thoroughly under running tap water to make them free of adhering soil particles. Brown coloured lesioned rootlets suspected to have penetrating rice-root nematode, *H. gracilis* were selected and cut into small bits of 0.5 to 1.0 cm length. The cut root bits were fixed in formalin-aceto-alcohol (FAA), which was prepared by mixing 90 ml of 50 per cent ethanol, 5 ml of glacial acetic acid and 5 ml of 40 per cent formaldehyde and care was taken for proper submergence of the root tissues. The fixation was carried out for 10 days. The fixed root bits were dehydrated by moving them stepwise through increasingly higher concentrations of alcohol, following the tertiary-butyl-alcohol (TBA) dehydration schedule and necessary precautions were followed to avoid the desiccation of the root tissues (Johansen, 1940). Dehydrated root tissues were

infiltrated with paraffin wax (58°C m p) to replace alcohol and then embedded in the paraffin wax of same melting point. Sectioning of paraffin block containing tissues was done on a rotary microtome (Spencers, USA make) and ribbons of 10 – 15 μ thickness were mounted on glass slides coated with Haupt's adhesive (mixture of 1 g powdered gelatin, 2 g phenol crystals, 15 ml glycerin and 100 ml distilled water) which was flooded with freshly prepared 2 per cent formalin followed by, mild heating of slides. Staining of microtomed sections of tissues on the slides was carried out by following the Sass safranin and fast green staining schedule in which 1 per cent aqueous safranin and 0.1 per cent fast green (in 95 per cent ethanol) were used for staining and counter staining respectively (Sass, 1951). The stained tissues on slides were mounted with cover slips by using D P X mountant (Jensen, 1962).

3.8 Statistical Analysis

The data generated from the glasshouse and field experiments were subjected to statistical analysis following the methods for randomised block design (Gomez and Gomez, 1984, Rangaswami, 1995).

EXPERIMENTAL RESULTS

CHAPTER IV

EXPERIMENTAL RESULTS

Glasshouse and field experiments were conducted on rice infected with *Hirschmanniella gracilis* either naturally or by inoculation. Performances of popular high yielding rice varieties and microbial inoculants against the nematode infection were evaluated. Physiological, biochemical and nutritional responses of host plants to the introduced microorganisms and histopathological changes of rice roots to the nematode infection were studied. The data and observations relating to the above aspects were recorded, analysed and presented in this chapter.

4.1 Screening of popular high yielding rice varieties for their reaction to the rice-root nematode, *H. gracilis*

A total of 91 short, medium and long duration high yielding rice varieties was screened against the infection of *H. gracilis*, evaluated and furnished in Table 3, 4 & 5 and Figure 3.

Out of twenty-seven short duration varieties screened for their reaction to *H. gracilis*, the lowest maximum nematode population of 29 nematodes per g root was observed in cv TKM 7 and the highest maximum population of 124 nematodes per g root was recorded in cv IR 50 30 days after inoculation. None of the variety was found to have less than 10 nematodes per g root. In cultivars TKM 5, TKM 7, TKM 8 and TKM 9 *H. gracilis* population per g root were recorded between 11 and 50, whereas varieties ASD 3, CO 41, IR 48 and IR 50 had more than 100 nematodes per g root. Remaining 19 cultivars recorded the maximum population of *H. gracilis* between 51 and 100 per g of fresh root at maximum tillering stage (Table 3).

Table 3. Reaction of short duration high yielding rice varieties to the rice-root nematode, *H. gracilis* at maximum tillering stage (45 DAS)

Sl. No.	Varieties	Maximum nematode population per g root*	Host reaction
1	ADT 7	67	Susceptible
2	ASD 3	102	Highly susceptible
3	Basmati 370	89	Susceptible
4	CO 9	68	Susceptible
5	CO 13	87	Susceptible
6	CO 20	69	Susceptible
7	CO 21	66	Susceptible
8	CO 28	84	Susceptible
9	CO 29	93	Susceptible
10	CO 31	66	Susceptible
11	CO 35	88	Susceptible
12	CO 41	108	Highly susceptible
13	IR 32	61	Susceptible
14	IR 36	79	Susceptible
15	IR 46	61	Susceptible
16	IR 48	104	Highly susceptible
17	IR 50	124	Highly susceptible
18	IR 56	88	Susceptible
19	IR 58	72	Susceptible
20	IR 60	59	Susceptible
21	MDU 1	78	Susceptible
22	TKM 2	58	Susceptible
23	TKM 3	86	Susceptible
24	TKM 5	46	Moderately resistant
25	TKM 7	29	Moderately resistant
26	TKM 8	42	Moderately resistant
27	TKM 9	37	Moderately resistant

* Among five replications

Table 4. Reaction of medium duration high yielding rice varieties to the rice-root nematode, *H. gracilis* at maximum tillering stage (60 DAS)

Sl. No.	Varieties	Maximum nematode population per g root*	Host reaction
1	ADT 1	56	Susceptible
2	ADT 2	63	Susceptible
3	ADT 5	71	Susceptible
4	ADT 8	55	Susceptible
5	ADT 10	68	Susceptible
6	ADT 11	71	Susceptible
7	ADT 12	61	Susceptible
8	ADT 21	75	Susceptible
9	ADT 22	90	Susceptible
10	ADT 25	74	Susceptible
11	ADT 39	92	Susceptible
12	ASD 1	139	Highly susceptible
13	ASD 7	68	Susceptible
14	ASD 8	46	Moderately resistant
15	ASD 16	93	Susceptible
16	ASD 18	58	Susceptible
17	ADT 19	85	Susceptible
18	Bhavan1	121	Highly susceptible
19	CO 1	69	Susceptible
20	CO 2	61	Susceptible
21	CO 3	84	Susceptible
22	CO 4	82	Susceptible
23	CO 6	58	Susceptible
24	CO 8	65	Susceptible
25	CO 12	116	Highly susceptible
26	CO 16	64	Susceptible
27	CO 25	68	Susceptible
28	CO 30	76	Susceptible
29	CO 38	109	Highly susceptible

Table 4 (Contd.)

Sl. No.	Varieties	Maximum nematode population per g root*	Host reaction
30	CO 42	127	Highly susceptible
31	CO 43	97	Susceptible
32	CO 44	125	Highly susceptible
33	CO 45	133	Highly susceptible
34	CO 46	204	Highly susceptible
35	IR 20	88	Susceptible
36	IR 34	109	Highly susceptible
37	IR 44	127	Highly susceptible
38	IR 54	84	Susceptible
39	IR 62	105	Highly susceptible
40	MDU 2	189	Highly susceptible
41	MDU 4	46	Moderately resistant
42	Ponni	124	Highly susceptible
43	PTB 12	48	Moderately resistant
44	PTB 15	43	Moderately resistant
45	PTB 16	59	Susceptible
46	PTB 18	68	Susceptible
47	PTB 20	56	Susceptible
48	PTB 23	47	Moderately resistant
49	PTB 33	52	Susceptible
50	PUSA 33	69	Susceptible
51	PUSA 167	88	Susceptible
52	PUSA 169	54	Susceptible
53	PUSA 205	108	Highly susceptible
54	PY 1	42	Moderately resistant
55	PY 2	63	Susceptible
56	TKM 1	58	Susceptible
57	TKM 6	75	Susceptible
58	TKM 10	54	Susceptible
59	TKM 11	83	Susceptible
60	White ponni	128	Highly susceptible

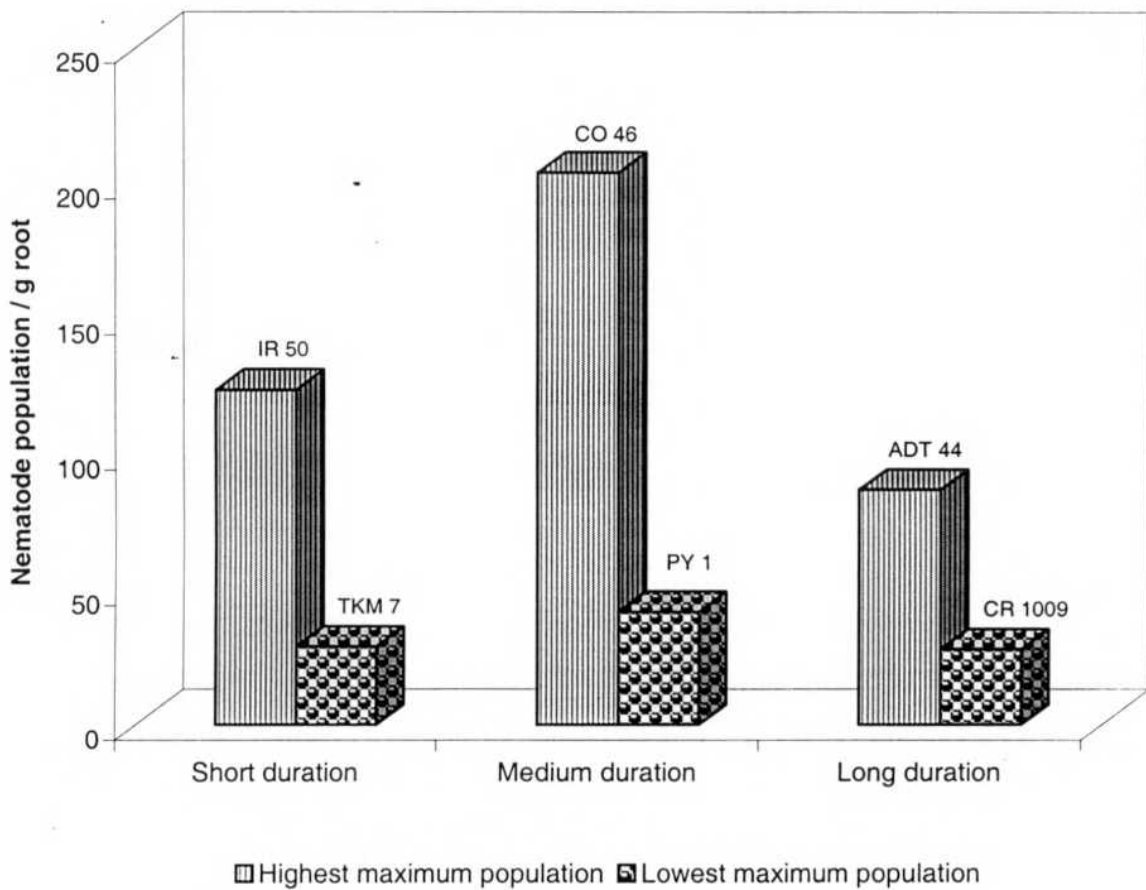
* Among five replications

Table 5. Reaction of long duration high yielding rice varieties to the rice-root nematode, *H. gracilis* at maximum tillering stage (90 DAS)

Sl. No.	Varieties	Maximum nematode population per g root*	Host reaction
1	ADT 40	72	Susceptible
2	ADT 44	87	Susceptible
3	CO 40	52	Susceptible
4	CR 1009	28	Moderately resistant

* Among five replications

Figure 3. Highest and lowest maximum population of the rice-root nematode *H.gracilis* among the short, medium and long duration popular high yielding rice varieties screened at maximum tillering stage



Among sixty medium duration rice cultivars evaluated for their reaction against the infection of *H. gracilis* 45 days after inoculation, the lowest maximum population (42/g root) was observed in cv PY 1 and the highest maximum population (204/g root) was in cv CO 46. Cultivars ASD 8, MDU 4, PTB 12, PTB 15, PTB 23 and PY 1 were found to contain maximum population of *H. gracilis* between 11 and 50 per g roots. Maximum nematode population of 100 and above per g root was recorded in varieties ASD 1, Bhavani, CO 12, CO 38, CO 42, CO 44, CO 45, CO 46, IR 34, IR 44, IR 62, MDU 2, Ponn1, PUSA 205 and White ponni. Rest of the 39 cultivars had maximum nematode population between 51 and 100 per g of fresh root at maximum tillering stage (Table 4).

In the four long duration high yielding rice varieties screened for their reaction to *H. gracilis* 75 days after inoculation, the lowest and highest maximum nematode population were recorded in cultivars CR 1009 and ADT 44 respectively with the nematode population of *H. gracilis* 28 and 87 per g root. The other two varieties viz, ADT 40 and CO 40 had maximum of 72 and 52 nematodes per g fresh root at maximum tillering stage (Table 5).

4.2 Studies on the management of the rice-root nematode, *H. gracilis* with microbial inoculants

4.2.1 Short duration cv. IR 50 (glasshouse study)

Plants of cv IR 50 replanted in pots with fixed inoculum of *H. gracilis* (1 nematode per g soil) were treated with microbial inoculants viz, *A. microphylla*, *A. lipoferum*, blue green algae, *B. megaterium*, *G. intraradices* and *P. fluorescens* either individually or in combination with either *G. intraradices* or *P. fluorescens* and were assessed for their performance against the infection of the nematode at panicle initiation stage (75 DAS). Observations on plant growth characters and population of nematode

Plate 3. Effect of *H. gracilis* infection on rice

Morphological differences between healthy (H) and infected (I) rice plants at inoculum level of one nematode per g root under glasshouse conditions

A. cv. IR 50

B. cv. ADT 44

C. Delayed flowering in untreated (UT) plot

Plate 3



both in root and soil were recorded, analysed and presented in Table 6 & 7, Figure 4, 5, 6 & 7 and Plate 4

4.2.1.1 Above ground plant growth parameters

Combined treatment of *P. fluorescens* with *G. intraradices* recorded maximum shoot length (76.1 cm), and fresh (32.2 g) and dry (18.6 g) shoot weight. Shoot length was on par with *P. fluorescens* combined inoculations with *A. microphylla* and *A. lipoferum*. Fresh shoot weight was statistically on par to combined inoculation of *P. fluorescens* with *A. lipoferum*, whereas dry shoot weight was significantly different. Dry shoot weight of combined treatment of *P. fluorescens* with *A. lipoferum* was the next best. Combined treatment of *P. fluorescens* with *A. lipoferum* recorded maximum total leaf area (482.7 cm²) and number of tillers (4.7) and both the said parameters were on par with only *P. fluorescens* combined with *G. intraradices* (Table 6 and Figure 4 & 5).

All the above ground plant growth parameters studied were minimum in untreated control. Significant reduction was noticed in fresh shoot weight (11.4 g), dry shoot weight (6.2 g) and total leaf area (136.3 cm²). Both the shoot length (55.2 cm) and number of tillers (2.1) were on par with *B. megaterium* individual treatment.

Uninoculated control registered maximum of all the above ground plant growth parameters.

4.2.1.2 Below ground plant growth parameters

All the below ground plant growth parameters such as, root length, fresh root weight, dry root weight and root volume of combined treatment of *P. fluorescens* with *G. intraradices* were enhanced maximum by 72.3, 295.9, 217.9 and 217.4 per cent respectively over untreated control. Root length and volume were on par with *P. fluorescens* combined inoculation with *A. lipoferum*, while other parameters differ

Table 6. Effect of microbial inoculants on above ground plant growth parameters of rice cv. IR 50 against the infection of *H. gracilis* at panicle initiation stage (75 DAS) under glasshouse conditions

Treatment	Shoot length (cm)		Fresh shoot weight (g)		Dry shoot weight (g)		Total leaf area (cm ²)		Number of tillers	
	%	%	%	%	%	%	%	%	%	%
T ₁ – <i>Azolla microphylla</i>	61.8	12.0	15.9	39.5	8.8	41.9	218.7	60.5	2.9	38.1
T ₂ – <i>Azospirillum lipoferum</i>	64.2	16.3	17.4	52.6	9.4	51.6	286.5	110.2	3.2	52.4
T ₃ – Blue green algae	62.4	13.0	16.2	42.1	8.9	43.5	222.8	63.5	2.6	23.8
T ₄ – <i>Bacillus megaterium</i>	55.9	1.3	15.1	32.5	8.4	35.5	214.3	57.2	2.4	14.3
T ₅ – <i>Glomus intraradices</i>	61.2	10.9	16.8	47.4	9.0	45.2	249.6	83.1	3.0	42.9
T ₆ – <i>A. microphylla</i> + <i>G. intraradices</i>	66.4	20.3	17.9	57.0	10.2	64.5	281.3	106.4	3.2	52.4
T ₇ – <i>A. lipoferum</i> + <i>G. intraradices</i>	68.7	24.5	19.1	67.5	10.9	75.8	327.5	140.3	3.5	66.7
T ₈ – Blue green algae + <i>G. intraradices</i>	67.9	23.0	17.1	50.0	9.8	58.1	252.1	85.0	3.1	47.6
T ₉ – <i>B. megaterium</i> + <i>G. intraradices</i>	61.4	11.2	16.9	48.2	9.6	54.8	250.0	83.4	3.0	42.9
T ₁₀ – <i>Pseudomonas fluorescens</i>	70.4	27.5	24.7	116.7	13.6	119.4	354.8	160.3	3.6	71.4
T ₁₁ – <i>A. microphylla</i> + <i>P. fluorescens</i>	73.9	33.9	28.0	145.6	15.8	154.8	412.2	202.4	4.0	90.5
T ₁₂ – <i>A. lipoferum</i> + <i>P. fluorescens</i>	73.7	33.5	31.7	178.1	17.4	180.6	482.7	254.1	4.7	123.8
T ₁₃ – Blue green algae + <i>P. fluorescens</i>	70.5	27.7	27.1	137.7	14.4	132.3	396.3	190.8	3.7	76.2
T ₁₄ – <i>B. megaterium</i> + <i>P. fluorescens</i>	71.1	28.8	26.5	132.5	14.1	127.4	401.5	194.6	3.9	85.7
T ₁₅ – <i>G. intraradices</i> + <i>P. fluorescens</i>	76.1	37.9	32.2	182.5	18.6	200.0	476.9	249.9	4.5	114.3
T ₁₆ – Carbofuran 3G	62.1	12.5	15.7	37.7	8.3	33.9	226.4	66.1	2.5	19.0
T ₁₇ – Untreated control	55.2	–	11.4	–	6.2	–	136.3	–	2.1	–
T ₁₈ – Uninoculated control	76.9	39.3	32.8	187.7	18.7	201.6	548.7	302.6	5.3	152.4
CD (P=0.05)	3.9		1.3		0.7		63.7		0.3	

% - Per cent increase over untreated control

Figure 4. Effect of *P.fluorescens* and its combinations on shoot length and fresh and dry shoot weights of rice cv. IR 50 against the infection of *H.gracilis* at panicle initiation stage (75 DAS) under glasshouse conditions

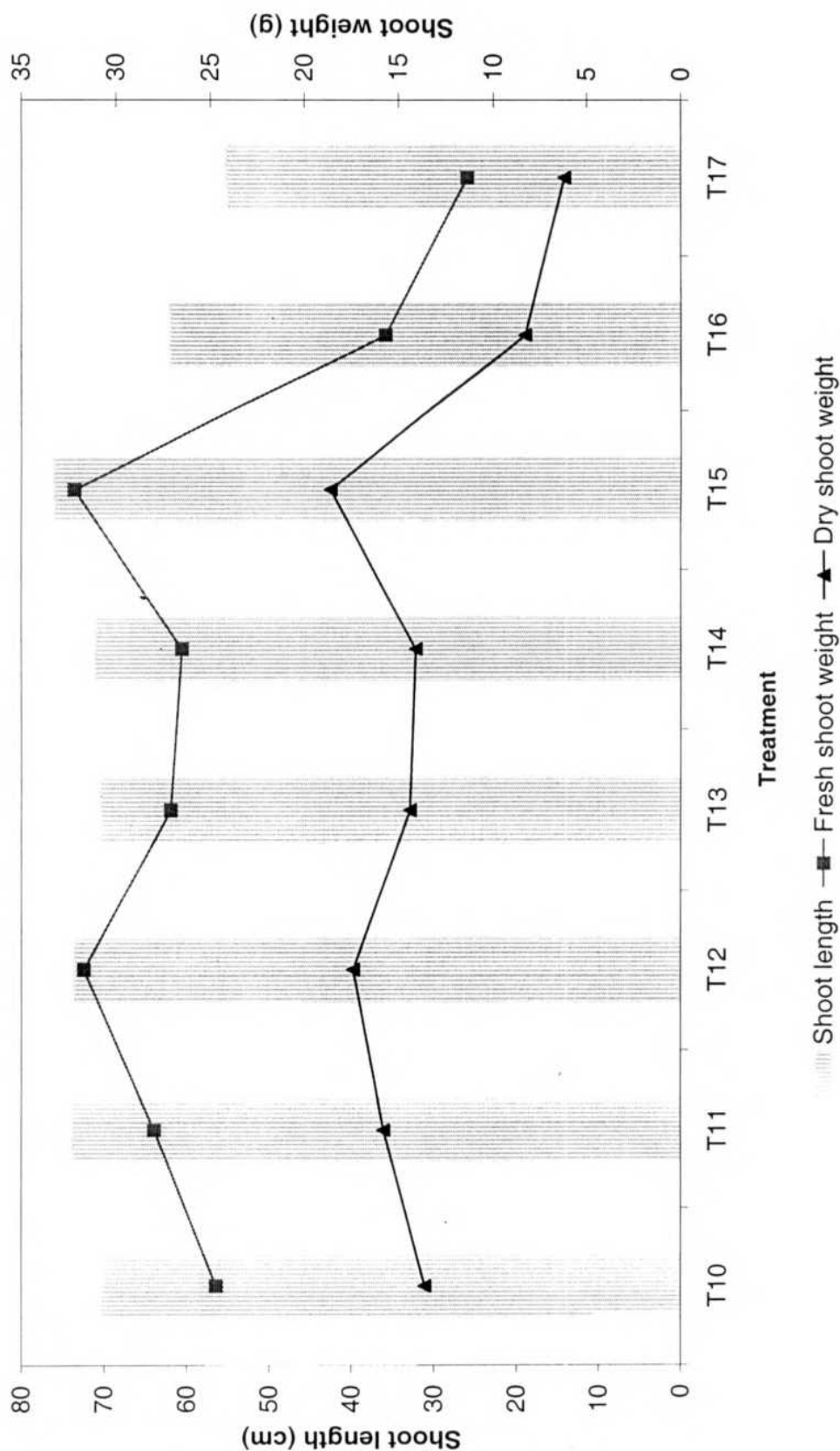


Figure 5. Effect of *P.fluorescens* and its combinations on total leaf area and number of tillers of rice cv. IR 50 against the infection of *H.gracilis* at panicle initiation stage (75 DAS) under glasshouse conditions

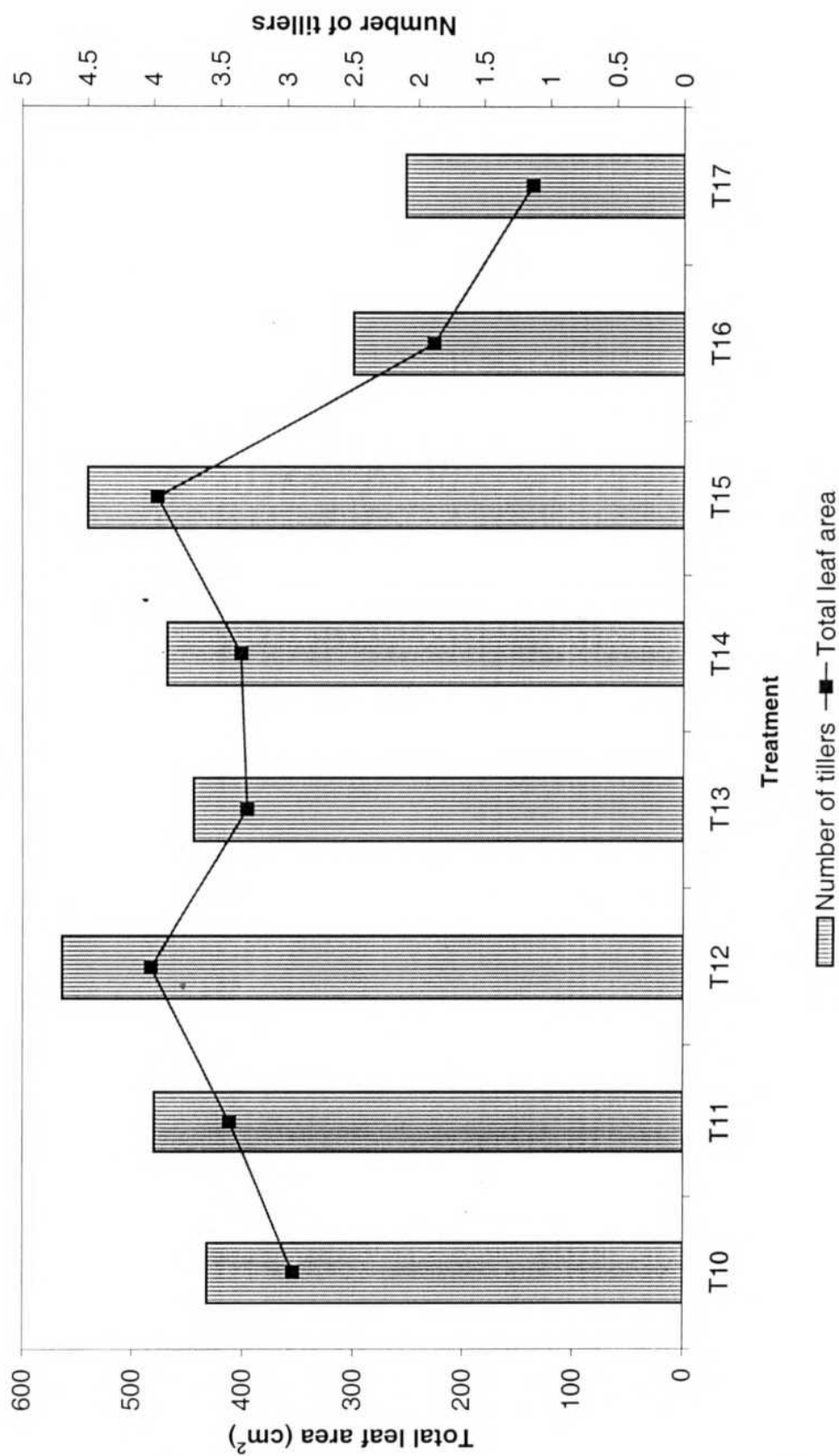


Table 7. Effect of microbial inoculants on below ground plant growth parameters and nematode population in root and soil of rice cv. IR 50 against the infection of *H. gracilis* at panicle initiation stage (75 DAS) under glasshouse conditions

Treatment	Root length (cm)		Fresh root weight (g)		Dry root weight (g)		Root volume (cc)		Nematode population/ g root		Nematode population/ 200 g soil	
	%	%	%	%	%	%	%	%	%	%	%	%
T ₁ – <i>Azolla microphylla</i>	14.6	30.4	7.4	51.0	3.3	17.9	10.4	50.7	164.2	-31.3	186.9	-38.2
T ₂ – <i>Azospirillum lipoferum</i>	17.7	58.0	8.8	79.6	4.1	46.4	12.2	76.8	152.7	-36.1	163.5	-45.9
T ₃ – Blue green algae	14.0	25.0	7.1	44.9	3.2	14.3	11.2	62.3	176.9	-26.0	176.2	-41.7
T ₄ – <i>Bacillus megaterium</i>	13.8	23.2	6.7	36.7	3.0	7.1	9.7	40.6	184.4	-22.8	194.7	-35.6
T ₅ – <i>Glomus intraradices</i>	13.8	23.2	9.0	83.7	4.3	53.6	13.4	94.2	161.7	-32.3	154.7	-48.8
T ₆ – <i>A. microphylla</i> + <i>G. intraradices</i>	18.2	62.5	9.8	100.0	4.7	67.9	14.7	113.0	142.4	-40.4	144.2	-52.3
T ₇ – <i>A. lipoferum</i> + <i>G. intraradices</i>	18.4	64.3	11.2	128.6	5.2	85.7	15.2	120.3	127.8	-46.5	138.9	-54.1
T ₈ – Blue green algae + <i>G. intraradices</i>	18.0	60.7	9.1	85.7	4.2	50.0	13.6	97.1	153.9	-35.6	146.7	-51.5
T ₉ – <i>B. megaterium</i> + <i>G. intraradices</i>	18.1	61.6	10.8	120.4	5.0	78.6	14.8	114.5	160.4	-32.9	149.4	-50.6
T ₁₀ – <i>Pseudomonas fluorescens</i>	17.9	59.8	14.5	195.9	6.6	135.7	19.9	188.4	121.5	-49.1	136.2	-55.0
T ₁₁ – <i>A. microphylla</i> + <i>P. fluorescens</i>	18.4	64.3	14.7	200.0	6.9	146.4	20.1	191.3	114.3	-52.2	128.2	-57.6
T ₁₂ – <i>A. lipoferum</i> + <i>P. fluorescens</i>	18.7	67.0	16.2	230.6	8.2	192.9	20.9	202.9	104.9	-56.1	125.8	-58.4
T ₁₃ – Blue green algae + <i>P. fluorescens</i>	18.2	62.5	16.2	230.6	8.0	185.7	20.6	198.6	120.2	-49.7	132.7	-56.1
T ₁₄ – <i>B. megaterium</i> + <i>P. fluorescens</i>	18.0	60.7	16.0	226.5	7.7	175.0	20.8	201.4	118.7	-50.3	130.4	-56.9
T ₁₅ – <i>G. intraradices</i> + <i>P. fluorescens</i>	19.3	72.3	19.4	295.9	8.9	217.9	21.9	217.4	103.7	-56.6	124.1	-59.0
T ₁₆ – Carbofuran 3G	14.3	27.7	9.1	85.7	4.1	46.4	13.8	100.0	153.9	-35.6	168.2	-44.4
T ₁₇ – Untreated control	11.2	–	4.9	–	2.8	–	6.9	–	238.9	–	302.4	–
T ₁₈ – Uninoculated control	18.9	68.8	19.7	302.0	9.1	225.0	22.4	224.6	–	–	–	–
CD (P=0.05)	0.7		0.5		0.2		1.0		6.9		8.2	

% - Per cent increase or decrease (-) over untreated control

Figure 6. Effect of *P.fluorescens* and its combinations on below ground plant growth parameters of rice cv. IR 50 against the infection of *H.gracilis* at panicle initiation stage (75 DAS) under glasshouse conditions

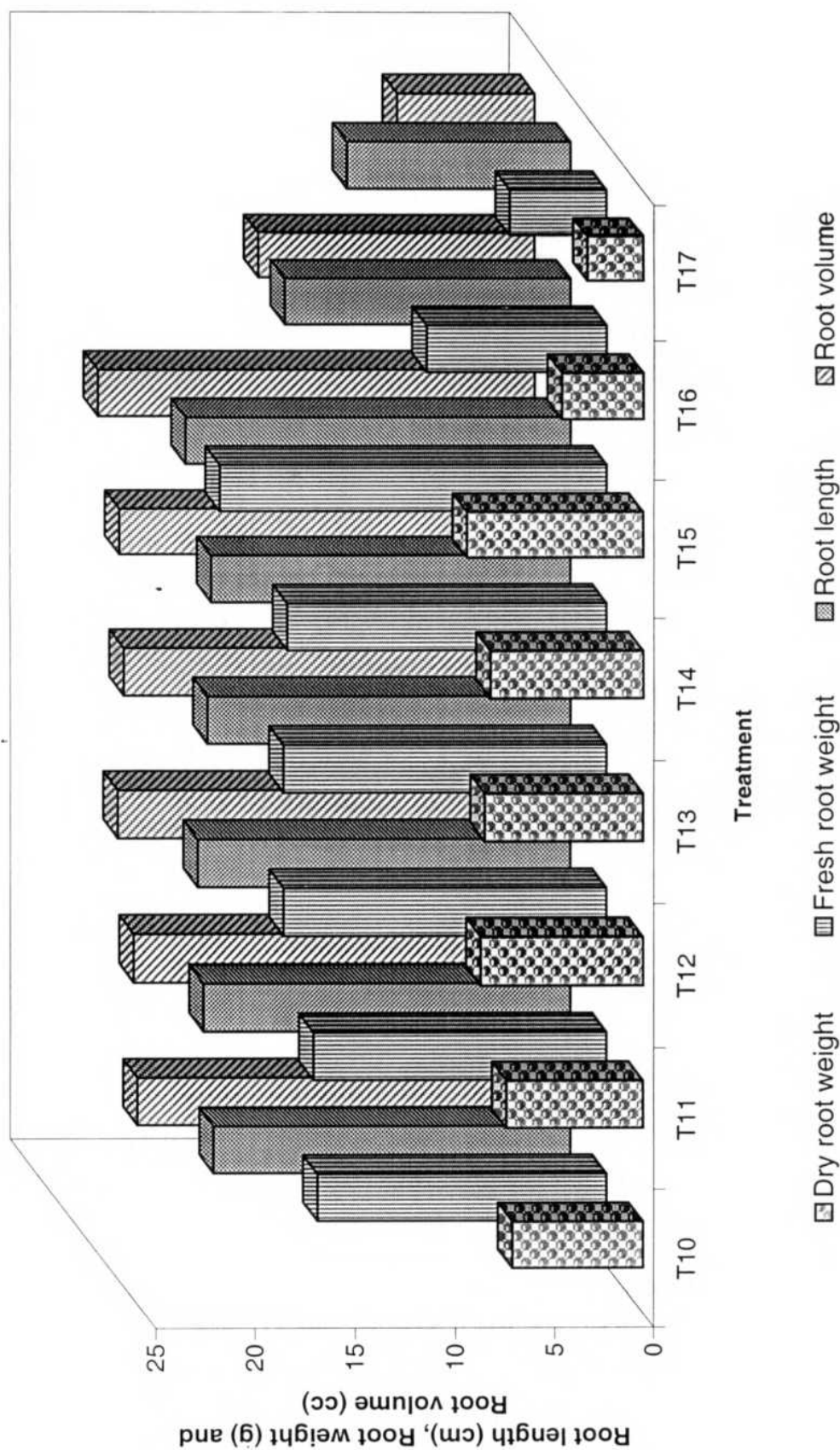


Figure 7. Effect of *P. fluorescens* and its combinations on nematode population in root and soil of rice cv. IR 50 against the infection of *H. gracilis* at panicle initiation stage (75 DAS) under glasshouse conditions

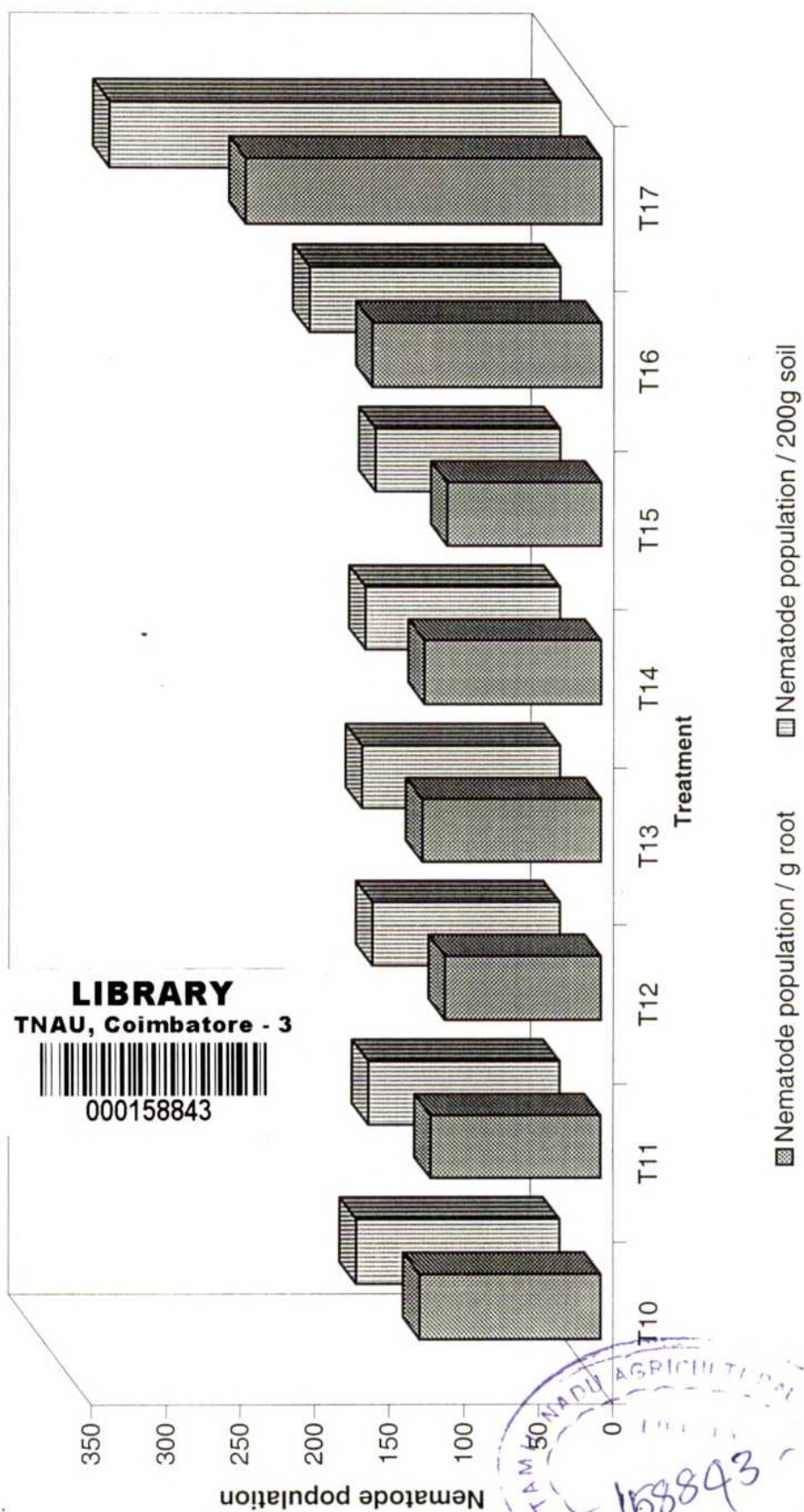


Plate 4. Effect of microbial inoculants on cv. IR 50 against the infection of the rice-root nematode, *H. gracilis* at panicle initiation stage (75 DAS) under glasshouse conditions

Individual and combined effect of treatments in comparison with carbofuran treatment, untreated and uninoculated control

A. Individual microbial inoculants

B. *G. intraradices*, *P. fluorescens* and their combination

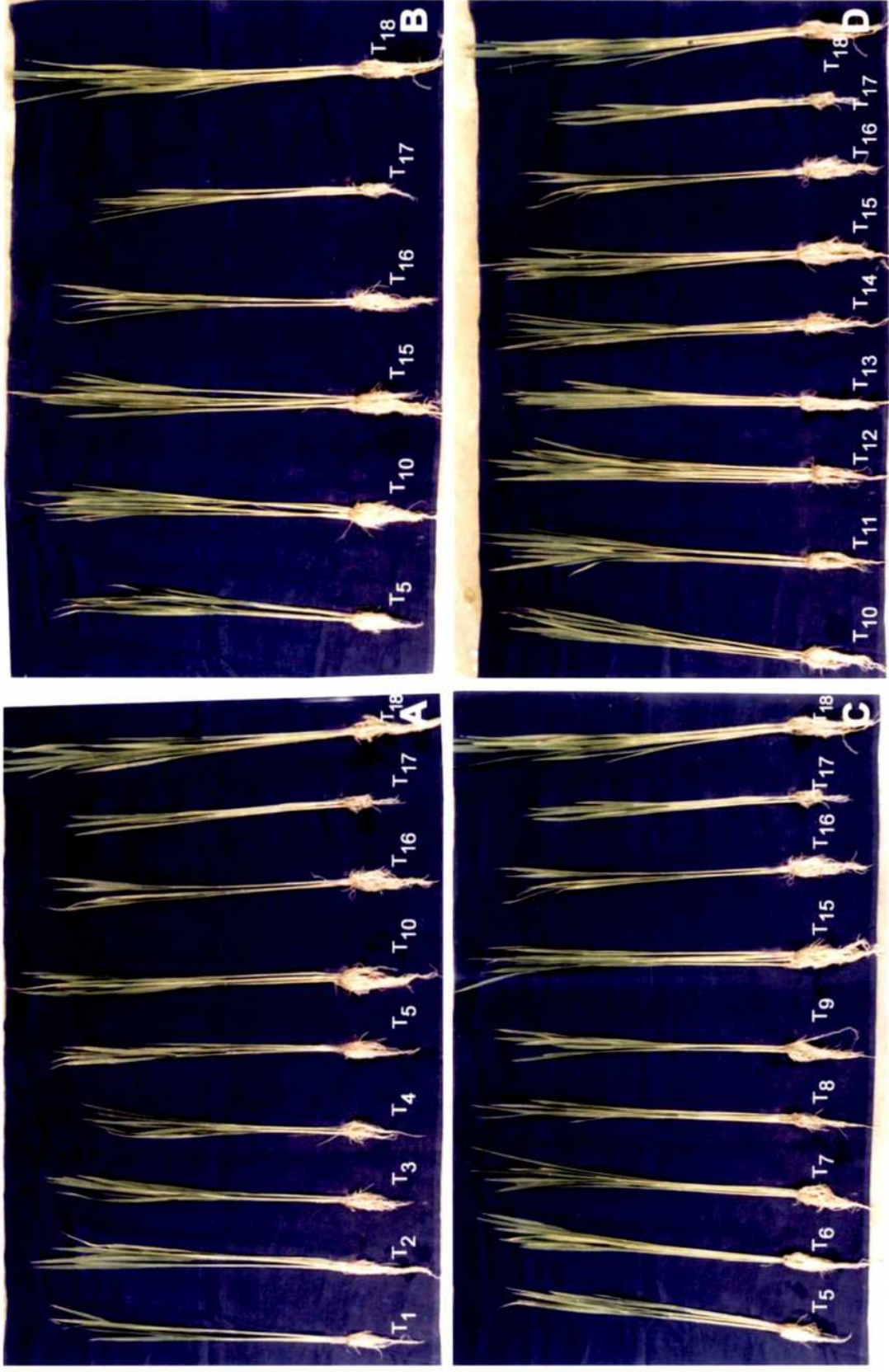
C. *G. intraradices* and its combinations

D. *P. fluorescens* and its combinations

Treatment

- T₁ – *Azolla microphylla*
- T₂ – *Azospirillum lipoferum*
- T₃ – Blue green algae
- T₄ – *Bacillus megaterium*
- T₅ – *Glomus intraradices*
- T₆ – *A. microphylla* + *G. intraradices*
- T₇ – *A. lipoferum* + *G. intraradices*
- T₈ – Blue green algae + *G. intraradices*
- T₉ – *B. megaterium* + *G. intraradices*
- T₁₀ – *Pseudomonas fluorescens*
- T₁₁ – *A. microphylla* + *P. fluorescens*
- T₁₂ – *A. lipoferum* + *P. fluorescens*
- T₁₃ – Blue green algae + *P. fluorescens*
- T₁₄ – *B. megaterium* + *P. fluorescens*
- T₁₅ – *G. intraradices* + *P. fluorescens*
- T₁₆ – Carbofuran 3G
- T₁₇ – Untreated control
- T₁₈ – Uninoculated control

Plate 4



significantly *P fluorescens* combined inoculations with both *A lipoferum* and blue green algae were the next best treatments with regard to fresh root weight, followed by co-inoculation of *P fluorescens* and *A. lipoferum* with respect to dry root weight (Table 7 and Figure 6)

Untreated control recorded minimum of all the below ground plant growth characters Dry root weight (2.8 g) was on par with *B megaterium* individual treatment, while all other parameters were significantly inferior in rest of the treatments Root length, fresh root weight, and root volume of untreated control were recorded as 11.2 cm, 4.9 g and 6.9 cc respectively

Uninoculated control registered maximum of all the below ground parameters, except the root length

4.2.1.3 Nematode population in root and soil

Population of *H gracilis* was reduced to the minimum both in root (103.7 per g) and soil (124.1 per 200 g) of dual inoculation of *P fluorescens* with *G intraradices* Root population of the nematode was on par with combined inoculation of *P fluorescens* with *A lipoferum*, whereas soil population of *H gracilis* was statistically on par in all the combined applications of *P fluorescens* except blue green algae (Table 7 and Figure 7)

Untreated control had significantly higher population of the rice-root nematode both in root (238.9 per g) and soil (302.4 per 200 g)

4.2.2 Long duration cv. ADT 44 (glasshouse study)

Data on both above and below ground plant growth characters, root and soil population of *H gracilis* in controlled glasshouse conditions were recorded for cv ADT 44 in response to the inoculation of microbial inoculants viz , *A microphylla*, *A lipoferum*, blue green algae, *B megaterium*, *G intraradices* and *P fluorescens* either

individually or in combination with either *P. fluorescens* or *G. intraradices*. The data collected during panicle initiation stage (120 DAS) were analysed and furnished in Table 8 & 9, Figure 8, 9, 10 & 11 and Plate 5

4.2.2.1 Above ground plant growth parameters

G. intraradices combined with *A. microphylla* recorded maximum shoot length (92.4 cm) and was on par with all the combined treatments and *P. fluorescens*, *A. microphylla* and blue green algae individual treatments. With all other above ground parameters viz., weight of fresh and dry shoot, total leaf area and tiller number, *P. fluorescens* combined treatment with *A. lipoferum* recorded maximum which were enhanced by 69.7, 75.5, 64.1 and 100.0 per cent respectively over untreated control. Weight of fresh and dry shoot were statistically similar to *P. fluorescens* combined treatments with *G. intraradices* and *A. microphylla*. Total leaf area was on par with all the combined treatments of *P. fluorescens* and tiller number was statistically similar to combined inoculation of *P. fluorescens* with *G. intraradices* (Table 8 and Figure 8 & 9)

Significantly reduced fresh (26.7 g) and dry (14.3 g) shoot weight, and total leaf area (453.2 cm²) were recorded in untreated control. Shoot length (75.8 cm) and number of tillers (2.6) were minimum and were on par with individual treatment of *B. megaterium*.

Uninoculated control registered maximum of all the parameters except shoot length.

4.2.2.2 Below ground plant growth parameters

P. fluorescens combined with *G. intraradices* recorded the maximum root length (22.4 cm), fresh (29.4 g) and dry (13.6 g) root weight, and root volume (23.6 cc) and were on par with *P. fluorescens* combined with *A. lipoferum* (Table 9 and Figure 10)

Table 8. Effect of microbial inoculants on above ground plant growth parameters of rice cv. ADT 44 against the infection of *H. gracilis* at panicle initiation stage (120 DAS) under glasshouse conditions.

Treatment	(Mean of three replications)									
	Shoot length (cm)	Fresh shoot weight (g)		Dry shoot weight (g)		Total leaf area (cm ²)	Number of tillers			
	%	%	%	%	%	%	%			
T ₁ – <i>Azolla microphylla</i>	86.4	14.0	37.9	41.9	21.2	48.3	631.2	39.3	3.2	23.1
T ₂ – <i>Azospirillum lipoferum</i>	85.8	13.2	36.2	35.6	19.9	39.2	627.9	38.5	3.4	30.8
T ₃ – Blue green algae	86.2	13.7	39.0	46.1	21.8	52.4	681.4	50.4	3.8	46.2
T ₄ – <i>Bacillus megaterium</i>	79.7	5.1	34.7	30.0	19.0	32.9	548.7	21.1	2.9	11.5
T ₅ – <i>Glomus intraradices</i>	82.7	9.1	38.1	42.7	21.5	50.3	649.3	43.3	3.4	30.8
T ₆ – <i>A. microphylla</i> + <i>G. intraradices</i>	92.4	21.9	38.9	45.7	22.1	54.5	671.5	48.2	3.8	46.2
T ₇ – <i>A. lipoferum</i> + <i>G. intraradices</i>	92.1	21.5	40.0	49.8	22.8	59.4	682.1	50.5	4.1	57.7
T ₈ – Blue green algae + <i>G. intraradices</i>	87.4	15.3	39.9	49.4	22.1	54.5	660.4	45.7	3.5	34.6
T ₉ – <i>B. megaterium</i> + <i>G. intraradices</i>	88.9	17.3	38.3	43.4	21.7	51.7	654.7	44.5	3.7	42.3
T ₁₀ – <i>Pseudomonas fluorescens</i>	87.6	15.6	39.1	46.4	22.2	55.2	664.7	46.7	3.9	50.0
T ₁₁ – <i>A. microphylla</i> + <i>P. fluorescens</i>	91.6	20.8	43.4	62.5	24.4	70.6	712.4	57.2	4.1	57.7
T ₁₂ – <i>A. lipoferum</i> + <i>P. fluorescens</i>	90.4	19.3	45.3	69.7	25.1	75.5	743.5	64.1	5.2	100.0
T ₁₃ – Blue green algae + <i>P. fluorescens</i>	89.7	18.3	42.5	59.2	23.8	66.4	697.2	53.8	4.0	53.8
T ₁₄ – <i>B. megaterium</i> + <i>P. fluorescens</i>	89.2	17.7	42.8	60.3	23.1	61.5	699.7	54.4	4.2	61.5
T ₁₅ – <i>G. intraradices</i> + <i>P. fluorescens</i>	89.1	17.5	44.5	66.7	24.8	73.4	721.2	59.1	4.9	88.5
T ₁₆ – Carbofuran 3G	86.3	13.9	38.0	42.3	21.3	49.0	638.4	40.9	3.3	26.9
T ₁₇ – Untreated control	75.8	–	26.7	–	14.3	–	453.2	–	2.6	–
T ₁₈ – Uninoculated control	89.8	18.5	48.6	82.0	27.4	91.6	763.3	68.4	5.8	123.1
CD (P=0.05)	6.2		2.3		1.2		47.4		0.4	

% - Per cent increase over untreated control

Figure 8. Effect of *P.fluorescens* and its combinations on shoot length and fresh and dry shoot weights of rice cv. ADT 44 against the infection of *H.gracilis* at panicle initiation stage (120 DAS) under glasshouse conditions

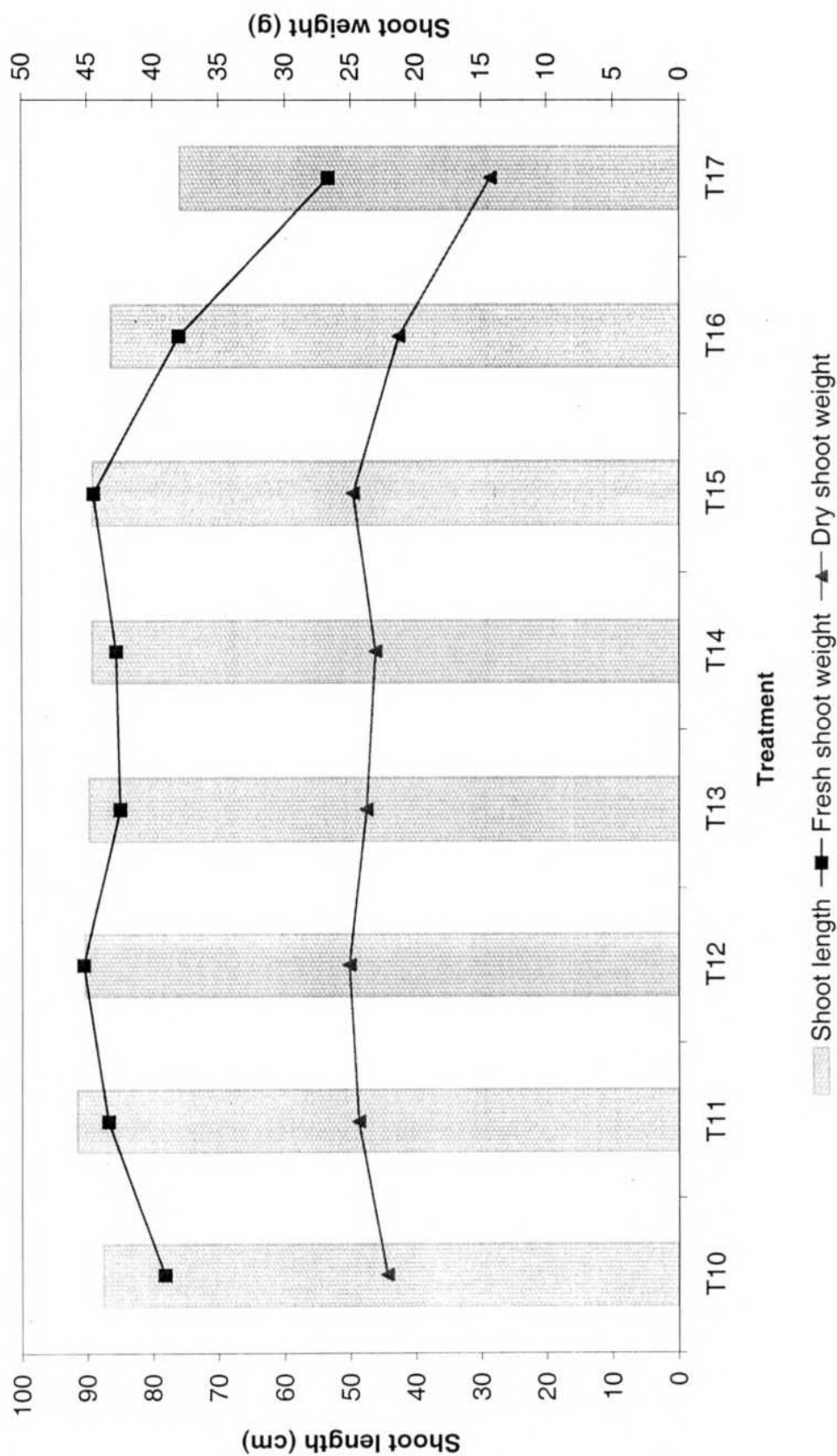


Figure 9. Effect of *P.fluorescens* and its combinations on total leaf area and number of tillers of rice cv. ADT 44 against the infection of *H.gracilis* at panicle initiation stage (120 DAS) under glasshouse conditions

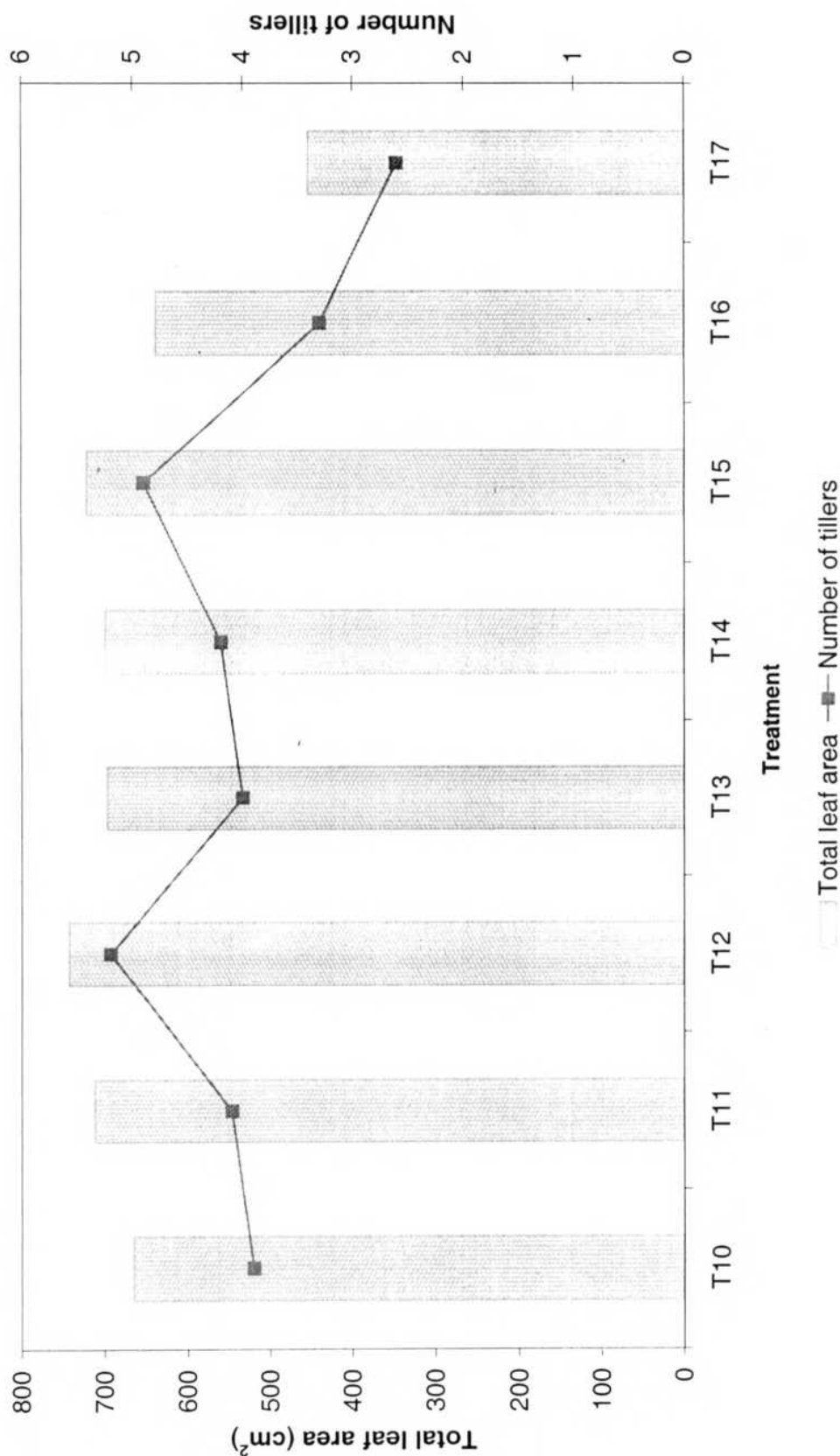


Table 9. Effect of microbial inoculants on below ground plant growth parameters and nematode population in root and soil of rice cv. ADT 44 against the infection of *H. gracilis* at panicle initiation stage (120 DAS) under glasshouse conditions

Treatment	(Mean of three replications)										
	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root volume (cc)	Nematode population/ g root	Nematode population/ 200 g soil	%	%	%	%	
T ₁ – <i>Azolla microphylla</i>	19.2	20.6	66.1	10.5	87.5	18.1	30.2	118.7	-37.4	168.8	-41.1
T ₂ – <i>Azospirillum lipoferum</i>	19.9	24.5	97.6	11.9	112.5	20.9	50.4	107.9	-43.1	148.9	-48.1
T ₃ – Blue green algae	19.1	20.2	62.9	10.7	91.1	17.4	25.2	128.6	-32.2	164.2	-42.7
T ₄ – <i>Bacillus megaterium</i>	19.4	20.8	67.7	9.4	67.9	18.2	30.9	136.7	-27.9	173.6	-39.4
T ₅ – <i>Glomus intraradices</i>	18.7	23.2	87.1	11.5	105.4	19.2	38.1	104.7	-44.8	143.9	-49.8
T ₆ – <i>A. microphylla</i> + <i>G. intraradices</i>	20.3	21.4	72.6	11.3	101.8	18.4	32.4	94.2	-50.3	139.4	-51.4
T ₇ – <i>A. lipoferum</i> + <i>G. intraradices</i>	20.1	24.9	100.8	12.1	116.1	21.2	52.5	84.9	-55.2	133.7	-53.4
T ₈ – Blue green algae + <i>G. intraradices</i>	19.2	23.4	88.7	11.4	103.6	19.4	39.6	91.8	-51.6	142.6	-50.3
T ₉ – <i>B. megaterium</i> + <i>G. intraradices</i>	19.5	24.1	94.4	11.7	108.9	20.2	45.3	88.5	-53.3	140.9	-50.9
T ₁₀ – <i>Pseudomonas fluorescens</i>	20.2	23.8	91.9	11.6	107.1	19.7	41.7	79.4	-58.1	120.7	-57.9
T ₁₁ – <i>A. microphylla</i> + <i>P. fluorescens</i>	19.6	22.5	81.5	11.8	110.7	18.7	34.5	71.6	-62.3	109.9	-61.7
T ₁₂ – <i>A. lipoferum</i> + <i>P. fluorescens</i>	22.3	27.8	124.2	13.1	133.9	22.5	61.9	68.7	-63.8	105.9	-63.1
T ₁₃ – Blue green algae + <i>P. fluorescens</i>	20.6	25.4	104.8	12.0	114.3	21.4	54.0	75.4	-60.3	118.8	-58.6
T ₁₄ – <i>B. megaterium</i> + <i>P. fluorescens</i>	19.8	24.3	96.0	11.9	112.5	20.4	46.8	76.3	-59.8	114.5	-60.1
T ₁₅ – <i>G. intraradices</i> + <i>P. fluorescens</i>	22.4	29.4	137.1	13.6	142.9	23.6	69.8	68.4	-63.9	106.7	-62.8
T ₁₆ – Carbofuran 3G	18.5	20.1	62.1	9.2	64.3	17.6	26.6	106.5	-43.9	132.9	-53.6
T ₁₇ – Untreated control	17.4	12.4	–	5.6	–	13.9	–	189.7	–	286.7	–
T ₁₈ – Uninoculated control	19.6	29.6	138.7	14.1	151.8	23.7	70.5	–	–	–	–
CD (P=0.05)	1.5	2.0	1.1	1.5	5.9	7.0					

% - Per cent increase or decrease (-) over untreated control

Figure 10 . Effect of *P.fluorescens* and its combinations on below ground plant growth parameters of rice cv. ADT 44 against the infection of *H.gracilis* at panicle initiation stage (120 DAS) under glasshouse conditions

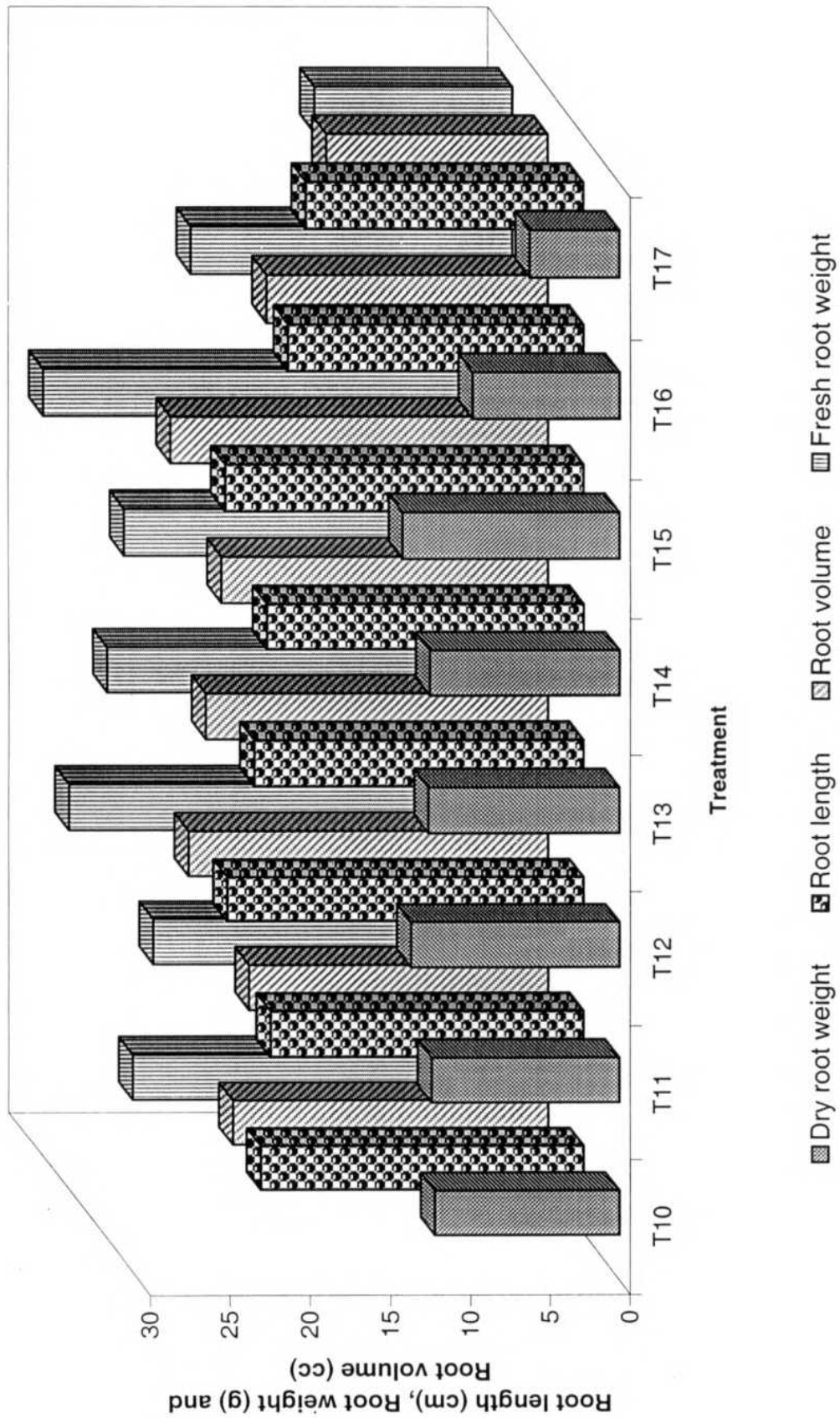


Figure 11. Effect of *P.fluorescens* and its combinations on nematode population in root and soil of rice cv. ADT 44 against the infection of *H.gracilis* at panicle initiation stage (120 DAS) under glasshouse conditions

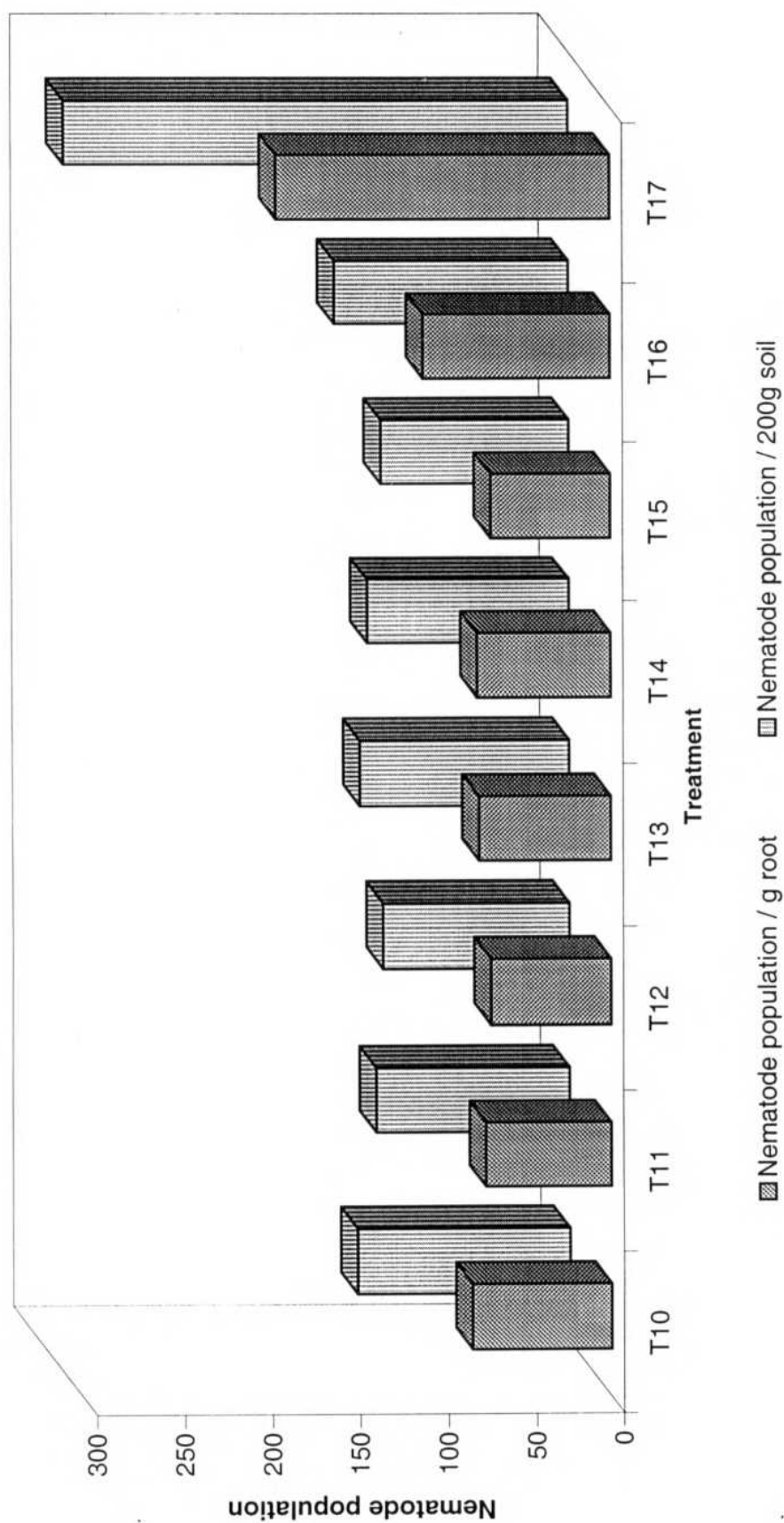


Plate 5. Effect of microbial inoculants on cv. ADT 44 against the infection of the rice-root nematode, *H. gracilis* at panicle initiation stage (120 DAS) under glasshouse conditions

Individual and combined effect of treatments in comparison with carbofuran treatment, untreated and uninoculated control

A. Individual microbial inoculants

B. *G. intraradices*, *P. fluorescens* and their combination

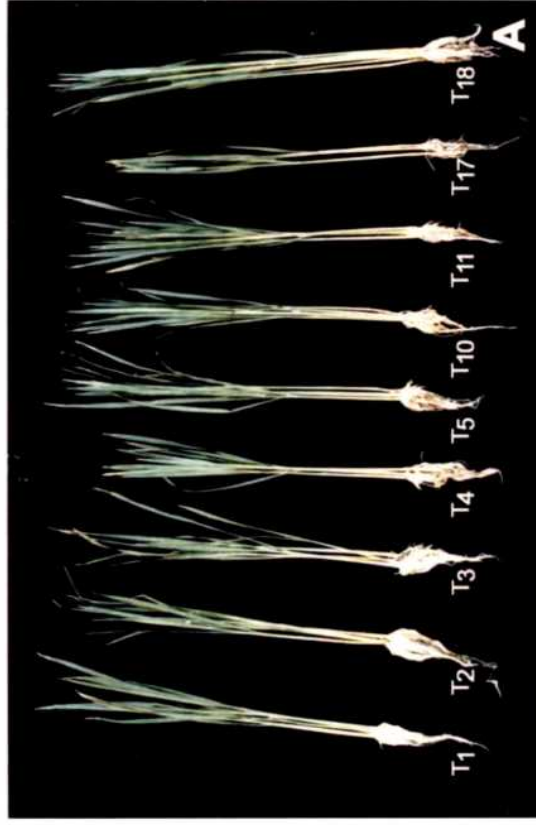
C. *G. intraradices* and its combinations

D. *P. fluorescens* and its combinations

Treatment

- T₁ – *Azolla microphylla*
- T₂ – *Azospirillum lipoferum*
- T₃ – Blue green algae
- T₄ – *Bacillus megaterium*
- T₅ – *Glomus intraradices*
- T₆ – *A. microphylla* + *G. intraradices*
- T₇ – *A. lipoferum* + *G. intraradices*
- T₈ – Blue green algae + *G. intraradices*
- T₉ – *B. megaterium* + *G. intraradices*
- T₁₀ – *Pseudomonas fluorescens*
- T₁₁ – *A. microphylla* + *P. fluorescens*
- T₁₂ – *A. lipoferum* + *P. fluorescens*
- T₁₃ – Blue green algae + *P. fluorescens*
- T₁₄ – *B. megaterium* + *P. fluorescens*
- T₁₅ – *G. intraradices* + *P. fluorescens*
- T₁₆ – Carbofuran 3G
- T₁₇ – Untreated control
- T₁₈ – Uninoculated control

Plate 5



Root length (17.4 cm) of untreated control was minimum and on par with *G intraradices* individual treatment. Weight of fresh (12.4 g) and dry (5.6 g) root, and root volume (13.9 cc) of untreated control was significantly reduced.

Uninoculated control registered maximum of all the below ground parameters except the root length.

4.2.2.3 Nematode population in root and soil

Maximum reduction of 63.9 per cent over untreated control of *H. gracilis* population in root was recorded in dual inoculation of *P. fluorescens* with *G. intraradices*. Combined treatment of *P. fluorescens* with *A. lipoferum* was the next best treatment in restricting the root population of *H. gracilis*. Dual inoculation of *P. fluorescens* with *A. lipoferum* was the most effective in suppressing soil population of *H. gracilis* to 105.9 nematodes per 200 g followed by *P. fluorescens* with *G. intraradices* (106.7 nematodes/200 g). *P. fluorescens* combined inoculations with *A. microphylla*, *A. lipoferum* and *G. intraradices* were on par with regard to *H. gracilis* population both in root and soil (Table 9 and Figure 11).

Significantly higher nematode population in root (189.7 per g) and soil (286.7 per 200 g) were recorded in untreated control.

4.2.3 Short duration cv. IR 50 (field study)

Analysed pooled data of field experiments conducted during summer and *kharif* seasons on plant growth parameters of short duration rice cv. IR 50 and *H. gracilis* population in root and soil at seedling stage (30 DAS) and panicle initiation stage (75 DAS), yield attributes at maturity stage (105 DAS), grain and straw yield and nematode population both in root and soil at harvest (110 DAS) are furnished in Table 10, 11, 12, 13 & 14, Figure 12, 13, 14, 15, 16, 17, 18 & 19 and Plate 6, 7, 8, 9, 10 & 11.

Plate 6. Effect of microbial inoculants on cvs. IR 50 and ADT 44 against the infection of the rice-root nematode, *H. gracilis* at seedling stage (30 DAS) under field conditions

Individual and combined effect of treatments in comparison with carbofuran treatment and untreated control

- A. Nursery bed view during summer, 2001 (cvs. IR 50 and ADT 44)**
- B. Nursery bed view during *kharif*, 2001 (cv. IR 50)**
- C. Seedling samples of cv. IR 50 (summer, 2001)**
- D. Seedling samples of cv. IR 50 (*kharif*, 2001)**
- E. Seedling samples of cv. ADT 44 (summer, 2001)**

Treatment

- 1 = T₁ – *Azospirillum lipoferum*
- 2 = T₂ – *Glomus intraradices*
- 3 = T₃ – *Pseudomonas fluorescens*
- 4 = T₄ – *A. lipoferum* + *G. intraradices*
- 5 = T₅ – *A. lipoferum* + *P. fluorescens*
- 6 = T₆ – *G. intraradices* + *P. fluorescens*
- 7 = T₇ – Carbofuran 3G
- C = T₈ – Untreated control

Plate 6



4.2.3.1 Seedling stage (30 DAS)

A total of eight treatments, as seed and nursery application were imposed prior to sowing and observations were recorded and analysed for the effectiveness of the treatments after 30 days of sowing before transplanting the seedlings to the main field (Plate 6)

4.2.3.1.1 Above ground plant growth parameters

All the above ground plant growth characters were recorded maximum in *P fluorescens* combined with *G intraradices* treatment. The parameters were enhanced by 35.0, 144.4, 325.0 and 58.6 per cent over untreated control in respect to shoot length, fresh shoot weight, dry shoot weight and total leaf area respectively. Shoot length was on par with *A lipoferum* combined applications to *P fluorescens* and *G intraradices*, whereas total leaf area was statistically similar to only combined treatment of *P fluorescens* with *A lipoferum*. Weight of fresh and dry shoot was significantly different from other treatments. Dual inoculation of *P fluorescens* with *A lipoferum* was the next best treatment with regard to all the above ground plant parameters studied (Table 10 and Figure 12)

Untreated control registered significantly reduced shoot length (27.7 cm), and total leaf area (23.2 cm²). Fresh shoot weight (0.9 g) was on par with individual treatments of *G intraradices* and *A lipoferum*, whereas dry shoot weight (0.4 g) was statistically similar to *A lipoferum* individual treatment.

4.2.3.1.2 Below ground plant growth parameters

Root length (12.4 cm), fresh root weight (1.2 g), dry root weight (0.8 g) and root volume (0.9 cc) of combined application of *P fluorescens* with *G intraradices* recorded maximum. Among the below ground plant parameters only the root volume of the treatment was on par with *A lipoferum* combined inoculations with *P fluorescens* and

Table 10. Effect of microbial inoculants on above and below ground plant growth parameters and nematode population in root and soil of rice cv. IR 50 against the infection of *H. gracilis* at seedling stage (30 DAS) under field conditions*

Treatment	(Mean of three replications)									
	Shoot length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Total leaf area (cm ²)	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root volume (cc)	Nematode population/g root	Nematode population/200 g soil
T ₁ - <i>Azospirillum lipoferum</i>	32.1 (15.9)	1.2 (33.3)	0.7 (75.0)	28.7 (23.7)	10.8 (36.7)	0.7 (250.0)	0.4 (300.0)	0.5 (150.0)	15.9 (-38.4)	118.4 (-19.1)
T ₂ - <i>Glomus intraradices</i>	31.6 (14.1)	1.0 (11.1)	0.8 (100.0)	27.4 (18.1)	10.5 (32.9)	0.7 (250.0)	0.5 (400.0)	0.6 (200.0)	12.8 (-50.4)	111.5 (-23.8)
T ₃ - <i>Pseudomonas fluorescens</i>	34.0 (22.7)	1.6 (77.8)	1.1 (175.0)	28.9 (24.6)	10.3 (30.4)	0.8 (300.0)	0.6 (500.0)	0.6 (200.0)	9.7 (-62.4)	88.9 (-39.2)
T ₄ - <i>A. lipoferum</i> + <i>G. intraradices</i>	35.7 (28.9)	1.7 (88.9)	1.1 (175.0)	32.7 (40.9)	11.0 (39.2)	0.8 (300.0)	0.6 (500.0)	0.7 (250.0)	10.8 (-58.1)	89.6 (-38.8)
T ₅ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	36.8 (32.9)	1.7 (88.9)	1.1 (175.0)	35.5 (53.0)	11.2 (41.8)	0.9 (350.0)	0.6 (500.0)	0.8 (300.0)	8.9 (-65.5)	75.5 (-48.4)
T ₆ - <i>G. intraradices</i> + <i>P. fluorescens</i>	37.4 (35.0)	2.2 (144.4)	1.7 (325.0)	36.8 (58.6)	12.4 (57.0)	1.2 (500.0)	0.8 (700.0)	0.9 (350.0)	8.5 (-67.1)	73.3 (-49.9)
T ₇ - Carbofuran 3G	31.1 (12.3)	1.0 (11.1)	0.6 (50.0)	26.7 (15.1)	8.8 (11.4)	0.4 (100.0)	0.2 (100.0)	0.4 (100.0)	15.6 (-39.5)	96.2 (-34.2)
T ₈ - Untreated control	27.7 -	0.9 -	0.4 -	23.2 -	7.9 -	0.2 -	0.1 -	0.2 -	25.8 -	146.3 -
CD (P=0.05)	2.0	0.3	0.3	1.5	0.8	0.1	0.1	0.1	1.8	9.5

Figures in parentheses indicate per cent increase or decrease (-) over untreated control

* Pooled data of both summer and *kharif* season experiments

Figure 12. Effect of microbial inoculants on above ground plant growth parameters of rice cv. IR 50 against the infection of *H.gracilis* at seedling stage (30 DAS) under field conditions

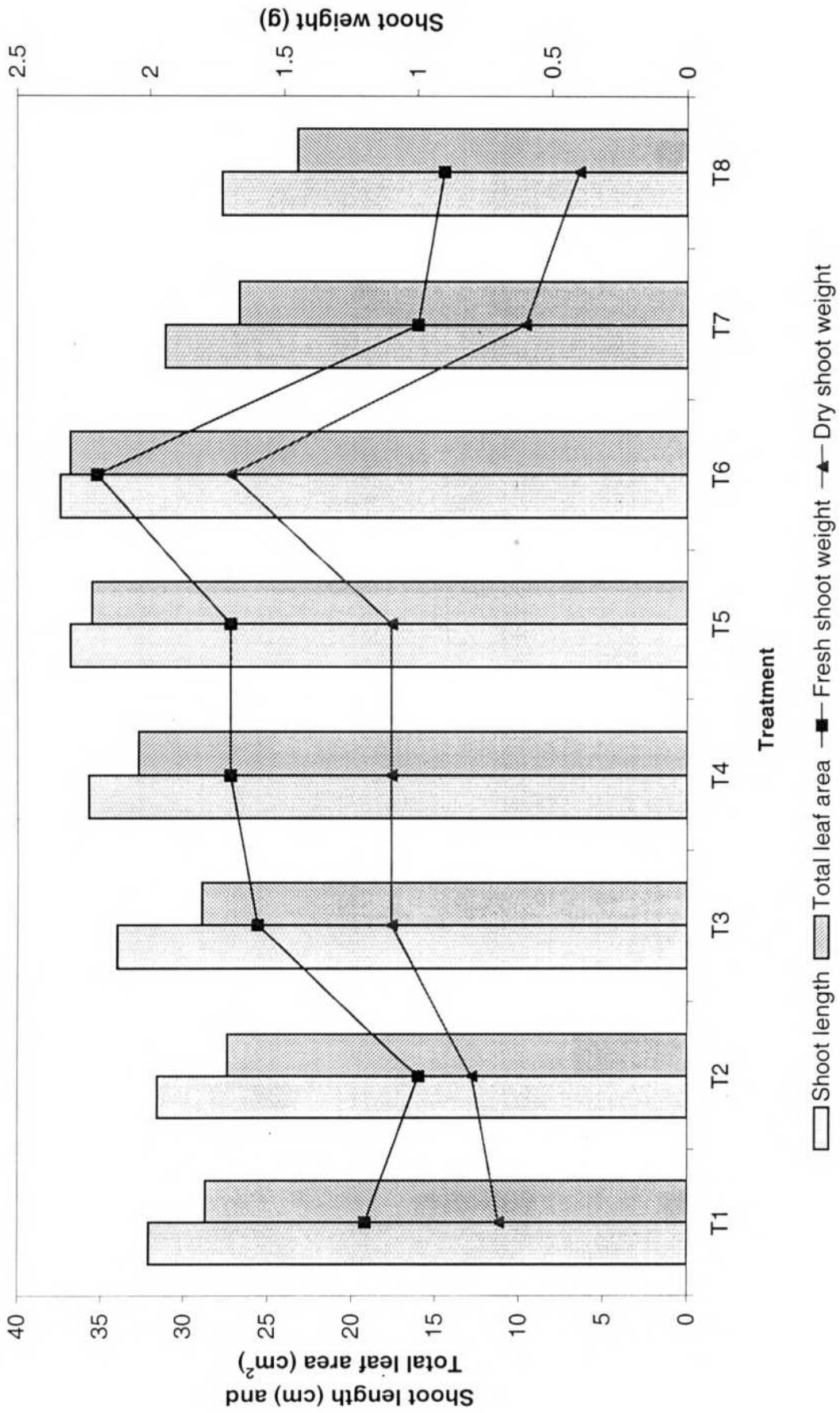


Figure 13. Effect of microbial inoculants on below ground plant growth parameters of rice cv. IR 50 against the infection of *H.gracilis* at seedling stage (30 DAS) under field conditions

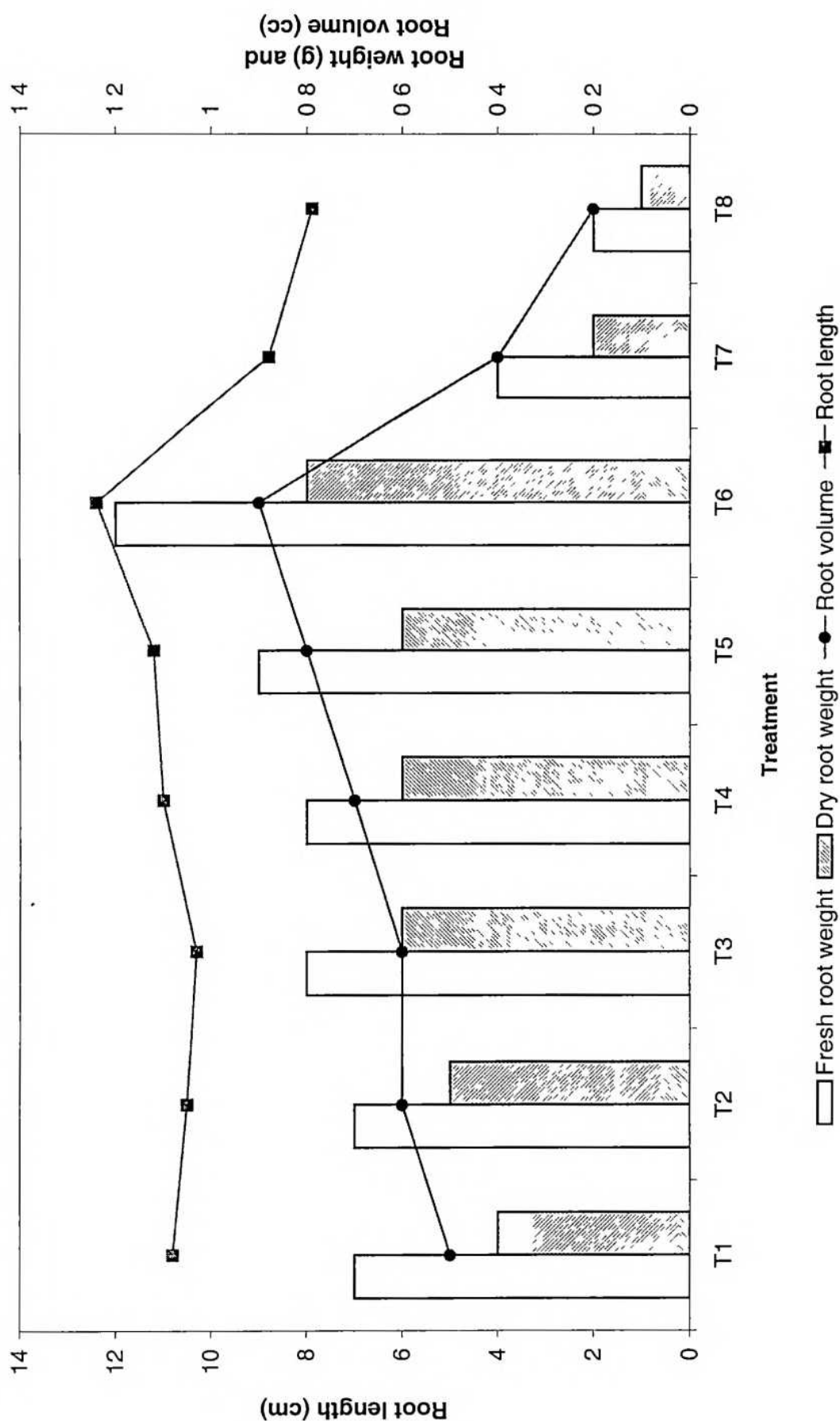
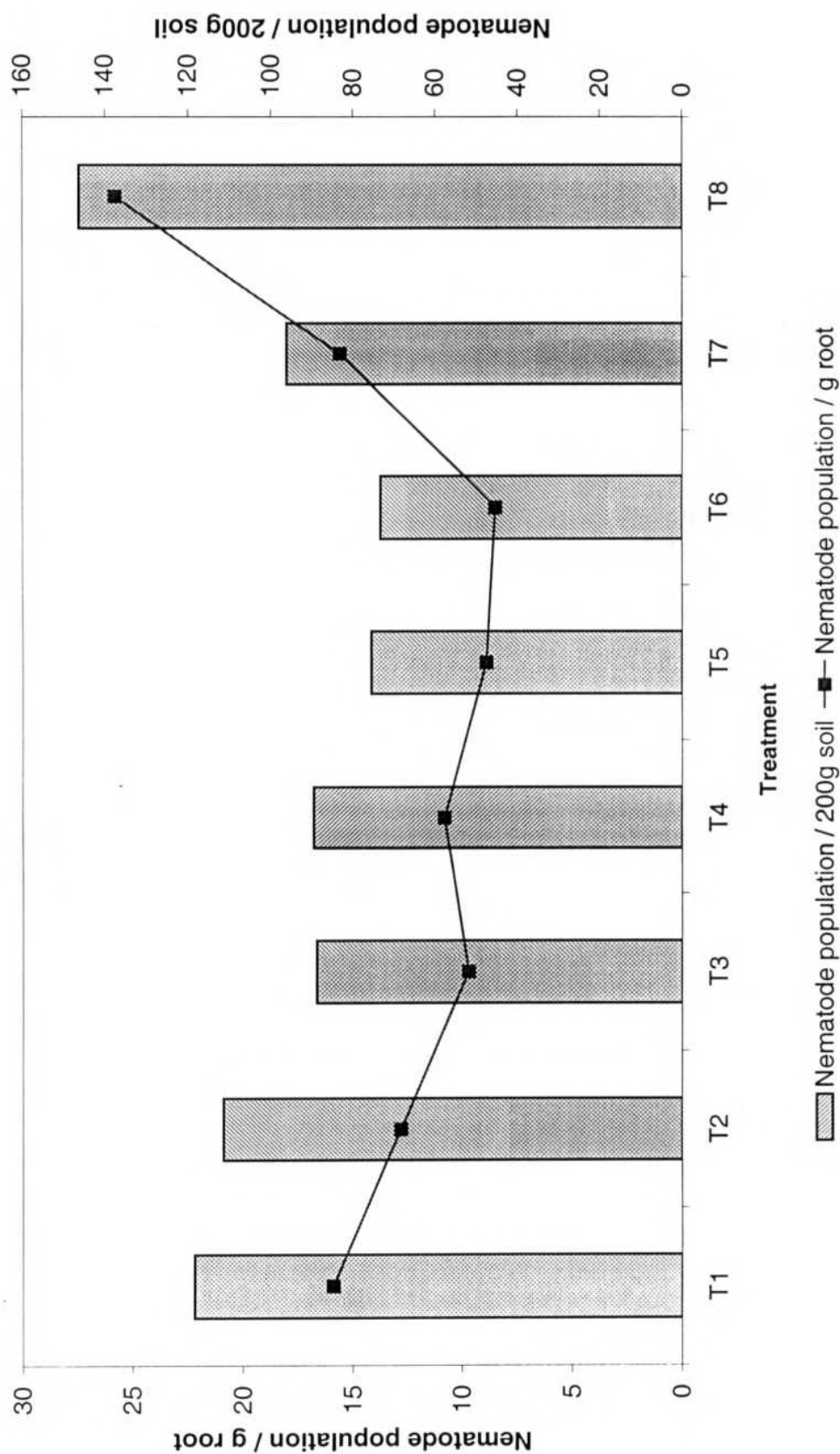


Figure 14 . Effect of microbial inoculants on nematode population in root and soil of rice cv. IR 50 against the infection of *H.gracilis* at seedling stage (30 DAS) under field conditions



G intraradices Co-inoculation of *P fluorescens* with *A lipoferum* was observed to be the next best treatment in respect of all the plant root characters (Table 10 and Figure 13)

Root length (7.9 cm), weight of fresh (0.2 g) and dry (0.1 g) root and root volume (0.2 cc) of untreated control were significantly recorded minimum

4.2.3.1.3 Nematode population in root and soil

Maximum reduction of both root and soil population of *H gracilis* was observed in the dual inoculation of *P fluorescens* with *G intraradices* and this was on par with *P fluorescens* individual and combined treatment with *A lipoferum* in case of root population, whereas soil population was statistically similar to *P fluorescens* combined with *A lipoferum*. The best treatment was able to reduce the nematode population in root and soil by 67.1 and 49.9 per cent respectively over untreated control (Table 10 and Figure 14)

Significantly higher population of *H gracilis* was recorded in untreated control both in root (25.8 per g) and soil (146.3 per 200 g)

4.2.3.2 Panicle initiation stage (75 DAS)

A total of seventeen treatments were imposed in the main field. Plant growth characters and population of nematode in root and soil of cv IR 50 were observed at panicle initiation stage (75 days after sowing) and analysed (Plate 7, 8 & 9)

4.2.3.2.1 Above ground plant growth parameters

Shoot length (78.8 cm) of combined inoculation of *P fluorescens* with *G intraradices* was the maximum. Shoot length of the best treatment was on par with individual and combined treatments of *P fluorescens* and *G intraradices*. Weight of fresh and dry shoot, leaf area index, tiller number per hill of dual application of *P fluorescens* with *A lipoferum* recorded maximum. Fresh shoot weight of the best

Plate 7. Management of the rice-root nematode, *H. gracilis* with microbial inoculants under field conditions (Main field view)

- A. cv. IR 50 at panicle initiation stage (summer, 2001)**
- B. cv. IR 50 at maturity stage (summer, 2001)**
- C. cv. IR 50 at panicle initiation stage (*kharif*, 2001)**
- D. cv. IR 50 at maturity stage (*kharif*, 2001)**
- E. cv. ADT 44 at panicle initiation stage (summer, 2001)**
- F. cv. ADT 44 at maturity stage (summer, 2001)**

Plate 7



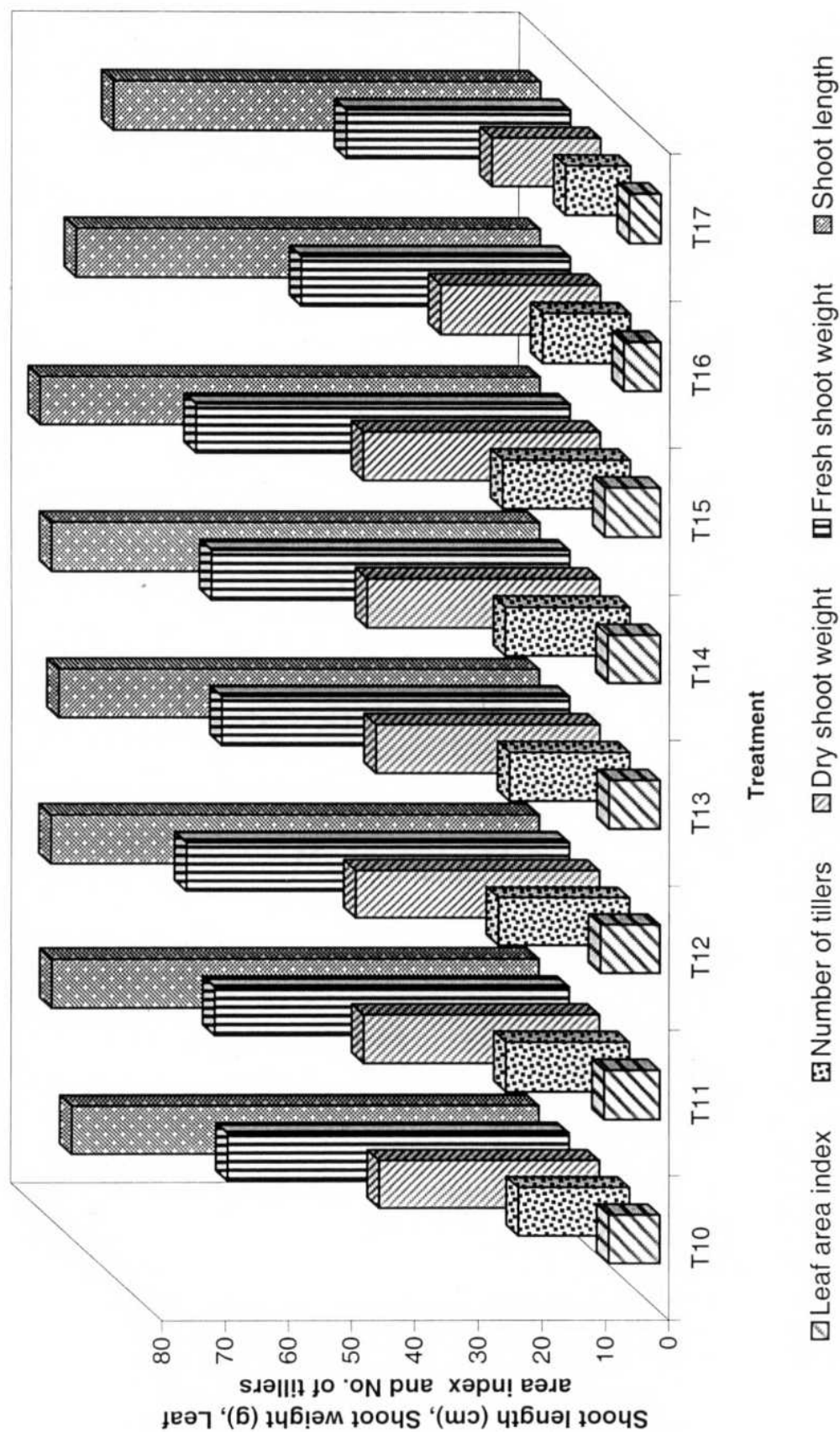
Table 11. Effect of microbial inoculants on above ground plant growth parameters of rice cv. IR 50 against the infection of *H. gracilis* at panicle initiation stage (75 DAS) under field conditions*

Treatment	(Mean of three replications)					
	Shoot length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Leaf area index	Number of tillers	%
T ₁ - <i>Azolla microphylla</i>	69.6	48.2	30.3	6.8	14.1	36.9
T ₂ - <i>Azospirillum lipoferum</i>	71.2	50.5	31.7	7.2	14.2	37.9
T ₃ - Blue green algae	72.2	47.9	30.3	7.2	13.8	34.0
T ₄ - <i>Bacillus megaterium</i>	69.1	47.9	31.1	6.8	14.3	38.8
T ₅ - <i>Glomus intraradices</i>	73.8	49.1	31.4	6.9	15.0	45.6
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	76.4	53.3	33.1	7.7	15.6	51.5
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	76.8	55.6	36.0	8.3	17.5	69.9
T ₈ - Blue green algae + <i>G. intraradices</i>	75.8	53.5	34.6	7.4	16.8	63.1
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	74.4	53.7	33.9	8.0	17.3	68.0
T ₁₀ - <i>Pseudomonas fluorescens</i>	73.7	53.9	34.6	8.1	17.5	69.9
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	76.9	56.0	37.1	8.8	19.5	89.3
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	77.1	60.5	38.4	9.4	20.7	101.0
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	75.9	54.9	35.2	8.1	19.0	84.5
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	77.0	56.6	36.6	8.3	19.7	91.3
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	78.8	59.1	37.3	8.8	20.2	96.1
T ₁₆ - Carbofuran 3G	73.2	42.4	25.2	5.8	13.9	35.0
T ₁₇ - Untreated control	67.4	35.3	17.2	5.0	10.3	-
CD (P=0.05)	5.0	3.9	2.3	0.7	1.5	

% - Per cent increase over untreated control

* Pooled data of both summer and *kharif* season experiments

Figure 15. Effect of *P. fluorescens* and its combinations on above ground plant growth parameters of rice cv. IR 50 against the infection of *H. gracilis* at panicle initiation stage (75 DAS) under field conditions



treatment, which was enhanced to 71.4 per cent over untreated control was on par with *P. fluorescens* combined inoculations with *G. intraradices* and *B. megaterium*, whereas dry shoot weight was statistically similar to these two treatments along with *P. fluorescens* dual application with *A. microphylla*. The parameters viz., dry shoot weight and number of tillers per hill of the best treatment was increased by 123.3 and 101.0 per cent respectively over untreated control. Leaf area index per hill of the best treatment was enhanced by 88.0 per cent over untreated control and was on par with *P. fluorescens* combined inoculation with *G. intraradices* and *A. microphylla*. Number of tillers per hill of the best treatment was statistically similar to *P. fluorescens* combined treatment with *G. intraradices*, blue green algae and *A. microphylla* (Table 11 and Figure 15).

All the above ground plant growth parameters were minimum in untreated control. Shoot length (67.4 cm) was on par with individual treatments of *A. microphylla*, *A. lipoferum*, blue green algae and *B. megaterium*. Fresh shoot weight per hill (35.3 g), dry shoot weight (17.2 g), leaf area index (5.0) and number of tillers (10.3) per hill were significantly reduced.

4.2.3.2.2 Below ground plant growth parameters

Root length of *P. fluorescens* combined inoculation with *G. intraradices* was enhanced to the maximum by 89.6 per cent over untreated control and was on par with dual inoculations of *P. fluorescens* with *A. lipoferum* and *A. microphylla*. Fresh root weight (34.6 g), dry root weight (16.9 g) and root volume (40.1 cc) per hill of *P. fluorescens* combined with *A. lipoferum* recorded maximum followed by dual application of *P. fluorescens* with *G. intraradices*. Fresh root weight was significantly different from rest of the treatments, while dry root weight was on par with *P. fluorescens* combined with *G. intraradices* and *A. microphylla*. Not much difference was observed

Table 12. Effect of microbial inoculants on below ground plant growth parameters and nematode population in root and soil of rice cv. IR 50 against the infection of *H. gracilis* at panicle initiation stage (75 DAS) under field conditions*

Treatment	(Mean of three replications)									
	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root volume (cc)	Nematode population/ g root	Nematode population/ 200 g soil	%	%	%	%
T ₁ - <i>Azolla microphylla</i>	15.3	14.2	27.3	35.1	13.9	52.7	33.3	31.6	82.9	-51.5
T ₂ - <i>Azospirillum lipoferum</i>	17.1	27.6	26.8	32.7	12.5	37.4	35.0	38.3	69.4	-59.4
T ₃ - Blue green algae	16.7	24.6	25.3	25.2	11.4	25.3	31.3	23.7	73.0	-57.3
T ₄ - <i>Bacillus megaterium</i>	15.7	17.2	25.3	25.2	11.0	20.9	33.2	31.2	79.3	-53.6
T ₅ - <i>Glomus intraradices</i>	17.2	28.4	27.3	35.1	13.0	42.9	35.1	38.7	71.9	-57.9
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	17.5	30.6	29.5	46.0	14.4	58.2	34.8	37.5	61.4	-64.1
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	18.7	39.6	30.6	51.5	15.0	64.8	36.2	43.1	57.1	-66.6
T ₈ - Blue green algae + <i>G. intraradices</i>	17.4	29.9	29.7	47.0	14.3	57.1	35.5	40.3	58.2	-65.9
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	17.6	31.3	27.8	37.6	13.1	44.0	34.3	35.6	60.1	-64.8
T ₁₀ - <i>Pseudomonas fluorescens</i>	22.0	64.2	30.1	49.0	14.6	60.4	37.2	47.0	46.9	-72.6
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	23.4	74.6	31.3	54.9	15.7	72.5	37.6	48.6	43.3	-74.7
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	24.1	79.9	34.6	71.3	16.9	85.7	40.1	58.5	34.3	-79.9
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	22.3	66.4	30.4	50.5	15.0	64.8	36.6	44.7	37.7	-77.9
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	22.8	70.1	31.5	55.9	15.4	69.2	37.1	46.6	37.0	-78.3
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	25.4	89.6	31.5	55.9	15.7	72.5	39.3	55.3	36.1	-78.9
T ₁₆ - Carbofuran 3G	13.7	2.2	23.6	16.8	10.9	19.8	30.8	21.7	80.2	-53.1
T ₁₇ - Untreated control	13.4	-	20.2	-	9.1	-	25.3	-	170.9	-
CD (P=0.05)	2.3	-	2.4	-	1.3	-	6.3	-	7.7	-

% - Per cent increase or decrease (-) over untreated control

* Pooled data of both summer and *kharif* season experiments

Figure 16 . Effect of *P.fluorescens* and its combinations on below ground plant growth parameters of rice cv. IR 50 against the infection of *H.gracilis* at panicle initiation stage (75 DAS) under field conditions

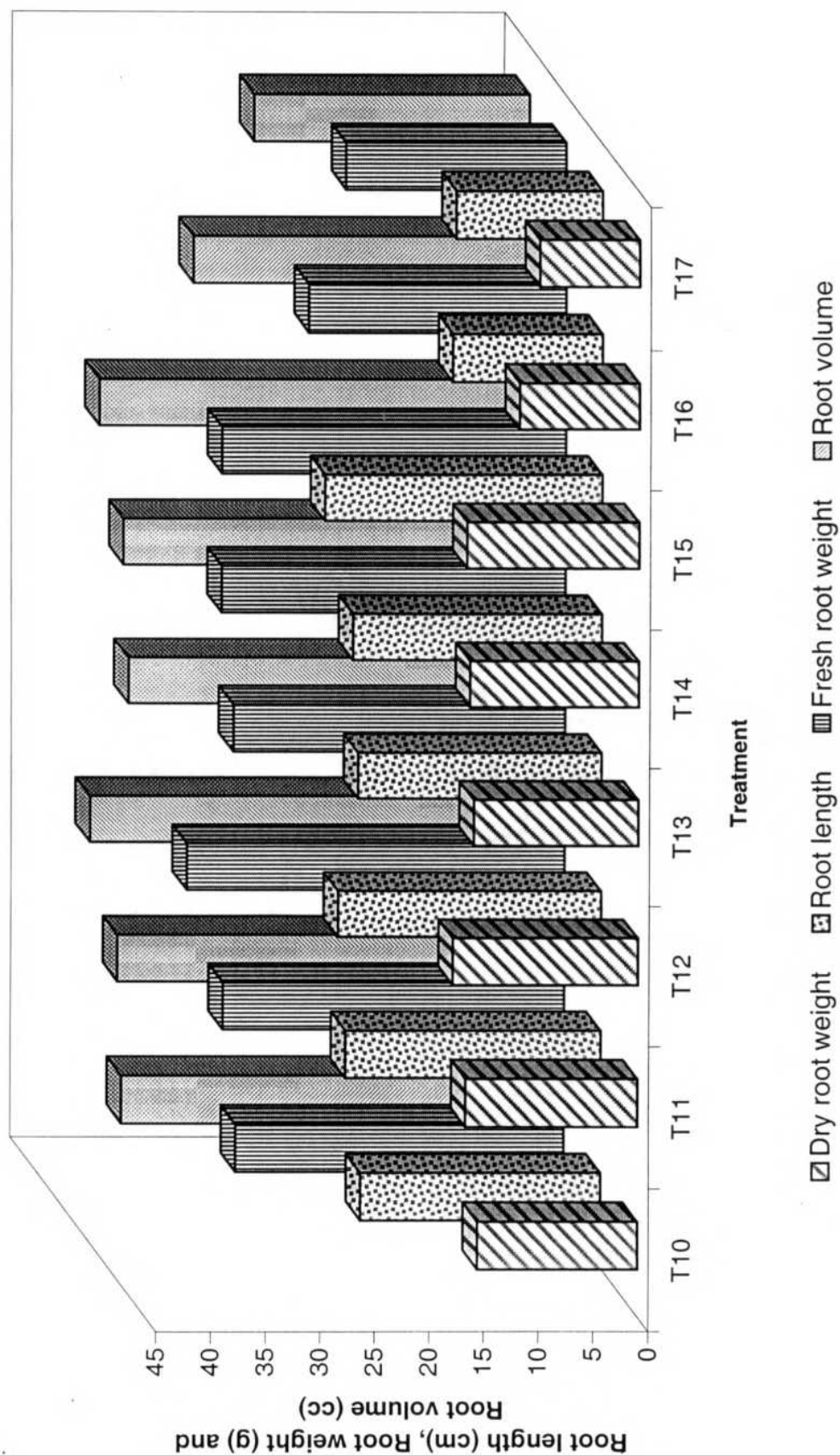


Figure 17 . Effect of *P.fluorescens* and its combinations on nematode population in root and soil of rice cv. IR 50 against the infection of *H.gracilis* at panicle initiation stage (75 DAS) under field conditions

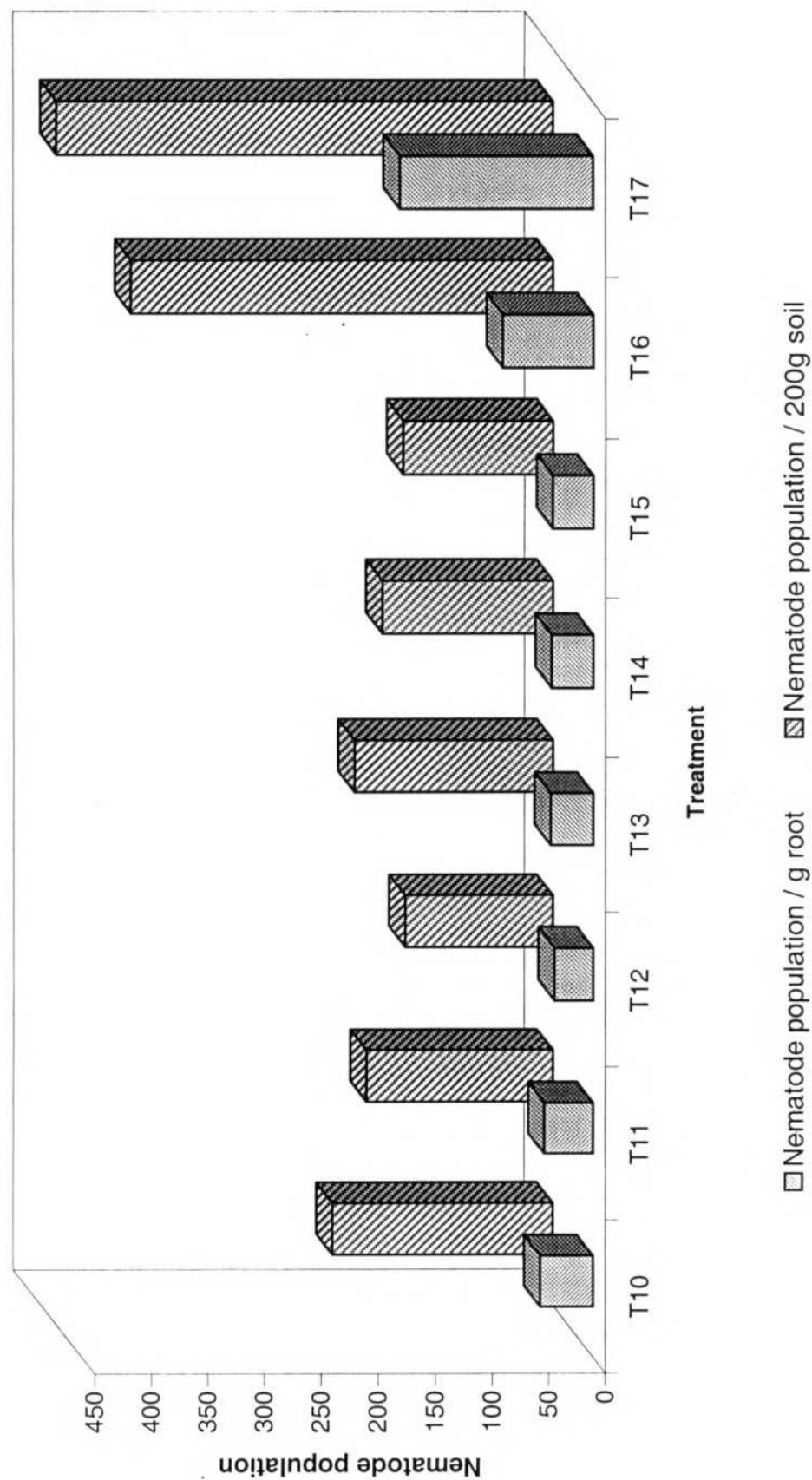


Plate 8. Effect of microbial inoculants on cv. IR 50 against the infection of the rice-root nematode, *H. gracilis* at panicle initiation stage (75 DAS) under field conditions during summer, 2001

Individual and combined effect of treatments in comparison with carbofuran treatment and untreated control

A. Individual microbial inoculants

B. *G. intraradices*, *P. fluorescens* and their combination

C. *G. intraradices* and its combinations

D. *P. fluorescens* and its combinations

Treatment

- T₁ – *Azolla microphylla*
- T₂ – *Azospirillum lipoferum*
- T₃ – Blue green algae
- T₄ – *Bacillus megaterium*
- T₅ – *Glomus intraradices*
- T₆ – *A. microphylla* + *G. intraradices*
- T₇ – *A. lipoferum* + *G. intraradices*
- T₈ – Blue green algae + *G. intraradices*
- T₉ – *B. megaterium* + *G. intraradices*
- T₁₀ – *Pseudomonas fluorescens*
- T₁₁ – *A. microphylla* + *P. fluorescens*
- T₁₂ – *A. lipoferum* + *P. fluorescens*
- T₁₃ – Blue green algae + *P. fluorescens*
- T₁₄ – *B. megaterium* + *P. fluorescens*
- T₁₅ – *G. intraradices* + *P. fluorescens*
- T₁₆ – Carbofuran 3G
- T₁₇ – Untreated control

Plate 8



Plate 9. Effect of microbial inoculants on cv. IR 50 against the infection of the rice-root nematode, *H. gracilis* at panicle initiation stage (75 DAS) under field conditions during *kharif*, 2001

Individual and combined effect of treatments in comparison with carbofuran treatment and untreated control

A. Individual microbial inoculants

B. *G. intraradices*, *P. fluorescens* and their combination

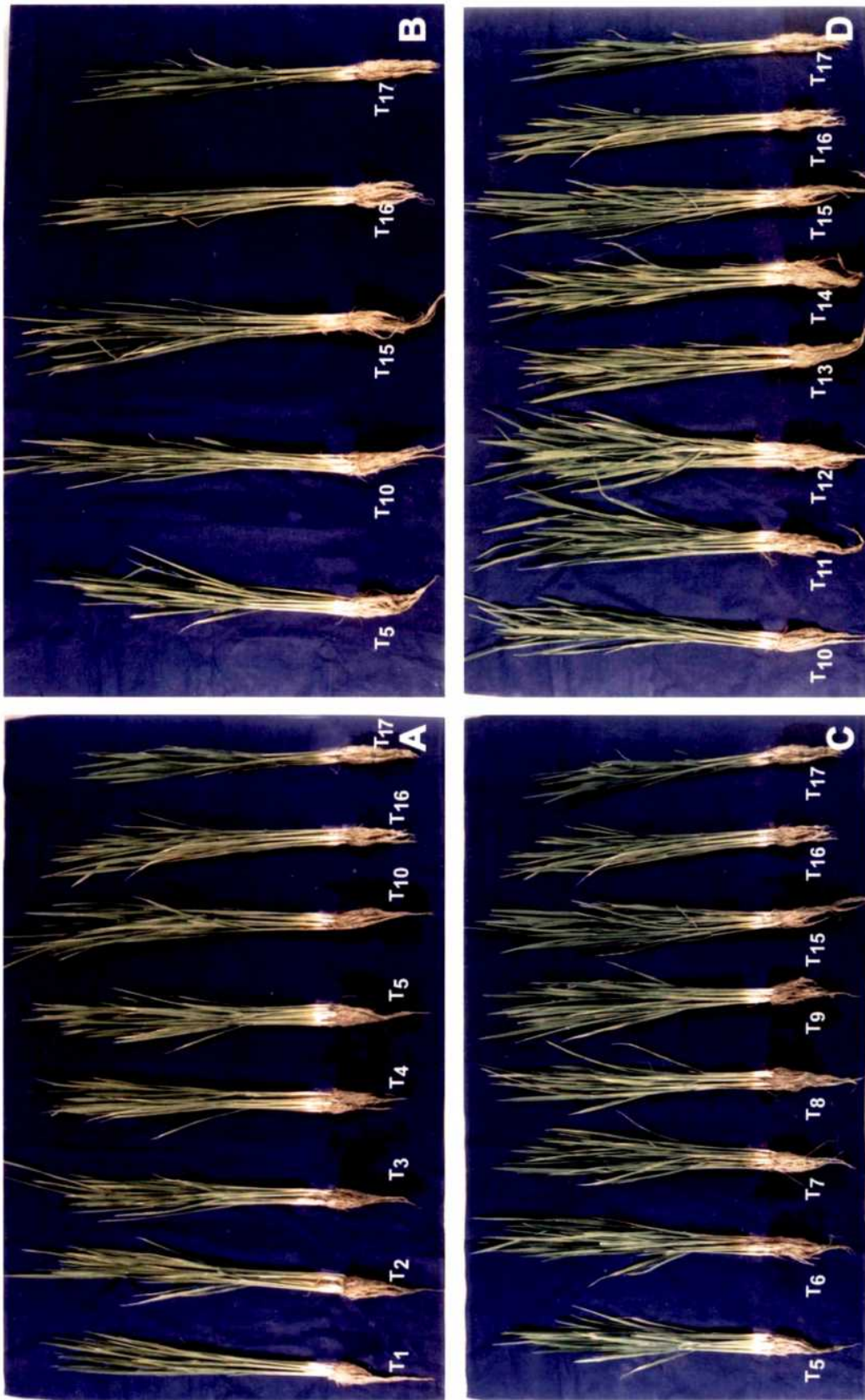
C. *G. intraradices* and its combinations

D. *P. fluorescens* and its combinations

Treatment

- T₁ – *Azolla microphylla*
- T₂ – *Azospirillum lipoferum*
- T₃ – Blue green algae
- T₄ – *Bacillus megaterium*
- T₅ – *Glomus intraradices*
- T₆ – *A. microphylla* + *G. intraradices*
- T₇ – *A. lipoferum* + *G. intraradices*
- T₈ – Blue green algae + *G. intraradices*
- T₉ – *B. megaterium* + *G. intraradices*
- T₁₀ – *Pseudomonas fluorescens*
- T₁₁ – *A. microphylla* + *P. fluorescens*
- T₁₂ – *A. lipoferum* + *P. fluorescens*
- T₁₃ – Blue green algae + *P. fluorescens*
- T₁₄ – *B. megaterium* + *P. fluorescens*
- T₁₅ – *G. intraradices* + *P. fluorescens*
- T₁₆ – Carbofuran 3G
- T₁₇ – Untreated control

Plate 9



among the treatments with regard to root volume per hill as the best treatment was on par with all the treatments except individual applications of *A microphylla*, *B megaterium*, blue green algae and untreated control (Table 12 and Figure 16)

Minimum root length (13.4 cm), fresh root weight (20.2 g), dry root weight (9.1 g) and root volume (25.3 cc) were registered in untreated control. Among these root parameters, root length was statistically similar to *A microphylla* and *B megaterium* individual applications and root volume was on par with blue green algae treatment. While weights of fresh and dry roots were significantly reduced in comparison to other treatments.

4.2.3.2.3 Nematode population in root and soil

Minimum *H gracilis* population in root (34.3 per g) and soil (130.4 per 200 g) of cv IR 50 was observed in *P fluorescens* combined with *A lipoferum*. All other *P fluorescens* combinations were on par with the best treatment, except *A microphylla* and blue green algae in case of root and soil population of *H gracilis* respectively. Combined application of *G intraradices* with *P fluorescens* was the next best treatment in restricting nematode population in root and soil (Table 12 and Figure 17).

Significantly higher population of the nematode were observed in root (170.9 per g) and soil (437.5 per 200 g) in untreated control.

4.2.3.3 Maturity stage (105 DAS)

Yield attributes were recorded at maturity stage (105 days after sowing) and analysed to ascertain the performances of microbial inoculant treatments (Plate 7, 10 & 11).

4.2.3.3.1 Yield attributes

Number of panicles per hill (13.9) and number of filled grains per panicle (43.6) were found to be maximum in combined inoculation of *P. fluorescens* with *A. lipoferum*. Number of panicles per hill is the best treatment and was on par with *P. fluorescens* combined treatments except with *A. microphylla*, whereas number of filled grains per panicle was statistically similar to above treatments and combined application of *G. intraradices* with *A. lipoferum*. Panicle length and thousand grain weight were maximum in *P. fluorescens* combinations with both *G. intraradices* and *A. lipoferum* and the attributes were enhanced by 17.0 and 5.2 per cent over untreated control respectively. Panicle length was on par with *P. fluorescens* combined inoculation with *B. megaterium* and thousand grain weight was statistically similar to all *P. fluorescens* combined treatments along with *G. intraradices* combined inoculation with *B. megaterium* (Table 13 and Figure 18).

Number of panicles per hill (6.2), panicle length (18.2 cm) and number of filled grains per panicle (37.5) were minimum in untreated control, whereas thousand grain weight (19.2 g) was minimum in both untreated control and *B. megaterium* individual treatment. All the yield attributes of untreated control was on par with microbial inoculant individual applications except *A. lipoferum* and *P. fluorescens* with respect to number of panicles per hill, *A. microphylla*, *B. megaterium*, and *P. fluorescens* in case of panicle length and *P. fluorescens* for thousand grain weight.

4.2.3.4 At harvest (110 DAS)

Grain and straw yield were recorded and *H. gracilis* population in root and soil were enumerated after 110 days of sowing during termination of the experiment and analysed.

Table 13. Effect of microbial inoculants on yield attributes of cv. IR 50 against the infection of *H. gracilis* at maturity stage (105 DAS) under field conditions*

Treatment	(Mean of three replications)					
	Number of panicles/ hill	Panicle length (cm)		Number of filled grains/ panicle	Thousand grain weight (g)	%
		%	%	%	%	%
T ₁ – <i>Azolla microphylla</i>	73	17.7	19.0	39.4	19.3	0.5
T ₂ – <i>Azospirillum lipoferum</i>	77	24.2	18.9	39.7	19.3	0.5
T ₃ – Blue green algae	70	12.9	18.4	39.0	19.4	1.0
T ₄ – <i>Bacillus megaterium</i>	72	16.1	19.0	39.0	19.2	0.0
T ₅ – <i>Glomus intraradices</i>	70	12.9	18.6	39.3	19.4	1.0
T ₆ – <i>A. microphylla</i> + <i>G. intraradices</i>	75	21.0	19.1	40.6	19.6	2.1
T ₇ – <i>A. lipoferum</i> + <i>G. intraradices</i>	93	50.0	19.7	41.8	19.8	3.1
T ₈ – Blue green algae + <i>G. intraradices</i>	87	40.3	19.3	40.2	19.8	3.1
T ₉ – <i>B. megaterium</i> + <i>G. intraradices</i>	89	43.5	19.5	40.5	20.0	4.2
T ₁₀ – <i>Pseudomonas fluorescens</i>	91	46.8	19.7	40.2	19.9	3.6
T ₁₁ – <i>A. microphylla</i> + <i>P. fluorescens</i>	98	58.1	20.1	40.9	20.1	4.7
T ₁₂ – <i>A. lipoferum</i> + <i>P. fluorescens</i>	139	124.2	21.3	43.6	20.2	5.2
T ₁₃ – Blue green algae + <i>P. fluorescens</i>	128	106.5	20.4	41.9	20.1	4.7
T ₁₄ – <i>B. megaterium</i> + <i>P. fluorescens</i>	134	116.1	20.9	42.1	20.1	4.7
T ₁₅ – <i>G. intraradices</i> + <i>P. fluorescens</i>	134	116.1	21.3	42.6	20.2	5.2
T ₁₆ – Carbofuran 3G	66	6.5	18.7	38.8	19.3	0.5
T ₁₇ – Untreated control	62	–	18.2	37.5	19.2	–
CD (P=0.05)	11	–	0.7	2.4	0.2	–

% - Per cent increase over untreated control

* Pooled data of both summer and *kharif* season experiments

Figure 18. Effect of *P.fluorescens* and its combinations on yield attributes of rice cv. IR 50 against the infection of *H.gracilis* at maturity stage (105 DAS) under field conditions

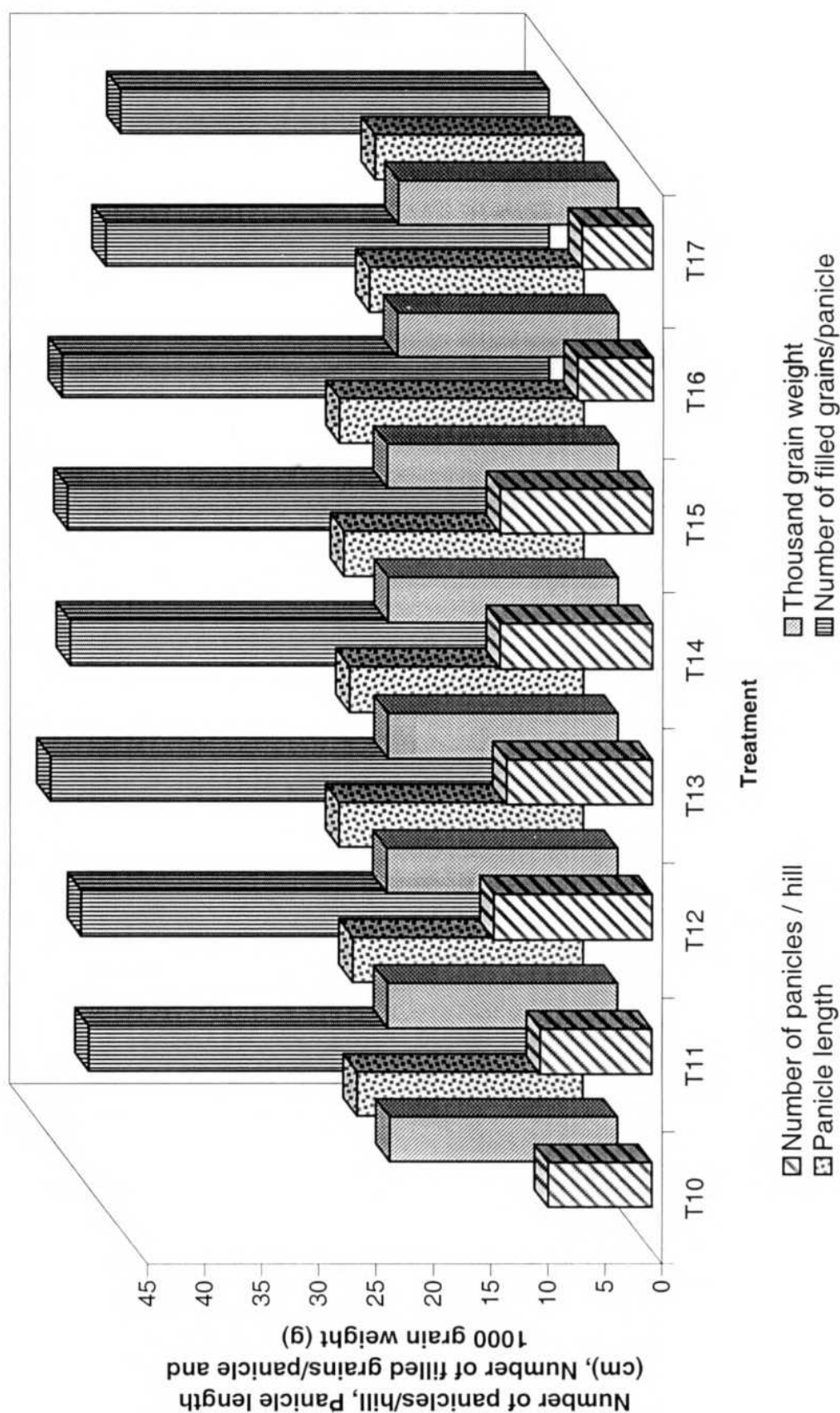


Plate 10. Effect of microbial inoculants on cv. IR 50 against the infection of the rice-root nematode, *H. gracilis* at maturity stage (105 DAS) under field conditions during summer, 2001

Individual and combined effect of treatments in comparison with carbofuran treatment and untreated control

A. Individual microbial inoculants

B. *G. intraradices*, *P. fluorescens* and their combination

C. *G. intraradices* and its combinations

D. *P. fluorescens* and its combinations

Treatment

- T₁ – *Azolla microphylla*
- T₂ – *Azospirillum lipoferum*
- T₃ – Blue green algae
- T₄ – *Bacillus megaterium*
- T₅ – *Glomus intraradices*
- T₆ – *A. microphylla* + *G. intraradices*
- T₇ – *A. lipoferum* + *G. intraradices*
- T₈ – Blue green algae + *G. intraradices*
- T₉ – *B. megaterium* + *G. intraradices*
- T₁₀ – *Pseudomonas fluorescens*
- T₁₁ – *A. microphylla* + *P. fluorescens*
- T₁₂ – *A. lipoferum* + *P. fluorescens*
- T₁₃ – Blue green algae + *P. fluorescens*
- T₁₄ – *B. megaterium* + *P. fluorescens*
- T₁₅ – *G. intraradices* + *P. fluorescens*
- T₁₆ – Carbofuran 3G
- T₁₇ – Untreated control

Plate 10

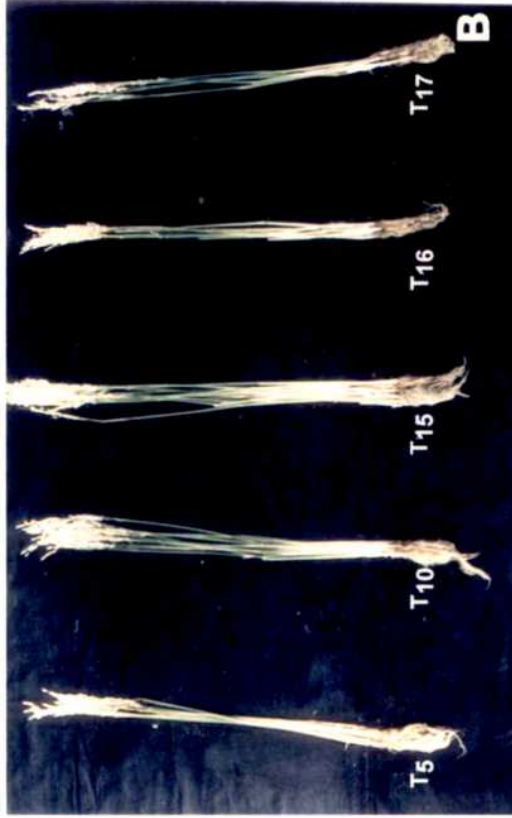
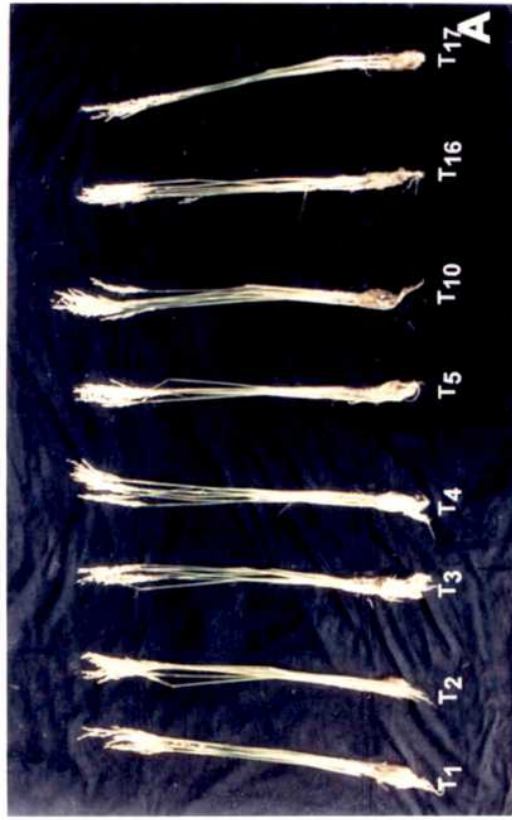


Plate 11. Effect of microbial inoculants on cv. IR 50 against the infection of the rice-root nematode, *H. gracilis* at maturity stage (105 DAS) under field conditions during *kharif*, 2001

Individual and combined effect of treatments in comparison with carbofuran treatment and untreated control

A. Individual microbial inoculants

B. *G. intraradices*, *P. fluorescens* and their combination

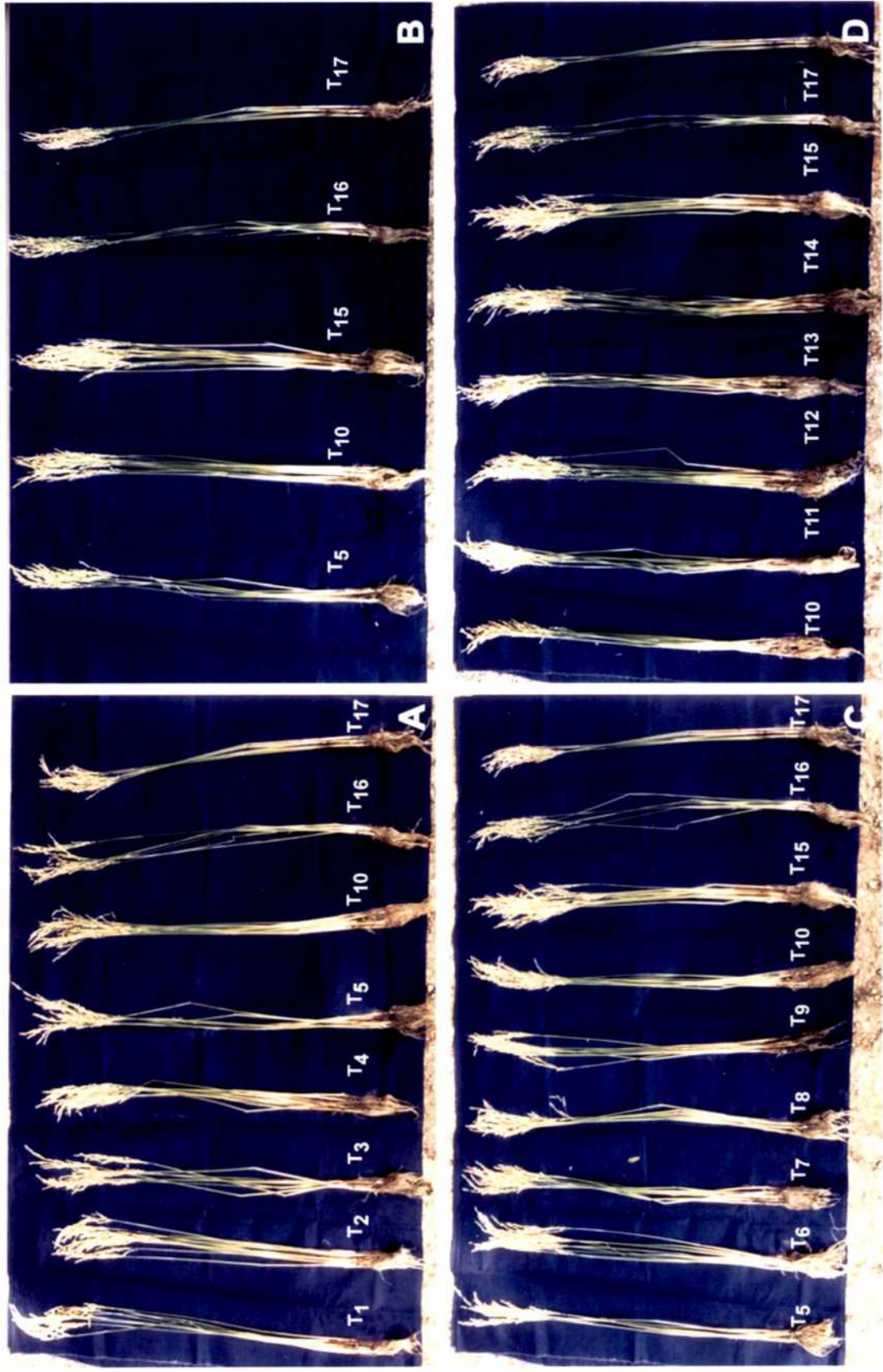
C. *G. intraradices* and its combinations

D. *P. fluorescens* and its combinations

Treatment

- T₁ – *Azolla microphylla*
- T₂ – *Azospirillum lipoferum*
- T₃ – Blue green algae
- T₄ – *Bacillus megaterium*
- T₅ – *Glomus intraradices*
- T₆ – *A. microphylla* + *G. intraradices*
- T₇ – *A. lipoferum* + *G. intraradices*
- T₈ – Blue green algae + *G. intraradices*
- T₉ – *B. megaterium* + *G. intraradices*
- T₁₀ – *Pseudomonas fluorescens*
- T₁₁ – *A. microphylla* + *P. fluorescens*
- T₁₂ – *A. lipoferum* + *P. fluorescens*
- T₁₃ – Blue green algae + *P. fluorescens*
- T₁₄ – *B. megaterium* + *P. fluorescens*
- T₁₅ – *G. intraradices* + *P. fluorescens*
- T₁₆ – Carbofuran 3G
- T₁₇ – Untreated control

Plate 11



4.2.3.4.1 Grain and straw yield

Maximum yield of both grain and straw were registered in *P fluorescens* combined inoculation with *A lipoferum* and the yield increased by 64.7 and 89.7 per cent over untreated control. Grain yield was on par with combined application of *P fluorescens* with *G intraradices*, while the straw yield was statistically similar to *P fluorescens* co-inoculations with *G intraradices*, *A microphylla* and *B megaterium* (Table 14 and Figure 19).

Significant reduction in both grain (3.4 ton h⁻¹) and straw (8.7 ton h⁻¹) yield were recorded in untreated control.

4.2.3.4.2 Nematode population in root and soil

Maximum reduction of *H. gracilis* in root (81.9%) and soil (78.2%) over untreated control was observed in dual application of *P fluorescens* with *A lipoferum*. Root population of the rice-root nematode, was on par with *P fluorescens* combined with *G intraradices*, blue green algae and *B megaterium*, while the soil population was statistically similar to co-inoculations of *P fluorescens* with *G intraradices* and *A microphylla* (Table 14 and Figure 19).

Significantly higher population of *H. gracilis* in root and soil was recorded in untreated control and the nematode population were registered as 170.5 per g root and 915.8 per 200 g soil respectively.

4.2.4 Long duration cv. ADT 44 (field study)

Data recorded on plant growth parameters and population of *H. gracilis* in root and soil of long duration cv. ADT 44 at seedling stage (30 DAS), panicle initiation stage (120 DAS), yield attributes at maturity stage (150 DAS), grain and straw yield, and *H. gracilis* population in root and soil at harvest (155 DAS) conducted during summer

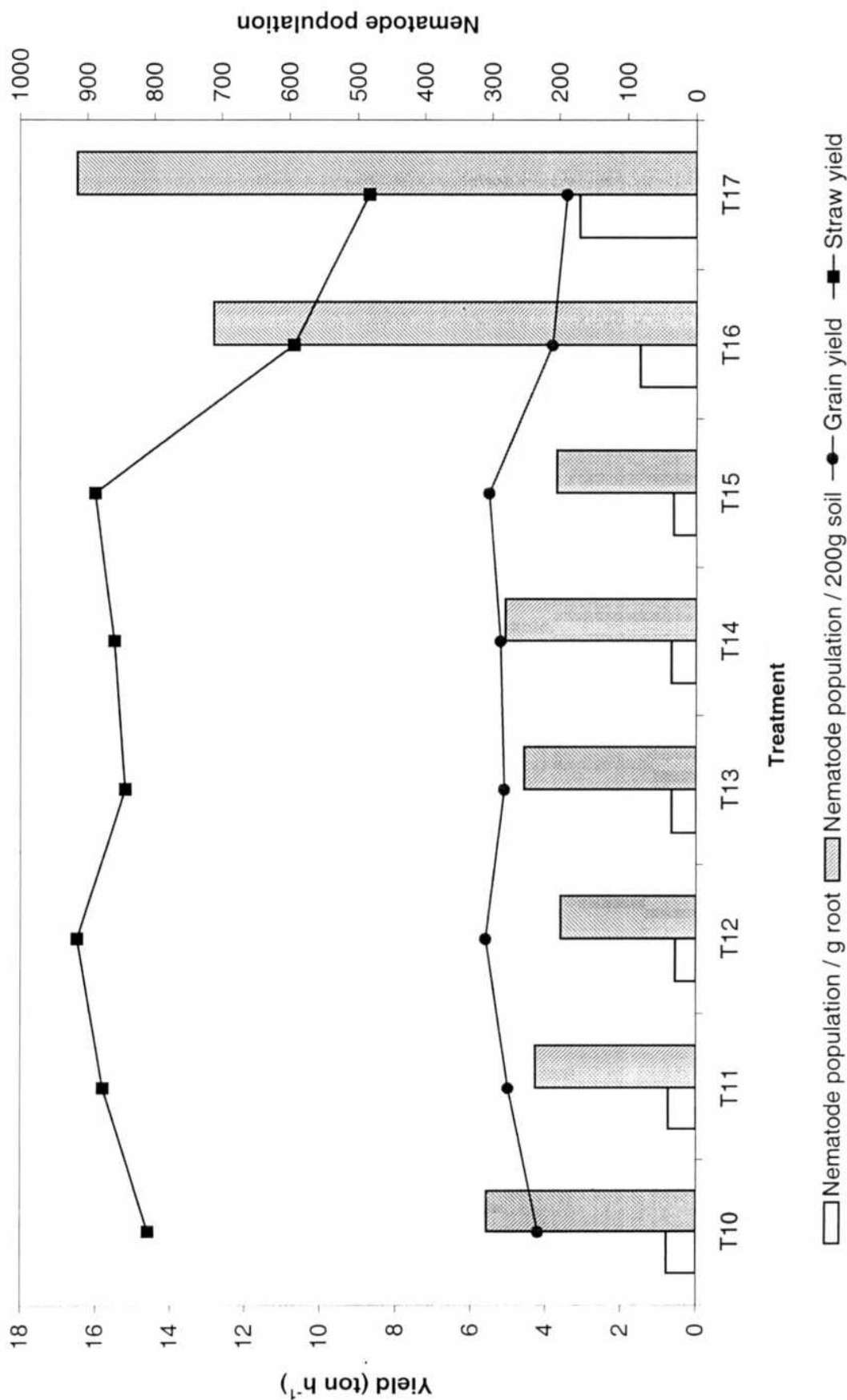
Table 14. Effect of microbial inoculants on grain and straw yield and nematode population in root and soil of rice cv. IR 50 against the infection of *H. gracilis* at harvest (110 DAS) under field conditions*

Treatment	(Mean of three replications)					
	Grain yield (ton h ⁻¹)	%	Straw yield (ton h ⁻¹)	%	Nematode population / g root	Nematode population / 200 g soil
T ₁ - <i>Azolla microphylla</i>	3.8	11.8	12.1	39.1	71.9	670.2
T ₂ - <i>Azospirillum lipoferum</i>	3.9	14.7	12.8	47.1	66.8	458.7
T ₃ - Blue green algae	3.8	11.8	12.9	48.3	80.0	611.4
T ₄ - <i>Bacillus megaterium</i>	3.8	11.8	12.7	46.0	78.2	525.4
T ₅ - <i>Glomus intraradices</i>	3.9	14.7	12.7	46.0	68.3	668.9
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	4.0	17.6	13.8	58.6	59.1	523.5
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	4.0	17.6	13.9	59.8	57.4	323.3
T ₈ - Blue green algae + <i>G. intraradices</i>	4.0	17.6	13.9	59.8	57.4	465.6
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	4.0	17.6	14.7	69.0	55.4	329.1
T ₁₀ - <i>Pseudomonas fluorescens</i>	4.2	23.5	14.6	67.8	43.4	310.0
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	5.0	47.1	15.8	81.6	40.4	237.3
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	5.6	64.7	16.5	89.7	30.8	199.5
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	5.1	50.0	15.2	74.7	35.9	253.4
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	5.2	52.9	15.5	78.2	36.5	281.8
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	5.5	61.8	16.0	83.9	33.2	204.7
T ₁₆ - Carbofuran 3G	3.8	11.8	10.7	23.0	82.8	712.7
T ₁₇ - Untreated control	3.4	-	8.7	-	170.5	915.8
CD (P=0.05)	0.3	-	1.2	-	8.6	42.5

% - Per cent increase or decrease (-) over untreated control

* Pooled data of both summer and *kharif* season experiments

Figure 19. Effect of *P. fluorescens* and its combinations on grain and straw yield and nematode population in root and soil of rice cv. IR 50 against the infection of *H. gracilis* at harvest (110 DAS) under field conditions



season were analysed and presented in Table 15, 16, 17, 18 & 19, Figure 20, 21, 22, 23, 24, 25, 26 & 27 and Plate 6, 7, 12 & 13

4.2.4.1 Seedling stage (30 DAS)

Seedlings in nursery beds were subjected to a total of eight treatments either by seed treatment or nursery bed applications and the efficiencies of these treatments were evaluated after 30 days of sowing before transplanting to the main field (Plate 6)

4.2.4.1.1 Above ground plant growth parameters

Shoot length, fresh shoot weight, dry shoot weight of the combined inoculation of *P. fluorescens* with *A. lipoferum* were enhanced maximum by 43.0, 228.6 and 375.0 per cent respectively over untreated control. Shoot length and fresh shoot weight were on par with *G. intraradices* co-inoculation with *A. lipoferum* and dry shoot weight was significantly different from other treatments and dual application of *G. intraradices* with *A. lipoferum* was the next best treatment. Total leaf area (94.3 cm²) was maximum in combined treatment of *P. fluorescens* with *G. intraradices* and on par with combined application of *P. fluorescens* with *A. lipoferum* (Table 15 and Figure 20)

Significantly reduced shoot length (28.6 cm), weight of fresh (0.7 g) and dry (0.4 g) shoot, and total leaf area (62.1 cm²) were recorded in untreated control

4.2.4.1.2 Below ground plant growth parameters

Significantly maximum root length (16.5 cm) was observed in combined treatment of *P. fluorescens* with *G. intraradices* followed by dual inoculation of *G. intraradices* with *A. lipoferum*. Combined application of *P. fluorescens* with *A. lipoferum* recorded maximum fresh root weight, dry root weight and root volume and the root characters were enhanced by 114.3, 200.0 and 175.0 per cent respectively over untreated control. Fresh root weight was on par with *G. intraradices* combined

Table 15. Effect of microbial inoculants on above and below ground plant growth parameters and nematode population in root and soil of rice cv. ADT 44 against the infection of *H. gracilis* at seedling stage (30 DAS) under field conditions*

Treatment	(Mean of three replications)									
	Shoot length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Total leaf area (cm ²)	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root volume (cc)	Nematode population/ g root	Nematode population/ 200 g soil
T ₁ - <i>Azospirillum lipoferum</i>	38.7 (35.3)	1.5 (114.3)	0.9 (125.0)	84.7 (36.4)	13.3 (27.9)	1.1 (57.1)	0.8 (100.0)	0.5 (25.0)	10.3 (-51.6)	112.4 (-19.0)
T ₂ - <i>Glomus intraradices</i>	37.4 (30.8)	1.6 (128.6)	1.1 (175.0)	86.9 (39.9)	14.2 (36.5)	1.2 (71.4)	0.9 (125.0)	0.8 (100.0)	9.8 (-54.0)	97.3 (-29.8)
T ₃ - <i>Pseudomonas fluorescens</i>	39.2 (37.1)	1.5 (114.3)	1.0 (150.0)	85.4 (37.5)	13.6 (30.8)	1.3 (85.7)	1.0 (150.0)	0.9 (125.0)	9.2 (-56.8)	86.4 (-37.7)
T ₄ - <i>A. lipoferum</i> + <i>G. intraradices</i>	40.1 (40.2)	2.1 (200.0)	1.6 (300.0)	88.9 (43.2)	15.2 (46.2)	1.4 (100.0)	1.0 (150.0)	1.0 (150.0)	7.9 (-62.9)	82.2 (-40.7)
T ₅ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	40.9 (43.0)	2.3 (228.6)	1.9 (375.0)	91.6 (47.5)	14.8 (42.3)	1.5 (114.3)	1.2 (200.0)	1.1 (175.0)	5.6 (-73.7)	65.8 (-52.6)
T ₆ - <i>G. intraradices</i> + <i>P. fluorescens</i>	39.6 (38.5)	1.7 (142.9)	1.2 (200.0)	94.3 (51.9)	16.5 (58.7)	1.4 (100.0)	1.1 (175.0)	0.9 (125.0)	3.8 (-82.2)	71.3 (-48.6)
T ₇ - Carbofuran 3G	34.8 (21.7)	1.1 (57.1)	0.7 (75.0)	80.4 (29.5)	12.3 (18.3)	0.9 (28.6)	0.6 (50.0)	0.6 (50.0)	12.6 (-40.8)	87.6 (-36.8)
T ₈ - Untreated control	28.6 —	0.7 —	0.4 —	62.1 —	10.4 —	0.7 —	0.4 —	0.4 —	21.3 —	138.7 —
CD (P=0.05)	1.2	0.2	0.1	4.7	0.7	0.1	0.1	0.1	1.8	40.1

Figures in parentheses indicate per cent increase or decrease (-) over untreated control

* Data of summer season experiment

Figure 20. Effect of microbial inoculants on above ground plant growth parameters of rice cv. ADT 44 against the infection of *H.gracilis* at seedling stage (30 DAS) under field conditions

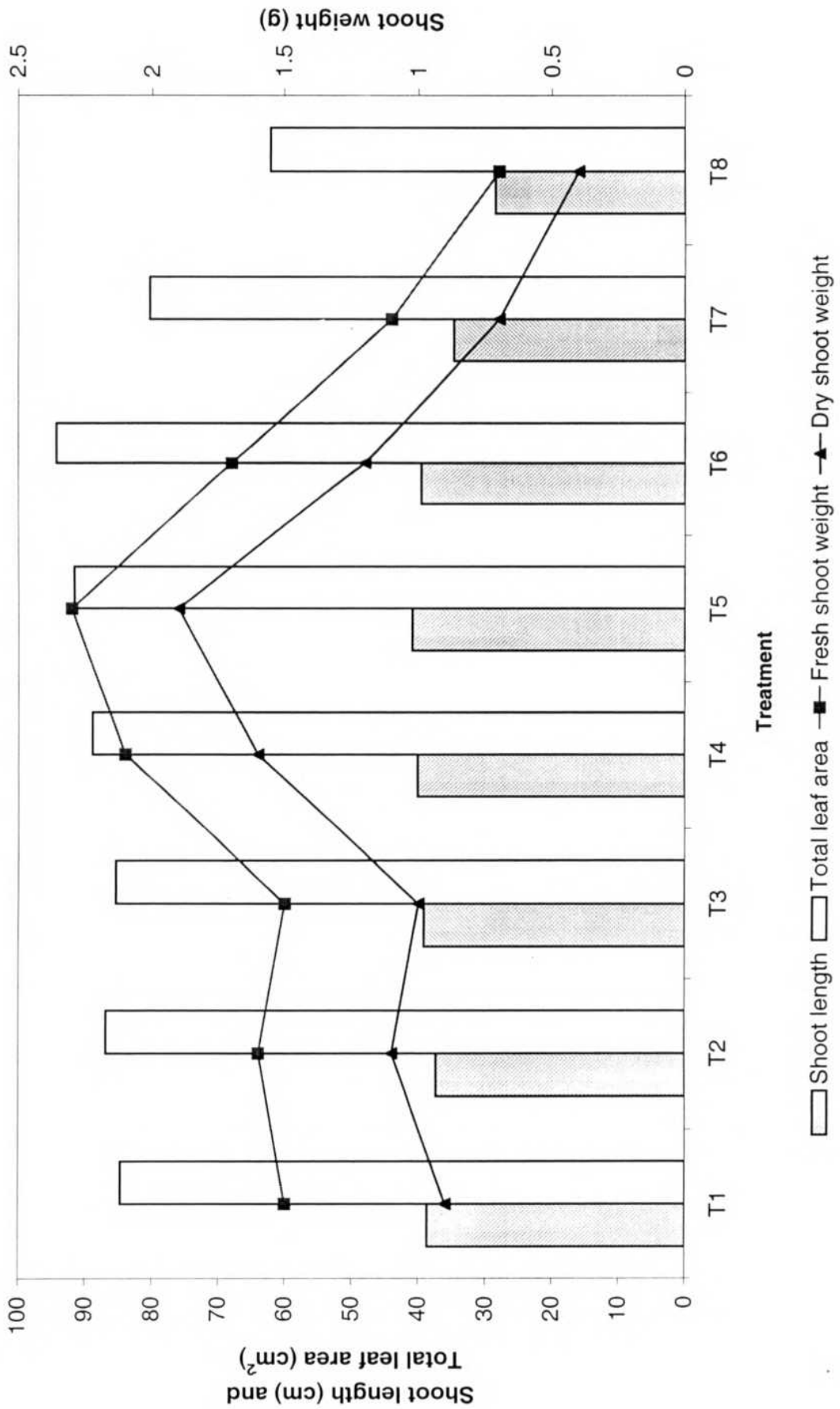


Figure 21. Effect of microbial inoculants on below ground plant growth parameters of rice cv. ADT 44 against the infection of *H.gracilis* at seedling stage (30 DAS) under field conditions

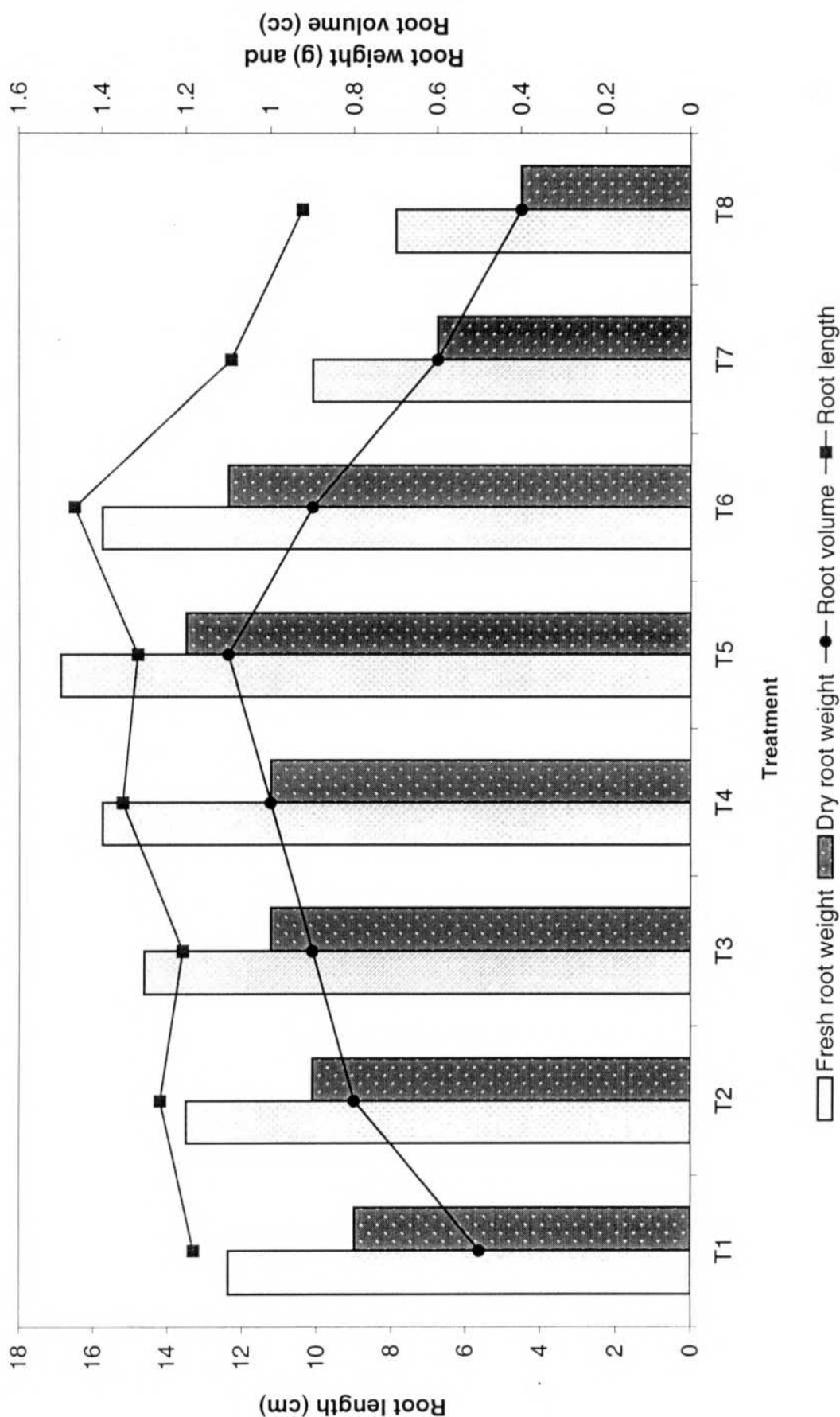
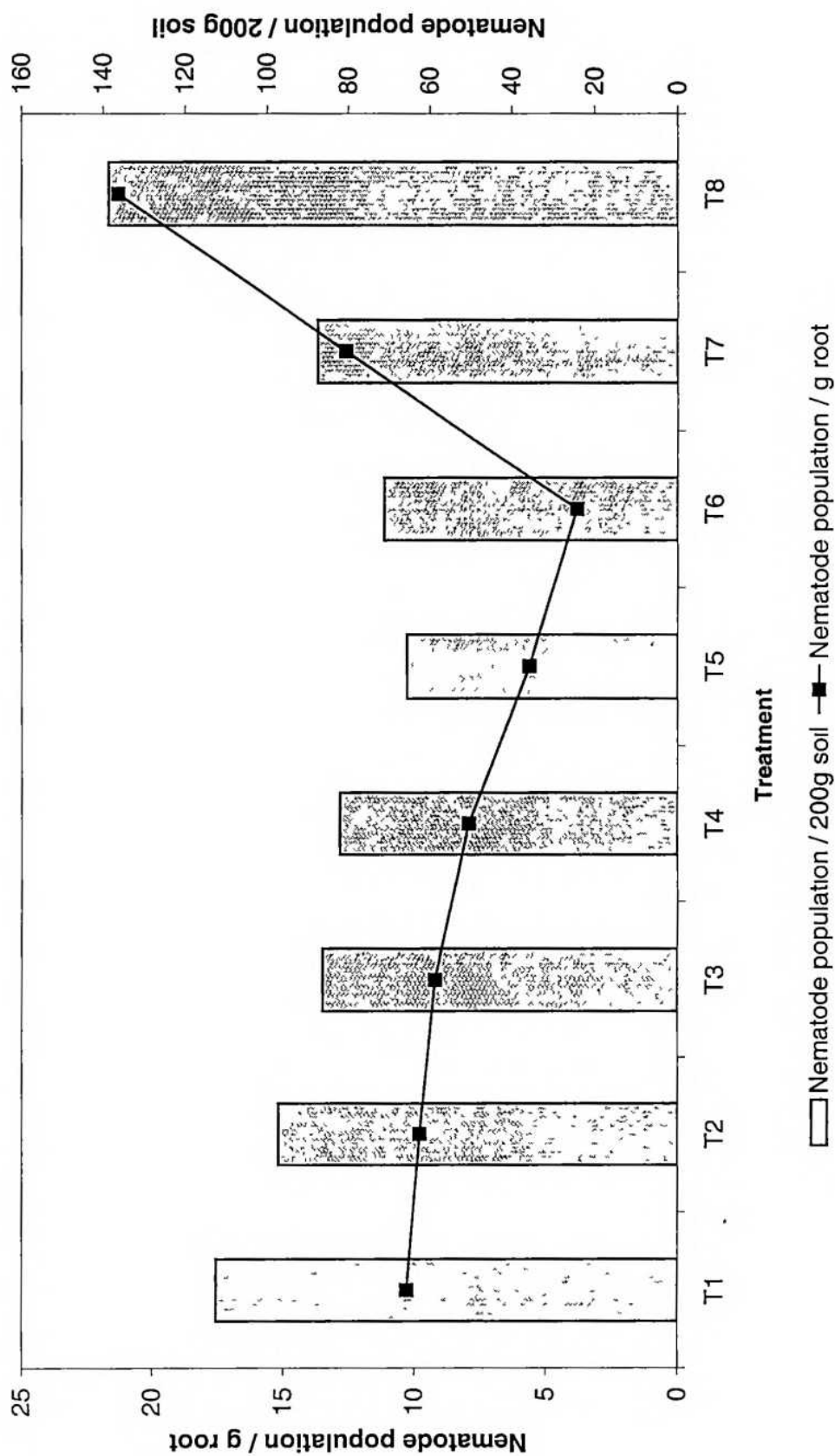


Figure 22. Effect of microbial inoculants on nematode population in root and soil of rice cv. ADT 44 against the infection of *H.gracilis* at seedling stage (30 DAS) under field conditions



inoculations with *P fluorescens* and *A lipoferum*. Dry root weight was statistically similar to co-inoculation of *P fluorescens* with *G intraradices*, whereas root volume was on par with *G intraradices* combined application with *A lipoferum* (Table 15 and Figure 21)

Root length (10.4 cm), and fresh (0.7 g) and dry (0.4 g) root weight of untreated control were recorded minimum. Root volume (0.4 cc) was on par with *A lipoferum* individual treatment.

4.2.4.1.3 Nematode population in root and soil

Maximum reduction (82.2%) of root population of *H gracilis* was observed in combined inoculation of *P fluorescens* with *G intraradices* and it was on par with combined treatment of *P fluorescens* with *A lipoferum*. Minimum population of *H gracilis* (65.8 per 200 g) in soil was recorded in *P fluorescens* combined inoculation with *A lipoferum* treated plots and was statistically similar to all other treatments except individual treatment of *A lipoferum* and untreated control (Table 15 and Figure 22)

Significantly higher population of *H gracilis* (21.3 per g) was observed in root of untreated control. Although the soil nematode population (138.7 per 200 g) was maximum in untreated control, it was on par with individual application of *A lipoferum*.

4.2.4.2 Panicle initiation stage (120 DAS)

At panicle initiation stage (120 days after sowing) data on the above and below ground plant growth parameters, and *H gracilis* population in root and soil of cv ADT 44 were recorded and analysed (Plate 12)

4.2.4.2.1 Above ground plant growth parameters

Maximum shoot length (108.4 cm) was recorded in combined treatment of *P fluorescens* with *A microphylla* and was on par with co-inoculation of *P fluorescens*

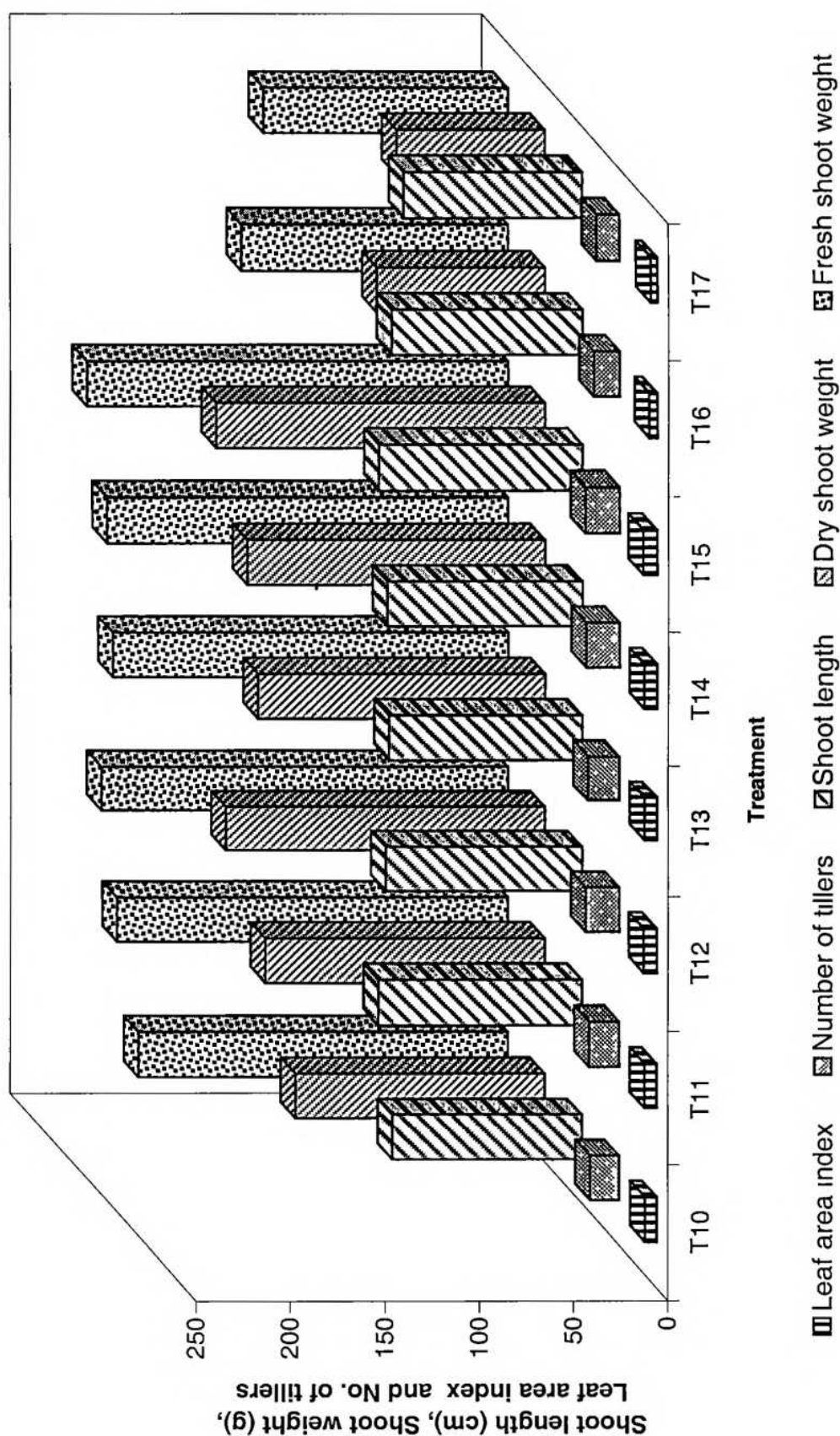
Table 16. Effect of microbial inoculants on above ground plant growth parameters of rice cv. ADT 44 against the infection of *H. gracilis* at panicle initiation stage (120 DAS) under field conditions*

Treatment	(Mean of three replications)					
	Shoot length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Leaf area index	Number of tillers	%
T ₁ - <i>Azolla microphylla</i>	98.4	164.3	99.7	4.8	14.2	15.4
T ₂ - <i>Azospirillum lipoferum</i>	96.4	174.7	113.2	5.2	15.1	22.8
T ₃ - Blue green algae	99.2	153.9	86.4	4.6	14.4	17.1
T ₄ - <i>Bacillus megaterium</i>	95.8	144.7	82.3	4.6	14.2	15.4
T ₅ - <i>Glomus intraradices</i>	99.7	172.9	111.6	5.8	14.5	17.9
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	102.4	184.3	118.9	6.1	15.1	22.8
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	102.6	203.7	137.4	6.8	16.3	32.5
T ₈ - Blue green algae + <i>G. intraradices</i>	103.5	198.6	124.2	6.4	15.8	28.5
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	104.3	192.4	127.7	5.9	14.8	20.3
T ₁₀ - <i>Pseudomonas fluorescens</i>	100.7	195.7	132.1	6.2	15.5	26.0
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	108.4	207.3	148.2	6.3	16.1	30.9
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	104.5	215.5	169.3	6.8	17.9	45.5
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	102.7	209.6	152.3	6.5	16.6	35.0
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	103.8	212.4	157.9	6.7	17.8	44.7
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	108.2	223.1	174.4	7.3	18.2	48.0
T ₁₆ - Carbofuran 3G	101.4	141.3	89.4	4.2	13.7	11.4
T ₁₇ - Untreated control	94.9	129.4	78.9	3.4	12.3	-
CD (P=0.05)	3.6	19.4	12.3	0.3	1.4	-

% - Per cent increase over untreated control

* Data of summer season experiment

Figure 23. Effect of *P.fluorescens* and its combinations on above ground plant growth parameters of rice cv. ADT 44 against the infection of *H.gracilis* at panicle initiation stage (120 DAS) under field conditions



with *G. intraradices*. All other above ground plant characters viz., weight of fresh & dry shoot, leaf area index and number of tillers per hill were enhanced by 72.4, 121.0, 114.7 and 48.0 per cent respectively over untreated control in combined treatment of *P. fluorescens* with *G. intraradices* followed by combined application of *P. fluorescens* with *A. lipoferum*. Fresh shoot weight per hill was on par with all *P. fluorescens* combined inoculations and *G. intraradices* combined application with *A. lipoferum*. Dry shoot weight per hill statistically similar to only dual inoculation of *P. fluorescens* with *A. lipoferum*, whereas number of tillers per hill was on par with *P. fluorescens* combined treatments with *A. lipoferum* and *B. megaterium*. Leaf area index per hill was significantly different from rest of the treatments (Table 16 and Figure 23).

Minimum of all the above ground plant growth characters was recorded in untreated control. Shoot length (94.9 cm) was statistically similar to individual treatments of *A. microphylla*, *A. lipoferum*, and *B. megaterium*. Fresh shoot weight per hill (129.4 g) was on par with *B. megaterium* individual treatment and dry shoot weight per hill (78.9 g) that was statistically similar to individual treatments of *B. megaterium* and blue green algae. Leaf area index (3.4) and number of tillers (12.3) per hill were significantly reduced.

4.2.4.2.2 Below ground plant growth parameters

Combined treatment of *P. fluorescens* with *G. intraradices* registered maximum root length (33.1 cm) and weight of fresh (137.9 g) and dry (59.7 g) root per hill. Root length was on par with combined application of *P. fluorescens* & *A. lipoferum* and *G. intraradices* co-inoculations with *A. lipoferum* and *A. microphylla*. Fresh root weight was significantly different from rest of the treatments followed by combined application of *P. fluorescens* with *A. lipoferum*. Dry root weight was statistically similar to combined treatment of *P. fluorescens* with *A. lipoferum*. Root volume (68.9 cc) of dual inoculation

Table 17. Effect of microbial inoculants on below ground plant growth parameters and nematode population in root and soil of rice cv. ADT 44 against the infection of *H. gracilis* at panicle initiation stage (120 DAS) under field conditions*
(Mean of three replications)

Treatment	Root length (cm)		Fresh root weight (g)		Dry root weight (g)		Root volume (cc)		Nematode population/ g root		Nematode population/ 200 g soil	
	%	%	%	%	%	%	%	%	%	%	%	%
T ₁ - <i>Azolla microphylla</i>	28.3	20.9	98.7	33.6	46.9	44.8	56.7	63.4	76.4	-53.5	235.6	-40.6
T ₂ - <i>Azospirillum lipoferum</i>	28.6	22.2	94.3	27.6	44.3	36.7	54.2	56.2	64.8	-60.6	215.9	-45.6
T ₃ - Blue green algae	25.7	9.8	89.4	21.0	42.6	31.5	51.7	49.0	73.8	-55.1	206.4	-48.0
T ₄ - <i>Bacillus megaterium</i>	24.2	3.4	84.3	14.1	40.9	26.2	48.2	38.9	76.8	-53.3	242.7	-38.8
T ₅ - <i>Glomus intraradices</i>	28.9	23.5	96.2	30.2	45.3	39.8	53.9	55.3	63.4	-61.4	210.9	-46.8
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	31.3	33.8	101.2	36.9	48.7	50.3	57.2	64.8	61.8	-62.4	201.5	-49.2
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	31.4	34.2	104.7	41.7	49.4	52.5	60.3	73.8	57.4	-65.1	183.7	-53.7
T ₈ - Blue green algae + <i>G. intraradices</i>	30.6	30.8	99.2	34.2	47.5	46.6	55.7	60.5	58.9	-64.2	189.5	-52.2
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	29.8	27.4	98.9	33.8	47.1	45.4	54.4	56.8	59.2	-64.0	195.4	-50.7
T ₁₀ - <i>Pseudomonas fluorescens</i>	28.2	20.5	102.7	39.0	50.3	55.2	59.7	72.0	44.7	-72.8	174.3	-56.1
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	30.6	30.8	114.7	55.2	53.4	64.8	64.4	85.6	41.9	-74.5	168.4	-57.5
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	32.9	40.6	121.2	64.0	56.7	75.0	68.9	98.6	33.4	-79.7	157.3	-60.3
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	29.6	26.5	106.9	44.7	51.9	60.2	61.3	76.7	40.7	-75.2	170.7	-57.0
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	29.8	27.4	108.5	46.8	52.4	61.7	63.7	83.6	36.2	-78.0	162.3	-59.1
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	33.1	41.5	137.9	86.6	59.7	84.3	68.4	97.1	32.6	-80.2	159.7	-59.7
T ₁₆ - Carbofuran 3G	28.4	21.4	81.4	10.1	38.6	19.1	46.4	33.7	79.2	-51.8	267.3	-32.6
T ₁₇ - Untreated control	23.4	-	73.9	-	32.4	-	34.7	-	164.3	-	396.7	-
CD (P=0.05)	2.0		12.8		4.0		3.2		8.5		30.0	

% - Per cent increase or decrease (-) over untreated control

* Data of summer season experiment

Figure 24. Effect of *P.fluorescens* and its combinations on below ground plant growth parameters of rice cv. ADT 44 against the infection of *H.gracilis* at panicle initiation stage (120 DAS) under field conditions

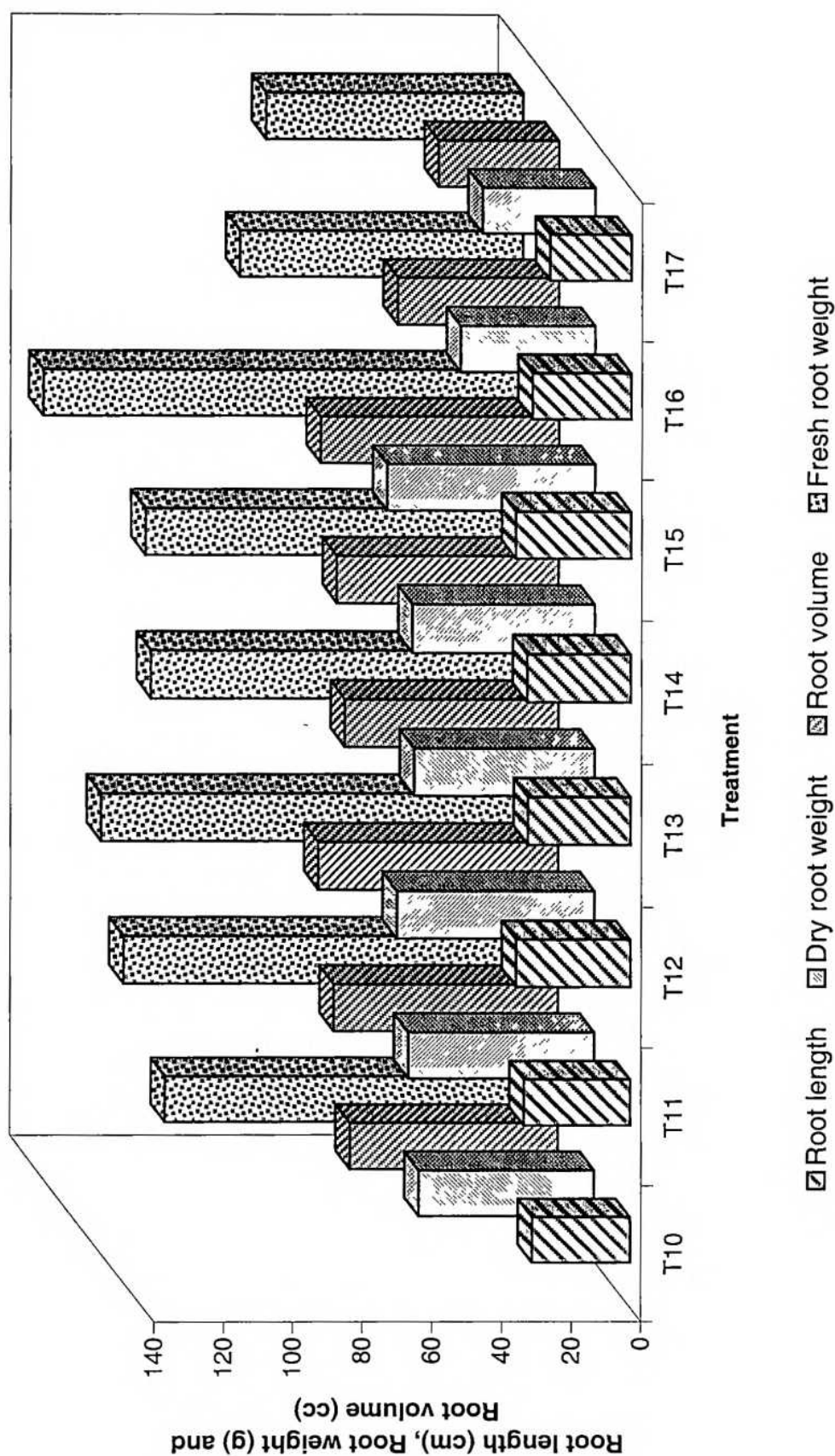


Figure 25. Effect of *P. fluorescens* and its combinations on nematode population in root and soil of rice cv. ADT 44 against the infection of *H. gracilis* at panicle initiation stage (120 DAS) under field conditions

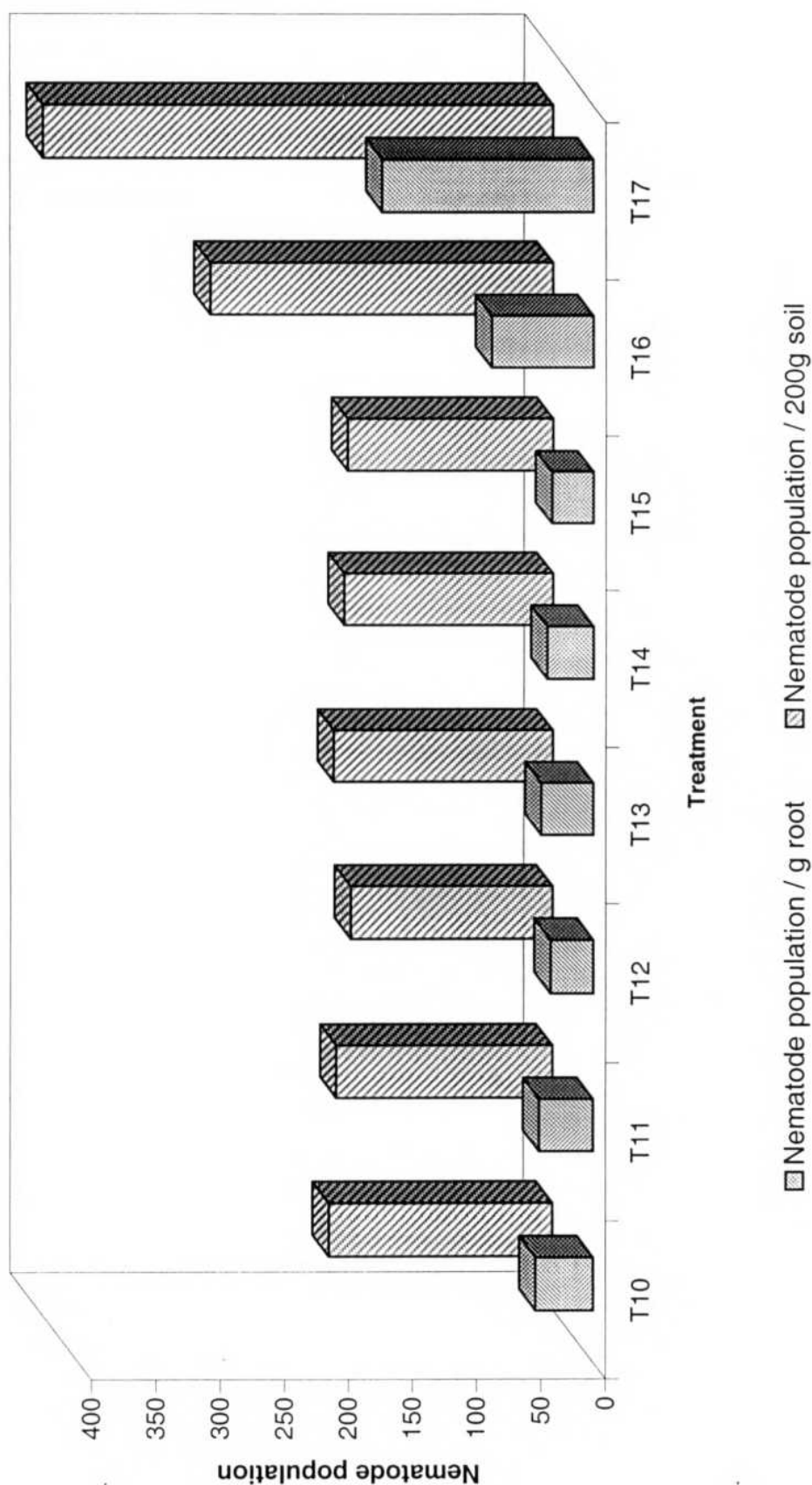


Plate 12. Effect of microbial inoculants on cv. ADT 44 against the infection of the rice-root nematode, *H. gracilis* at panicle initiation stage (120 DAS) under field conditions during summer, 2001

Individual and combined effect of treatments in comparison with carbofuran treatment and untreated control

A. Individual microbial inoculants

B. *G. intraradices*, *P. fluorescens* and their combination

C. *G. intraradices* and its combinations

D. *P. fluorescens* and its combinations

Treatment

- T₁ – *Azolla microphylla*
- T₂ – *Azospirillum lipoferum*
- T₃ – Blue green algae
- T₄ – *Bacillus megaterium*
- T₅ – *Glomus intraradices*
- T₆ – *A. microphylla* + *G. intraradices*
- T₇ – *A. lipoferum* + *G. intraradices*
- T₈ – Blue green algae + *G. intraradices*
- T₉ – *B. megaterium* + *G. intraradices*
- T₁₀ – *Pseudomonas fluorescens*
- T₁₁ – *A. microphylla* + *P. fluorescens*
- T₁₂ – *A. lipoferum* + *P. fluorescens*
- T₁₃ – Blue green algae + *P. fluorescens*
- T₁₄ – *B. megaterium* + *P. fluorescens*
- T₁₅ – *G. intraradices* + *P. fluorescens*
- T₁₆ – Carbofuran 3G
- T₁₇ – Untreated control



of *P. fluorescens* with *A. lipoferum* was the maximum and on par with only *P. fluorescens* combined treatment with *G. intraradices* (Table 17 and Figure 24)

All the root parameters were minimum in untreated control. Root length (23.4 cm) and fresh root weight (73.9 g) per hill were on par with individual treatment of *B. megaterium*. Both dry root weight (32.4 g) and root volume (34.7 cc) per hill was significantly reduced.

4.2.4.2.3 Nematode population in root and soil

Maximum reduction (80.2%) in root population of *H. gracilis* was observed in *P. fluorescens* combined with *G. intraradices* and the nematode population was statistically similar to all other *P. fluorescens* combined inoculations except with *A. microphylla*. Minimum soil population of *H. gracilis* (157.3 per 200 g) was recorded in *P. fluorescens* combined with *A. lipoferum* and was on par with individual and combined treatments of *P. fluorescens* and combined treatment of *G. intraradices* with *A. lipoferum*. Combined inoculations of *P. fluorescens* with *A. lipoferum* and *G. intraradices* were the next best treatments with regard to reduction of root & soil population of *H. gracilis* respectively (Table 17 and Figure 25).

Significantly higher root (164.3 per g) & soil (396.7 per 200 g) population of *H. gracilis* was recorded in untreated control.

4.2.4.3 Maturity stage (150 DAS)

Yield attributes, like number of panicles/hill, panicle length, number of filled grains/panicle and thousand grain weight were recorded at maturity stage i.e., 150 days after sowing (Plate 7 & 13).

4.2.4.3.1 Yield attributes

Yield attributes such as number of panicles per hill, number of filled grains per panicle, and thousand grain weight were increased to maximum by 85.3, 16.4 and 8.8 per

Table 18. Effect of microbial inoculants on yield attributes of rice cv. ADT 44 against the infection of *H. gracilis* at maturity stage (150 DAS) under field conditions*

Treatment	(Mean of three replications)					
	Number of panicles/ hill	Panicle length (cm)	Number of filled grams/ panicle	Thousand grain weight (g)	%	%
T ₁ - <i>Azolla microphylla</i>	8.8	25.3	73.3	22.2	7.2	2.8
T ₂ - <i>Azospirillum lipoferum</i>	9.6	26.4	74.6	22.6	9.1	4.6
T ₃ - Blue green algae	7.9	25.8	73.8	22.3	7.9	3.2
T ₄ - <i>Bacillus megaterium</i>	7.4	25.2	72.9	22.1	6.6	2.3
T ₅ - <i>Glomus intraradices</i>	9.2	26.3	74.1	22.8	8.3	5.6
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	9.4	26.4	74.8	22.9	9.4	6.0
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	10.2	26.8	76.2	22.9	11.4	6.0
T ₈ - Blue green algae + <i>G. intraradices</i>	9.9	26.6	75.6	22.6	10.5	4.6
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	9.3	26.5	75.1	22.4	9.8	3.7
T ₁₀ - <i>Pseudomonas fluorescens</i>	10.3	27.1	76.4	23.1	11.7	6.9
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	10.8	27.4	77.6	23.3	13.5	7.9
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	12.4	28.8	78.9	23.4	15.4	8.3
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	11.5	27.2	78.1	23.2	14.2	7.4
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	12.3	28.1	78.4	23.4	14.6	8.3
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	12.6	28.2	79.6	23.5	16.4	8.8
T ₁₆ - Carbofuran 3G	8.2	24.6	73.1	21.8	6.9	0.9
T ₁₇ - Untreated control	6.8	24.2	68.4	21.6	-	-
CD (P=0.05)	2.2	1.3	5.1	0.7		

% - Per cent increase over untreated control

* Data of summer season experiment

Figure 26. Effect of *P.fluorescens* and its combinations on yield attributes of rice cv. ADT 44 against the infection of *H.gracilis* at maturity stage (150 DAS) under field conditions

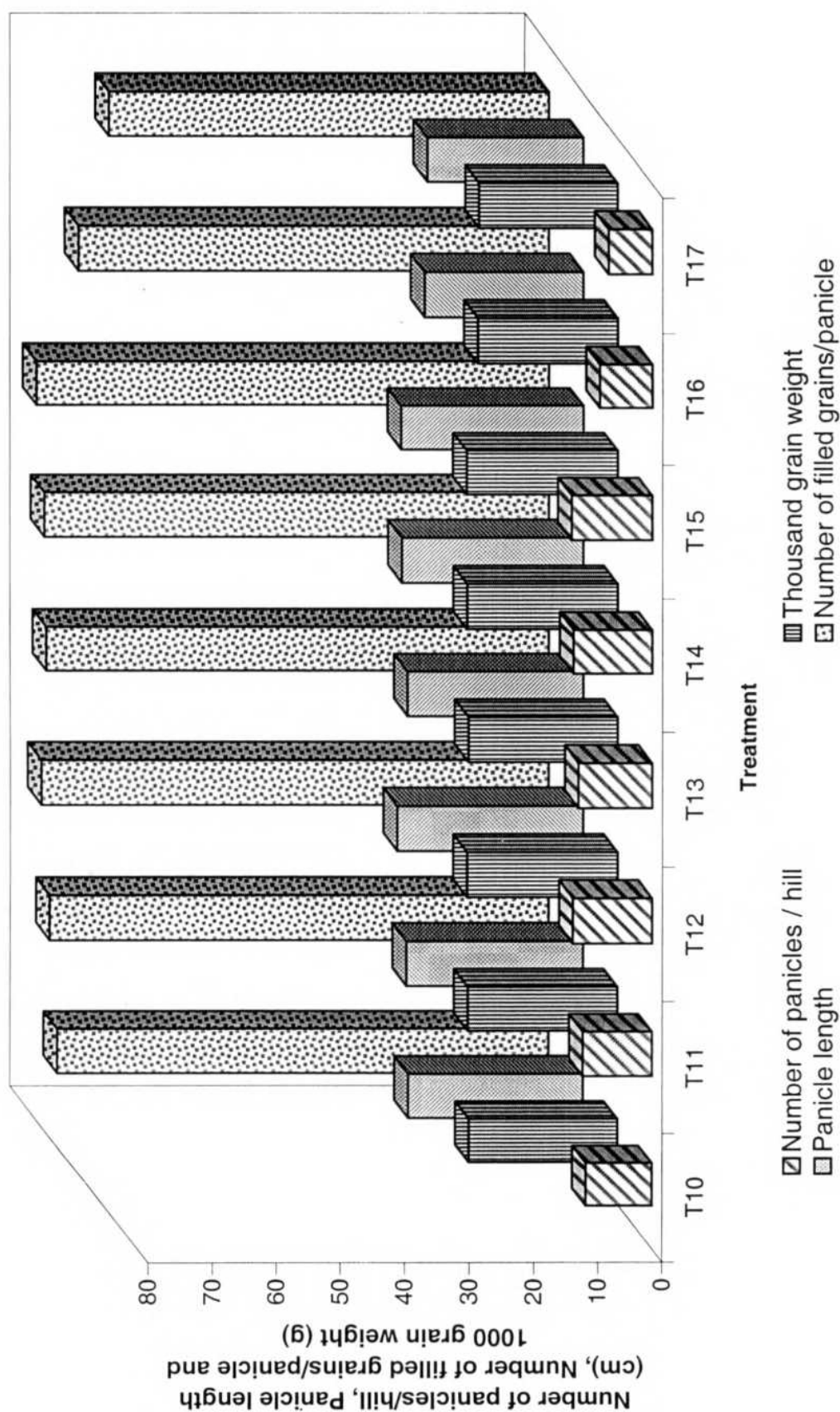


Plate 13. Effect of microbial inoculants on cv. ADT 44 against the infection of the rice-root nematode, *H. gracilis* at maturity stage (150 DAS) under field conditions during summer, 2001

Individual and combined effect of treatments in comparison with carbofuran treatment and untreated control

A. Individual microbial inoculants

B. *G. intraradices*, *P. fluorescens* and their combination

C. *G. intraradices* and its combinations

D. *P. fluorescens* and its combinations

Treatment

- T₁ – *Azolla microphylla*
- T₂ – *Azospirillum lipoferum*
- T₃ – Blue green algae
- T₄ – *Bacillus megaterium*
- T₅ – *Glomus intraradices*
- T₆ – *A. microphylla* + *G. intraradices*
- T₇ – *A. lipoferum* + *G. intraradices*
- T₈ – Blue green algae + *G. intraradices*
- T₉ – *B. megaterium* + *G. intraradices*
- T₁₀ – *Pseudomonas fluorescens*
- T₁₁ – *A. microphylla* + *P. fluorescens*
- T₁₂ – *A. lipoferum* + *P. fluorescens*
- T₁₃ – Blue green algae + *P. fluorescens*
- T₁₄ – *B. megaterium* + *P. fluorescens*
- T₁₅ – *G. intraradices* + *P. fluorescens*
- T₁₆ – Carbofuran 3G
- T₁₇ – Untreated control

Plate 13



cent over untreated control in *P fluorescens* combined treatment with *G intraradices* followed by dual inoculation of *P fluorescens* with *A lipoferum*. Number of panicles per hill in the best treatment was statistically similar to *P fluorescens* combined treatments, whereas number of filled grains per panicle was on par with all the combined and individual treatments of *P fluorescens* and *G intraradices*. Similar to number of filled grains per panicle not much difference was observed among the treatment with respect to thousand grain weight and the best treatment was statistically similar to all the combined treatments, except *G intraradices* combined inoculations with blue green algae and *B megaterium* and with individual treatment of *P fluorescens*. Panicle length (28.8 cm) was maximum in combined application of *P fluorescens* with *A' lipoferum*, which was on par with *P fluorescens* co-inoculations with *G intraradices* and *B megaterium* (Table 18 and Figure 26).

Minimum of all the yield attributes was registered in untreated control. Number of panicles per hill (6.8) was on par with individual treatments of *B megaterium*, blue green algae and *A microphylla*. Panicle length (24.2 cm) and number of filled grains per panicle (68.4) were statistically similar to individual applications of *A microphylla* and *B megaterium*, whereas thousand grain weight (21.6 g) was on par with individual treatments of *A microphylla*, blue green algae and *B megaterium*.

4.2.4.4 At harvest (155 DAS)

During the termination of experiment (155 days after sowing) both grain and straw yield, and *H. gracilis* population in root and soil were estimated.

4.2.4.4.1 Grain and straw yield

Significantly maximum grain yield of 7.8 ton h⁻¹ was observed in both *P fluorescens* combined treatments with *A lipoferum* and *G intraradices*. Straw yield in combined application of *P fluorescens* with *G intraradices* was enhanced to the

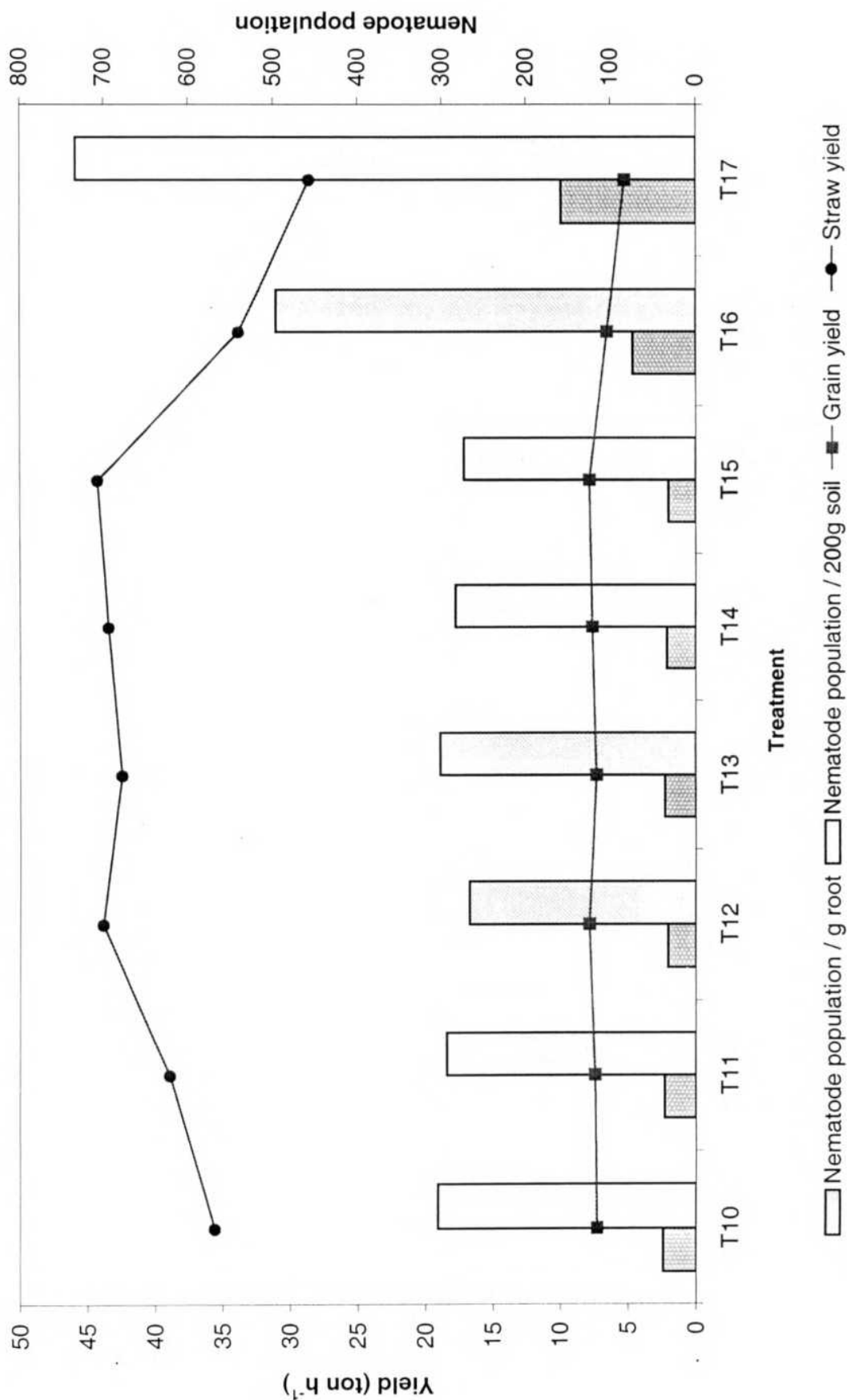
Table 19. Effect of microbial inoculants on grain and straw yield and nematode population in root and soil of rice cv. ADT 44 against the infection of *H. gracilis* at harvest (155 DAS) under field conditions*

Treatment	(Mean of three replications)			
	Grain yield (ton h ⁻¹)	Straw yield (ton h ⁻¹)	Nematode population / g root	Nematode population / 200 g soil
	%	%	%	%
T ₁ - <i>Azolla microphylla</i>	6.8	32.9	69.5	439.2
T ₂ - <i>Azospirillum lipoferum</i>	7.0	38.2	58.1	384.7
T ₃ - Blue green algae	6.9	33.6	68.4	398.2
T ₄ - <i>Bacillus megaterium</i>	6.5	33.2	69.7	476.9
T ₅ - <i>Glomus intraradices</i>	7.0	34.8	57.2	348.2
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	7.0	38.5	54.3	328.7
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	7.2	39.4	52.9	311.4
T ₈ - Blue green algae + <i>G. intraradices</i>	7.1	37.4	53.2	321.8
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	7.1	36.4	54.6	337.2
T ₁₀ - <i>Pseudomonas fluorescens</i>	7.3	35.6	38.9	305.4
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	7.4	38.9	36.7	294.8
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	7.8	43.8	32.4	267.7
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	7.3	42.4	36.0	302.3
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	7.6	43.4	33.8	284.1
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	7.8	44.2	31.7	273.9
T ₁₆ - Carbofuran 3G	6.5	33.8	73.9	496.5
T ₁₇ - Untreated control	5.2	28.6	158.4	733.4
CD (P=0.05)	0.1	2.5	7.2	89.9

%- Per cent increase or decrease (-) over untreated control

* Data of summer season experiment

Figure 27. Effect of *P.fluorescens* and its combinations on grain and straw yield and nematode population in root and soil of rice cv. ADT 44 against the infection of *H.gracilis* at harvest (155 DAS) under field conditions



maximum by 54.5 per cent over untreated control followed by *P. fluorescens* co-inoculation with *A. lipoferum*. The straw yield of the best treatment was on par with *P. fluorescens* combined treatments except with *A. microphylla* (Table 19 and Figure 27)

Significantly reduced grain (5.2 ton ha⁻¹) and straw (28.6 ton ha⁻¹) yield were recorded in untreated control

4.2.4.4.2 Nematode population in root and soil

Maximum reduction of *H. gracilis* population in root (80.0%) was registered in dual inoculation of *P. fluorescens* with *G. intraradices*. This was on par with *P. fluorescens* individual and combined treatments. Minimum *H. gracilis* population (267.7 per 200 g) in soil was recorded in *P. fluorescens* combined application with *A. lipoferum* followed by co-inoculation of *P. fluorescens* with *G. intraradices* (273.9 g/200 g). The soil population of *H. gracilis* of the best treatment was on par with individual and combined inoculations of *P. fluorescens* and *G. intraradices* (Table 19 and Figure 27)

H. gracilis population in root (158.4 per g) and soil (733.4 per 200 g soil) of untreated control were significantly higher

4.3 Physiological, biochemical and nutritional responses of cvs. IR 50 and ADT 44 to the infection of rice-root nematode, *H. gracilis* in consortium with microbial inoculants

4.3.1 Physiological parameters

Data on CO₂ gas exchange parameters, indicators of degree of stress countered by the plants viz., leaf temperature, transpiration rate, photosynthetic active radiation and diffusive resistance of cvs. IR 50 and ADT 44 were recorded at panicle initiation stage i.e., 75 and 120 days after sowing in cvs. IR 50 and ADT 44 respectively and were analysed and presented in Table 20 & 21 and Figure 28 & 29

4.3.1.1 Leaf temperature

Combined treatment of *P. fluorescens* with *A. lipoferum* recorded minimum leaf temperature of 29.80°C in cv. IR 50 and was on par with *P. fluorescens* individual and combined treatments and dual inoculation of *G. intraradices* with *A. lipoferum*. In cv. ADT 44, minimum leaf temperature (29.28°C) was registered in *P. fluorescens* combined inoculation with *G. intraradices* and was statistically similar to individual and combined treatments of *P. fluorescens* and combined inoculation of *G. intraradices* with blue green algae (Table 20 and Figure 28 & 29).

Leaf temperature (30.26°C) of untreated control in cv. IR 50 was maximum and on par with *B. megaterium* individual treatment. Significantly higher leaf temperature (30.01°C) of untreated control was noticed in cv. ADT 44.

Uninoculated control registered minimum leaf temperature in both the cultivars.

4.3.1.2 Transpiration rate

Transpiration rate was minimum in both the varieties in *P. fluorescens* co-inoculation with *A. lipoferum* and it was reduced by 12.6 and 18.0 per cent over untreated control in cv. IR 50 and cv. ADT 44 respectively and was statistically similar to individual and combined treatments of *P. fluorescens* (Table 20 and Figure 28 & 29).

Untreated control registered maximum transpiration rate in cv. IR 50 (5.94 $\mu\text{g cm}^{-2} \text{sec}^{-1}$) and cv. ADT 44 (4.78 $\mu\text{g cm}^{-2} \text{sec}^{-1}$). Transpiration rate of untreated control in cv. IR 50 was on par with all the individual treatments except *P. fluorescens* and *G. intraradices* combined inoculations except with *P. fluorescens* and in cv. ADT 44 it was statistically similar to all the individual applications of microbial inoculants except *P. fluorescens*.

Table 20. Effect of microbial inoculants on leaf temperature and transpiration rate of rice cvs. IR 50 and ADT 44 against the infection of *H. gracilis* at panicle initiation stage under glasshouse conditions

Treatment	(Mean of two replications)					
	Leaf temperature (°C)			Transpiration rate ($\mu\text{g cm}^{-2} \text{ sec}^{-1}$)		
	IR 50 (75 DAS)	ADT 44 (120 DAS)	%	IR 50 (75 DAS)	ADT 44 (120 DAS)	%
T ₁ - <i>Azolla microphylla</i>	30.16	29.64	-0.3	5.87	4.57	-1.2
T ₂ - <i>Azospirillum lipoferum</i>	30.08	29.60	-0.6	5.74	4.52	-3.4
T ₃ - Blue green algae	30.04	29.61	-0.7	5.71	4.54	-3.9
T ₄ - <i>Bacillus megaterium</i>	30.21	29.68	-0.2	5.78	4.62	-2.7
T ₅ - <i>Glomus intraradices</i>	30.12	29.60	-0.5	5.77	4.51	-2.9
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	29.96	29.44	-1.0	5.71	4.39	-3.9
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	29.89	29.41	-1.2	5.69	4.27	-4.2
T ₈ - Blue green algae + <i>G. intraradices</i>	29.90	29.37	-1.2	5.73	4.22	-3.5
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	29.92	29.40	-1.1	5.76	4.33	-3.0
T ₁₀ - <i>Pseudomonas fluorescens</i>	29.86	29.36	-1.3	5.32	4.14	-10.4
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	29.84	29.33	-1.4	5.28	4.06	-11.1
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	29.80	29.30	-1.5	5.19	3.92	-12.6
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	29.81	29.32	-1.5	5.24	4.01	-11.8
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	29.86	29.34	-1.3	5.30	4.09	-10.8
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	29.85	29.28	-1.4	5.22	3.95	-12.1
T ₁₆ - Carbofuran 3G	30.14	29.71	-0.4	5.73	4.61	-3.5
T ₁₇ - Untreated control	30.26	30.01	-	5.94	4.78	-
T ₁₈ - Uninoculated control	29.77	29.26	-1.6	5.12	3.81	-13.8
CD (P=0.05)	0.09	0.09		0.44	0.29	

% - Per cent decrease (-) over untreated control

Uninoculated control had the minimum transpiration rates in both the rice varieties.

4.3.1.3 Photosynthetic active radiation

In cv IR 50 photosynthetic active radiation was maximum ($342 \mu\text{E m}^{-2} \text{sec}^{-1}$) in combined treatment of *P. fluorescens* with *G. intraradices*. It was on par with *P. fluorescens* and *G. intraradices* combined inoculations and *P. fluorescens* individual treatment except co-treatment of *G. intraradices* with *A. microphylla*. Photosynthetic active radiation of combined treatment of *P. fluorescens* and *A. lipoferum* was enhanced to maximum by 56.1 per cent over untreated control in cv ADT 44. The treatment was statistically similar to all the combined and individual treatments of *P. fluorescens* and *G. intraradices* (Table 21 and Figure 28 & 29).

In both the varieties minimum photosynthetic active radiation was noticed in untreated control and recorded as 227 and $244 \mu\text{E m}^{-2} \text{sec}^{-1}$ in cv. IR 50 & cv ADT 44 respectively. In cv IR 50, the untreated control was on par with combined inoculation of *G. intraradices* with *A. microphylla* and all the individual treatments except *P. fluorescens* and in cv ADT 44 it was statistically similar to individual inoculations except *P. fluorescens* and *G. intraradices*.

4.3.1.4 Diffusive resistance

Combined treatment of *P. fluorescens* with *G. intraradices* had maximum diffusive resistance of 7.19 and 8.86 sec cm^{-1} in cvs IR 50 and ADT 44 respectively. In cv IR 50, the treatment was statistically similar to individual and combined treatments of *P. fluorescens* and combined inoculation of *G. intraradices* with *A. lipoferum*, whereas in cv ADT 44 it was on par with combined treatments of *P. fluorescens* and *G. intraradices* and individual inoculation of *P. fluorescens* (Table 21 and Figure 28 & 29).

Table 21. Effect of microbial inoculants on photosynthetic active radiation and diffusive resistance of rice cvs. IR 50 and ADT 44 against the infection of *H. gracilis* at panicle initiation stage under glasshouse conditions
(Mean of two replications)

Treatment	Photosynthetic active radiation ($\mu\text{E m}^{-2} \text{ sec}^{-1}$)				Diffusive resistance (sec cm^{-1})			
	IR 50 (75 DAS)		ADT 44 (120 DAS)		IR 50 (75 DAS)		ADT 44 (120 DAS)	
	%		%		%		%	
T ₁ - <i>Azolla microphylla</i>	243	7.0	284	16.4	6.26	6.3	7.30	0.1
T ₂ - <i>Azospirillum lipoferum</i>	264	16.3	304	24.6	6.32	7.3	7.32	0.4
T ₃ - Blue green algae	263	15.9	296	21.3	6.26	6.3	7.31	0.3
T ₄ - <i>Bacillus megaterium</i>	238	4.8	282	15.6	6.08	3.2	7.30	0.1
T ₅ - <i>Glomus intraradices</i>	252	11.0	316	29.5	6.13	4.1	7.32	0.4
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	279	22.9	324	32.8	6.24	5.9	7.62	4.5
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	299	31.7	329	34.8	6.38	8.3	7.89	8.2
T ₈ - Blue green algae + <i>G. intraradices</i>	293	29.1	327	34.0	6.22	5.6	7.94	8.9
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	287	26.4	319	30.7	6.14	4.2	7.54	3.4
T ₁₀ - <i>Pseudomonas fluorescens</i>	314	38.3	344	41.0	6.69	13.6	8.42	15.5
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	329	44.9	357	46.3	6.85	16.3	8.64	18.5
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	337	48.5	381	56.1	6.92	17.5	8.84	21.3
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	326	43.6	362	48.4	6.86	16.5	8.76	20.2
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	318	40.1	373	52.9	6.76	14.8	8.63	18.4
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	342	50.7	378	54.9	7.19	22.1	8.86	21.5
T ₁₆ - Carbofuran 3G	248	9.3	305	25.0	6.28	6.6	7.35	0.8
T ₁₇ - Untreated control	227	-	244	-	5.89	-	7.29	-
T ₁₈ - Uninoculated control	363	59.9	396	62.3	7.27	23.4	9.74	33.6
CD (P=0.05)	55		66		0.81		1.33	

% - Per cent increase over untreated control

Figure 28. Effect of *P. fluorescens* and its combinations on physiological parameters of rice cv. IR 50 against the infection of *H. gracilis* at panicle initiation stage (75 DAS) under glasshouse conditions

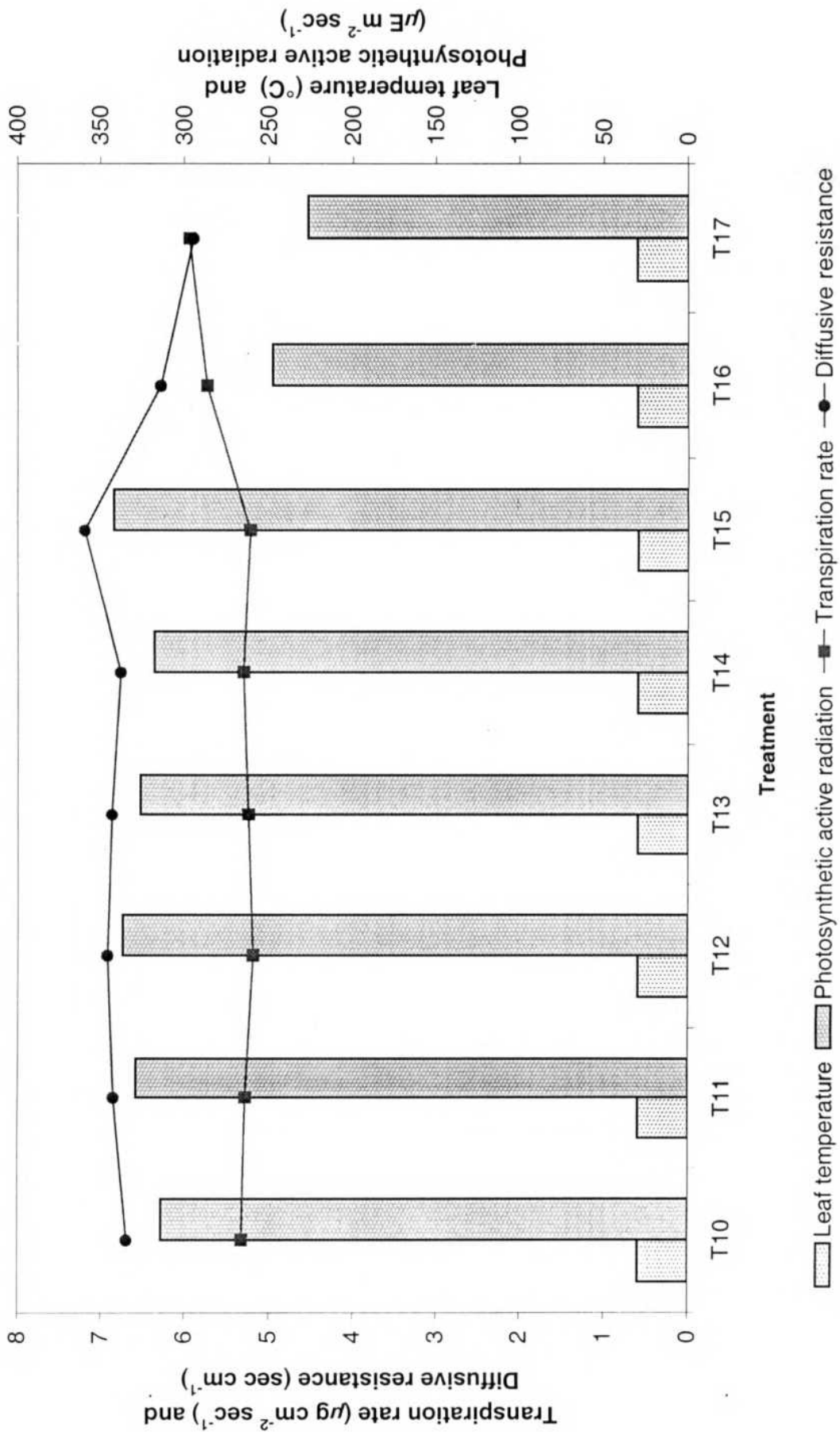
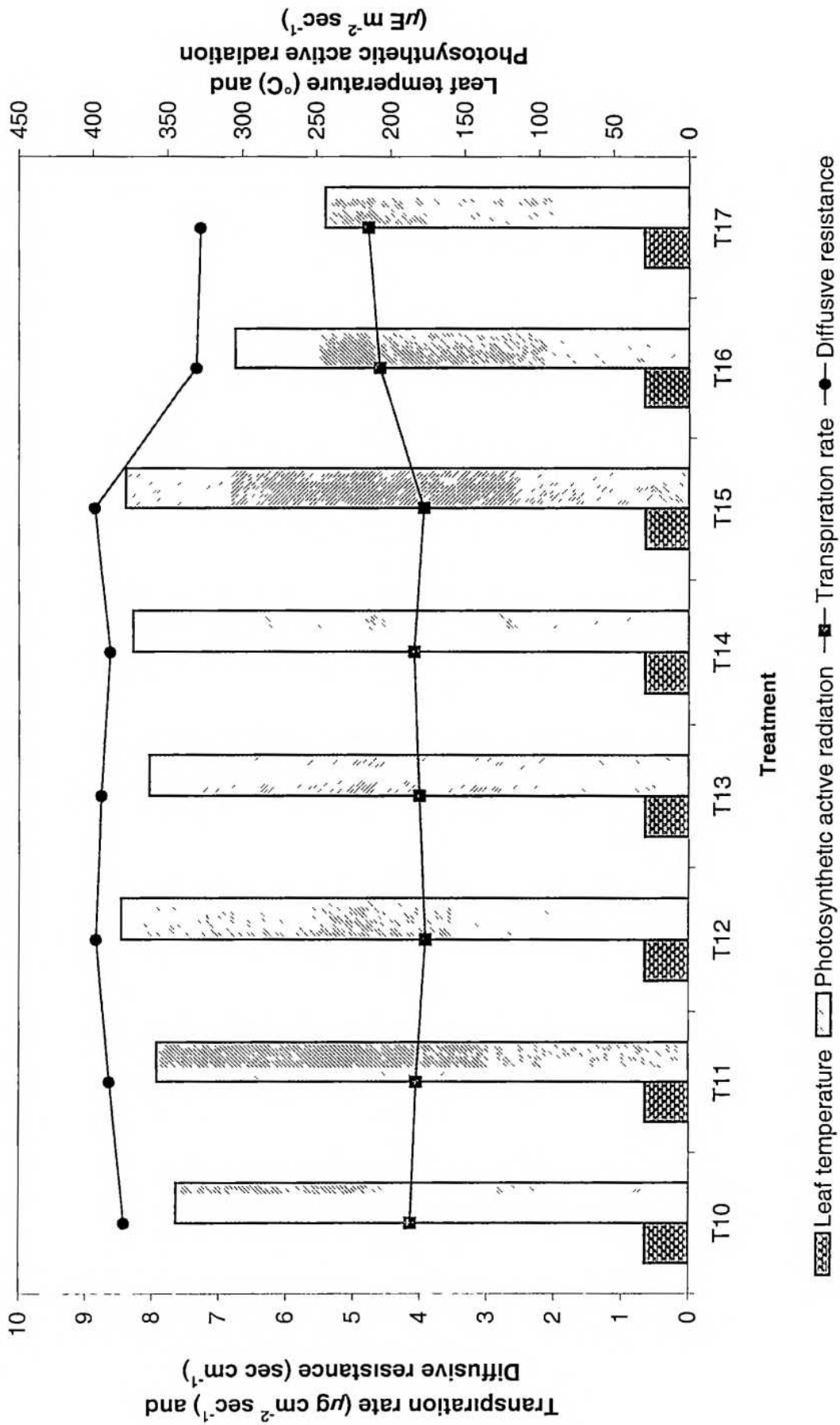


Figure 29. Effect of *P.fluorescens* and its combinations on physiological parameters of rice cv. ADT 44 against the infection of *H.gracilis* at panicle initiation stage (120 DAS) under glasshouse conditions



Untreated control recorded minimum diffusive resistance in both cv IR 50 (5.89 sec cm⁻¹) and cv ADT 44 (7.29 sec cm⁻¹). Diffusive resistance of untreated control was on par with all the individual and *G intraradices* combined treatments except with *P fluorescens* in both the varieties.

Maximum diffusive resistance was noticed in uninoculated control of both the varieties.

4.3.2 Biochemical attributes

Plant biochemistry provides an insight into plant-microbe interactions through the changes in the quantities of important constituents and their activities. Synthesis of these biochemicals impletes the knowledge of plant response to the prevailing environment. Some of the vital biochemical components such as, total free amino acids, soluble proteins, total and reducing sugars, total and ortho dihydroxy phenols, chlorophyll 'a', chlorophyll 'b' and total chlorophyll were quantified and peroxidase and catalase enzyme activities of roots of cvs IR 50 and ADT 44 were determined during the course of study, analysed and furnished in Table 22, 23, 24, 25 & 26, and Figure 30, 31, 32, 33, 34 & 35.

4.3.2.1 Quantitative assessment of biochemical components

4.3.2.1.1 Total free amino acids

Maximum increase of total free amino acids in roots were recorded in *P fluorescens* combined inoculation with *A lipoferum* in both the varieties and were enhanced by 65.7 and 77.5 per cent over untreated control in cvs IR 50 and ADT 44 respectively. Total free amino acid content of the best treatment was statistically similar to all the *P fluorescens* combined inoculations in cv IR 50 and in cv ADT 44 it was on par with combined treatments of *P fluorescens* except with *A microphylla* (Table 22 and Figure 30 & 31).

Table 22. Effect of microbial inoculants on total free amino acid and soluble protein contents of rice cvs. IR 50 and ADT 44 against the infection of *H. gracilis* at panicle initiation stage under glasshouse conditions

Treatment	(Mean of two replications)			
	Total free amino acids (mg g ⁻¹ fresh root weight)		Soluble proteins (mg g ⁻¹ fresh root weight)	
	IR 50 (75 DAS)	ADT 44 (120 DAS)	IR 50 (75 DAS)	ADT 44 (120 DAS)
	%	%	%	%
T ₁ - <i>Azolla microphylla</i>	0.367	0.382	3.354	3.549
T ₂ - <i>Azospirillum lipoferum</i>	0.386	0.414	3.584	3.812
T ₃ - Blue green algae	0.369	0.371	3.441	3.582
T ₄ - <i>Bacillus megaterium</i>	0.372	0.364	3.492	3.613
T ₅ - <i>Glomus intraradices</i>	0.394	0.462	3.529	3.594
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	0.428	0.497	3.693	3.843
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	0.448	0.539	3.772	4.208
T ₈ - Blue green algae + <i>G. intraradices</i>	0.407	0.482	3.748	3.895
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	0.452	0.484	3.761	3.929
T ₁₀ - <i>Pseudomonas fluorescens</i>	0.492	0.546	3.872	3.924
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	0.514	0.569	4.074	4.168
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	0.542	0.614	4.217	4.276
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	0.528	0.573	4.124	4.213
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	0.507	0.578	4.163	4.227
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	0.540	0.604	4.297	4.253
T ₁₆ - Carbofuran 3G	0.374	0.397	3.373	3.594
T ₁₇ - Untreated control	0.327	0.346	4.526	4.329
T ₁₈ - Uninoculated control	0.582	0.724	3.341	3.478
CD (P=0.05)	0.041	0.041	0.520	0.393

% - Per cent increase or decrease (-) over untreated control

Total free amino acid content of untreated control of cv IR 50 (0 327 mg g⁻¹ fresh root) was on par with only individual treatment of *A. microphylla*, whereas in cv ADT 44 (0 346 mg g⁻¹ fresh root) it was statistically similar to individual treatments except *P. fluorescens* and *G. intraradices*

Uninoculated control had the maximum total free amino acids in roots of both the rice cultivars

4.3.2.1.2 Soluble proteins

Among the microbial inoculant treatments, soluble protein content was maximum in the roots of *P. fluorescens* combined inoculation with *G. intraradices* in cv IR 50 (4 297 mg g⁻¹ fresh root), while in cv ADT 44 maximum of soluble proteins (4 276 mg g⁻¹ fresh root) was recorded in combined inoculation of *P. fluorescens* with *A. lipoferum*. In cv. IR 50, the treatment having maximum soluble proteins was on par with individual and combined inoculations of *P. fluorescens*. Individual inoculation of *P. fluorescens* and combined treatments of *P. fluorescens* and *G. intraradices* except combined application of *G. intraradices* with *A. microphylla* were statistically similar to dual inoculation of *P. fluorescens* and *A. lipoferum* in cv ADT 44 (Table 22 and Figure 30 & 31)

Untreated control registered maximum soluble proteins viz, 4 526 and 4 329 mg g⁻¹ fresh root in cvs IR 50 and ADT 44 respectively. Soluble protein content of untreated control was on par with all the *P. fluorescens* combined treatments in cv IR 50, whereas in cv ADT 44 the soluble protein content was statistically similar to all the combined treatments of *P. fluorescens* and dual inoculation of *G. intraradices* and *A. lipoferum*

Uninoculated control recorded minimum soluble proteins in both the rice varieties

4.3.2.1.3 Sugars

4.3.2.1.3.1 Total sugars

Maximum enhancement of total sugars was recorded in combined inoculation of *P. fluorescens* with *G. intraradices* by 51.5 and 26.3 per cent over untreated control in cvs IR 50 and ADT 44 respectively. The total sugar content of the above treatment was statistically different and *P. fluorescens* combined with *A. lipoferum* was the next best in cv. IR 50, whereas in cv. ADT 44 it was statistically similar to the combined inoculation of *P. fluorescens* with *A. lipoferum* (Table 23 and Figure 30 & 31).

Total sugar content of untreated control was the minimum in both the varieties. Untreated control of cvs. IR 50 and ADT 44 registered total sugars 2.547 and 3.724 mg g⁻¹ of fresh root and were statistically similar to *A. microphylla* individual treatment.

Uninoculated control registered maximum total sugars in cv. ADT 44.

4.3.2.1.3.2 Reducing sugars

Reducing sugar content of cv. IR 50 (0.507 mg g⁻¹ fresh root) was maximum in combined treatment of *P. fluorescens* with *G. intraradices* and was statistically similar to *P. fluorescens* combined treatments. The same treatment had maximum reducing sugars (0.521 mg g⁻¹ fresh root) in cv. ADT 44 and was on par with combined treatments of *P. fluorescens* except with *A. microphylla* (Table 23 and Figure 30 & 31).

Minimum reducing sugar content was noticed in untreated control in both the varieties. In cv. IR 50 it was recorded as 0.323 mg g⁻¹ fresh root and it was on par with *A. microphylla* individual treatment. In cv. ADT 44 reducing sugars of untreated control was 0.358 mg g⁻¹ fresh root and was statistically similar to individual treatments of *A. microphylla* and blue green algae.

Uninoculated control recorded minimum reducing sugars in both the cultivars.

Table 23. Effect of microbial inoculants on total and reducing sugar contents of rice cvs. IR 50 and ADT 44 against the infection of *H. gracilis* at panicle initiation stage under glasshouse conditions

Treatment	(Mean of two replications)						
	Total sugars (mg g ⁻¹ fresh root weight)			Reducing sugars (mg g ⁻¹ fresh root weight)			
	IR 50 (75 DAS)	ADT 44 (120 DAS)	IR 50 (75 DAS)	ADT 44 (120 DAS)	IR 50 (75 DAS)	ADT 44 (120 DAS)	
	%	%	%	%	%	%	
T ₁ – <i>Azolla microphylla</i>	2 556	0 4	3 732	0 2	0 336	0 372	3 9
T ₂ – <i>Azospirillum lipoferum</i>	3 125	22 7	3 975	6 7	0 389	0 423	18 2
T ₃ – Blue green algae	2 642	3 7	3 771	1 3	0 351	0 381	6 4
T ₄ – <i>Bacillus megaterium</i>	3 045	19 6	3 890	4 5	0 384	0 421	17 6
T ₅ – <i>Glomus intraradices</i>	3 179	24 8	4 015	7 8	0 412	0 437	22 1
T ₆ – <i>A. microphylla</i> + <i>G. intraradices</i>	3 312	30 0	4 156	11 6	0 436	0 454	26 8
T ₇ – <i>A. lipoferum</i> + <i>G. intraradices</i>	3 505	37 6	4 309	15 7	0 453	0 472	31 8
T ₈ – Blue green algae + <i>G. intraradices</i>	3 427	34 6	4 219	13 3	0 448	0 458	27 9
T ₉ – <i>B. megaterium</i> + <i>G. intraradices</i>	3 489	37 0	4 374	17 5	0 462	0 461	28 8
T ₁₀ – <i>Pseudomonas fluorescens</i>	3 179	24 8	4 568	22 7	0 489	0 463	29 3
T ₁₁ – <i>A. microphylla</i> + <i>P. fluorescens</i>	3 617	42 0	4 652	24 9	0 494	0 477	33 2
T ₁₂ – <i>A. lipoferum</i> + <i>P. fluorescens</i>	3 843	50 9	4 682	25 7	0 501	0 516	44 1
T ₁₃ – Blue green algae + <i>P. fluorescens</i>	3 796	49 0	4 379	17 6	0 498	0 492	37 4
T ₁₄ – <i>B. megaterium</i> + <i>P. fluorescens</i>	3 745	47 0	4 467	20 0	0 496	0 503	40 5
T ₁₅ – <i>G. intraradices</i> + <i>P. fluorescens</i>	3 859	51 5	4 703	26 3	0 507	0 521	45 5
T ₁₆ – Carbofuran 3G	2 897	13 7	3 809	2 3	0 359	0 394	10 1
T ₁₇ – Untreated control	2 547	-	3 724	-	0 323	0 358	-
T ₁₈ – Unmuculated control	3 346	31 4	4 867	30 7	0 189	0 241	-32 7
CD (P=0.05)	0.014		0.037		0.014	0.035	

% - Per cent increase or decrease (-) over untreated control

4.3.2.1.4 Phenolics

4.3.2.1.4.1 Total phenols

Maximum increase of total phenols by 74.8 and 118.1 per cent over untreated control was recorded in combined treatment of *P. fluorescens* with *G. intraradices* of cv IR 50 and combined inoculation of *P. fluorescens* with *A. lipoferum* of cv ADT 44. Total phenol content of the best treatment was on par with individual and combined inoculations of *P. fluorescens* in cv IR 50 and in cv ADT 44 only combined treatments of *P. fluorescens* were statistically similar (Table 24 and Figure 30 & 31).

Total phenols of untreated control was minimum in cv IR 50 (0.923 mg g⁻¹ fresh root) and cv ADT 44 (0.814 mg g⁻¹ fresh root). In cv IR 50 the untreated control was statistically similar to individual treatments except *P. fluorescens* and in cv ADT 44 it was significantly reduced.

Uninoculated control registered minimum total phenols in both the cultivars.

4.3.2.1.4.2 Ortho dihydroxy (OD) phenols

Combined treatment of *P. fluorescens* with *G. intraradices* had maximum of ortho dihydroxy phenols recording 0.514 and 0.674 mg g⁻¹ fresh root in cvs IR 50 and ADT 44 respectively. The OD phenol content of the best treatment of both the varieties were on par with combined inoculation of *P. fluorescens* with *A. lipoferum* (Table 24 and Figure 30 & 31).

Ortho dihydroxy phenols of untreated control had minimum of 0.364 and 0.423 mg g⁻¹ fresh root in cvs IR 50 and ADT 44 respectively and were significantly reduced.

Minimum OD phenols were noticed in uninoculated control of both the varieties.

Figure 30. Effect of *P.fluorescens* and its combinations on total free amino acid, soluble protein, total and reducing sugar and total and ortho dihydroxy phenol contents of rice cv. IR 50 against the infection of *H.gracilis* at panicle initiation stage (75 DAS) under glasshouse conditions

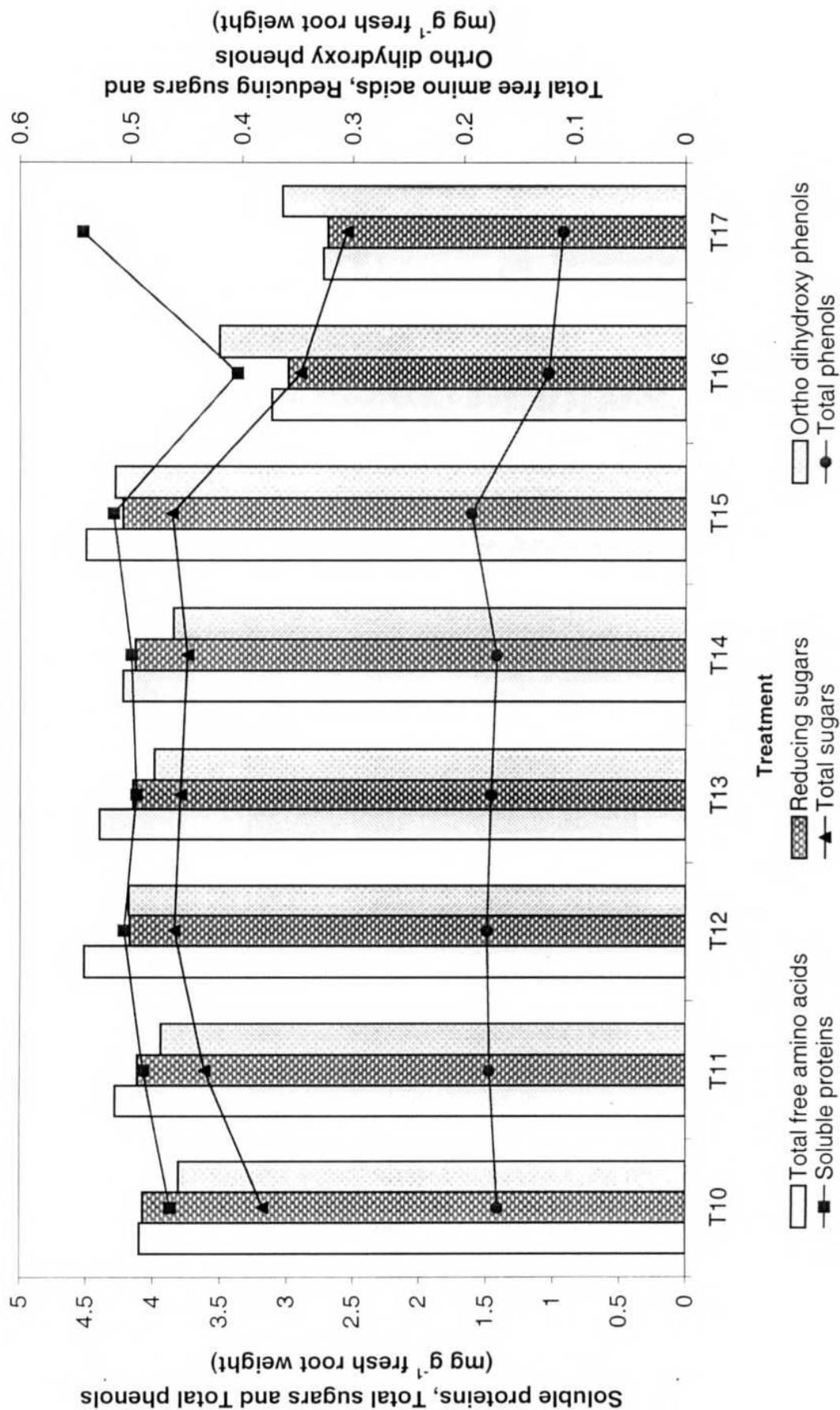
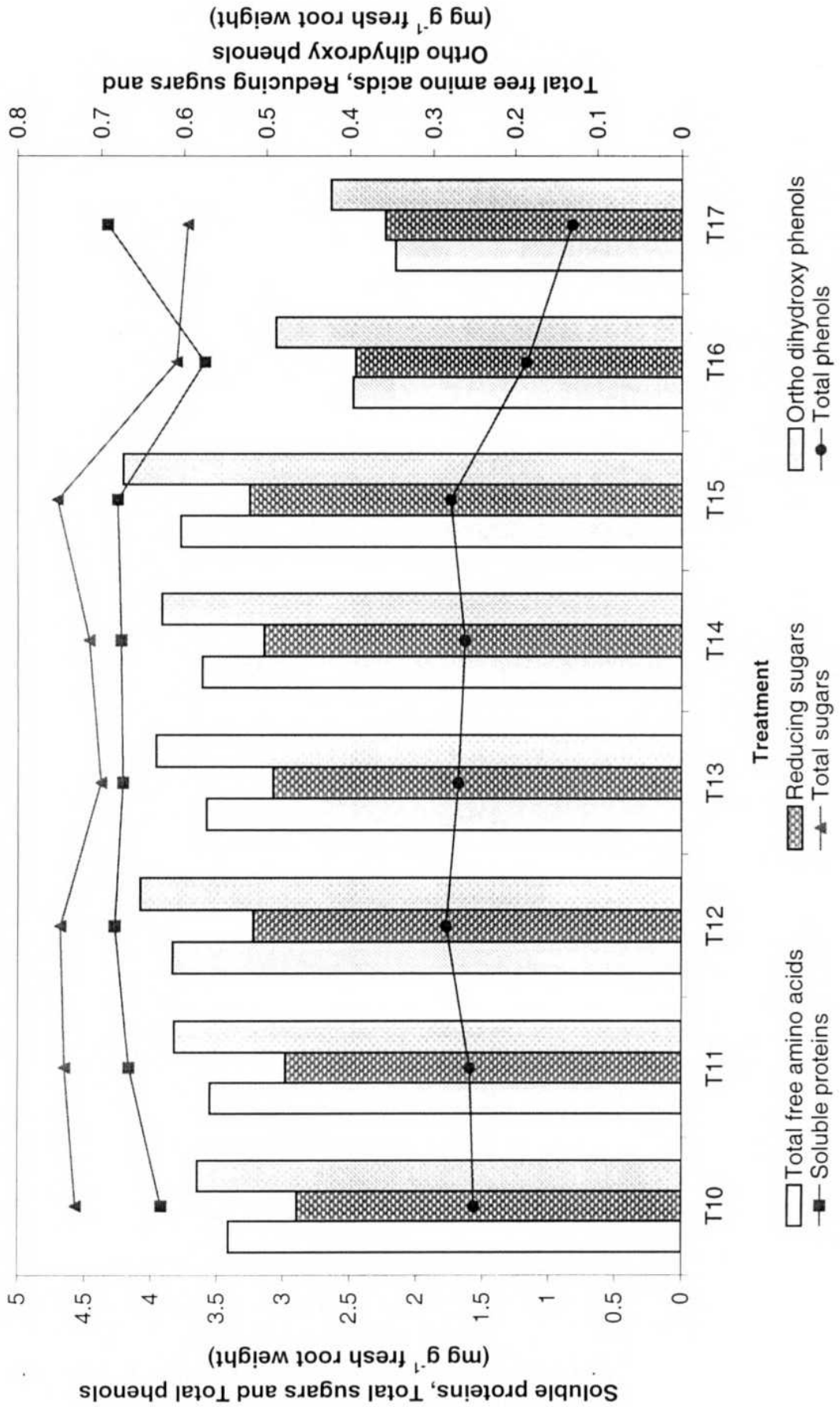


Figure 31. Effect of *P.fluorescens* and its combinations on total free amino acid, soluble protein, total and reducing sugar and total and ortho dihydroxy phenol contents of rice cv. ADT 44 against the infection of *H.gracilis* at panicle initiation stage (120 DAS) under glasshouse conditions



4.3.2.1.5 Chlorophylls

4.3.2.1.5.1 Chlorophyll 'a'

Maximum enhancement of chlorophyll 'a' by 70 per cent over untreated control was recorded in combined inoculation of *P. fluorescens* with *G. intraradices* in cv IR 50 and was statistically similar to *P. fluorescens* combined inoculations with *A. lipoferum* and *A. microphylla*. Combined treatment of *P. fluorescens* with *A. lipoferum* recorded maximum chlorophyll 'a' of 1 459 mg g⁻¹ fresh leaf in cv ADT 44 and was significantly different. Co-inoculation of *P. fluorescens* with *A. microphylla* was the next best treatment (Table 25 and Figure 32 & 33)

Untreated control registered minimum of chlorophyll 'a' of 1 363 and 1 368 mg g⁻¹ fresh leaf weight in cvs. IR 50 and ADT 44 respectively and were significantly reduced

4.3.2.1.5.2 Chlorophyll 'b'

Combined treatment of *P. fluorescens* with *A. lipoferum* of both the cultivars had maximum chlorophyll 'b' and were recorded as 0 599 and 0 689 mg g⁻¹ fresh leaf weight in cvs. IR 50 and ADT 44 respectively. The best treatment in cv IR 50 was on par with *P. fluorescens* combined inoculations with *G. intraradices*, *B. megaterium* and *G. intraradices* co-inoculation with *A. lipoferum* and in cv ADT 44 it was statistically similar to *P. fluorescens* combined applications with *G. intraradices*, *B. megaterium* and *A. microphylla* (Table 25 and Figure 32 & 33)

Chlorophyll 'b' content of untreated control in cv IR 50 (0 422) and cv ADT 44 (0.562) mg g⁻¹ fresh leaf were significantly reduced

4.3.2.1.5.3 Total chlorophyll

Maximum enhancement of total chlorophyll by 14.5 and 10.7 per cent over untreated control was recorded in combined inoculation of *P. fluorescens* with *A. lipoferum* in cvs IR 50 and ADT 44 respectively. The treatment was on par with

Table 25. Effect of microbial inoculants on chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents of cvs. IR 50 and ADT 44 against the infection of *H. gracilis* at panicle initiation stage under glasshouse conditions
(Mean of two replications)

Treatment	Chlorophyll 'a' (mg g ⁻¹ fresh leaf weight)			Chlorophyll 'b' (mg g ⁻¹ fresh leaf weight)			Total chlorophyll (mg g ⁻¹ fresh leaf weight)					
	IR 50 (75 DAS)	ADT 44 (120 DAS)	%	IR 50 (75 DAS)	ADT 44 (120 DAS)	%	IR 50 (75 DAS)	ADT 44 (120 DAS)	%			
T ₁ – <i>Azolla microphylla</i>	1 416	3 9	1 413	3 3	0 556	31 8	0 667	18 7	1 974	10 7	2 073	6 7
T ₂ – <i>Azospirillum lipoferum</i>	1 419	4 1	1 419	3 7	0 584	38 4	0 672	19 6	1 992	11 7	2 089	7 6
T ₃ – Blue green algae	1 407	3 2	1 394	1 9	0 537	27 3	0 659	17 3	1 881	5 4	2 048	5 5
T ₄ – <i>Bacillus megaterium</i>	1 376	0 9	1 379	0 8	0 574	36 0	0 671	19 4	1 951	9 4	2 051	5 6
T ₅ – <i>Glomus intraradices</i>	1 394	2 3	1 402	2 5	0 565	33 9	0 668	18 9	1 963	10 0	2 077	7 0
T ₆ – <i>A. microphylla</i> + <i>G. intraradices</i>	1 427	4 7	1 428	4 4	0 578	37 0	0 673	19 8	1 998	12 0	2 089	7 6
T ₇ – <i>A. lipoferum</i> + <i>G. intraradices</i>	1 422	4 3	1 431	4 6	0 597	41 5	0 681	21 2	2 004	12 3	2 110	8 7
T ₈ – Blue green algae + <i>G. intraradices</i>	1 410	3 4	1 416	3 5	0 571	35 3	0 669	19 0	1 942	8 9	2 096	7 9
T ₉ – <i>B. megaterium</i> + <i>G. intraradices</i>	1 398	2 6	1 405	2 7	0 584	38 4	0 676	20 3	1 994	11 8	2 084	7 3
T ₁₀ – <i>Pseudomonas fluorescens</i>	1 416	3 9	1 421	3 9	0 568	34 6	0 665	18 3	1 984	11 2	2 093	7 8
T ₁₁ – <i>A. microphylla</i> + <i>P. fluorescens</i>	1 452	6 5	1 449	5 9	0 582	37 9	0 683	21 5	2 023	13 4	2 125	9 4
T ₁₂ – <i>A. lipoferum</i> + <i>P. fluorescens</i>	1 454	6 7	1 459	6 7	0 599	41 9	0 689	22 6	2 042	14 5	2 149	10 7
T ₁₃ – Blue green algae + <i>P. fluorescens</i>	1 434	5 2	1 435	4 9	0 576	36 5	0 681	21 2	2 007	12 5	2 114	8 9
T ₁₄ – <i>B. megaterium</i> + <i>P. fluorescens</i>	1 427	4 7	1 426	4 2	0 592	40 3	0 685	21 9	2 018	13 1	2 103	8 3
T ₁₅ – <i>G. intraradices</i> + <i>P. fluorescens</i>	1 458	7 0	1 437	5 0	0 594	40 8	0 686	22 1	2 032	13 9	2 131	9 7
T ₁₆ – Carbofuran 3G	1 389	1 9	1 384	1 2	0 533	26 3	0 647	15 1	1 922	7 7	2 029	4 5
T ₁₇ – Untreated control	1 363	-	1 368	-	0 422	-	0 562	-	1 784	-	1 942	-
T ₁₈ – Uninoculated control	1 418	4 0	1 424	4 1	0 541	28 2	0 613	9 1	1 957	9 7	2 054	5 8
CD (P=0.05)	0.008		0.007		0.009		0.007		0.014		0.012	

Figure 32. Effect of *P.fluorescens* and its combinations on chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents of rice cv. IR 50 against the infection of *H.gracilis* at panicle initiation stage (75 DAS) under glasshouse conditions

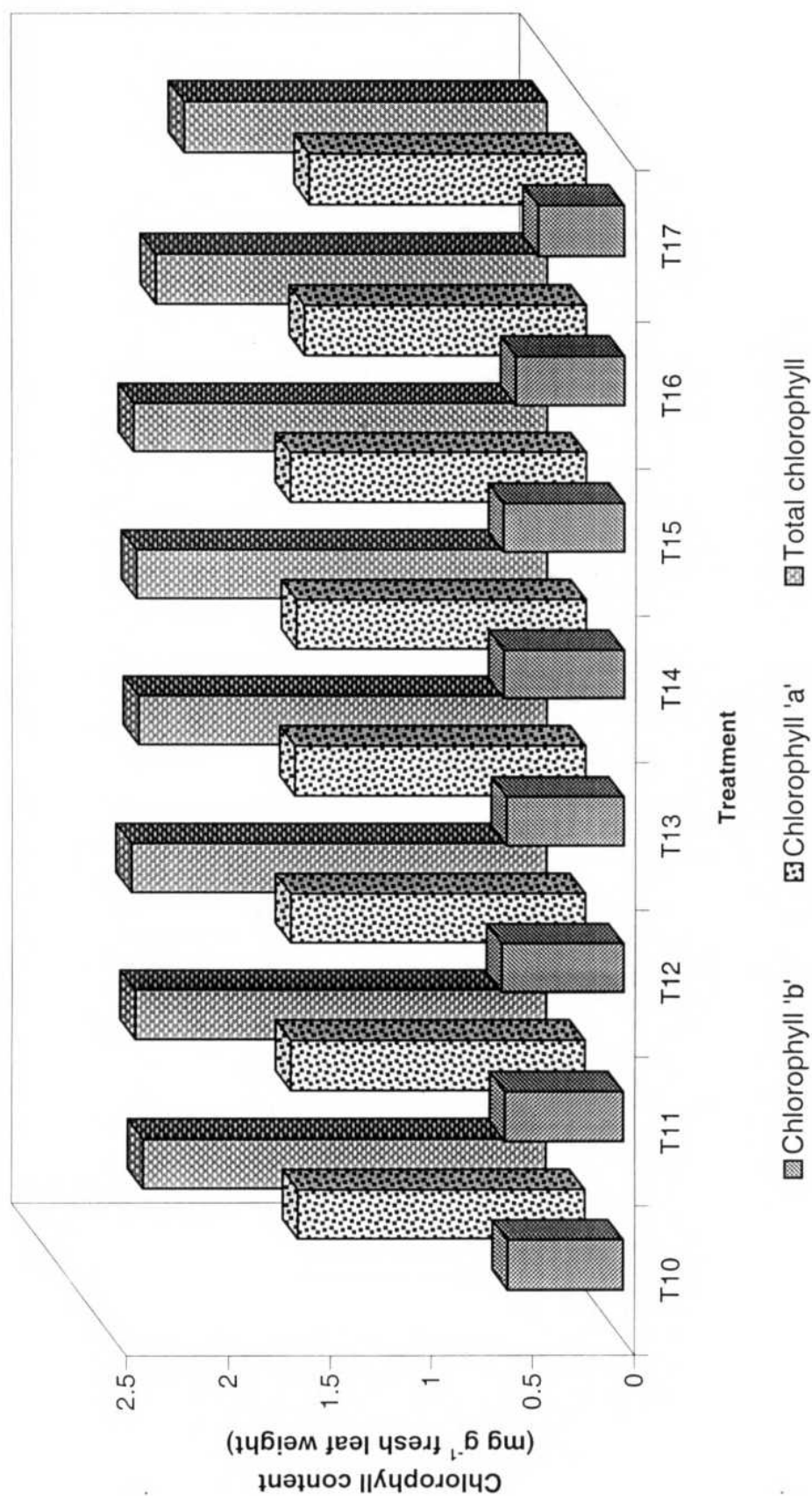
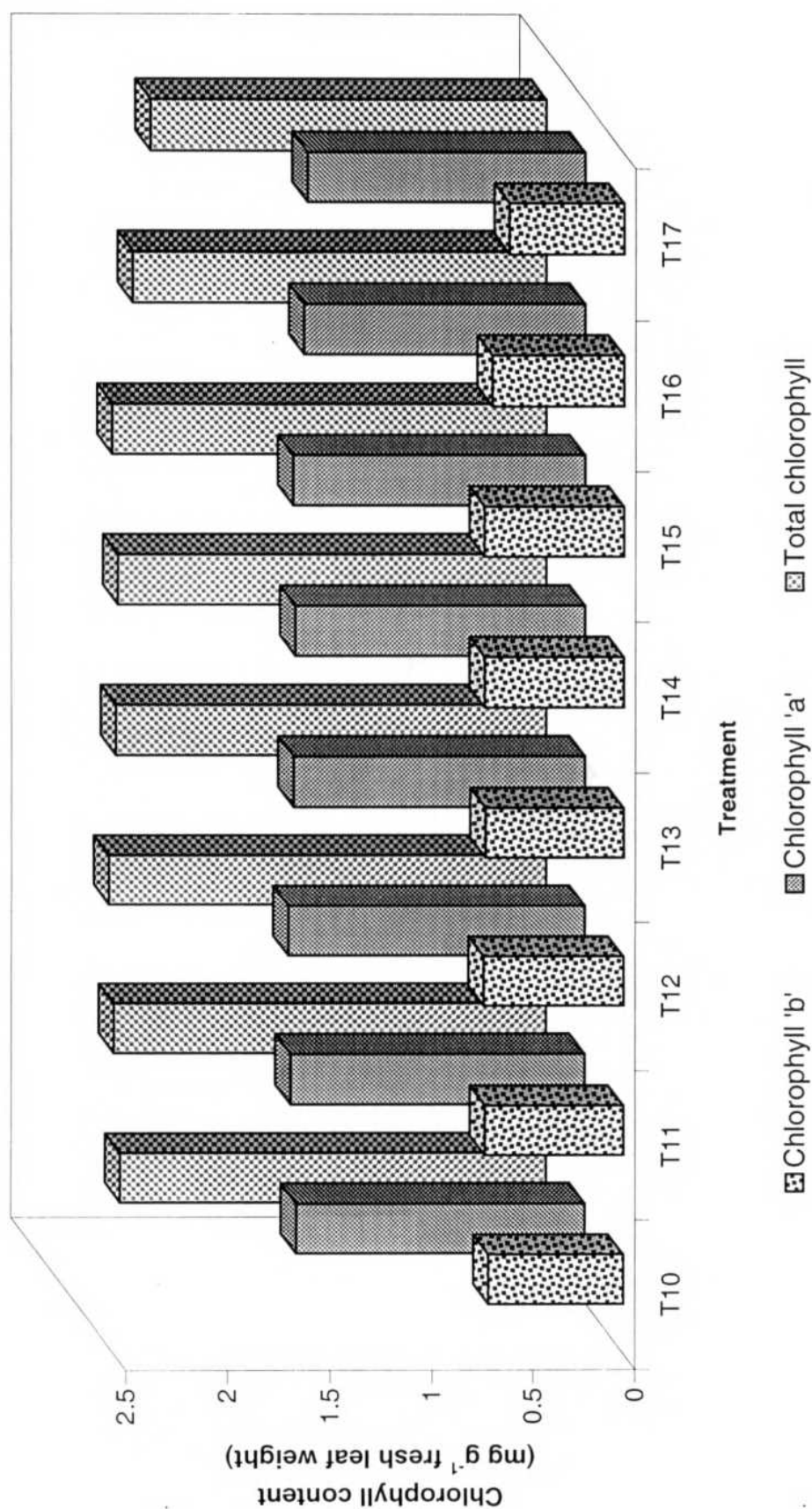


Figure 33. Effect of *P.fluorescens* and its combinations on chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents of rice cv. ADT 44 against the infection of *H.gracilis* at panicle initiation stage (120 DAS) under glasshouse conditions



combined treatment of *P. fluorescens* with *G. intraradices* in cv IR 50. The best treatment was significantly different in cv ADT 44 and combined application of *P. fluorescens* with *G. intraradices* was the next best treatment (Table 25 and Figure 32 & 33).

Significantly minimum total chlorophyll in cvs IR 50 (1 784) and ADT 44 (1 942) mg g⁻¹ fresh leaf were registered in untreated control

4.3.2.2 Enzyme activity

Activities of oxidative enzymes such as peroxidase and catalase that have importance in phytonematode pathogenesis, were estimated in the roots of cvs IR 50 and ADT 44, analysed and furnished in Table 26 and Figure 34 & 35

4.3.2.2.1 Peroxidase

Maximum increase in peroxidase activities by 72.9 and 58.2 per cent over untreated control was recorded in combined treatment of *P. fluorescens* and *A. lipoferum* of cvs IR 50 and ADT 44 respectively. This was statistically similar to *P. fluorescens* combined treatments in cv IR 50, whereas in cv ADT 44 it was on par with individual and combined treatments of *P. fluorescens* (Table 26 and Figure 34 & 35)

Untreated control had significantly reduced peroxidase activities of 32.5 and 38.8 enzyme units h⁻¹ in cvs IR 50 and ADT 44 respectively

Uninoculated control registered minimum peroxidase activities in the root of both the rice cultivars

4.3.2.2.2 Catalase

Combined inoculation of *P. fluorescens* with *G. intraradices* had maximum (97.6 and 102.7 µg of H₂O₂ g⁻¹ min⁻¹) of catalase activities in cvs IR 50 and ADT 44

Table 26. Effect of microbial inoculants on peroxidase and catalase activities of cvs. IR 50 and ADT 44 against the infection of *H. gracilis* at panicle initiation stage under glasshouse conditions

Treatment	(Mean of two replications)					
	Peroxidase (enzyme units h ⁻¹)			Catalase (µg of H ₂ O ₂ g ⁻¹ min ⁻¹)		
	IR 50 (75 DAS)	ADT 44 (120 DAS)	IR 50 (75 DAS)	ADT 44 (120 DAS)	IR 50 (75 DAS)	ADT 44 (120 DAS)
	%	%	%	%	%	%
T ₁ - <i>Azolla microphylla</i>	44.3	49.5	77.3	76.9	13.0	6.7
T ₂ - <i>Azospirillum lipoferum</i>	45.2	50.4	84.7	91.6	23.8	27.0
T ₃ - Blue green algae	44.6	49.8	79.2	78.4	15.8	8.7
T ₄ - <i>Bacillus megaterium</i>	44.4	49.1	83.4	81.5	21.9	13.0
T ₅ - <i>Glomus intraradices</i>	46.7	52.6	87.3	94.4	27.6	30.9
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	49.3	54.2	87.9	95.1	28.5	31.9
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	50.4	55.4	91.3	98.2	33.5	36.2
T ₈ - Blue green algae + <i>G. intraradices</i>	45.3	53.8	88.1	95.6	28.8	32.6
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	50.2	53.2	89.4	96.2	30.7	33.4
T ₁₀ - <i>Pseudomonas fluorescens</i>	51.6	56.8	86.6	94.7	26.6	31.3
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	55.1	57.6	87.2	95.8	27.5	32.9
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	56.2	61.4	91.6	99.4	33.9	37.9
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	55.4	58.3	88.2	95.7	28.9	32.7
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	54.3	58.1	90.5	96.3	32.3	33.6
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	55.8	61.2	97.6	102.7	42.7	42.4
T ₁₆ - Carbofuran 3G	44.8	49.6	81.8	88.0	19.6	22.1
T ₁₇ - Untreated control	32.5	38.8	68.4	72.1	-	-
T ₁₈ - Uninoculated control	22.2	26.9	56.2	66.7	-17.8	-7.5
CD (P=0.05)	4.0	4.9	11.1	9.6		

% - Per cent increase or decrease (-) over untreated control

Figure 34. Effect of *P.fluorescens* and its combinations on peroxidase and catalase activities of rice cv. IR 50 against the infection of *H.gracilis* at panicle initiation stage (75 DAS) under glasshouse conditions

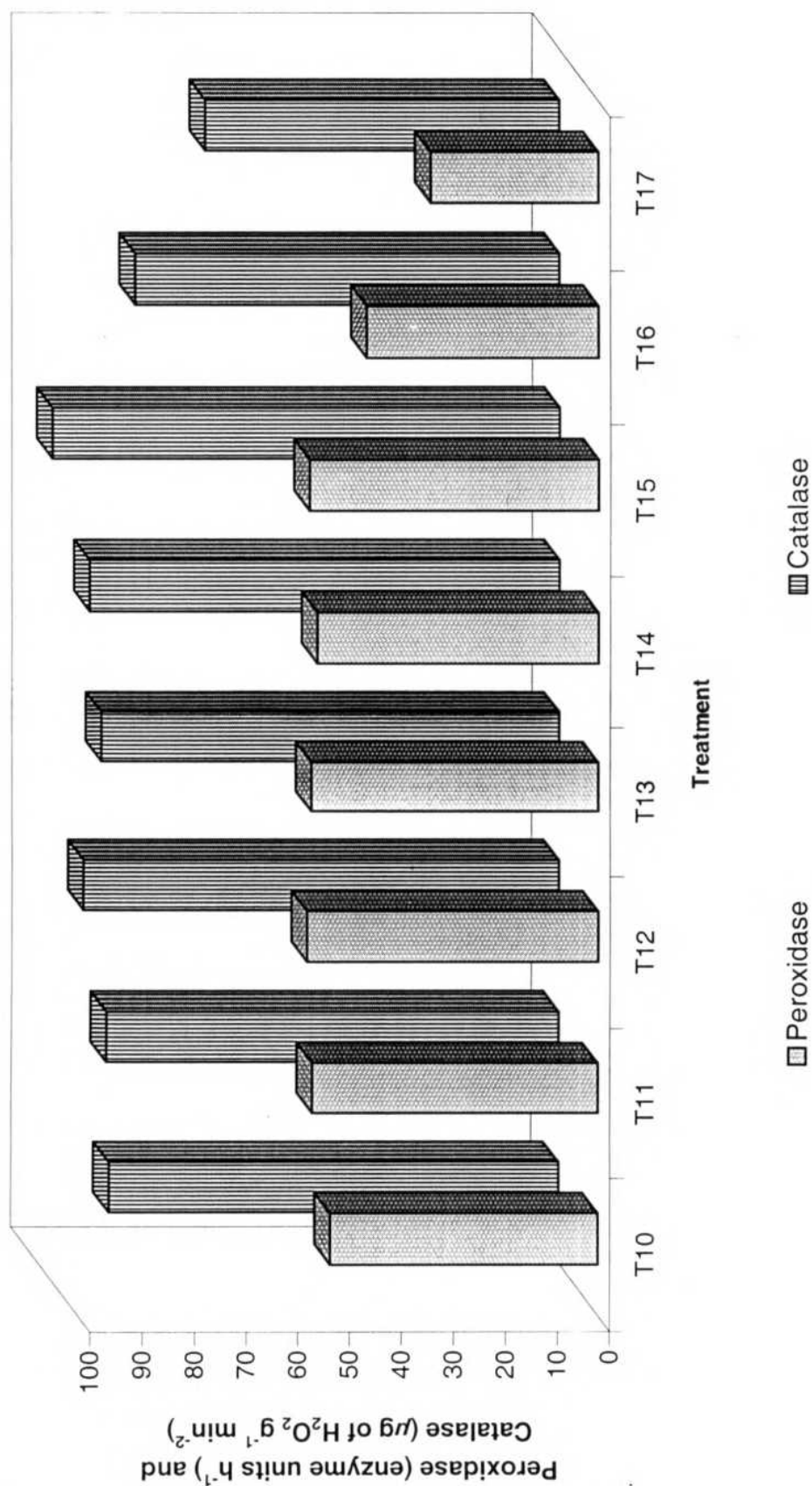
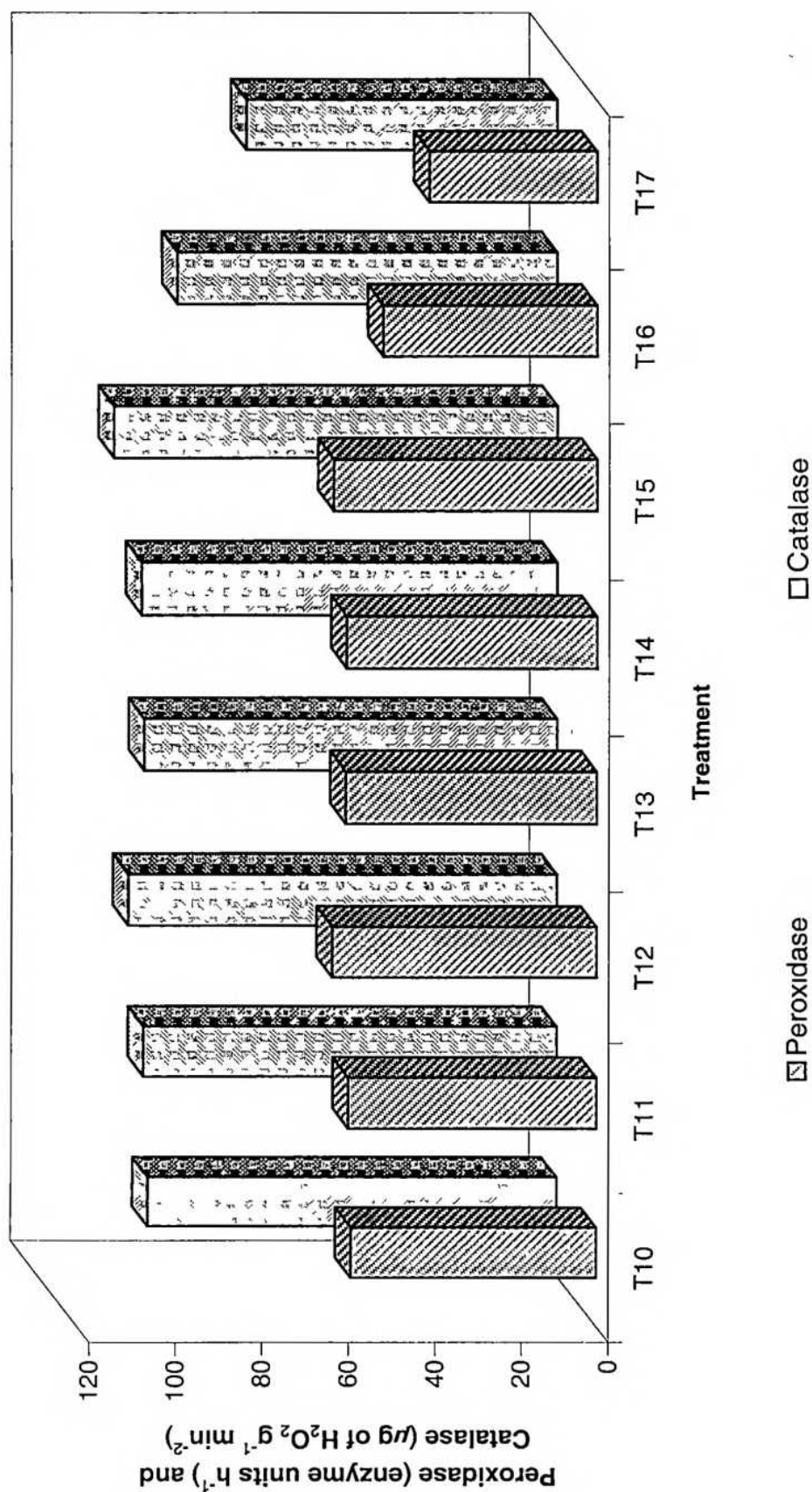


Figure 35. Effect of *P.fluorescens* and its combinations on peroxidase and catalase activities of rice cv. ADT 44 against the infection of *H.gracilis* at panicle initiation stage (120 DAS) under glasshouse conditions



respectively. In both the cultivars, the treatment was on par with combined and individual treatments of both *P. fluorescens* and *G. intraradices* (Table 26 and Figure 34 & 35)

Catalase activities recorded in cvs IR 50 (68.4) and ADT 44 (72.1 $\mu\text{g of H}_2\text{O}_2 \text{ g}^{-1} \text{ min}^{-1}$) in untreated control was minimum. In cv IR 50, the untreated control was statistically similar to individual treatments of *A. microphylla* and blue green algae, whereas in cv ADT 44 it was on par with individual applications of *A. microphylla*, blue green algae and *B. megaterium*.

Uninoculated control registered minimum of root catalase activities in both the rice varieties.

4.3.3 Nutritional status

Macronutrient (nitrogen, phosphorus and potassium) and micronutrient (iron, copper, zinc and manganese) contents of shoot and root of cvs IR 50 and ADT 44 were estimated to ascertain the degree of damage caused to the infected plants by the rice-root nematode, *H. gracilis* and analysed and furnished in Table 27, 28, 29, 30, 31, 32 & 33 and Figure 36, 37, 38, 39, 40, 41, 42 & 43.

4.3.3.1 Macronutrients

4.3.3.1.1 Total nitrogen

Combined treatment of *P. fluorescens* with *A. lipoferum* recorded maximum total nitrogen content in the shoot of both the rice varieties and were increased by 39.0 and 25.4 per cent over untreated control in cvs IR 50 and ADT 44 respectively. This was on par with combined treatments of both *P. fluorescens* and *G. intraradices* and individual treatments of *A. microphylla* and *A. lipoferum* in cv IR 50. Individual inoculations except, *B. megaterium* and all the combined treatments were statistically similar to total nitrogen content of shoot of the treatment in cv ADT 44 (Table 27 and Figure 36 & 37).

Table 27. Effect of microbial inoculants on total nitrogen content of shoot and root of cvs. IR 50 and ADT 44 against the infection of *H. gracilis* at panicle initiation stage under glasshouse conditions

Treatment	(Mean of two replications)					
	Shoot (per cent dry weight basis)			Root (per cent dry weight basis)		
	IR 50 (75 DAS)	ADT 44 (120 DAS)	%	IR 50 (75 DAS)	ADT 44 (120 DAS)	%
T ₁ - <i>Azolla microphylla</i>	1.56	1.59	26.8	1.23	1.46	31.5
T ₂ - <i>Azospirillum lipoferum</i>	1.63	1.68	32.5	1.46	1.50	35.1
T ₃ - Blue green algae	1.52	1.53	23.6	1.30	1.37	23.4
T ₄ - <i>Bacillus megaterium</i>	1.48	1.49	20.3	1.29	1.34	20.7
T ₅ - <i>Glomus intraradices</i>	1.53	1.52	24.4	1.31	1.38	24.3
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	1.68	1.71	36.6	1.45	1.49	34.2
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	1.61	1.71	30.9	1.48	1.51	36.0
T ₈ - Blue green algae + <i>G. intraradices</i>	1.65	1.68	34.1	1.36	1.39	25.2
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	1.63	1.67	32.5	1.33	1.38	24.3
T ₁₀ - <i>Pseudomonas fluorescens</i>	1.51	1.54	22.8	1.28	1.31	18.0
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	1.57	1.70	27.6	1.41	1.46	31.5
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	1.71	1.73	39.0	1.53	1.66	49.5
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	1.60	1.69	30.1	1.35	1.37	23.4
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	1.58	1.68	28.5	1.33	1.36	22.5
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	1.62	1.70	31.7	1.40	1.42	27.9
T ₁₆ - Carbofuran 3G	1.47	1.40	19.5	1.06	1.12	0.9
T ₁₇ - Untreated control	1.23	1.38	-	0.93	1.11	-
T ₁₈ - Uninoculated control	1.63	1.72	32.5	1.47	1.58	42.3
CD (P=0.05)	0.17	0.22		0.16	0.16	

% - Per cent increase over untreated control

Figure 36. Effect of *P. fluorescens* and its combinations on total nitrogen content of shoot and root of rice cv. IR 50 against the infection of *H. gracilis* at panicle initiation stage (75 DAS) under glasshouse conditions

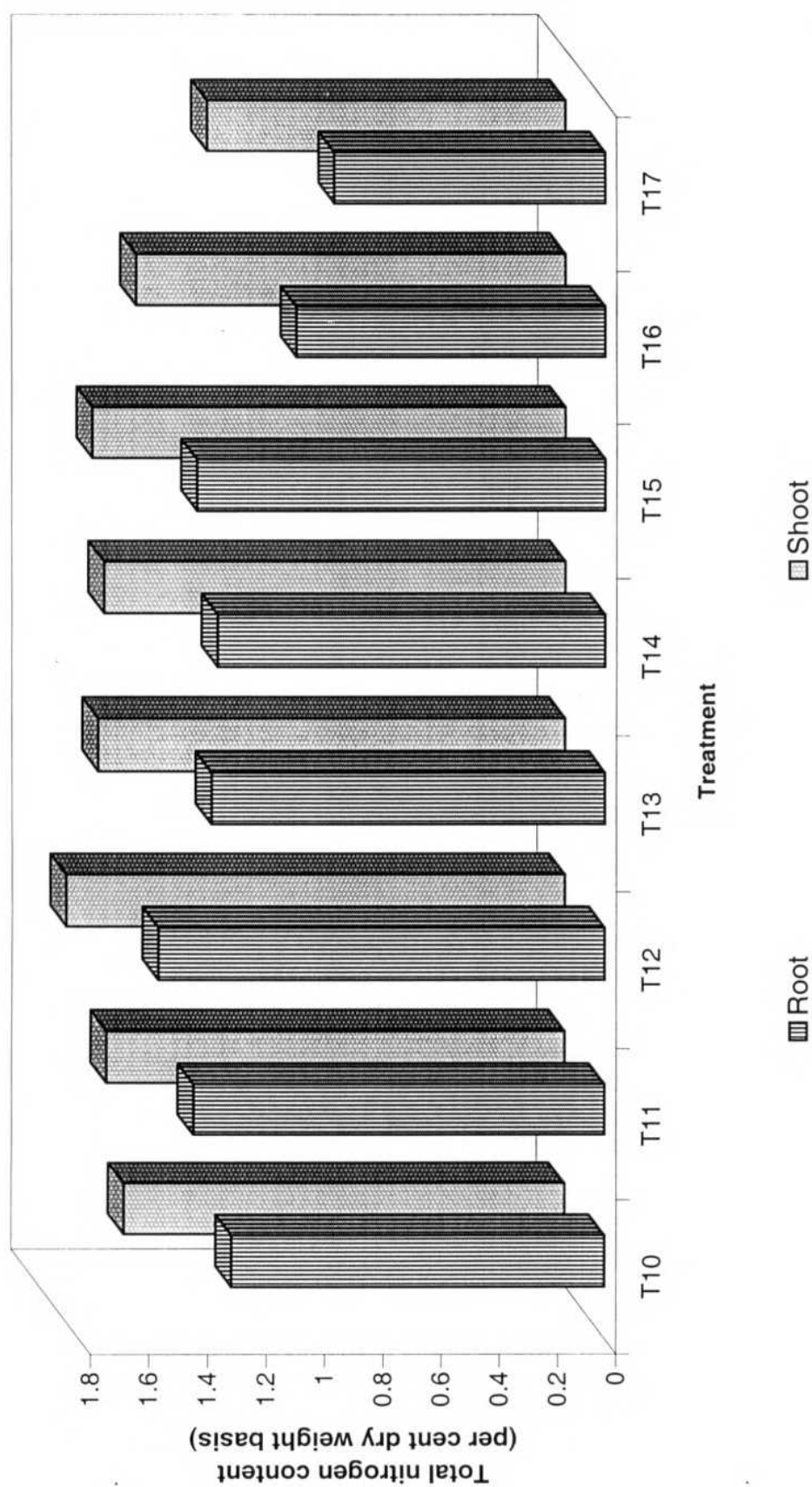
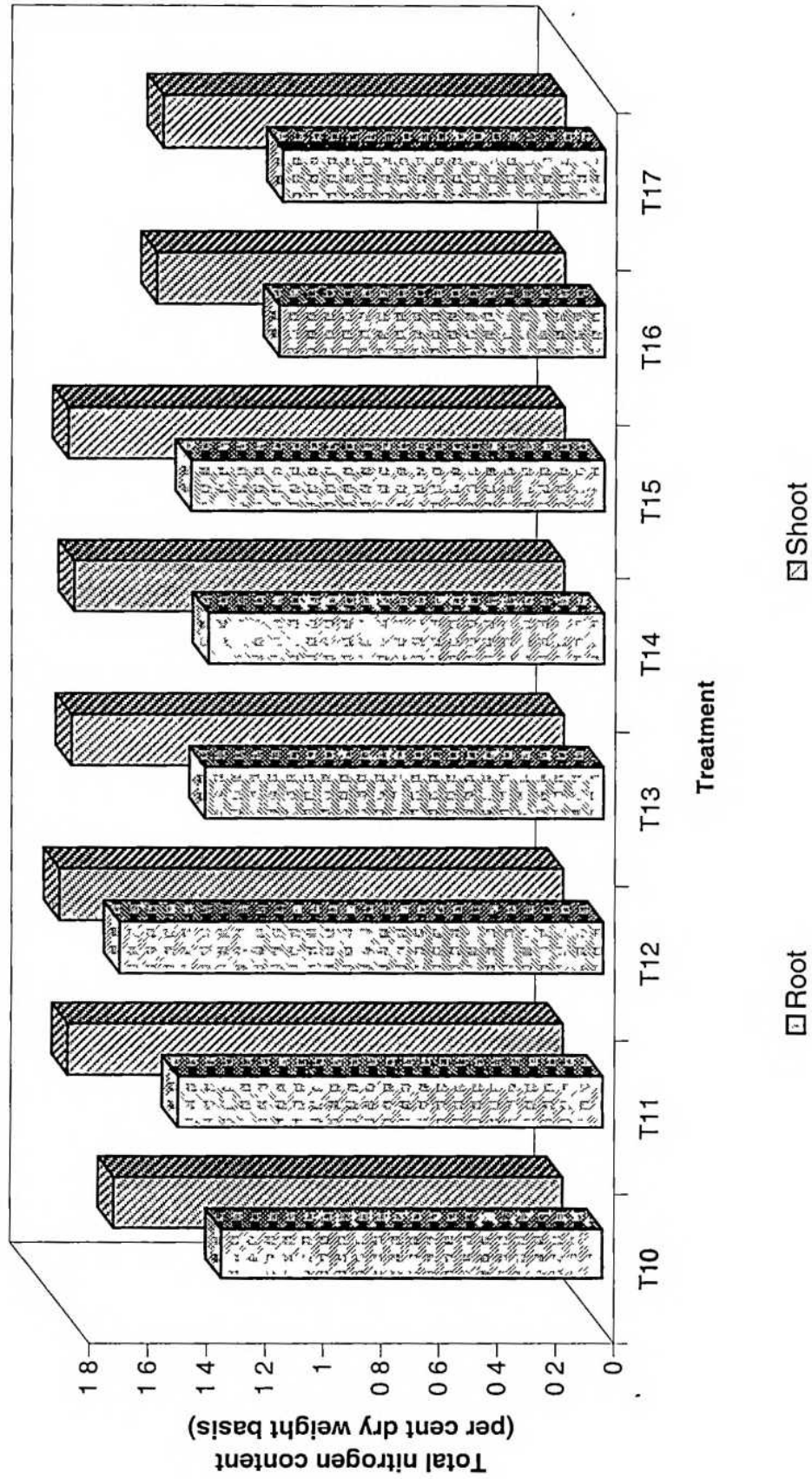


Figure 37. Effect of *P.fluorescens* and its combinations on total nitrogen content of shoot and root of rice cv. ADT 44 against the infection of *H.gracilis* at panicle initiation stage (120 DAS) under glasshouse conditions



Untreated control of cv IR 50 had significantly reduced total nitrogen (1.23% of shoot on dry weight basis). In cv. ADT 44 untreated control registered minimum of shoot total nitrogen (1.38% on dry weight basis) and was on par with all the individual treatments except *A. lipoferum*.

Similar to shoot nitrogen, root nitrogen contents of combined inoculation of *P. fluorescens* and *A. lipoferum* were maximum (1.53 and 1.66% on dry weight basis) in cvs. IR 50 and ADT 44 respectively. This was statistically similar to individual treatment of *A. lipoferum*, *G. intraradices* combined inoculations with *A. microphylla*, *A. lipoferum* and combined treatments of *P. fluorescens* with *A. microphylla* and *G. intraradices* in cv. IR 50. Root nitrogen content of the best treatment of cv ADT 44 was on par with *A. lipoferum* individual treatment and dual inoculation with *G. intraradices* (Table 27 and Figure 36 & 37).

Untreated control registered significantly minimum root nitrogen content in both the rice varieties and were found to be 0.93 and 1.11 per cent on dry weight basis in cvs. IR 50 and ADT 44 respectively.

4.3.3.1.2 Phosphorus

Shoot phosphorus contents of cvs IR 50 and ADT 44 were enhanced to maximum by 200.0 and 107.1 per cent over untreated control respectively in co-inoculation of *G. intraradices* with *B. megaterium*. This was statistically similar to combined treatments of both *P. fluorescens* and *G. intraradices* except *P. fluorescens* combination with *A. microphylla* and blue green algae in cv IR 50. In cv ADT 44, the treatment was on par with combined treatments of *B. megaterium* with *P. fluorescens* and *G. intraradices* with blue green algae (Table 28 and Figure 38 & 39).

Table 28. Effect of microbial inoculants on phosphorus content of shoot and root of cvs. IR 50 and ADT 44 against the infection of *H. gracilis* at panicle initiation stage under glasshouse conditions

Treatment	(Mean of two replications)					
	Shoot (per cent dry weight basis)			Root (per cent dry weight basis)		
	IR 50 (75 DAS)	ADT 44 (120 DAS)	%	IR 50 (75 DAS)	ADT 44 (120 DAS)	%
T ₁ – <i>Azolla microphylla</i>	0 14	0 16	55 6	0 17	0 31	47 6
T ₂ – <i>Azospirillum lipoferum</i>	0 16	0 17	77 8	0 16	0 27	28 6
T ₃ – Blue green algae	0 12	0 15	33 3	0 16	0 29	38 1
T ₄ – <i>Bacillus megaterium</i>	0 18	0 21	100 0	0 24	0 40	90 5
T ₅ – <i>Glomus intraradices</i>	0 19	0 23	111 1	0 29	0 39	85 7
T ₆ – <i>A. microphylla</i> + <i>G. intraradices</i>	0 21	0 24	133 3	0 31	0 41	95 2
T ₇ – <i>A. lipoferum</i> + <i>G. intraradices</i>	0 20	0 26	122 2	0 31	0 42	100 0
T ₈ – Blue green algae + <i>G. intraradices</i>	0 20	0 27	122 2	0 30	0 41	95 2
T ₉ – <i>B. megaterium</i> + <i>G. intraradices</i>	0 27	0 29	200 0	0 36	0 44	109 5
T ₁₀ – <i>Pseudomonas fluorescens</i>	0 14	0 20	55 6	0 19	0 33	57 1
T ₁₁ – <i>A. microphylla</i> + <i>P. fluorescens</i>	0 15	0 20	66 7	0 21	0 36	71 4
T ₁₂ – <i>A. lipoferum</i> + <i>P. fluorescens</i>	0 21	0 22	133 3	0 23	0 39	85 7
T ₁₃ – Blue green algae + <i>P. fluorescens</i>	0 16	0 23	77 8	0 19	0 37	76 2
T ₁₄ – <i>B. megaterium</i> + <i>P. fluorescens</i>	0 26	0 27	188 9	0 32	0 40	90 5
T ₁₅ – <i>G. intraradices</i> + <i>P. fluorescens</i>	0 24	0 23	166 7	0 30	0 43	104 8
T ₁₆ – Carbofuran 3G	0 10	0 16	11 1	0 14	0 26	23 8
T ₁₇ – Untreated control	0 09	0 14	–	0 11	0 21	–
T ₁₈ – Uninoculated control	0 28	0 29	211 1	0 46	0 44	109 5
CD (P=0.05)	0.07	0.02		0.03	0.05	

% - Per cent increase over untreated control

Figure 38. Effect of *G.intraradices* and its combinations on phosphorus content of shoot and root of rice cv. IR 50 against the infection of *H.gracilis* at panicle initiation stage (75 DAS) under glasshouse conditions

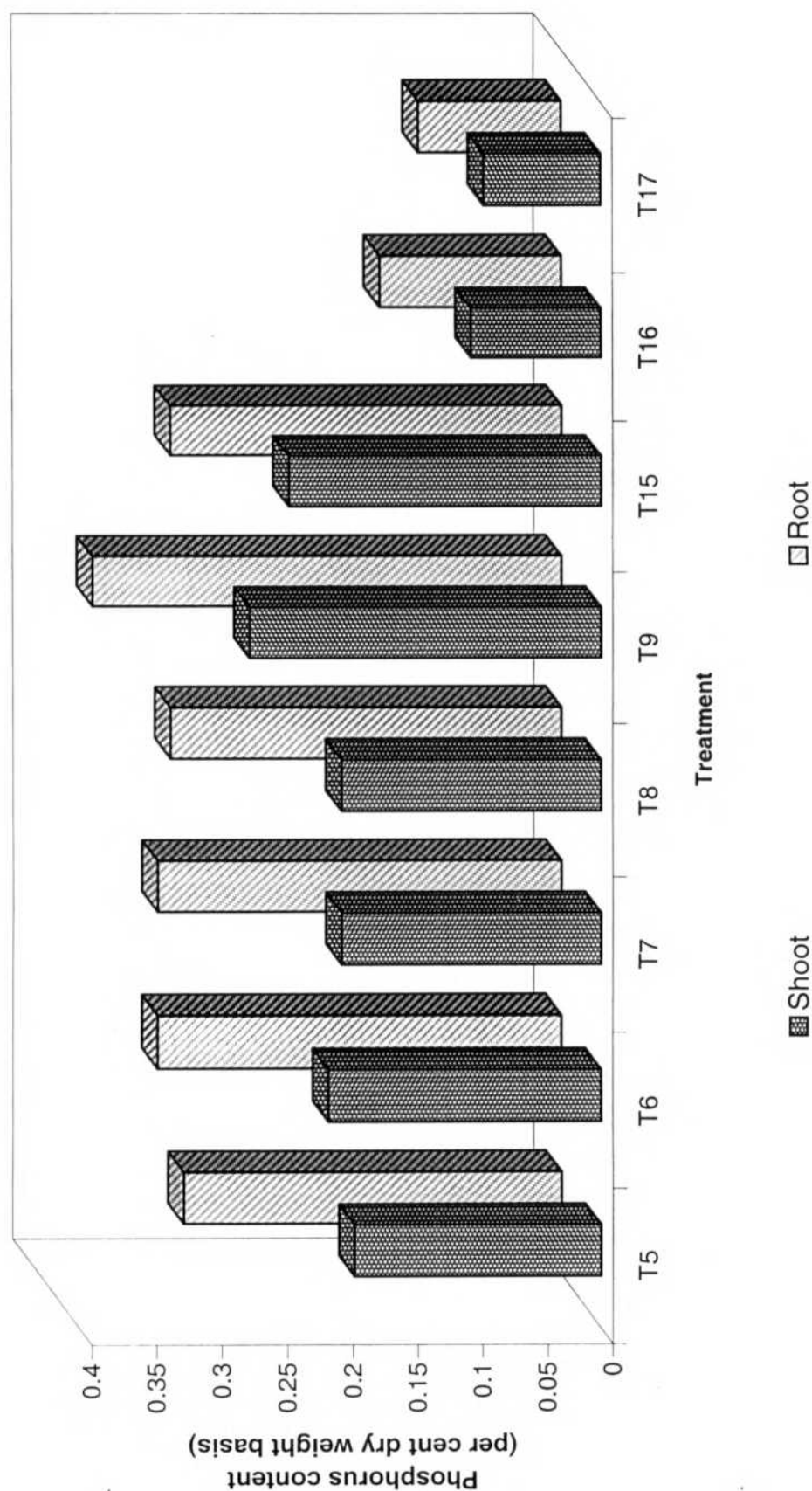
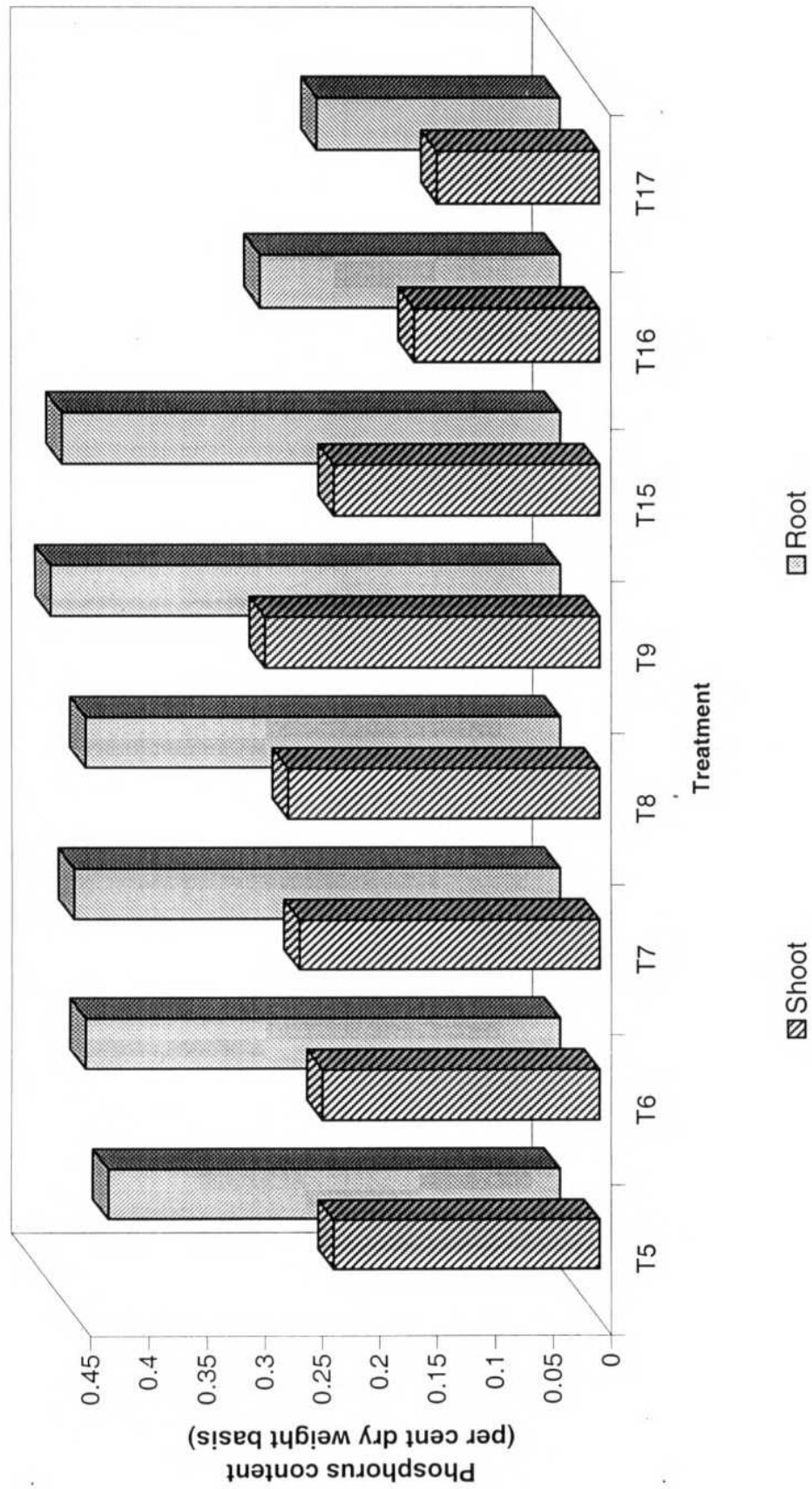


Figure 39. Effect of *G.intraradices* and its combinations on phosphorus content of shoot and root of rice cv. ADT 44 against the infection of *H.gracilis* at panicle initiation stage (120 DAS) under glasshouse conditions



Maximum reduction of shoot phosphorus was observed in untreated control of both the cultivars. In cv. IR 50, shoot phosphorus content (0.09 per cent of dry weight basis) of untreated control was on par with *P. fluorescens* individual and in combinations with *A. microphylla* and blue green algae and individual inoculations of *A. microphylla*, *A. lipoferum* and blue green algae. Untreated control of cv. ADT 44 had 0.14 per cent shoot phosphorus on dry weight basis and was statistically similar to individual treatments of *A. microphylla* and blue green algae.

Uninoculated control had maximum of shoot phosphorus in both the rice cultivars.

Root phosphorus content was recorded maximum in combined treatment of *G. intraradices* with *B. megaterium* in cv. IR 50 (0.36) and cv. ADT 44 (0.44% on dry weight basis). In cv. IR 50, the root phosphorus content of the best treatment was significantly enhanced. *B. megaterium* in combination with *P. fluorescens* was the next best treatment. Root phosphorus content of the best treatment of cv. ADT 44 was statistically similar to individual treatment of *B. megaterium*, individual and combined treatments of *G. intraradices* and *P. fluorescens* combined treatments with *A. lipoferum* and *B. megaterium* (Table 28 and Figure 38 & 39).

Root phosphorus content in both the cultivars was significantly reduced and it was 0.11 and 0.21 per cent on dry weight in cvs. IR 50 and ADT 44 respectively.

In both the cultivars uninoculated control had maximum root phosphorus content.

4.3.3.1.3 Potassium

Maximum enhancement of potassium content in shoot by 43.8 per cent over control was noticed in combined inoculation of *P. fluorescens* with *G. intraradices* in cv. IR 50 and potassium content in shoot (1.26 per cent on dry weight basis) of combined treatment of *P. fluorescens* with *A. lipoferum* was maximum in cv. ADT 44. All the

Table 29. Effect of microbial inoculants on potassium content of shoot and root of cvs. IR 50 and ADT 44 against the infection of *H. gracilis* at panicle initiation stage under glasshouse conditions

Treatment	(Mean of two replications)					
	Shoot (per cent dry weight basis)			Root (per cent dry weight basis)		
	IR 50 (75 DAS)	ADT 44 (120 DAS)	%	IR 50 (75 DAS)	ADT 44 (120 DAS)	%
T ₁ - <i>Azolla microphylla</i>	0.81	1.02	10.0	0.72	0.84	2.4
T ₂ - <i>Azospirillum lipoferum</i>	0.85	1.07	16.4	0.75	0.86	4.9
T ₃ - Blue green algae	0.84	1.06	15.1	0.71	0.84	2.4
T ₄ - <i>Bacillus megaterium</i>	0.82	1.08	12.3	0.70	0.85	3.7
T ₅ - <i>Glomus intraradices</i>	0.91	1.10	24.7	0.76	0.89	8.5
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	0.94	1.14	28.8	0.81	0.91	11.0
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	0.93	1.19	27.4	0.83	0.93	13.4
T ₈ - Blue green algae + <i>G. intraradices</i>	0.94	1.20	28.8	0.80	0.92	12.2
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	0.93	1.19	27.4	0.82	0.93	13.4
T ₁₀ - <i>Pseudomonas fluorescens</i>	1.01	1.21	38.4	0.84	0.94	14.6
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	1.02	1.23	39.7	0.87	0.95	15.9
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	1.04	1.26	42.5	0.88	0.95	15.9
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	1.03	1.22	41.1	0.84	0.94	14.6
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	1.03	1.22	41.1	0.85	0.94	14.6
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	1.05	1.24	43.8	0.89	0.96	17.1
T ₁₆ - Carbofuran 3G	0.83	0.99	13.7	0.73	0.84	2.4
T ₁₇ - Untreated control	0.73	0.89	-	0.68	0.82	-
T ₁₈ - Uninoculated control	1.09	1.28	49.3	0.88	0.93	13.4
CD (P=0.05)	0.03	0.09		0.04	0.04	

% - Per cent increase over untreated control

Figure 40. Effect of *P.fluorescens* and its combinations on potassium content of shoot and root of rice cv. IR 50 against the infection of *H.gracilis* at panicle initiation stage (75 DAS) under glasshouse conditions

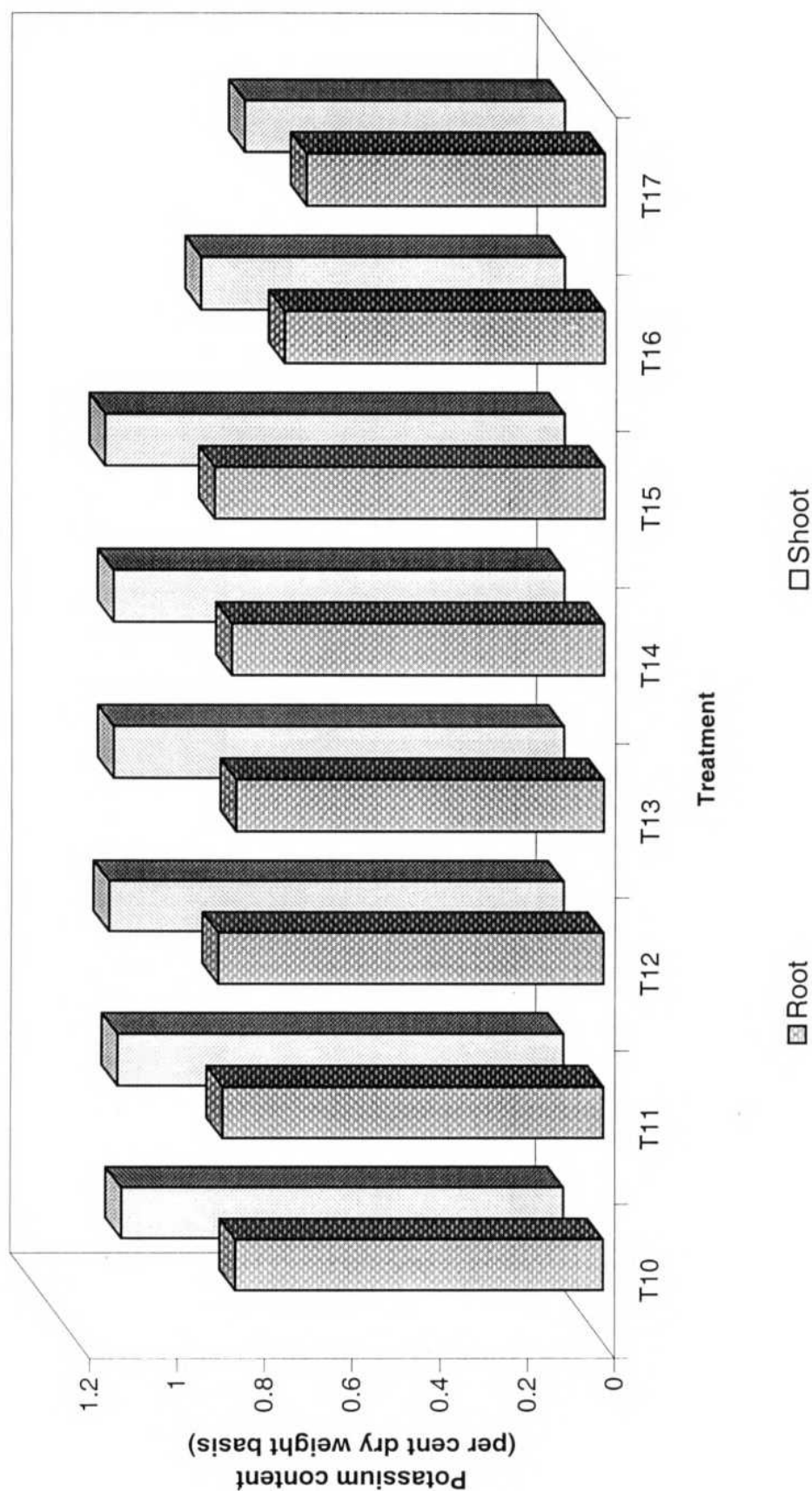
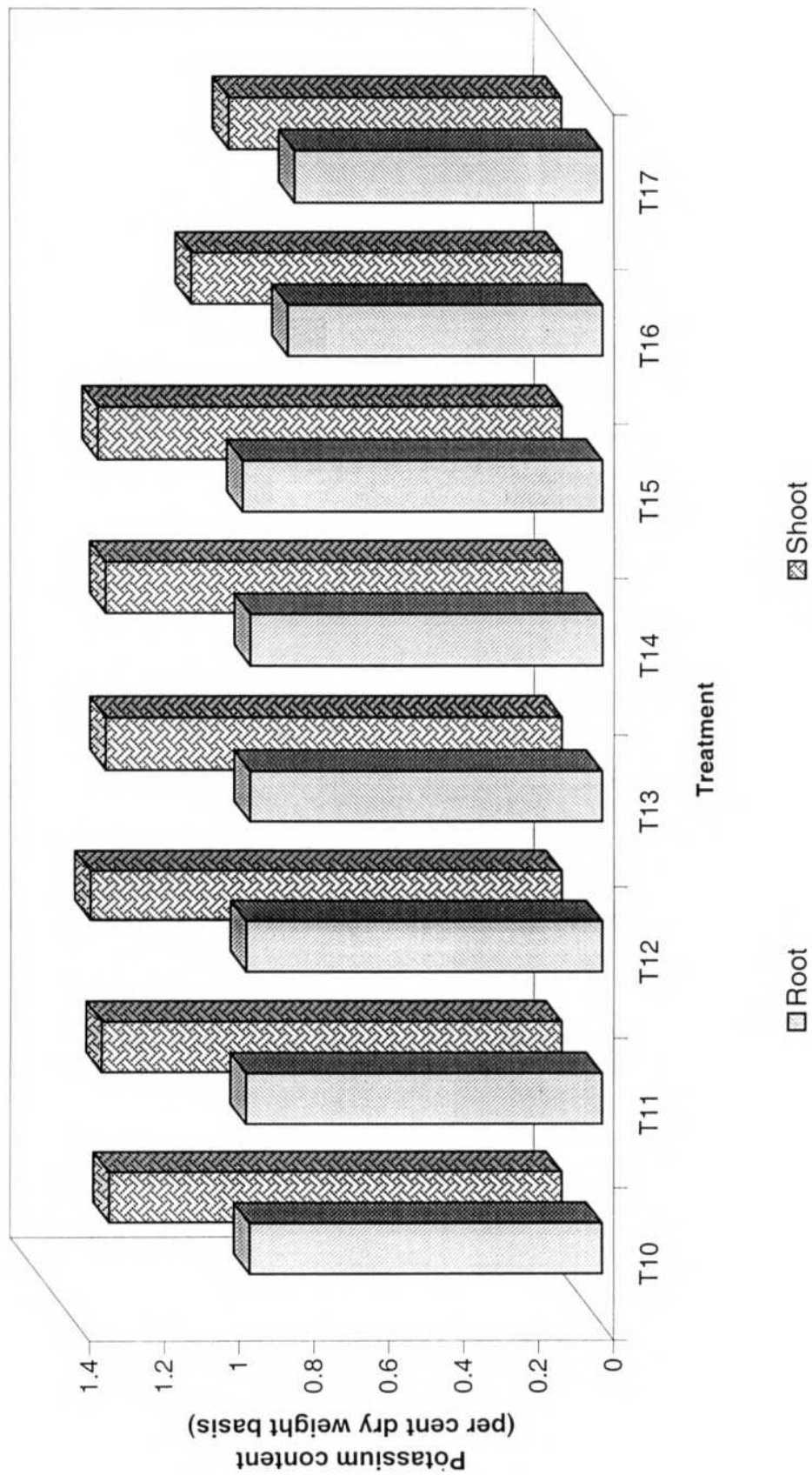


Figure 41. Effect of *P.fluorescens* and its combinations on potassium content of shoot and root of rice cv. ADT 44 against the infection of *H.gracilis* at panicle initiation stage (120 DAS) under glasshouse conditions



combined treatments of *P. fluorescens* were on par with regard to potassium content of shoot in cv. IR 50. In cv. ADT 44, shoot potassium content of the best treatment was statistically similar to individual and combined treatments of *P. fluorescens* and combined inoculations of *G. intraradices* except with *A. microphylla* (Table 29 and Figure 40 & 41)

Untreated control recorded 0.73 and 0.89 per cent dry weight of shoot potassium and was significantly reduced.

Uninoculated control recorded maximum of shoot potassium in both the rice cultivars

Root potassium content of dual inoculation of *P. fluorescens* with *G. intraradices* was maximum in cvs IR 50 and ADT 44 recording 0.89 and 0.96 per cent on dry weight basis. In cv IR 50 this was statistically similar to *P. fluorescens* combined treatments except with blue green algae, whereas in cv ADT 44 this was on par with *P. fluorescens* individual and combined inoculations of *P. fluorescens* and *G. intraradices* except co-inoculation of *G. intraradices* with *A. microphylla* (Table 29 and Figure 40 & 41)

Maximum reduction in root potassium of untreated control was recorded in both the varieties. Untreated control of cv IR 50 had 0.68 per cent of root potassium on dry weight and it was statistically similar to individual treatments of *A. microphylla*, blue green algae and *B. megaterium*. Root potassium content (0.82% on dry weight basis) of untreated control of cv ADT 44 was on par with individual treatments except *P. fluorescens* and *G. intraradices*.

4.3.3.2 Micronutrients

4.3.3.2.1 Iron

Maximum enhancement of shoot iron content of cvs. IR 50 (139.7) and ADT 44 (159.4% over control) was recorded in combined treatment of *P. fluorescens* with *G. intraradices*. Shoot iron content of this treatment in cv IR 50 was significantly

Table 30. Effect of microbial inoculants on iron content of shoot and root of cvs. IR 50 and ADT 44 against the infection of *H. gracilis* at panicle initiation stage under glasshouse conditions

Treatment	(Mean of two replications)					
	Shoot (ppm dry weight basis)			Root (ppm dry weight basis)		
	IR 50 (75 DAS)	ADT 44 (120 DAS)	%	IR 50 (75 DAS)	ADT 44 (120 DAS)	%
T ₁ - <i>Azolla microphylla</i>	93.7	148.3	37.2	135.2	156.9	74.7
T ₂ - <i>Azospirillum lipoferum</i>	98.2	178.6	43.8	142.4	184.3	105.2
T ₃ - Blue green algae	92.5	146.1	35.4	136.7	151.2	68.4
T ₄ - <i>Bacillus megaterium</i>	91.9	143.9	34.6	132.9	149.8	66.8
T ₅ - <i>Glomus intraradices</i>	94.3	154.5	38.1	140.5	158.7	76.7
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	98.3	192.7	43.9	144.3	195.4	117.6
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	114.7	194.4	67.9	159.6	198.1	120.6
T ₈ - Blue green algae + <i>G. intraradices</i>	99.2	189.6	45.2	141.2	193.2	115.1
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	95.4	187.3	39.7	133.5	192.8	114.7
T ₁₀ - <i>Pseudomonas fluorescens</i>	124.0	198.5	81.6	168.7	201.8	124.7
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	148.3	204.6	117.1	173.3	207.3	130.8
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	153.1	209.3	124.2	181.5	211.7	135.7
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	135.3	202.4	98.1	172.1	202.8	125.8
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	131.7	201.3	92.8	170.5	202.6	125.6
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	163.7	217.1	139.7	182.9	232.4	158.8
T ₁₆ - Carbofuran 3G	88.3	121.3	29.3	92.8	125.4	39.6
T ₁₇ - Untreated control	68.3	83.7	-	71.4	89.8	-
T ₁₈ - Uninoculated control	89.8	119.7	31.5	105.3	127.3	41.8
CD (P=0.05)	6.6	8.6		7.1	11.7	

% - Per cent increase over untreated control

enhanced, whereas in cv. ADT 44 this was on par with combined treatment of *P. fluorescens* with *A. lipoferum*. Dual inoculation of *P. fluorescens* with *A. lipoferum* was next best in cv. IR 50 (Table 30 and Figure 42 & 43)

Significantly reduced quantities of iron in shoot was recorded in untreated control of cv. IR 50 (68.3) and cv. ADT 44 (83.7 ppm on dry weight basis)

Iron content in roots were found maximum in cv. IR 50 (182.9) and cv. ADT 44 (232.4 ppm on dry weight basis) in combined treatment of *P. fluorescens* with *G. intraradices*. In cv. IR 50, this treatment was statistically similar to *P. fluorescens* combined inoculation with *A. lipoferum*, and in cv. ADT 44 it was significantly enhanced and dual inoculation of *P. fluorescens* with *A. lipoferum* was the next best (Table 30 and Figure 42 & 43)

Untreated control of both the varieties had significantly minimum root iron content (71.4 and 89.8 ppm on dry weight basis)

4.3.3.2.2 Copper

Both shoot and root copper contents of the two rice varieties were maximum in combined treatment of *P. fluorescens* with *G. intraradices*.

Copper content of shoot of the treatment was recorded as 7.9 and 18.8 ppm on dry weight basis in cvs. IR 50 and ADT 44 respectively. This treatment was on par with *P. fluorescens* combined inoculations with *A. lipoferum* and *B. megaterium* in cv. IR 50, whereas in cv. ADT 44 it was statistically similar to co-inoculation of *P. fluorescens* with *A. lipoferum* (Table 31 and Figure 42 & 43)

Untreated control had significantly reduced quantities of copper in shoots of cv. IR 50 (4.1) and cv. ADT 44 (9.4 ppm on dry weight basis)

Table 31. Effect of microbial inoculants on copper content of shoot and root of cvs. IR 50 and ADT 44 against the infection of *H. gracilis* at panicle initiation stage under glasshouse conditions

Treatment	(Mean of two replications)					
	Shoot (ppm dry weight basis)			Root (ppm dry weight basis)		
	IR 50 (75 DAS)	ADT 44 (120 DAS)	%	IR 50 (75 DAS)	ADT 44 (120 DAS)	%
T ₁ - <i>Azolla microphylla</i>	4.9	13.6	19.5	9.9	37.5	19.7
T ₂ - <i>Azospirillum lipoferum</i>	5.3	14.5	29.3	9.8	36.1	20.4
T ₃ - Blue green algae	5.6	12.7	36.6	9.8	36.1	18.8
T ₄ - <i>Bacillus megaterium</i>	5.8	12.9	41.5	10.2	41.7	20.2
T ₅ - <i>Glomus intraradices</i>	5.9	15.2	43.9	10.1	40.3	21.8
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	6.1	16.2	48.8	10.8	50.0	23.9
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	6.6	18.0	61.0	11.9	65.3	33.4
T ₈ - Blue green algae + <i>G. intraradices</i>	6.3	16.8	53.7	10.5	45.8	22.0
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	6.4	17.4	56.1	11.8	63.9	24.7
T ₁₀ - <i>Pseudomonas fluorescens</i>	6.4	17.7	56.1	10.2	41.7	24.2
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	6.9	18.0	68.3	10.9	51.4	28.7
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	7.4	18.2	80.5	11.8	63.9	33.4
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	6.8	17.6	65.9	11.0	52.8	27.3
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	7.6	17.9	85.4	11.9	65.3	33.2
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	7.9	18.8	92.7	12.3	70.8	33.7
T ₁₆ - Carbofuran 3G	4.5	11.8	9.8	9.7	34.7	18.6
T ₁₇ - Untreated control	4.1	9.4	-	7.2	-	17.9
T ₁₈ - Uninoculated control	5.2	12.1	26.8	9.6	33.3	28.6
CD (P=0.05)	0.5	0.5		0.7	0.5	

% - Per cent increase over untreated control

Maximum enhancement of 70.8 and 88.3 per cent over control was recorded in combined inoculation of *G. intraradices* with *P. fluorescens* in cvs IR 50 and ADT 44 respectively. In cv IR 50, this treatment was statistically similar to both *P. fluorescens* and *G. intraradices* combined treatments with *A. lipoferum* and *B. megaterium*. Combined inoculations of *P. fluorescens* with *A. lipoferum* and *B. megaterium*, and dual inoculation of *G. intraradices* with *A. lipoferum* were on par with the treatment having maximum copper contents in roots of cv ADT 44 (Table 31 and Figure 42 & 43).

Significantly reduced quantities of copper were recorded in the roots of untreated control of cv IR 50 (7.2) and cv ADT 44 (17.9 ppm on dry weight).

4.3.3.2.3 Zinc

Combined treatment of *P. fluorescens* and *G. intraradices* registered maximum increase in zinc contents in shoot and root of both the rice cultivars.

In cv IR 50, the shoot of the best treatment had 39.7 ppm of zinc on dry weight basis and it was on par with *P. fluorescens* combined inoculation with *A. lipoferum* and combined treatments of *G. intraradices* with *A. lipoferum* and *B. megaterium*. The best treatment had 62.3 ppm shoot zinc content on dry weight basis in cv ADT 44 and was statistically similar to co-inoculation of *P. fluorescens* with *A. lipoferum* (Table 32 and Figure 42 & 43).

Untreated control of both the varieties had significantly reduced shoot zinc contents recording 28.9 and 40.5 ppm on dry weight in cvs IR 50 and ADT 44 respectively.

Zinc contents of root were enhanced to maximum by 24.5 and 57.9 per cent over untreated control in cv IR 50 and cv ADT 44 respectively. This was on par with *P. fluorescens* combined with *A. lipoferum* and *B. megaterium* and *G. intraradices* combined with *A. lipoferum* in cv IR 50, whereas in cv ADT 44 the root copper contents

Table 32. Effect of microbial inoculants on zinc content of shoot and root of cvs. IR 50 and ADT 44 against the infection of *H. gracilis* at panicle initiation stage under glasshouse conditions

Treatment	Shoot (ppm dry weight basis)				Root (ppm dry weight basis)			
	IR 50 (75 DAS)		ADT 44 (120 DAS)		IR 50 (75 DAS)		ADT 44 (120 DAS)	
	%		%		%		%	
T ₁ - <i>Azolla microphylla</i>	36.9	27.7	41.8	3.2	25.7	1.6	30.8	10.8
T ₂ - <i>Azospirillum lipoferum</i>	37.9	31.1	50.2	24.0	26.9	6.3	34.3	23.4
T ₃ - Blue green algae	36.9	27.7	49.0	21.0	26.5	4.7	32.1	15.5
T ₄ - <i>Bacillus megaterium</i>	37.0	28.0	48.8	20.5	26.8	5.9	31.7	14.0
T ₅ - <i>Glomus intraradices</i>	37.2	28.7	49.9	23.2	28.4	12.3	35.8	28.8
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	37.4	29.4	50.2	24.0	29.2	15.4	36.2	30.2
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	39.4	36.3	53.7	32.6	31.2	23.3	43.3	55.8
T ₈ - Blue green algae + <i>G. intraradices</i>	38.4	32.9	51.9	28.1	30.0	18.6	38.8	39.6
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	39.6	37.0	50.5	24.7	29.7	17.4	37.4	34.5
T ₁₀ - <i>Pseudomonas fluorescens</i>	38.2	32.2	52.1	28.6	29.1	15.0	38.5	38.5
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	38.4	32.9	54.2	33.8	29.4	16.2	39.7	42.8
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	39.6	37.0	61.2	51.1	31.4	24.1	43.5	56.5
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	38.8	34.3	58.8	45.2	30.6	20.9	41.8	50.4
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	38.6	33.6	57.5	42.0	31.2	23.3	40.4	45.3
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	39.7	37.4	62.3	53.8	31.5	24.5	43.9	57.9
T ₁₆ - Carbofuran 3G	36.5	26.3	42.4	4.7	25.7	1.6	29.9	7.6
T ₁₇ - Untreated control	28.9	-	40.5	-	25.3	-	27.8	-
T ₁₈ - Uninoculated control	26.6	-8.0	38.7	-4.4	25.1	-0.8	27.1	-2.5
CD (P=0.05)	0.4		1.1		0.3		0.6	

% - Per cent increase or decrease (-) over untreated control

of this treatment was statistically similar to combined inoculations of both *P fluorescens* and *G intraradices* with *A lipoferum* (Table 32 and Figure 42 & 43)

Root of untreated control had significantly reduced quantities of zinc in cvs IR 50 (25.3) and ADT 44 (27.8 ppm on dry weight basis)

Uninoculated control recorded minimum zinc contents in shoot and root of both the cultivars

4.3.3.2.4 Manganese

Combined inoculation of *P fluorescens* and *G intraradices* had maximum shoot manganese content of 52.3 and 64.8 ppm on dry weight basis in shoot of cvs IR 50 and ADT 44 respectively. *P fluorescens* and *G intraradices* combined inoculations with *A lipoferum* was statistically similar to the above treatment in cv IR 50. The best treatment was on par with co-inoculation of *P fluorescens* with *A lipoferum* in cv ADT 44 (Table 33 and Figure 42 & 43)

Untreated control registered minimum of 41.7 and 45.1 ppm of shoot manganese contents in cvs IR 50 and ADT 44, respectively and were significantly reduced

Root manganese contents in cvs IR 50 and ADT 44 were enhanced to maximum in dual inoculation of *P fluorescens* with *A lipoferum* by 30.5 and 23.9 per cent over untreated control in cvs IR 50 and ADT 44 respectively. The treatment was on par with combined treatments of both *P fluorescens* and *G intraradices* with *A lipoferum* and *B megaterium* and co-inoculation of *P fluorescens* with *A microphylla* in both the varieties (Table 33 and Figure 42 & 43)

Significantly minimum root manganese contents was recorded in untreated control of cv IR 50 (55.7) and cv ADT 44 (71.4 ppm on dry weight basis)

Table 33. Effect of microbial inoculants on manganese content of shoot and root of cvs. IR 50 and ADT 44 against the infection of *H. gracilis* at panicle initiation stage under glasshouse conditions

Treatment	(Mean of two replications)					
	Shoot (ppm dry weight basis)			Root (ppm dry weight basis)		
	IR 50 (75 DAS)	ADT 44 (120 DAS)	%	IR 50 (75 DAS)	ADT 44 (120 DAS)	%
T ₁ - <i>Azolla microphylla</i>	43.2	51.4	3.6	59.6	78.9	10.5
T ₂ - <i>Azospirillum lipoferum</i>	44.6	55.2	7.0	61.8	80.3	12.5
T ₃ - Blue green algae	43.8	52.9	5.0	61.3	72.7	1.8
T ₄ - <i>Bacillus megaterium</i>	44.1	50.8	5.8	62.4	76.2	6.7
T ₅ - <i>Glomus intraradices</i>	44.3	53.9	6.2	62.1	79.8	11.8
T ₆ - <i>A. microphylla</i> + <i>G. intraradices</i>	46.0	57.6	10.3	62.9	80.6	12.9
T ₇ - <i>A. lipoferum</i> + <i>G. intraradices</i>	51.7	61.4	24.0	70.2	87.3	22.3
T ₈ - Blue green algae + <i>G. intraradices</i>	46.3	58.7	11.0	64.5	80.4	12.6
T ₉ - <i>B. megaterium</i> + <i>G. intraradices</i>	45.4	54.3	8.9	70.1	86.4	21.0
T ₁₀ - <i>Pseudomonas fluorescens</i>	45.9	59.6	10.1	63.7	82.0	14.8
T ₁₁ - <i>A. microphylla</i> + <i>P. fluorescens</i>	48.3	60.2	15.8	70.3	86.8	21.6
T ₁₂ - <i>A. lipoferum</i> + <i>P. fluorescens</i>	51.8	64.2	24.2	72.7	88.5	23.9
T ₁₃ - Blue green algae + <i>P. fluorescens</i>	49.5	61.8	18.7	68.8	83.7	17.2
T ₁₄ - <i>B. megaterium</i> + <i>P. fluorescens</i>	47.6	59.3	14.1	70.4	88.0	23.2
T ₁₅ - <i>G. intraradices</i> + <i>P. fluorescens</i>	52.3	64.8	25.4	72.2	88.2	23.5
T ₁₆ - Carbosufuran 3G	42.2	49.3	1.2	58.2	72.1	1.0
T ₁₇ - Untreated control	41.7	45.1	-	55.7	71.4	-
T ₁₈ - Uninoculated control	60.5	68.7	45.1	80.2	89.6	25.5
CD (P=0.05)	0.6	0.7		2.8	2.2	

% Per cent increase over untreated control

58843

Figure 42. Effect of *P.fluorescens* and its combinations on iron, copper, zinc and manganese contents of shoot and root of rice cv. IR 50 against the infection of *H.gracilis* at panicle initiation stage (75 DAS) under glasshouse conditions

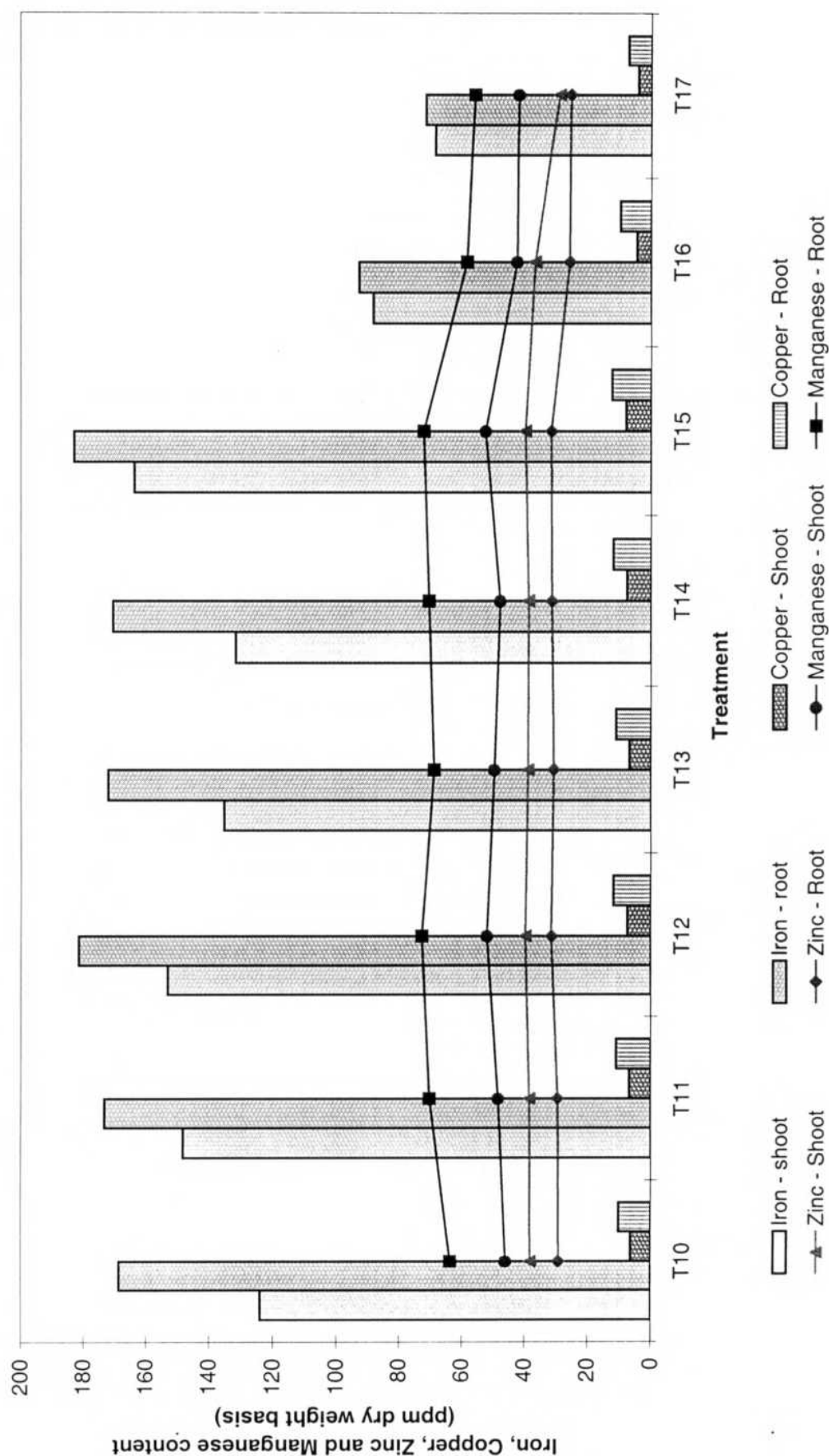
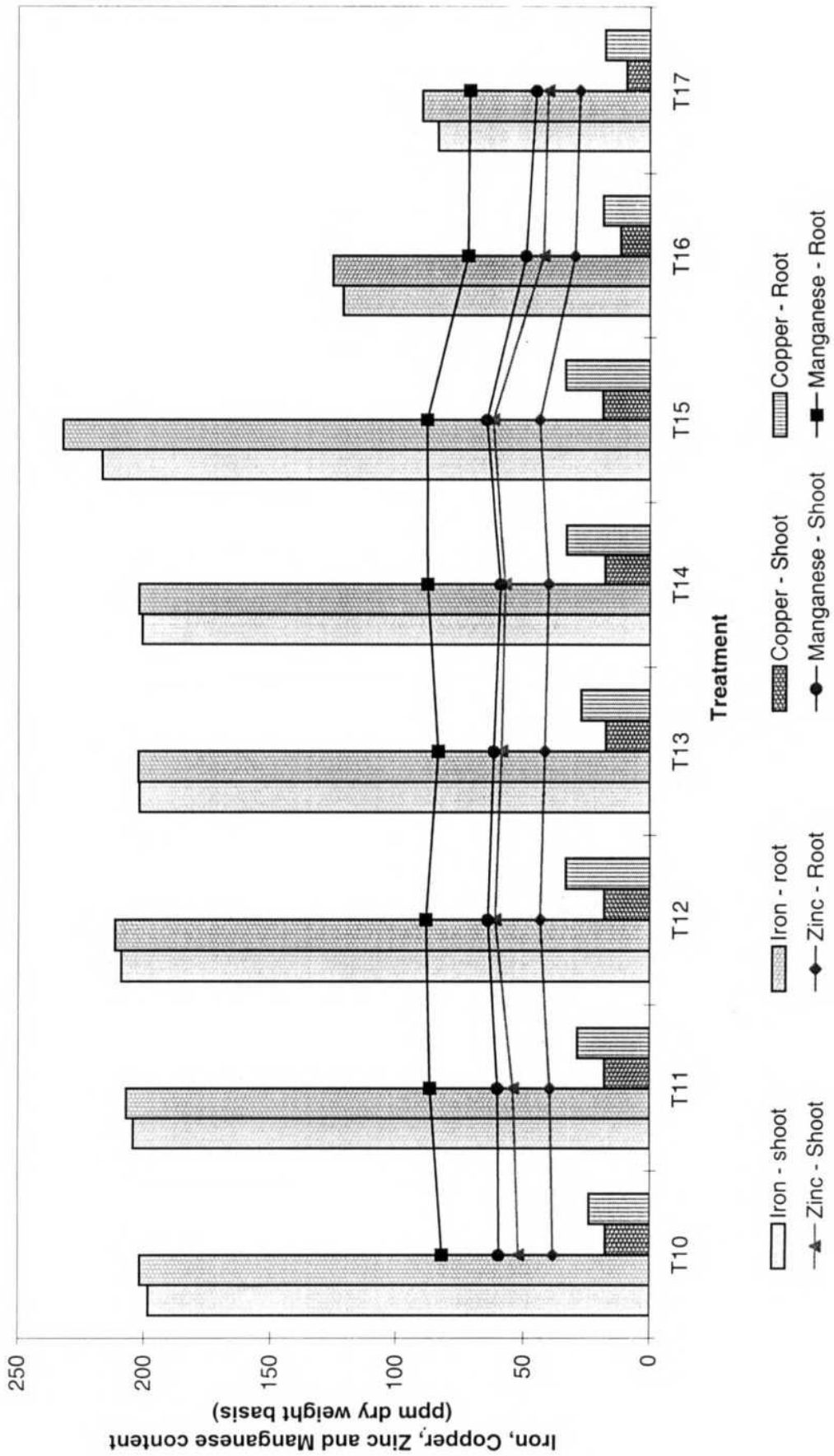


Figure 43. Effect of *P.fluorescens* and its combinations on iron, copper, zinc and manganese contents of shoot and root of rice cv. ADT 44 against the infection of *H.gracilis* at panicle initiation stage (120 DAS) under glasshouse conditions



Maximum manganese content in shoot and root were noticed in uninoculated control of both the rice varieties

4.4 Histopathological changes of rice roots to the infection of *H. gracilis*

Following penetration, the rice-root nematode, *H. gracilis* was observed to feed on cortical parenchymatous cells by lying almost parallel to the long axis of infected rice roots (Plate 14A and 14B) and formed large cavities due to breakdown of cell walls around the nematode as evident from longitudinal root sections

Cross sectional microscopic view of infected roots showed that nematodes were feeding on cortical cells and anatomical changes in the form of damaged cortical cells (Plate 14C, 14D, 14E and 14F) Thickening and dark colouration in the broken cell walls were also observed in damaged cortical cells The nematode was found to be confined to epidermal and cortical regions of infected rice roots, never reaching the central vascular core

**Plate 14. Histopathological studies of the rice-root nematode,
H. gracilis infected rice roots**

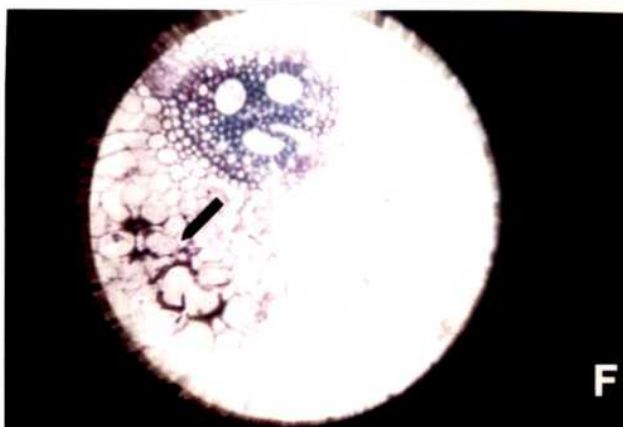
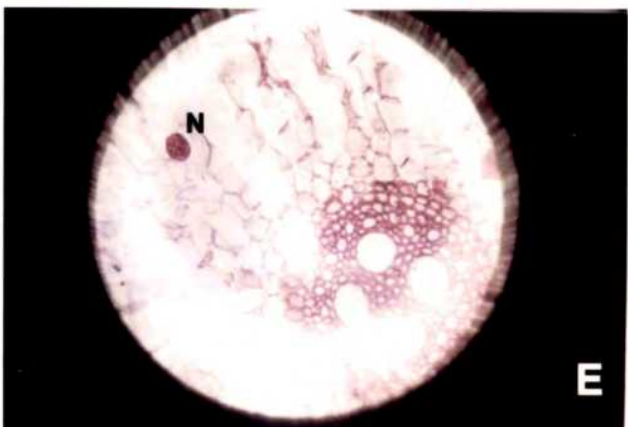
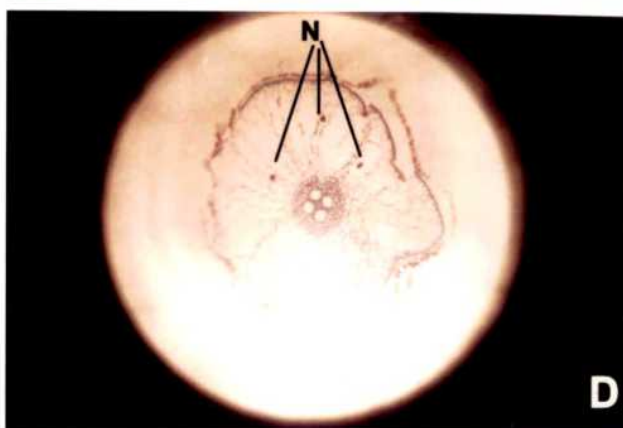
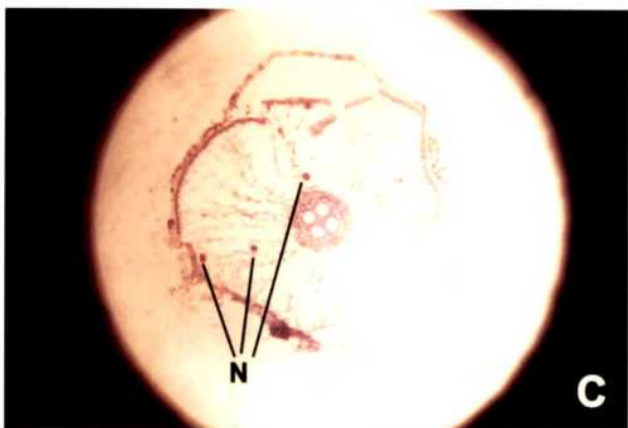
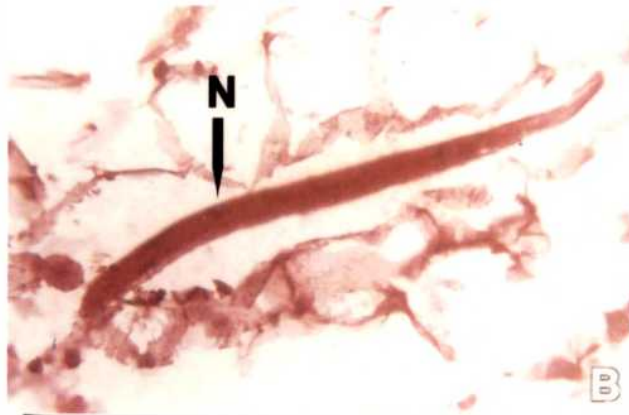
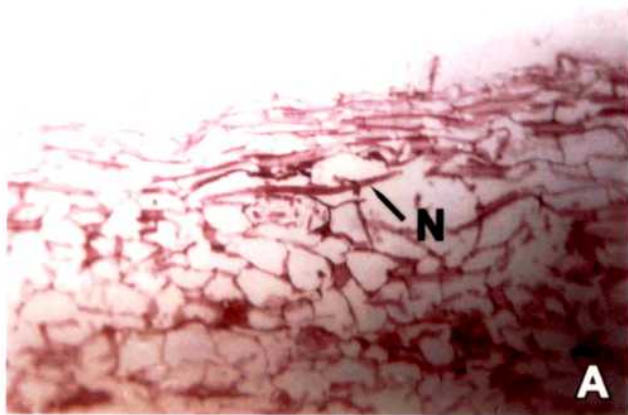
Nematode feeding on cortical cells

A & B. Longitudinal sections

C, D & E. Transverse sections

F. Damaged cortical cells

Plate 14



DISCUSSION

CHAPTER V

DISCUSSION

The present investigation was undertaken with the principal objective of studying efficiencies of popular high yielding rice cultivars and microbial inoculants in the suppression of the rice-root nematode, *Hirschmanniella gracilis*. The results of the above aspects along with the physiological, biochemical and nutritional responses by the microorganism inoculated in the *H. gracilis* infected rice plants and histopathological changes of the nematode infected plant roots are highlighted in this chapter in accordance with the objectives set forth with appropriate and logistic discussion.

5.1 Screening of popular high yielding rice varieties for their reaction to the rice-root nematode, *H. gracilis*

Ying *et al.* (1996) proposed the injury level of *H. oryzae* to rice as 15 nematodes per g root at the end of maximum tillering stage and that could be applied as an economic threshold level. Prasad *et al.* (1987) observed that at low levels of nematode infection (below 50/g root) there was no appreciable reduction in important biochemical components like chlorophyll, starch and proteins in plants indicating no apparent change in metabolic processes. Park *et al.* (1970) rated the varieties, which had more than 1,000 nematodes per 10 g root as susceptible to *Hirschmanniella oryzae*. In the current study it was found that when the nematode population was more than 100 nematodes per g root, the nematode aliquots was dominated by juveniles, and it might be due to high rate of reproduction of *H. gracilis* in the favoured varieties and accompanied by discernible yellowish brown necrotic lesions on the roots (Plate 2B).

Keeping in view the above facts and figures, a simplified and quicker rating index (1 to 5 scale) of rice varieties at maximum tillering stage for resistance against the infection of the rice-root nematode, *Hirschmanniella* spp. is proposed.

Rating index:

Scale	Nematode population per g root	Host reaction
1	0	Immune (I)
2	1 – 10	Highly resistant (HR)
3	11 – 50	Moderately resistant (MR)
4	51 – 100	Susceptible (S)
5	> 100	Highly susceptible (HS)

According to the above mentioned scale TKM 5, TKM 7, TKM 8 and TKM 9 were categorised as moderately resistant among the short duration cultivars screened. The rice varieties ASD 3, CO 41, IR 48, and IR 50 were found to be highly susceptible. The remaining 19 varieties were rated susceptible as the maximum nematode population recorded was between 51 to 100 per g of their roots.

Among the medium duration varieties screened, ASD 8, MDU 4, PTB 12, PTB 15, PTB 23 and PY 1 were categorised as moderately resistant having nematode population between 11 to 50 as per the proposed rating index for the rice-root nematode. Cultivars ASD 1, Bhavani, CO 12, CO 38, CO 42, CO 44, CO 45, CO 46, IR 34, IR 44, IR 62, MDU 2, Ponni, PUSA 205 and White Ponni were observed to be highly susceptible to *H. gracilis* with the maximum population range reaching beyond 100 nematode per g root. The remaining 39 varieties were rated susceptible with the maximum population range of 51 to 100 per g root.

Of the four long duration varieties screened only CR 1009 was observed to be moderately resistant and rest of the three varieties, viz., ADT 40, ADT 44 and CO 40 were rated as susceptible at their maximum tillering stage as per the rating index proposed.

Previously screened variety TKM 6 which was found to be less susceptible to the rice-root nematode, *H. mucronata* (Israel and Rao, 1971), was found to be susceptible in the present rating scale. Cultivars IR 50 and TKM 9 were observed to be susceptible and resistant respectively to the rice-root nematode, *H. oryzae* (Ramakrishnan *et al.*, 1984). In the present rating index, IR 50 was found to be highly susceptible and TKM 9 moderately resistant to the rice-root nematode, *H. gracilis*. Cultivar Paiyur 1 (= PY 1) previously screened as susceptible to *H. oryzae* (Ramakrishnan *et al.*, 1984) was observed as moderately resistant. PTB 12 susceptible to *H. oryzae* (Gokulapalan and Nair, 1983), IR 20 susceptible against *H. oryzae* (Ramakrishnan *et al.*, 1984), Basmati 370 found to have higher population build up of *H. oryzae* (Randhawa *et al.*, 1991; 1992), were rated as susceptible to *H. gracilis* in the present study also.

No rice variety among 91 accessions screened was observed to be either immune or highly resistant to *H. gracilis*. The reasons attributed were less specialised parasitic relationship of the nematode with host crop (Roberts, 1982), and the possible existence of pathotypes of differing virulence within the species of rice-root nematode (Whitehead, 1998).

Maximum varieties, short (70.4 per cent), medium (65.0 per cent) and long (75.0 per cent) were observed to be susceptible to *H. gracilis* infection. This result was in accordance with the findings of Park *et al.* (1970).

5.2 Studies on the management of the rice-root nematode, *H. gracilis* with microbial inoculants

5.2.1 Effect of microbial inoculants on plant growth of short and long duration rice cvs. IR 50 and ADT 44

5.2.1.1 Glasshouse study

All the microbial inoculants were able to enhance both above and below ground plant growth parameters of cvs. IR 50 and ADT 44 against the infection of *H. gracilis* (1 nematode per g soil) at panicle initiation stage. The combined treatments were more effective than individual applications. Overall plant growth was better in *P. fluorescens* combinations.

Combined inoculation of *P. fluorescens* with *G. intraradices* was observed to be most efficient treatment to increase shoot and root length, fresh and dry weight of shoot and root and root volume of cv. IR 50, and all the below ground plant growth parameters of cv. ADT 44. Dual inoculation of *P. fluorescens* with *A. lipoferum* was the most effective treatment in increasing total leaf area and number of tillers of cv. IR 50, and all the above ground plant growth parameters except the shoot length in cv. ADT 44, for which combined treatment of *G. intraradices* with *A. microphylla* was the most effective.

The treatments comprising of *P. fluorescens* in combinations with *G. intraradices* and *A. lipoferum* were the most efficient and equally effective in improving overall plant growth of the *H. gracilis* infected rice varieties viz., IR 50 and ADT 44 under glasshouse conditions at the population level of 1000 nematodes per kg soil.

5.2.1.2 Field study

5.2.1.2.1 Seedling stage

Combined inoculation of *P. fluorescens* with *G. intraradices* was most effective to increase all the plant growth parameters of cv. IR 50, and total leaf area and root length

of cv. ADT 44. Dual inoculation of *P. fluorescens* with *A. lipoferum* was the most efficient treatment with respect to enhancement of shoot length, fresh and dry weight of shoot and root, and root volume of cv. ADT 44.

Both the above mentioned treatments were found to be equally effective in the enhancement of majority of the plant growth parameters of the cultivars.

5.2.1.2.2 Panicle initiation stage

Combined inoculation of *P. fluorescens* with *G. intraradices* was the most effective treatment to increase shoot and root length of cv. IR 50, and all the plant growth parameters except shoot length and root volume of cv. ADT 44. Dual inoculation of *P. fluorescens* with *A. lipoferum* was most effective in enhancement of fresh and dry weight of shoot and root, leaf area index, number of tillers and root volume per hill of cv. IR 50, and root volume per hill of cv. ADT 44. *P. fluorescens* in combination with *A. microphylla* was most efficient in increasing the shoot length of cv. ADT 44.

P. fluorescens combined treatments with *G. intraradices* and *A. lipoferum* were most efficient and equally effective in enhancing the overall growth of both the rice cultivars.

5.2.2 Effect of microbial inoculants on yield attributes and grain and straw yield of short and long duration rice cvs. IR 50 and ADT 44

All the microbial inoculant treatments were capable of enhancing yield attributes of both the varieties at maturity stage and combined treatments were more effective. In general, among the combined treatments *P. fluorescens* combinations were comparatively more efficient.

As the grain and straw yield are directly dependent on the yield attributes and plant growth parameters, thus they followed a similar pattern.

P. fluorescens combined inoculation with *G. intraradices* was the most effective treatment in enhancing the yield attributes such as number of panicles per hill, number of filled grains per panicle and thousand grain weight of cv. ADT 44. Combined inoculation of *P. fluorescens* with *A. lipoferum* was the most desired treatment for increased number of panicles per hill and number of filled grains per panicle of cv. IR 50, and panicle length of cv. ADT 44.

Both the treatments comprising of *P. fluorescens* with *G. intraradices* and *A. lipoferum* were the most favoured treatments in increasing panicle length and thousand grain weight of cv. IR 50, and were equally effective in enhancing all the yield attributes of both the varieties.

P. fluorescens in combinations with *G. intraradices* and *A. lipoferum* were equally the most efficient treatments to increase grain yield of cv. ADT 44. Combined treatment of *P. fluorescens* with *G. intraradices* was most effective to increase the straw yield of cv. ADT 44. Combined inoculation of *P. fluorescens* with *A. lipoferum* was the most efficient treatment in the enhancement of grain and straw yield of cv. IR 50.

P. fluorescens combined inoculations with *G. intraradices* and *A. lipoferum* were equally effective in increasing the grain and straw yield of both the cvs. IR 50 and ADT 44.

In agreement to the results of the present study, combined inoculation of *P. fluorescens* and VAM fungi have been reported to increase plant growth and / or yield of several crops like banana (Alonso Reyes *et al.*, 1995), barley (Fester *et al.*, 1998), beans and cotton (Burla *et al.*, 1996), carrot (Bianciotto *et al.*, 2001), chickpea (Siddiqui and Irshad Mahmood, 2001), coffee (Rivera *et al.*, 1997), cucumber (Ravnskov and Jackobsen, 1999; Ravnskov *et al.*, 1999), grapevine (Bavaresco and Fogher, 1992; 1996a;

1996b; Bavaresco *et al.*, 1995), leek (Edwards *et al.*, 1998), maize (Attia, 1999), pepper (Marschner and Crowley, 1996a; 1996b; Marschner *et al.*, 1997), pigeonpea (Siddiqui *et al.*, 1998), potato (Klyuchnikov and Kozhevin, 1990), radish (Shabaev *et al.*, 1998a), raspberry (Lemoine *et al.*, 2000), soyabean (Shabaev *et al.*, 1992; 1996; Shabaev and Smolin, 1992; Shabaev *et al.*, 1998b), tomato (Edwards *et al.*, 1998) and wheat (Walley and Germida, 1997; Fester *et al.*, 1998). *P. fluorescens* and *G. intraradices* when applied in combination improved the growth of tomato plants (Nemec, 1997).

Both *P. fluorescens* and vesicular arbuscular mycorrhizal fungi were bioenhancers. Simultaneous inoculation by both of them had been observed to be mutualistic. *P. fluorescens* markedly stimulated fungal root colonization of VAM fungi and acted as mycorrhizal helper bacteria (MHB) through blumenin accumulation (Fester *et al.*, 1998). The plant growth promoting rhizobacterium presented in soil or added with vesicular-arbuscular mycorrhiza (VAM fungi) either to soil or as seed treatment improved mycorrhizal colonization of roots (Meyer and Linderman, 1986; Paulitz and Linderman, 1989; Staley *et al.*, 1992; Nemec, 1997). *P. fluorescens* was also observed to enhance hyphal length density (Ravnskov and Jakobsen, 1999). Bacterial cultures of *P. fluorescens* stimulated greater spore numbers of VAM fungi and their germination, more active growth resulted in higher viability and colonization of mycelium both *in vitro* and *in vivo* (Klyuchnikov and Geltser, 1990; Burla *et al.*, 1996; Marschner and Crowley, 1996b; Attia, 1999), and improved establishment and mycorrhizal growth parameters of VAM fungi (Bavaresco and Fogher, 1996c; Parveen Kumar and Bagyaraj, 1998). On the other hand VAM fungi enhanced the development of *P. fluorescens* population on the surface of extra radicular mycelium (Klyuchnikov and Kozhevin, 1990) and improved physiological status of *P. fluorescens* during establishment phase as measured by lag phase of bioluminescens that led to better establishment of the rhizobacterium (Marschner *et al.*, 1997). VAM fungus enhanced propagules and their

survival of *P. fluorescens* by increasing root surface area (Nemec, 1997) and enhanced population of *P. fluorescens* in the rhizospheres (Edwards *et al.*, 1998). Acidic extracellular polysaccharides released by *P. fluorescens* played an important role in the formation of bacterial biolayers, thus anchoring the rhizobacterium on mycorrhizal roots as well as on extra radical mycelium of the VAM fungi forming a dense and patchy bacterial layer on the roots and fungal structures. Increased adhesive properties led to stable interactions between rhizobacterium and VAM fungi (Bianciotto *et al.*, 2001). There observed to be no spatial competition between *P. fluorescens* and VAM fungi as *P. fluorescens* occupied exorhizospheres and the VAM fungi endorhizospheres (Klyuchnikov and Kozhevin, 1990). Both mycorrhizal colonization and bacterial sporulation were enhanced in rhodes grass, when combinedly inoculated with *P. fluorescens* and VAM fungus *G. mosseae*, resulted in improvement in plant biomass (Parveen Kumar and Bagyaraj, 1998).

Probable reasons cited for improvement in host plant growth and yield of crops upon combined treatment with *P. fluorescens* and VAM fungi were, increase in mineral nutrient concentrations of both plant and rhizosphere particularly 'N', 'P' and ' Fe^{+2} ' (Bavaresco and Fogher, 1992; Shabaev and Smolin, 1992; Smolin and Shabaev, 1992; Bavaresco *et al.*, 1995; Alonso Reyes *et al.*, 1995; Bavaresco and Fogher, 1996a; 1996b; Edwards *et al.*, 1998; Shabaev *et al.*, 1998a; Ravnskov and Jackobsen, 1999), improvement in physiological functions such as nitrogen fixation, photosynthetic rate and CO_2 gas exchange parameters (Shabaev *et al.*, 1992; 1996; 1998b), enhanced levels of plant growth promoting substances (Shabaev *et al.*, 1996), increased mineral fertilizer use efficiency by intake of applied fertilizer nutrients from soil (Shabaev and Smolin, 1992; Smolin and Shabaev, 1992; Attia, 1999), enhanced leaf chlorophyll and protein contents (Bavaresco *et al.*, 1995; Bavaresco and Fogher, 1996a; 1996b, Attia, 1999) and reduced transient transfer shock (Varma and Schiepp, 1994).

Both *P. fluorescens* and *Azospirillum* are gram negative rhizobacteria and are capable of improving plant growth and yield. The combined inoculation of *P. fluorescens* and *Azospirillum* enhanced growth and/or yield of chickpea (Vidyasekharan and Muthamilan, 1995; Siddiqui and Irshad Mahmood, 2001), gerbera and pink carnation (Hernandez *et al.*, 2000), pea (Beshir and El Ghany, 1997; Deubel *et al.*, 2000), soyabean (Amara and Nasr, 1995), tomato (Sanhita *et al.*, 1995) and wheat (Christiansen Weniger and Veen, 1991; Christiansen Weniger, 1992; Bashan and Gonzalez, 1999).

A number of authors have reported the mutualistic relationship between *P. fluorescens* and *Azospirillum* in the rhizosphere of crop plants. Both NO_3^- and NH_4^+ repressed nitrogenase activity and N_2 fixation of *Azospirillum* that helped in improvement of survival and density of *P. fluorescens* in the rhizosphere (Christiansen Weniger and Veen, 1991; Amara and Nasr, 1995); phosphate dissolution by *P. fluorescens* helped in root growth and hence, improvement in *Azospirillum* root colonization (Amara and Nasr, 1995). There observed to be no spatial competition between the rhizobacteria as active population of *P. fluorescens* were found in exorhizospheres and development of *Azospirillum* occurred in the rhizoplane and endorhizospheres (Klyuchnikov and Kozhevin, 1990). Enhancement of root growth by the rhizobacteria in turn helped in their colonization along with the elongating root with no interference for nitrogen fixation, auxin production and nitrate reduction among themselves (Christiansen Weniger, 1992). Both the rhizobacteria had similar optimum preferences for temperature, pH, oxygen supply, carbon and nitrogen sources and were able to control plant diseases within that optimum range (Cho and Chung, 1997). Both were proved mutualistic even in alginate-bead up to fourteen years at ambient temperature and retained their original physiological features (Bashan and Gonzalez, 1999). Acidification of rhizospheres by both the rhizobacteria helped in total bacterial counts of *P. fluorescens* and *Azospirillum* (Beshir and El Ghany, 1997) and both of them had differential preferences to different forms of

sugars for their nourishment as sugars of lower molecular weight such as pentoses were preferred by *P. fluorescens* (Deubel *et al.*, 2000).

The enhancement of plant growth and yield were the results of nitrogen fixation and nitrate reduction, nitrogenase activity of *Azospirillum* (Christiansen Weniger and Veen, 1991; Christiansen Weniger, 1992), auxin production by both the rhizobacteria (Christiansen Weniger, 1992), increased seedling emergence rate and enhanced release of ¹⁴C-labelled endogenous carbonaceous compounds in plants (Sanhita *et al.*, 1995), increase in nutritional status of host plants (Amara and Nasr, 1995), increased seedling survival, nodulation and protein percentage (Beshir and El Ghany, 1997) and shifting of sugar patterns by dissolution of tertiary calcium phosphate to lower pentoses along with low-molecular mono-, di- and tricarboxylic acids to solubilize 'P' in rhizospheres to enhance 'P' content of host plants (Deubel *et al.*, 2000).

5.2.3 Effect of microbial inoculants on nematode population in root and soil of short and long duration rice cvs. IR 50 and ADT 44

Similar trend as in the case of plant growth parameters, yield attributes, grain and straw yield was observed in the reduction of *H. gracilis* population in root and soil.

5.2.3.1 Glasshouse study

Combined inoculation of *P. fluorescens* with *G. intraradices* was the most effective treatment in reducing both root and soil population of *H. gracilis* in cv. IR 50, and root nematode population in cv. ADT 44. *P. fluorescens* in combination with *A. lipoferum* was most effective in suppression of the nematode population in soil of cv. ADT 44. Both the treatments were equally effective in the reduction of *H. gracilis* population in root and soil of both the rice varieties.

5.2.3.2 Field study

5.2.3.2.1 Seedling stage

Pooled data on two season trials of cv. IR 50 revealed that combined inoculation of *P. fluorescens* with *G. intraradices* was the most effective treatment in the suppression of both root and soil population of *H. gracilis* in the nursery beds in both summer and *kharif* season, and root population of cv. ADT 44 during summer season. Dual inoculation of *P. fluorescens* with *A. lipoferum* was the most efficient treatment in reducing the soil population of the nematode in cv. ADT 44. Both the treatments were equally capable of reducing the root and soil population of both the cultivars.

5.2.3.2.2 Panicle initiation stage

Combined treatment of *P. fluorescens* with *G. intraradices* was most effective in the suppression of root population of *H. gracilis* of cv. ADT 44.

Dual inoculation of *P. fluorescens* with *A. lipoferum* was the most potential treatment in restricting both root and soil population of *H. gracilis* in summer and *kharif* season as ascertained from pooled data of cv. IR 50, and also for soil population of *H. gracilis* in cv. ADT 44. Both the treatments were equally efficient in the reduction of root and soil population of *H. gracilis* in both the cultivars.

5.2.3.2.3 At harvest

In cv. ADT 44, combined inoculation of *P. fluorescens* with *G. intraradices* was most effective in reducing the root population of *H. gracilis*. Dual inoculation of *P. fluorescens* with *A. lipoferum* was the most efficient treatment in suppression of root and soil population of *H. gracilis* in cv. IR 50, and soil population of the nematode in cv. ADT 44. Both the treatments were equally effective in the reduction of *H. gracilis* population in root and soil of both the rice varieties.

In the present study, it was observed that, there existed an inverse relationship between plant growth and the population of *H. gracilis* both in root and soil of rice. In accordance to the present investigation, it was pointed out that the significant improvement of rice plant growth was directly related to the decline in population of *H. gracilis* (Ahmad *et al.*, 1984).

Apart from improving plant growth and yield, *P. fluorescens* in combination with VAM fungi successfully reduced galling and nematode reproduction in chickpea infected with *M. javanica* (Siddiqui and Irshad Mahmood, 2001).

Disease suppression ability of the combined treatment was primarily due to *de novo* synthesis of isoprenoid cyclohexenone derivatives with blumenin produced by VAM fungi resulting in accumulation of hydroxycinnamate amides (4-coumaroylagmatine and – putrescine) and close association of isoprenoid and the phenylpropanoid metabolism (Fester *et al.*, 1998) and enhanced levels of phosphorus in host plants (Siddiqui *et al.*, 1998).

Along with plant growth promotion, the rhizobacterium, *P. fluorescens*, produced iron chelating siderophores (Kloepper *et al.*, 1980; Leong, 1986), antibiotics and hydrogen cyanide (Ahl *et al.*, 1986) and these compounds were proved to suppress deleterious rhizospheric microorganisms, creating an environment favourable for root growth (Perveen *et al.*, 1998). Siderophore activity, antibiotic production, plant hormone production, toxin development and induced systemic resistance were the possible causes against plant diseases (Weller, 1988). Induced systemic resistance was primarily due to structural and ultra structural cell wall modifications and biochemical and physiological changes in host plants (Ramamoorthy *et al.*, 2001).

Earlier reports have proved the effectiveness of *P. fluorescens* in suppression of a number of economically important phytonematodes both *in vitro* and *in vivo*. Significant reduction in root and soil population of the rice-root nematode, *H. gracilis* was obtained when rice seeds were bacterized with *P. fluorescens* before sowing (Ramakrishnan *et al.*, 1998a; 1998b and Ramakrishnan and Sivakumar, 1999). Another important species of the rice-root nematode, *H. oryzae* was also successfully managed following the inoculation of *P. fluorescens* (Swarnakumari, 1996; Sreenivasan *et al.*, 2000a).

Several mechanisms were attributed to the suppression of phytonematodes by the application of *P. fluorescens*. The rhizobacterium induced systemic resistance against nematode pests (Oostendrop and Sikora, 1990; Sikora, 1992; Sikora and Hoffman Hergarten, 1992; Aalten *et al.*, 1998) thereby inhibiting early root penetration of phytonematodes by alteration of specific root exudates such as polysaccharides and amino acids which modify nematode behaviour as the bacterium has the ability to envelop or bind the root surface with carbohydrate-lectin, thereby interfering normal host recognition by plant parasitic nematodes (Oostendrop and Sikora, 1989; 1990; Racke and Sikora, 1992). *P. fluorescens* produced nematicidal compounds such as ammonia and hydrogen cyanide (Becker *et al.*, 1988), and altered the disease development or sex ratio infected root tissues (Wyss, 1989). The rhizobacterium *P. fluorescens* had the potentiality to reduce hatching and invasion of phytonematodes by the production of toxic metabolites like protease and collagenase (Spiegel *et al.*, 1991). The turnover of immobilized N₂ and organic matter were accelerated by the introduction of *P. fluorescens* resulting in higher availability of inorganic nitrogen to plants for providing protection against plant parasitic nematodes inhabited in soil (Brimcombe *et al.*, 1999). Secondary metabolites such as 2,4-diacetylphloroglucinol (PHL) and lytic enzymes produced by *P. fluorescens* F113 strain were chitinolytic in nature and were responsible for increase in hatching of eggs and subsequent reduction in viability and mobility of juveniles of cyst

nematode, *G. rostochiensis* both under field and *in vitro* conditions (Elsherif and Grossmann, 1996; Dunne *et al.*, 1998). The *vgr* G gene, the two-component sensor kinase protein, *rtp* A (*gac* S) analogue, a UDP-glucose 4-epimerase gene, a siderophore receptor protein and the 50 S ribosomal protein, L 21 genes of *P. fluorescens* mutants were found to be involved in the ovicidal activity of the ring nematode (Wechter *et al.*, 2001). Increase in chitinase enzyme activity and phenolic content were responsible for reduction of *H. oryzae* infecting rice (Swarnakumari, 1996).

So far there has been no report on the control of any phytophagous nematodes of rice, by any of the species of VAM fungi. However, several nematologists have successfully used *G. intraradices* against a wide range of pathogenic nematodes mainly in perennial plantation and fruit crops like banana and pear (Pinochet *et al.*, 1997), cantaloupe (Herald *et al.*, 1989), cherry (Pinochet *et al.*, 1995b), grapevine (Linderman and Davis, 2001), plum (Pinochet *et al.*, 1998), quince (Calvet *et al.*, 1995) and rough lemon (Smith and Kaplan, 1988 and in few annuals such as cotton (Smith *et al.*, 1984; 1986a; 1986b) and soyabean (Price *et al.*, 1995). The VAM species were mostly found to be effective against endoparasitic nematodes. Migratory endoparasites (*P. brachyurus*, *P. vulnus* and *R. similis*) and sedentary endoparasites (*H. glycines*, *M. javanica* and *M. incognita*) were successfully reduced in various experiments by the VAM fungus (*Glomus* spp.).

VAM fungi as potential biocontrol agent against nematode infection and their probable roles have appeared in several research papers. The primary cause was related to the increase of host tolerance against phytonematodes (Hassan and Jain, 1998). Improvement in 'P' nutrition in mycorrhizal plants resulting in better plant vigour has largely been considered as one of the major factor of host tolerance (Schonbeck, 1979; Hussey and Roncadori, 1982; Hassan and Jain, 1987). Presence of fungal symbiont

altered root attractiveness, reduced larval penetration and impeded larval development and reproduction through increase of root growth and function, nutritional effects, alteration in root exudation, competition for host photosynthates, production of nematostatic compounds (hormones, amino acids, polysaccharides, phospholipids) and competition for space. Thus, VAM fungi suppressed plant parasitic nematodes primarily by altering host physically and physiologically rather than direct interaction (Sikora, 1979; Smith, 1987). Lignification of root tissues by VAM fungi at endo-and exodermis had proven to prevent the entry of ingress plant nematodes (Dehne and Schonbeck, 1979).

A few number of reports had been cited for the management of plant parasitic nematodes infecting crops like grapevine (Sivakumar and Vadivelu, 1998), maize (Rajeswari Sundara Babu *et al.*, 1998), mungbean (Khan and Kaunsar, 2000), okra (Ramakrishnan *et al.*, 1996; Sharma and Pankaj, 2000) and wheat (Bansal *et al.*, 1999) by *Azospirillum* and the phytonematode controlled mainly belong to genera *Meloidogyne*, *Heterodera*, *Pratylenchus*, *Radopholus* and *Rotylenchulus*.

Out of different properties of rhizobacteria including *Azospirillum* like nitrogen fixation, ammonia excretion, indole acetic acid, siderophore and ammonia production, all but siderophores and IAA were responsible for the control of nematode (Bansal *et al.*, 1999).

Apart from increasing growth and yield of plants various combinations of rhizobacteria were capable of reducing galling and nematode multiplication in chickpea infected with *M. javanica* (Siddiqui and Irshad Mahmood, 2001). The capability of biotic stress suppression by the combination of rhizobacteria was related to, improvement of seedling emergence rate due to enhanced release of ¹⁴C-labelled endogenous carbonaceous compounds (Sanhita *et al.*, 1995), increase in nutritional status of host plants particularly 'P' nutrition (Amara and Nasr, 1995; Deubel *et al.*, 2000),

enhancement of protein percentages of plant parts and increased seedling survival (Beshir and El Ghany, 1997) and improvement of plant growth (Siddiqui and Irshad Mahmood, 2001).

5.2.4 General observations

5.2.4.1 Field observations

Appreciable differences in plant growth between microbial inoculants treated and untreated *H. gracilis* infected seedlings were observed in nursery beds. However, in mainfield at later stages not much difference among them was recorded, which might be due to the result of compensation of plant growth by untreated plants. Babatola and Bridge (1979) and Fortuner and Merny (1979) made similar observations.

Delayed flowering by one to three days in *Hirsmanniella* spp. infected rice plants was observed by Fortuner (1974), and Babatola and Bridge (1979). Advancement of flowering date was reported in microbial inoculant treated rice plants (Hegde *et al.*, 1999). In the present study also, a difference of five to eight days of flowering dates was visually observed between microbial inoculants treated and untreated rice varieties (Plate 3C). In combined treatments quicker flowering was observed in both the rice cultivars.

5.2.4.2 Individual plant observations

At seedling stage, distinguishable differences in plant growth parameters were observed between treated and untreated rice plants except shoot and root length. However, not much difference was observed at panicle initiation stage except number of tillers, leaf area index per hill and number of panicles per hill at maturity stage of the rice varieties. Supporting the observations of the present study, similar reports were made by Fortuner (1974), Muthukrishnan *et al.* (1977), Babatola and Bridge (1979), McGowan (1979), Venkitesan *et al.* (1979), Babatola (1984), and Cho *et al.* (1994).

Infected plant roots had individual small yellowish lesions at a distance from root tip at initial stages of growth, which later turned to yellowish brown larger necrotic lesions (Plate 2B), whereas in microbial inoculant treated plants although browning of roots and lesions were observed they were not as conspicuous as in untreated plants. The results of the study were in line of the earlier findings of Sivakumar and Seshadri (1969) and Lee and Park (1975).

5.3 Physiological, biochemical and nutritional responses of cvs. IR 50 and ADT 44 to the infection of rice-root nematode, *H. gracilis* in consortium with microbial inoculants

5.3.1 Physiological parameters

Carbon dioxide (CO₂) gas exchange parameters such as leaf temperature, transpiration rate, photosynthetic active radiation and diffusive resistant play key role in limiting water loss from plants under stress conditions even in nematode infections (Wallace, 1987; Ramakrishnan and Rajendran, 1999) and are related to all the photosynthetic activities and are the indicators of physiological functioning of plants (Sen *et al.*, 1989).

There was an overall improvement in physiological functions of plants upon treatment with microbial inoculants.

P. fluorescens in combination with VAM fungus increased the intensity of photosynthesis of soyabean (Shabaev *et al.*, 1996) & radish (Shabaev *et al.*, 1998a; 1998b) and were related to increase in N₂ fixation which was due to enhanced plant phenology (leaf area) by the presence of growth promoting substances and accompanied by enhanced soil water absorption. These results are in accordance with the results of the present study relating to maximum reduction of leaf temperature of cv. ADT 44 and enhancement of photosynthetic active radiation of cv. IR 50, also increase of diffusive

resistance of both the cultivars observed in *P. fluorescens* combined inoculation with *G. intraradices*.

According to Pechurkin *et al.* (1997) reduction of transpiration rate of *P. fluorescens* inoculated wheat plants was due to increase in organic nitrogen in the biomass. Similar observations were also made by Shabaev *et al.* (1992) in soyabean plants.

Colonization of VAM fungus, inhibited accumulation of net carbon assimilation and elevated CO₂, which stimulate carbon flow below ground and some amount of carbon respired by the fungus to enhance the photosynthesis of *Plantago lanceolata* (Staddon *et al.*, 1999). The function of VAM fungus was to absorb extra amount of water and nutrients specially under stress condition resulting in an increase of CO₂ exchange rate and stomatal conductance (Ruiz Lozano and Azcsn, 1995).

Several workers have reported the enhancement of photosynthesis rate following the inoculation of *Azospirillum* in crop plants like alfalfa (Kvves Pichy *et al.*, 1998), oil palm (Amir *et al.*, 2001) and wheat (Panwar *et al.*, 1990; Elanchezhian and Panwar, 1997; Panwar and Elanchezhian, 1998). This was due to nitrate reductase and glutamine synthetase activities (Panwar, 1991; 1992), translocation of current photosynthates (¹⁴C-sucrose) towards the roots (Elanchezhian and Panwar, 1997), enhancement of nutritional status of plants and plant growth (Panwar and Elanchezhian, 1998; Amir *et al.*, 2001), increase of phenomenological electron transport (Kvves Pichy *et al.*, 1998).

Minimum of leaf temperature of cv. IR 50 and transpiration rates of both the cvs. IR 50 and ADT 44 and maximum of photosynthetic active radiation of cv. ADT44 were obtained in dual inoculation of *P. fluorescens* with *A. lipoferum* are in line with the

enhancement of physiological functions by individual *P. fluorescens* and *A. lipoferum* inoculations.

5.3.2 Biochemical attributes

5.3.2.1 Quantitative assessment of biochemical components

5.3.2.1.1 Total free amino acids

Total free amino acids of plants normally get affected upon nematode infections (Jayaprakash *et al.*, 1979, 1981; Mahmood and Siddiqui, 1993). In the present study treatments with microbial inoculants were able to enhance it.

Maximum enhancement was in combined treatment of *P. fluorescens* with *A. lipoferum* in both the varieties. In general, all the *P. fluorescens* combinations were equally effective.

Due to lack of report on combined effect of *P. fluorescens* with *A. lipoferum*, individual effect may be correlated.

Several workers have observed, increased levels of total free amino acids in plants such as potato (Ciampi *et al.*, 1996), spinach (Futamata *et al.*, 1998) and tomato (Simons *et al.*, 1996; 1997) following the application of *P. fluorescens*. Upon colonising on host plant root system *P. fluorescens* modified the root exudation and check amino acid leakage (Futamata *et al.*, 1998) or by virtue of its ability, *P. fluorescens* releases amino acids from its own intercellular solutes under stress conditions (Halveron *et al.*, 2000) which are the probable reasons for enhancement of total free amino acids by the rhizobacterium.

Azospirillum was able to enhance the total amino acid contents of winter rye, *Lonium perenne* and *Bromus inermis* by increasing the biologically fixed nitrogen in roots through enhanced 'N' assimilation (Mal'tseva *et al.*, 1995). Two adjacent genes

sal A and sal B, conferring *in vitro* glucosidase activity of *Azospirillum* were responsible for releasing amino acids (Faure *et al.*, 1999).

5.3.2.1.2 Soluble proteins

Reductions in soluble proteins were observed in microbial inoculant treatments in both the varieties. Among the microbial inoculant treatments minimum reduction was found in combined inoculation of *P. fluorescens* with *G. intraradices* of cv. IR 50 and in dual inoculation of *P. fluorescens* with *A. lipoferum* of cv. ADT 44 in response to combined effect of treatments and nematode infection. Not much difference was observed among the *P. fluorescens* combined treatments. Contradicting the results of the study, Attia *et al.* (1999) reported the enhancement of soluble protein contents of maize plants in response to combined inoculation of *Glomus* sp. and *P. fluorescens*. But, report of Domey *et al.* (1998) was partially in line with the results of the study, as soluble protein contents of shoot and root of *Panax ginseng* plants were reduced by application of *G. intraradices* due to induction of polypeptides with high molecular weights.

Not agreeing to the results of the study, *Azospirillum* was also found to enhance the soluble protein contents of crop plants *viz.*, maize (Pelaez Abellan *et al.*, 1992; Purcino *et al.*, 1996), and sugarcane (Gonzalez *et al.*, 1995). In response to application of *Azospirillum*, several enzyme activities *viz.*, nitrate reductase, phenylalanine ammonia lyase (Pelaez Abellan *et al.*, 1992), ribulose-1, 5-biphosphate carboxylase (RUBISCO), phosphoenolpyruvate carboxylase (PEPC), ferredoxin-glutamase synthetase (Fd-GOGAT) and glutamine synthetase (GS) (Purcino *et al.*, 1996) get activated in host plants which might be resulting in higher nitrogen metabolism and photosynthetic activity ultimately resulting in the increase in soluble proteins.

Report of Panwar (1991) showed that *Azospirillum* had no effect on protein content, while vesicular-arbuscular mycorrhiza increase the same in wheat due to enhanced photosynthetic rate and activities of nitrate reductase and glutamine synthetase.

Increased levels of proteins observed in diseased plants immediately following treatments with biocontrol agents are mainly pathogenesis related (PR) proteins (Alström, 1995; Liu *et al.*, 1995; Duiff *et al.*, 1998; Meena *et al.*, 2000; Srivastava *et al.*, 2001).

According to Srivastava *et al.* (2001) chitinase and glucanase enzyme activities and pathogenesis related proteins decreased gradually over advancement of time as post-treatment effect of *P. fluorescens* on *Macrophomina phaseolina*. Similar trend of decrease of acid phosphatase, alkaline phosphatase, superoxide dismutase, chitinase enzyme activities and proteins were observed in *Glomus* spp. inoculated *Pennisetum pedicellatum* plants by Ramesh *et al.* (2000). In the study, the enhancement of soluble proteins by microbial inoculants might not be sufficient enough to mitigate the reduction of soluble proteins at panicle initiation stage, or microbial inoculants individually or in combinations had not enhanced enough of soluble protein content of rice plants as post-treatment effects against *H. gracilis* infection to match up with extra amount of soluble protein synthesized by additional number of ingressed nematodes in untreated control as nematode infection leads to increase of plant soluble proteins (Tayal and Agarwal, 1982; Ahuja *et al.*, 1987).

5.3.2.1.3 Total and reducing sugars

Both the total and reducing sugars were in increasing trends in microbial inoculant treatments, with maximum enhancement in *P. fluorescens* with *G. intraradices* combined treatment in both the varieties. Enhancement of reducing sugars was more in comparison to total sugars. In general, more enhancements of sugars were recorded in cv. IR 50.

Upon treatment with *P. fluorescens*, enhanced sugars was recorded from seed and root extracts and root exudates of soyabean (Rao and Johri, 1999) and tomato (Muzafar and Duss, 1998; Lugtenberg *et al.*, 1999). The probable reasons might be, modification of root exudate sugar pattern to restrict the release of sugars of high molecular weight through colonization by the rhizobacterium (Muzafar and Duss, 1998) or by the ability of *P. fluorescens* to release its own 60 per cent of low molecular weight neutral sugar from intercellular solute pool specifically under stress conditions (Halverson *et al.*, 2000). However, in collected literature there was no specific mention about enhancement of different categories of sugar by *P. fluorescens*.

Inoculation of *G. fasciculatum* resulted in enhancement of total and reducing sugar contents of *M. incognita* infected tomato plants (Suresh and Bagyaraj, 1984). Similar reports were also recorded in a number of plants under various conditions (Krishna and Bagyaraj, 1991; Augi *et al.*, 1992; Ezz and Nawar, 1994; Subramanian *et al.*, 1997; Boucher *et al.*, 1999).

As post-application effects, mycorrhizal fungi were able to change in cell-type specific expression of a transporter (Harrison, 1996), decrease in amount of root exudates by mechanical blocking (Isobe and Tsuboki, 1998) or by enhancement of VAM sugar content by insoluble alkaline phosphatase involved in sugar metabolism (Ezawa *et al.*, 1999).

The individual effect of *P. fluorescens* with *G. intraradices* in increasing the sugar content may hold good to corroborate the findings of the present study.

5.3.2.1.4 Total and ortho dihydroxy phenols

Enhancement of both total and ortho dihydroxy phenols was observed by microbial inoculant treatments and in general, rates of increase were more in cv. ADT 44.

P. fluorescens in combination with *G. intraradices* was most effective in the enhancement of both total and ortho dihydroxy (OD) phenols of cv. IR 50 and OD phenols of cv. ADT 44. Combined inoculation of *P. fluorescens* and *A. lipoferum* was the most efficient treatment in increasing the total phenols of cv. ADT 44. In general, *P. fluorescens* combined treatments were found to be equally effective in case of total phenols. Above two treatments had similar potential in enhancement of both total and ortho dihydroxy phenols. Report pertaining to enhancement of phenolics by combined treatments is lacking. However, there are a number of reports on improvement of phenol contents of crop plants by the rhizobacterium, *P. fluorescens* in chickpea (Srivastava, 2001), maize (Shabaev *et al.*, 1999) and rice (Meena *et al.*, 1999 and 2000). Only one report on enhancement of total and ortho dihydroxy phenols following *P. fluorescens* application was made by Swarnakumari (1996) in rice plants infected with the rice-root nematode, *H. oryzae*. In diseased plants phenols generally get increased by defense mechanism of plants and linked with phenylpropanoid pathway. *P. fluorescens* by its capacity releases antimicrobial factors including the lytic enzymes which leads to the accumulation of phenolics (Bagnasco *et al.*, 1998; Meena *et al.*, 1999; 2000) by secretion of indole acetic acid that induced phenol metabolism in plants (Shabaev *et al.*, 1999).

VAM fungi were capable of raising concentrations of phenolics in plants. In response to mycorrhizal applications, total phenol contents of *Populus beijingensis* (Tang *et al.*, 2000), *Zea mays* (Chhabra *et al.*, 1992) and *Ziziphus xylopyrus* (Mathur and Vyas, 1996a) were found to be increased and also in *M. incognita* infected tomato plants (Singh *et al.*, 1991) and *H. cajani* infected cowpea plants (Nageswari, 1997). There is no mention about enhancement of ortho dihydroxy phenols by VAM fungi in any literature. VAM fungi are also capable of increasing enzymes involved in plant defense mechanisms and might be responsible for the increase of phenol contents of mycorrhizal plants (Mathur and Vyas, 1996a) and that might be the result of the involvement of plant

mRNA encoding enzymes of the phenyl propanoid pathway (Franken and Gnadinger, 1994).

Potato plants cv. Alpha when treated with *Azospirillum* had higher content of total phenolics compounds and was related to higher antioxidant activities of the rhizobacterium resulting in the increase of linoleic acid oxidation (El Moorsi *et al.*, 2000). The report on increase of ortho dihydroxy phenols by *Azospirillum* is wanting.

5.3.2.1.5 Chlorophylls

Microbial inoculants were able to raise the concentrations of chlorophylls of the rice cultivars against reducing ability of *H. gracilis* in rice (Wu, 1999) as observed in the present study. Cv. IR 50 was observed to be more receptive than cv. ADT 44.

Combined treatment of *P. fluorescens* with *G. intraradices* increased maximum chlorophyll 'a' in cv. IR 50. Dual inoculation of *P. fluorescens* with *A. lipoferum* was the most efficient treatment in increasing the chlorophyll 'a' of cv. ADT 44, and chlorophyll 'b' and total chlorophyll of both the varieties.

A series of experiments conducted in grafted grapevine rootstocks with the application of *P. fluorescens* and VAM fungus, *G. mosseae*, have proved their combined capacity to improve the leaf iron (Fe^{+2}) concentrations and thereby reducing the lime-induced chlorosis with no mention of chlorophyll fractions (Bavaresco and Fogher, 1992; 1996a; 1996b). VAM fungi, *G. intraradices*, and *G. sporum* were able to increase chlorophyll to a small fraction in sour orange and nodulated bean respectively (Ezz and Nawar, 1994; Izaguirre Mayoral *et al.*, 2000). The findings of these experiments were in supportive line with the results of the present study. The enhancement of chlorophyll by combined treatment was the product of accumulation of leaf mineral composition specifically the ferrus ion (Bavaresco and Fogher, 1992; 1996a; 1996b). Increase in

chlorophyll content upon VAM fungi application resulted in increase in sugar, starch, carbohydrate and mineral nutrient concentrations accompanied with higher rates of photosynthesis and decrease in photosynthetic water consumption as observed by the above authors.

For want of report on combined effect of *P. fluorescens* with *A. lipoferum* on chlorophyll concentrations of plant leaves, individual influence may be corroborated. *P. fluorescens* prevented loss of chlorophyll by controlling the diseases caused by *Sclerotinia homeocarpa* and *Bipolaris sorokiniana* in *Poa pratensis* (Hodges *et al.*, 1994) and improved the chlorophyll concentrations of grapevine rootstocks against lime-induced chlorosis by increasing leaf mineral element concentrations (Bavaresco *et al.*, 1995) and particularly 'P', 'K' and 'Fe' mineral elements (Bavaresco *et al.*, 2000). A number of plants *viz.*, banana (Tiwari *et al.*, 1999), cauliflower (Bambal *et al.*, 1998), lettuce (Agwah and Shahaby, 1993), maize (Hamadia and El Komy, 1998), rice (Stancheva *et al.*, 1999; Fang *et al.*, 2001), wheat (Mishra and Sen, 1993; Elanchezhian and Panwar, 1997; Panwar and Elanchezhian, 1998; Panwar and Singh, 2000) and yellow lupins (Ivanov *et al.*, 1993) had higher concentrations of leaf chlorophylls as post-application effects of *Azospirillum*, without mention of chlorophyll 'a' and chlorophyll 'b'. The enhancement of chlorophyll concentrations in plant leaves by *Azospirillum* was due to increase in nitrate reductase and glutamine synthetase activities (Panwar, 1991; 1992), decrease in leaf ion leakage (Panwar, 1993), increase of root development and active absorbing surface accompanied by enhancement of respiration rate (Ivanov *et al.*, 1993), improvement in physiological functions of plants by increasing photosynthetic rate and stomatal conductance (Elanchezhian and Panwar, 1997; Panwar and Elanchezhian, 1998), improvement in electron transport activity per absorption of roots (Stancheva *et al.*, 1999) and enhancement of nutritional status (Agwah and Shahaby, 1993; Hamadia and El Komy, 1998; Bambal *et al.*, 1998; Tiwari *et al.*, 1999; Fang *et al.*, 2001).

5.3.2.2 Enzyme activity

Oxidative enzymes play a decisive role in plant resistance to external biotic aggression. There are many instances on activities of enhanced peroxidase and catalase enzymes against nematode infection to protect the plants (Kaplan and Davis, 1987; Rajasekhar *et al.*, 1997; Trivedi, 1998).

Microbial inoculants enhanced both the enzyme activities. Peroxidase activity was comparatively more in cv. IR 50 than cv. ADT 44 and vice versa in case of catalase.

5.3.2.2.1 Peroxidase

P. fluorescens and *A. lipoferum* combined inoculation was the most effective treatment in the increase of peroxidase activities in both the varieties. The results of the study seem to be the first record, which may be correlated to individual effect.

P. fluorescens was capable of enhancing peroxidase activity of plants particularly against the diseased ones. The enzyme activity was enhanced in scots pine infected with root rot fungus (*Heterobasidion annosum*), fine root fungus (*Gibberella avenacea*), and wood decay fungus (*Phlebia gigantea*) (Asiegbu *et al.*, 1999), rice infected with blight pathogens (*Rhizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae*) (Nandakumar *et al.*, 2001; Vidyasekharan *et al.*, 2001) in response to the application of *P. fluorescens*. *P. fluorescens* application resulted in rapid accumulation of hydrogen peroxide (H₂O₂) and was responsible for enhanced peroxidase activity (Asiegbu *et al.*, 1999). Sharp increase in lignification by *P. fluorescens* enhanced the enzyme activity (Vidyasekharan *et al.*, 2001). *P. fluorescens* induce systemic resistance against diseases by activating defense genes which are expressed in the form of increase in peroxidase activity (Nandakumar *et al.*, 2001).

Azospirillum population in soil was correlated with enhancement of peroxidase activities of *Cajanus cajan*, *Vigna radiata*, *V. mungo*, *V. catjung* and *Glycine max* (Saralabai and Vivekanandan, 1995). Inoculation of *Azospirillum* to hydroponic and quartz sand cultures with toxic cadmium concentration enhanced peroxidase activity (Belimov and Dietz, 2000). The increase of peroxidase activity following the application of *Azospirillum* was related to increase in H_2O_2 and O_2 concentrations (Saralabai and Vivekanandan, 1995) and accumulation of sulphhydryl (SH) compounds and associative enzyme activities (Saralabai and Vivekanandan, 1995; Belimov and Dietz, 2000).

5.3.2.2.2 Catalase

Catalase activity was increased in combined inoculation of *P. fluorescens* and *G. intraradices* of both the varieties and all the combined treatments were equally effective. Due to want of combined effect, individual effect of the involved inoculants may be corroborated.

Reports on increase of catalase activity by *P. fluorescens* were confined to *in vitro* conditions. As a result of applications of chloroform (Pounder and Anderson, 1994), oxy free radicals such as H_2O_2 and O_2 (Kiraly *et al.*, 1993; Fiorenza and Ward, 1997) on cultures, *P. fluorescens* was able to release catalase from its own cells might be the cause of increase of catalase in the plants, as these compounds and radicals have already been observed in the process of pathogenesis as reported by the above authors.

Mycorrhizal plants reported to have higher catalase activity as observed in tobacco (Blilou *et al.*, 2000), and the salicylic acid content in roots increased the catalase activity.

5.3.3 Nutrient content

Microbial inoculants were able to increase the nutritional status of shoot and root of both the rice cultivars against the reducing tendency of *H. gracilis* except zinc.

In general, cv. IR 50 was more responsive to the enhancement of macronutrients (nitrogen, phosphorus and potassium), whereas micronutrients (iron, copper, zinc and manganese) were increased relatively more in cv. ADT 44 except root copper and manganese contents. Combined treatments were more effective.

P. fluorescens combined inoculation with *A. lipoferum* was able to enhance shoot and root nitrogen concentration of both the varieties, shoot potassium content of cv. ADT 44 and concentrations of root manganese of both the varieties. The results of the study are in line of the findings of Amara and Nasr (1995) and Rodelas *et al.* (1999) in soyabean and faba bean respectively. *Azospirillum* has greater nitrogenase (NO_3^- and NH_4^+) activities in root and soil which leads to the improvement of N_2 fixing potential of plants (Christiansen Weniger and Veen, 1991), nitrogen fixation, auxine production and nitrate reduction in the rhizosphere that tends to increase in physiological functioning of plants including absorption of water and nutrients (Christiansen Weniger, 1992). Inoculation of microorganisms in soil tends to produce and synthesis growth regulators more particularly IAA and was responsible for enhanced growth and functioning of roots (Hernandez *et al.*, 1996). *P. fluorescens* produced phytohormones (auxins and cytokinins) that enhance nitrogenase, nitrate reductase, 'P' mobilization, pectinase and cellulase activities and are related to accumulation of nutrients in plants (Hvfligh and Kihn, 1996). Increased nutrient recovery by the *P. fluorescens* inoculated plants was as a result of enhanced functional activities of the roots and were related to an increased secretion of phenols (Shabaev *et al.*, 1999).

Both shoot and root phosphorus concentrations were recorded maximum in *G. intraradices* with *B. megaterium* treatment of both the varieties. The results are in accordance to the reports of Delorenzini *et al.* (1979) and Sankaranarayanan (1995) in clover and blackgram respectively. VAM fungi mobilise phosphorus from beyond phosphorus depletion zone around the root and phosphobacterium by its capacity solubilizes the insoluble inorganic phosphorus from soil and made available to the mycorrhizal plant roots (Delorenzini *et al.*, 1979; Raj *et al.*, 1981).

Dual inoculation of *P. fluorescens* with *G. intraradices* was the most efficient treatment in increasing the shoot potassium content of cv. IR 50 and concentrations of root potassium of both the varieties, concentrations of iron, copper, zinc and manganese of shoot and root of both the varieties except root manganese content of cvs. IR 50 and ADT 44. Results of the study is in confirmation of the findings of Smolin and Shabaev (1992) on soyabean plants. VAM fungi improved nutritional status of plants by enhanced activity of phosphorylase in the soil and dehydrogenase activity in the roots, thus promote the absorption of nutrients (He *et al.*, 1994; Wang *et al.*, 1997), through colonization that impart ability to explore larger volumes of soil and thereby increase uptake of nutrients (Gerdemann, 1968; Harley and Smith, 1983), control of water loss and adjustment of leaf and root osmotic potentials (Hernandez Sebasti *et al.*, 1999). *P. fluorescens* and VAM fungi (*G. intraradices* or *G. deserticola*) improved physiological status and functioning, by mechanical blocking of root exudation and thereby enhanced nutritional status of inoculated plants (Marschner *et al.*, 1997).

5.4 Histopathological changes of rice-roots to the infection of *H. gracilis*

Longitudinal and transverse sections of nematode affected roots showed feeding of the nematode on cortical tissues. Disruption of cell walls and formation of cavities were also observed, these results of the study are in accordance to the observations made

by Keoboornrueng (1972), and Mathur and Prasad (1972a; 1972b) in *H. oryzae* infected rice roots. Similar to the findings of Hollis and Keoboornrueng (1984), the rice-root nematode (*H. gracilis*) was oriented parallel to the long axis of roots.

Cross sectional microscopic views of *H. gracilis* infected roots had thickened broken and dark coloured damaged cell walls in cortical region and the same were observed by Hollis and Keoboornrueng (1984) and Vovlas *et al.* (1996) in case of *H. caudacrena* and *H. oryzae* infecting rice roots and the damage was confined to epidermal and cortical cells.

The thickening of affected parenchymatous cells may be due to deposition of suberin as this substance being abundant in the hypodermal cells of rice roots (Juliano and Aldama, 1937). Since the histopathological studies have indicated the breakdown of cell walls probably after thickening may be related to dissolution of cell walls by the enzymes such as cellulase and others which are generally secreted by the nematodes which cause the release of polygalacturonase by the infected plants as observed in *D. dipsaci* and *P. penetrans* infected cotton roots (Krusberg and Sasser, 1956). Browning of the cell walls is the result of necrosis following death of the cells due to nematode feeding and the same was observed by Sivakumar (1964) in *H. oryzae* infected rice roots.

5.5. Future research thrusts

Several microorganisms were well documented to replace hazardous and costly agrochemicals by several research workers and also in the present study. Some of their combinations were synergistic in improving plant growth & yield and reducing the severity of pest and disease incidences. There is further scope for study on these microbial inoculants and their various combinations. Very little information is available regarding their effect on all the prevailing pests and diseases, therefore situation specific holistic approach of investigations have to be carried out.

SUMMARY

CHAPTER VI

SUMMARY

Rice is the most important food crop of human kind. In most of the rice growing region plant parasitic nematodes appear to have significant impact on production. *H. gracilis* is one of the endemic and potential pests of irrigated, lowland and deep water paddy in major rice growing areas of Indian subcontinent. So far, only chemical nematicides have been recommended to control the rice-root nematode. Although spectacular successes were demonstrated with chemical control measures, high cost, toxicity, residual and environmental problems have prompted the switch over to economically viable and ecofriendly nematode management strategies. A multitude of alternate innovative approaches was experimented with some degree of success. Recently, some plant growth promoting microorganisms were observed either nematostatic or nematocidal. Till date, very few attempts were made to evaluate the combined effects of these plant growth enhancers against rice nematodes. One of the important aspects and cheapest way of increasing crop production is by growing high yielding, nematode resistant varieties in endemic areas. Realising the above facts, studies were undertaken to evaluate the performances of popular high yielding rice varieties and microbial inoculants either individually or in combinations with *G. intraradices* or *P. fluorescens* both in glasshouse and field conditions on short and long duration rice cultivars. Physiological, biochemical and nutritional responses of these microorganism inoculated rice varieties against the infection of *H. gracilis* were investigated. Histopathological studies of *H. gracilis* infected rice roots were made. The inferences of the results of these studies are summarised below.

1. A simplified and quicker rating index (1-5 scale) of the rice varieties for resistance against the infection of *Hirschmanniella* spp. was proposed as follows:

Rating index:

Scale	Nematode population per g root	Host reaction
1	0	Immune (I)
2	1 – 10	Highly resistant (HR)
3	11 – 50	Moderately resistant (MR)
4	51 – 100	Susceptible (S)
5	> 100	Highly susceptible (HS)

2. None of the short, medium and long duration popular high yielding rice varieties screened was observed to be either immune or highly resistant to *H. gracilis*. Among the short duration varieties TKM 5, TKM 7, TKM 8 and TKM 9 were rated moderately resistant and most (70.4 per cent) of the cultivars were found susceptible. Medium duration varieties viz., ASD 8, MDU 4, PTB 12, PTB 15, PTB 23 and PY 1 were moderately resistant and 65 per cent of the varieties were susceptible. Only cv. CR 1009 was moderately resistant among the long duration varieties tested and the other three viz., ADT 40, ADT 44 and CO 40 were susceptible.
3. All the microbial inoculants evaluated were able to enhance both above and below ground plant growth parameters and reduced root and soil *H. gracilis* population of cvs. IR 50 and ADT 44 under glasshouse conditions in response to the infection of *H. gracilis* at an inoculum level of one nematode per g soil. Among them *P. fluorescens* combinations were more efficient.
4. Combined inoculation *P. fluorescens* with *G. intraradices* recorded maximum shoot and root length, fresh and dry weight of shoot and root and root volume of cv. IR 50, and root length, fresh and dry weight of shoot and root, total leaf area, number of tillers and root volume of cv. ADT 44 and minimum root and soil population of *H. gracilis* in cv. IR 50, and root population of cv. ADT 44.

5. *P. fluorescens* in combination with *A. lipoferum* recorded maximum total leaf area and number of tillers in cv. IR 50, and suppressed the soil population of *H. gracilis* to the maximum in cv. ADT 44.
6. Combined treatment *G. intraradices* with *A. microphylla* had maximum shoot length in cv. ADT 44.
7. Under field conditions all the treated microbial inoculants individually or in combinations increased above and below ground plant growth parameters and reduced *H. gracilis* population in root and soil of cvs. IR 50 and ADT 44 at seedling stage. Combined treatments were more effective.
8. Combined inoculation of *P. fluorescens* with *G. intraradices* had maximum shoot and root lengths, fresh and dry weight of shoot and root, total leaf area, and root volume of cv. IR 50, and total leaf area and root length of cv. ADT 44, and maximum reduction of root and soil population of *H. gracilis* in cv. IR 50, and root population in cv. ADT 44.
9. Dual inoculation of *P. fluorescens* with *A. lipoferum* enhanced shoot length, fresh and dry shoot and root weight and root volume to a maximum and reduced soil population of *H. gracilis* to a minimum in cv. ADT 44.
10. At panicle initiation stage, all the microbial inoculants either individually or in combinations improved both above and below ground plant growth parameters of cvs. IR 50 and ADT 44 naturally infected with *H. gracilis* and reduced nematode population in root and soil. Combined treatments performed better than individual treatments and *P. fluorescens* combinations were more efficient.
11. Combined inoculation of *P. fluorescens* with *G. intraradices* recorded maximum shoot and root lengths in cv. IR 50, and root length, fresh and dry weight of shoot and

root, leaf area index and number of tillers per hill and minimum *H. gracilis* root population in cv. ADT 44.

12. Dual inoculation of *P. fluorescens* with *A. lipoferum* had maximum fresh and dry weight of shoot and root, leaf area index, number of tillers and root volume per hill of cv. IR 50, and root volume of cv. ADT 44. The treatment reduced root and soil *H. gracilis* population of cv. IR 50, and soil population in cv. ADT 44 to a minimum.
13. Combined treatment of *P. fluorescens* with *A. microphylla* had maximum shoot length in cv. ADT 44.
14. Microbial inoculants were able to increase yield attributes of cvs. IR 50 and ADT 44. Not much difference were observed with respect to panicle length, number of grains per panicle and thousand grain weight. Only distinguishable difference observed was the number of panicles per hill. *P. fluorescens* combined treatments were comparatively more effective.
15. *P. fluorescens* in combinations with *G. intraradices* and *A. lipoferum* had maximum panicle length and thousand grain weight in cv. IR 50.
16. Maximum increase in number of panicles per hill, number of filled grains per panicle and thousand grain weight of cv. ADT 44 were observed in combined inoculation of *P. fluorescens* with *G. intraradices*.
17. Combined inoculation of *P. fluorescens* with *A. lipoferum* recorded maximum number of panicles per hill and number of filled grains per panicle in cv. IR 50, and panicle length in cv. ADT 44.

18. Increase of grain and straw yield and reduction of root and soil *H. gracilis* population at harvest in cvs. IR 50 and ADT 44 were obtained by the application of microbial inoculants. *P. fluorescens* combined treatments were more effective.
19. *P. fluorescens* in combinations with *G. intraradices* and *A. lipoferum* had maximum grain yield in cv. ADT 44.
20. Maximum straw yield and reduction of *H. gracilis* root population of cv. ADT 44, and reduction of *H. gracilis* root and soil population on cv. IR 50 were obtained in dual inoculation of *P. fluorescens* with *G. intraradices*.
21. *P. fluorescens* combined inoculation with *A. lipoferum* recorded maximum grain and straw yield in cv. IR 50, and reduced *H. gracilis* soil population in cv. ADT 44 to minimum.
22. Microbial inoculants had more influence on plant growth in *H. gracilis* infected seedlings in nursery beds than the later stages in main fields. Appreciable differences were observed only in number of tillers and leaf area index per hill at panicle initiation stage and number of panicles at maturity stage.
23. Treatments with microbial inoculants advanced flowering of both the cvs. IR 50 and ADT 44. Combined treatments were more efficient. Upon treatments with microbial inoculants, nematode infected rice cultivars had inconspicuous symptoms of nematode damage on roots such as presence of lesions and discolouration of root surface.
24. Microbial inoculants were able to decrease leaf temperature, transpiration rate and enhanced photosynthetic active radiation and diffusive resistance. Combined treatments of *P. fluorescens* were more effective. Combined inoculation of *P. fluorescens* with *G. intraradices* had maximum photosynthetic active radiation and

diffusive resistance in cv. IR 50, and minimum leaf temperature and maximum diffusive resistance of cv. ADT 44. *P. fluorescens* in combination with *A. lipoferum* recorded minimum leaf temperature and transpiration rate in cv. IR 50, and minimum transpiration rate and maximum photosynthetic active radiation in cv. ADT 44.

25. Microbial inoculants were able to enhance total free amino acid content in the roots of *H. gracilis* infected rice cvs. IR 50 and ADT 44. *P. fluorescens* combined treatments were more effective. Maximum increase was in *P. fluorescens* combined inoculation with *A. lipoferum* in both the rice varieties.
26. Microbial inoculants have failed to increase the soluble protein contents in the roots of rice cvs. IR 50 and ADT 44 infected with *H. gracilis*. Minimum reduction was observed in *P. fluorescens* combinations with *G. intraradices* and *A. lipoferum* in cvs. IR 50 and ADT 44 respectively.
27. Enhancement of total and reducing sugars of roots was observed in *H. gracilis* infected rice cvs. IR 50 and ADT 44. Upon treatments with microbial inoculants and quantities of enhancement were more in cv. IR 50. *P. fluorescens* combined treatments were more effective. *P. fluorescens* in combination with *G. intraradices* had maximum total and reducing sugars in both the varieties.
28. Individual and combined treatments of microbial inoculants had enhanced quantities of total and ortho dihydroxy phenols in the roots of *H. gracilis* infected cvs. IR 50 and ADT 44. Rate of enhancement of the phenolics was comparatively more in cv. ADT 44. *P. fluorescens* combined treatments were more efficient. *P. fluorescens* combined inoculation with *G. intraradices* had maximum phenolics in cv. IR 50, and ortho dihydroxy phenols of cv. ADT 44. Maximum enhancement of total phenols of cv. ADT 44 was obtained in dual inoculation of *P. fluorescens* with *A. lipoferum*.

29. Chlorophyll contents (chlorophyll 'a', chlorophyll 'b' and total chlorophyll) were increased in microbial inoculant treated rice cvs. IR 50 and ADT 44 infected with *H. gracilis*. Rates of increase were more in cv. IR 50. *P. fluorescens* combinations were more effective. Combined inoculation of *P. fluorescens* with *G. intraradices* had maximum chlorophyll 'a' in cv. IR 50. Dual inoculation of *P. fluorescens* with *A. lipoferum* recorded maximum chlorophyll 'b' and total chlorophyll in cv. IR 50, and chlorophylls in cv. ADT 44.
30. Activities of oxidative enzymes (peroxidase and catalase) were enhanced in *H. gracilis* infected rice cvs. IR 50 and ADT 44 following the treatments with microbial inoculants. Rate of enhancement of peroxidase activity was more in cv. IR 50 than cv. ADT 44 and vice versa in case of catalase. *P. fluorescens* combined treatments were more effective. *P. fluorescens* in combination with *A. lipoferum* recorded maximum peroxidase activity in both the cultivars. Maximum enhanced activity of catalase was in combined inoculation of *P. fluorescens* with *G. intraradices* in both the rice varieties.
31. Macronutrient (nitrogen, phosphorus and potassium) contents of shoot and root of *H. gracilis* infected cvs. IR 50 and ADT 44 were increased by the application of microbial inoculants individually or in combinations. Rates of enhancement were more in cv. IR 50. *P. fluorescens* combinations were more efficient except for phosphorus content. Dual inoculation of *P. fluorescens* with *G. intraradices* recorded maximum concentrations of shoot potassium of cv. IR 50, and root potassium of both the varieties. Combined inoculation of *G. intraradices* with *B. megaterium* had maximum quantities of shoot and root phosphorus of both the varieties. *P. fluorescens* in combination with *A. lipoferum* had maximum total nitrogen content of shoot and root of the rice varieties and shoot potassium content in cv. ADT 44.

32. Concentration of micronutrients (iron, copper, zinc and manganese) of shoot and root of *H. gracilis* infected rice cvs. IR 50 and ADT 44 were enhanced upon treatments with microbial inoculants. Rates of increase were relatively more in cv. ADT 44 except copper and manganese contents of roots. Combined treatments were more effective. *P. fluorescens* in combination with *G. intraradices* had maximum concentrations of shoot and root iron, copper and zinc, and shoot manganese contents of both the rice varieties. Combined inoculation of *P. fluorescens* with *A. lipoferum* recorded maximum root manganese content in the rice varieties.
33. *H. gracilis* lying parallel to the long axis inside the infected roots, formed intercellular cavities both in epidermal and cortical cells. Cortical root parenchyma cells were damaged upon feeding by the nematode, which resulted in thickening, dissolution and necrosis of cell walls.

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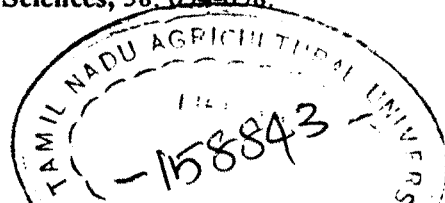
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* - Originals not seen.

