ABSTRACT

STUDIES ON THE ROOT-KNOT NEMATODE Meloidogyne graminicola GOLDEN AND BIRCHFIELD, 1965 INFECTING RICE IN TAMILNADU

by

B.ANITA

Degree : Doctor of Philosophy (Agriculture) in Plant Nematology

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The root knot nematode causing severe damage to rice crop in Tamil Nadu was identified as Meloidogyne graminicola Golden and Birchfield, 1965 based on the morphological and taxonomic characters, enzyme phenotypes and root symptoms. Studies on the biology of M. graminicola in rice revealed that the nematode completed one life cycle from egg to egg stage in 22 days under glass house conditions. Histopathogenesis of roots infested with M. graminicola indicated presence of eggs, juveniles, immature and adult female stages inside the root galls. Eggs hatched within the root and inter and intra cellular migration of juveniles within the root cortex caused disruption and hypertrophy of cells. At the site of larval establishment groups of 7 - 9 multinucleate giant cells with granular cytoplasm were found. Abnormal xylem proliferated around the giant cells causing swelling of vascular cylinder, limiting the uptake of nutrients and
The avoidable yield loss in rice cv. ASD 16 due to *M. graminicola* was 5.99 per cent under field conditions. Root-knot nematode infected seedlings turned chlorotic and remained stunted in the nursery resulting in patchiness. There was significant reduction in seedling weight and plant height. Roots of infested plants were severely galled with characteristic hooked terminal galls. Spindle or club or nodule shaped galls were also observed in infected roots.

Most of the high yielding rice varieties screened for their reaction to *M. graminicola* were found susceptible. Commonly occurring weeds in rice fields viz., *Echinochloa colonum* and *Cynodon dactylon* were categorized as excellent alternate hosts. Other weed hosts which supported the development and reproduction of *M. graminicola* were *Panicum repens*, *Ammania baccifera*, *Ipomea aquatica*, *Fimbristylis mileacea*, *Eclipta alba*, *Eclipta prostrata* and *Chloris barbata*. Among crop hosts, tomato and brinjal were highly susceptible, while onion and pulse crops such as redgram, greengram, blackgram and cowpea were not infected by *M. graminicola*.

Among the different methods of application of *Pseudomonas fluorescens*, dual application through both seed treatment and soil application proved to be highly effective in reducing the damage caused by *M. graminicola* in rice leading to increased growth of seedlings. Increased accumulation of phenols and induction of defense enzymes *viz.*, peroxidase, polyphenoloxidase, phenylalanine ammonialyase and chitinase in response to nematode invasion in roots treated with *P. fluorescens* collectively contributed to induced systemic resistance. Native PAGE analysis revealed strong and unique induction of various isoforms of PO, PPO and SOD in bacterized plants inoculated with *M. graminicola*.

Under field conditions soil application of *P. fluorescens* @ 2.5 kg ha\(^{-1}\) at the time of sowing in the nursery was as effective as application of carbofuran 3G @ 1 kg a.i. ha\(^{-1}\) in reducing the
nematode population and enhancing seedling growth in the nursery. Integration of *P. fluorescens* (2.5 kg ha\(^{-1}\), neem cake (1 t ha\(^{-1}\)) and carbofuran 3 G (1 kg a.i. ha\(^{-1}\)) was found to improve plant growth both in the nursery and main field resulting in increased grain yield. All the three treatments were compatible with each other and caused significant reduction in the population of *M. graminicola*. 
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(B. ANITA)
ABSTRACT


The species of root knot nematode infecting rice in Tamilnadu was identified as *Meloidogyne graminicola*. The life cycle of the nematode from egg to egg was 22 days at a temperature range of a maximum of 32 ±1°C and a minimum of 25±1°C. The roots of infected rice plants were severely galled with characteristically hooked terminal galls and profuse proliferation of roots with numerous hair like lateral growth on the galls. The rice root knot nematode caused about 5.99 per cent avoidable yield loss in rice under field conditions. In addition to rice, some commonly occurring wetland weeds such as *Echinochloa colonum*, *Cyanodon dactylon*, *Panicum repens*, *Fimbristylis miliaceae*, *Ipomea aquatica* and *Eclipta alba* and crop plants such as tomato and brinjal were also recorded as hosts for *M. graminicola*.

Regarding the management of the nematode, dual application of *P. fluorescens* both as seed treatment and soil application was found to significantly enhance plant growth and reduce nematode population. The study also revealed that *P. fluorescens* increased accumulation of phenols and defense enzymes such as peroxidase, chitinase, phenoloxidase and phenylalanine ammonialyase which induced systemic resistance against *M. graminicola* in treated plants. Application of *P. fluorescens* @ 2.5 kg ha\(^{-1}\) in the nursery prior to sowing was found to be as effective as application of carbofuran 3G @ 1kg a.i. ha\(^{-1}\). *P. fluorescens* was also found to be compatible with neem cake and carbofuran 3G, increasing the grain yield by 12.57 per cent under field conditions.
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STUDIES ON THE ROOT-KNOT NEMATODE *Meloidogyne graminicola* GOLDEN AND BIRCHFIELD, 1965 INFECTING RICE IN TAMILNADU

Thesis submitted in part fulfillment of the requirements for the award of the degree of
DOCTOR OF PHILOSOPHY (AGRICULTURE) in PLANT NEMATOLOGY
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By
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2004
CERTIFICATE

This is to certify that the thesis entitled "STUDIES ON THE ROOT-KNOT NEMATODE Meloidogyne graminicola GOLDEN AND BIRCHFIELD, 1965 INFECTING RICE IN TAMILNADU" submitted in part fulfillment of the requirements for the award of the degree of DOCTOR OF PHILOSOPHY (AGRICULTURE) in PLANT NEMATOLOGY to the Tamil Nadu Agricultural University, Coimbatore, is a record of bonafide research work carried out by Mrs.B.ANITA under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

Place : Coimbatore
Date : 02-07-2004

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Place : Coimbatore
Date :
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Dedicated to

My beloved Father Late Dr. J. Bellie

who was a living God to all his countless patients and who now rests with his own
God in His Heavenly abode.
CHAPTER I
INTRODUCTION

Rice *Oryza sativa* L. is the most important cereal crop, which is grown and consumed all over the world (Hawksworth, 1985) and it ranks third after wheat and maize in terms of worldwide production. In India rice occupies one quarter of the total cropped area, contributes to about 40 to 43 per cent of total food grain and continues to play a vital role in national food security (Krishnaiah and Shobharani, 2000).

Plant parasitic nematodes are important pest elements in the rapidly changing rice production system and the pest potential of many nematodes is often under estimated and their damage is misdiagnosed (Coyne and Plowright, 2000). According to estimates out of 346 million hectares of rice grown in the world, around 263 million hectares are infested with damaging levels of plant parasitic nematodes (Hollis and Keo Boonrueng, 1984). It has roughly been estimated that the world wide annual yield loss of rice due to plant parasitic nematodes ranges from 10 to 25 per cent (Jairajpuri and Baqri, 1991).

Over 144 different species of stylet bearing nematodes occur in association with rice culture (Gedder and Smart, 1987). They belong to several groups including ectoparasites on roots, ectoparasites of foliage, migratory endoparasites and sedentary endoparasites of roots.

Root-knot nematodes, *Meloidogyne* spp. are obligate endoparasites having a wide range of hosts including rice. They are reported to infect rice crop growing in well drained, sandy loam, laterite or alluvial soils. These areas occupy 16 per cent of the total acreage of 41 million hectares in India (Panwar and Rao, 1998).

Among them the root-knot nematode, *Meloidogyne graminicola* Golden and Birchfield (1965) is the most widely distributed serious pest of rice in the sub-tropics and tropics and has
been considered economically important in all rice ecosystems (Golden and Birchfield, 1965). It has also been reported from all rice growing regions in India.

The loss in grain yield has been estimated to be 16 to 32 per cent due to *M. graminicola* and may extend up to 50 per cent under severe infestation (Biswas and Rao, 1971; Rao and Biswas, 1973).

Severe crop damage associated with high levels of infestation by root-knot nematode was observed in the rice belt of Cauvery delta in Tamil Nadu and areas adjoining Kerala. Due to decrease in water availability for agricultural use and increase in costs for irrigation and transplanting, many farmers in Tamil Nadu follow wet seeding, delayed flooding and intermittent irrigation. The deviation from the usual cropping pattern may be the major cause for the high buildup of root-knot nematode population in these regions. Therefore it appears important to identify the species of root-knot nematode infecting rice in Tamil Nadu and study the biology and host parasite relationship, which are essential to formulate suitable management strategies.

Alternative cropping systems may be the key for the management of these nematodes, as they prefer monocot crops, while a number of cultivable dicot crops may not support their population. Many weed plants may also serve as alternate hosts for this nematode in the absence of the main crop. Information on host range of rice root-knot nematode among crops and weeds would be of great significance in planning cropping systems in rice ecosystems. The reaction of high yielding rice varieties commercially cultivated in Tamil Nadu to the root-knot nematode ought to be studied for identifying crop varieties suitable for nematode endemic areas.

To reduce the dependence on chemical crop protectants in agriculture, bio-agents, antagonists of pests are receiving increased attention. Resistance inducing rhizobacteria offer an excellent alternative in providing a natural, effective, safe, persistent and durable protection.
However crop protection based on biological agents alone is not always reliable and is seldom as effective as chemical treatments. Hence, different pest management practices may be integrated and combinations of bio-control agents with other complimentary control strategies may further reduce losses due to nematodes.

In view of the increasing importance of root-knot nematodes in rice based cropping systems in the changing agricultural scenario, the present investigation was carried out with the following objectives.

1. Identification of root-knot nematode species infecting rice in Tamil Nadu
2. Biology of rice root-knot nematode *M. graminicola* in rice
3. Histopathology of rice roots infected by root-knot nematode *M. graminicola*
4. Assessment of avoidable yield loss in rice due to *M. graminicola*
5. Reaction of popular high yielding rice varieties to the rice root-knot nematode *M. graminicola*
6. Identification of alternate hosts for the rice root-knot nematode *M. graminicola*
7. Integrated management of rice root-knot nematode *M. graminicola* using bio-control agents, organic amendments and nematicides
CHAPTER II

REVIEW OF LITERATURE

Root-knot nematodes *Meloidogyne* spp. are ubiquitous in the tropics and reports indicate that they constitute an environmental stress to the rice crop (Mian *et al.*, 1999; Greco *et al.*, 2000). Polyphagous populations of *Meloidogyne* have been observed in the rhizosphere of rice roots in many countries. Among the root-knot nematodes infecting rice, *M. graminicola* is recognized as the most important pest of rice, widely distributed in all rice ecosystems.

2.1 Distribution of *M. graminicola*

The rice root-knot nematode *M. graminicola* was first reported by Golden and Birchfield (1965) in the roots of barnyard grass *Echinochloa colonum* L. growing in a field at Baton Rouge, Lousiana. Subsequently it has been reported from different rice growing regions of the world (Table 1). In India, rice root-knot nematode was first isolated in upland soils by Israel *et al.* (1963). The distribution of *M. graminicola* in India is given in table 2.

Table 1. Worldwide distribution of rice root-knot nematode, *M. graminicola*

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**Table 2. Distribution of *M. graminicola* in India**

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<td>3.</td>
<td>Assam</td>
<td>Subramanian and Velayutham, 1983</td>
</tr>
<tr>
<td>6.</td>
<td>Sikkim</td>
<td>Baqri and Ahmad, 2000</td>
</tr>
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</table>
2.2. Biology

The life cycle of *M. graminicola* varies depending on the environment and season. Patnaik (1969) reported that *M. graminicola* took 26 - 51 days to complete its life cycle according to the temperature and climatic conditions prevailing during the year. Incubation of eggs in egg mass or in soil was about 6 - 7 days. Second stage juveniles invaded the roots and established inside the roots in about 5 days. The duration of third stage and fourth stage juveniles was 3 and 7 days, respectively. Males developed in about 3 days and development of egg masses took about 11 days. The life cycle was completed in 24 to 27 days during the early summer months, while the period was extended by 5 days in June, 12 days in October to December and 10 days from July to November. However, in continuous rice culture, nematodes complete 10 - 13 generations annually (Rao and Israel, 1973; Rao *et al.*, 1984a).

*Meloidogyne graminicola* from Bangladesh had a very short life cycle on rice of less than 19 days at temperatures of 22 - 29°C (Bridge and Page, 1982) and an isolate from the USA completed its life cycle in 23 - 27 days at 26°C (Yik and Birchfield, 1979).

Soil temperature of 18 to 23.5°C at 5 cm depth was favourable for root-knot development and from 18.5 to 20.5°C for egg mass production (Prasad *et al.*, 1985). Soomro (1989) reported that second stage juveniles of *M. graminicola* could survive and remain viable in soil without a host plant for up to 5 months at temperatures up to 26°C. Survival of the rice root-knot nematode was greater at lower temperatures (Soomro, 1994) and in flooded soils (Padgham *et al.*, 2003).
2.3. **Host parasite relationship**

Establishment and development of feeding site is typical of the genus. Penetration of *M. graminicola* into roots took a maximum of 41 hours when hypertrophy and hyperplasia began in cortical cells, galls being formed in 72 hours (Patnaik 1969).

Padhi and Patnaik (1988) reported that the second stage juveniles were infective, making their point of entry at the zone of elongation just behind the root cap and occasionally through the root cap, damaged root tip or at the juncture of developing rootlets at 4 hours after inoculation. After penetration, the juveniles entered into the cortex, oriented their bodies parallel to the stele, and moved in the cortical layers of cells in the direction of the apical meristem within 20 - 24 hours after invasion and commenced feeding. Juveniles fixed themselves in the region of the apical meristem 24 - 42 hours after inoculation. Within 42 - 72 hours after inoculation there was extreme hypertrophy of cortical cells accompanied by hyperplasia. At 3 days, a root gall was formed around the site of establishment of the nematode. Giant cells were formed in the stelar region and nematodes fixed their heads around the nearest metaxylem vessel. Mechanical disruption of metaxylem vessels interfered with the uptake of water and nutrients in the seedlings.

*Meloidogyne graminicola* was found to incite five to eight giant cells causing swelling at stele. In the roots, hypertrophy and hyperplasia in meristem, cortex, endodermis and xylem caused gall formation (Jairajpuri and Baqri, 1991).

2.4. **Pathogenecity and yield loss**

The rice root-knot nematode has established as a pest of rice and can cause yield reductions up to 50 per cent under severe infestation (Biswas and Rao, 1971). Rao and Biswas
(1973) reported that inoculation of *M. graminicola* at 1000 to 8000 per plant decreased yield by 2.6 to 8 per cent per 1000 nematodes. The soil borne nematode *M. graminicola* can cause up to 21 per cent yield loss in rainfed or well drained soils throughout the country (Prasad *et al.*, 1987).

Rice cv. IR 36 was severely damaged by *M. graminicola* in pot experiments representing upland conditions. A high initial population density of 80 infective juveniles per ml of soil caused seedling death at 10 days after sowing and 80 per cent of seedlings died after 32 days (Plowright and Bridge, 1990).

Prasad *et al.* (1990) reported high seedling mortality when inoculated with *M. graminicola* at 2-nematodes/g soil in rice varieties CN 492, CR 1018, CR 1030, FR 13A and Jaladhi-1. Plant height was reduced by 5.1 to 19 per cent and 4.2 to 55.4 per cent in the nematode infected plants at 5 and 15 days after submergence.

Growth and development of rice seedlings were adversely affected by *M. graminicola* at different inoculum levels with plant growth decreasing as the nematode inoculum increased. Major differences were recorded 30 days after inoculation when shoot and root weights and the number of tillers were significantly smaller (Soomro and Hague, 1993).

The rice root-knot nematode *M. graminicola* caused yield losses in upland rice and rainfed lowland rice (Jairajpuri and Baqri, 1991; Prot *et al.*, 1994). It was also frequently observed in irrigated rice (Pot and Matias, 1995).

Mian and Khan (1995) observed significantly higher numbers of galls, developing larvae and mature females with eggs in rice roots when inoculated with *M. graminicola* at the rate of 1000 larvae per plant.

**2.5. Symptoms of damage**
The above ground symptoms due to infection by *M. graminicola* are similar to that of other root-knot nematodes. Roy (1973) reported that symptoms appeared as yellowing of the upper portion of the leaves, which gradually extended towards the base. The plants were stunted with few chaffy grains on the panicles. There was profuse proliferation of roots which were, however, very slender and fluffy. Galls developed in a characteristic ring form and there were numerous hair-like lateral growths on the convex side of the curvature. Occasionally, other types of galls, such as spindle shaped, clubbed or nodule like also developed. In addition, symptoms such as smaller tillers, earlier heading and deformed grains were found (Patnaik, 1969). In Laos, the main attack was in nurseries and the seedlings turned yellow, brown and dry out. Galls were formed on upland rice but not been observed on irrigated rice although they appeared as soon as the irrigation water was removed (Fortuner and Merny, 1979).

Infestation by *M. graminicola* affected the entire rice plant producing damage symptoms on foliage and the roots. On the foliage, tip drying was observed. Leaf bronzing developed from the tip downward and from the margin toward the midrib of the leaf blade. Plants were chlorotic. There was considerable distortion in leaf emergence and growth. The leaves were crinkled. New tillers had reduced height. Severely infected plants flowered and matured early. Emerging panicles were crinkled, grain setting was poor and chaffiness was observed in the panicles. Characteristic knots appeared in a string on the fibrous roots. Roots showed profuse development of small, slender, lateral roots, resulting in a hairy root system. Galls were beaded, club or spindle shaped. In cases of heavy infection, they coalesced and formed peculiar shapes. The minimum size galls, which appeared 4 days after inoculation, were 300-500 μm long and 150-450 μm wide. Each gall was as big as 13 mm long and 2.29 mm wide after egg production (Patnaik and Padhi, 1987).
Soomro (1988) and Soomro and Hague (1992) reported significant reduction in the root growth of rice due to *M. graminicola* and the reduction in total root length was related to inoculum density of the nematode.

### 2.6. Nutritional imbalances in rice due to infestation by *M. graminicola*

Infestation of rice by *M. graminicola* caused a reduction in nitrogen, phosphorus, iron and reducing sugars in the shoots and roots. Potassium, magnesium and manganese decreased in shoots and increased in the roots. There was an increase in total sugars, protein, DNA and RNA in the *M. graminicola* infested plants. Infestation by *M. graminicola* caused nutritional disorders limiting the uptake of nitrogen and phosphorus and chlorophyll of leaves and resulted in nutritional disorders within the plants after 45 days (Rao *et al.*., 1986, 1988).

Swain and Prasad (1989) reported that the photosynthetic rate was reduced in *M. graminicola* infected rice plants compared to non-infected plants.

### 2.7. Varietal resistance to *M. graminicola*

There is a considerable literature on testing rice cultivars resistant to *M. graminicola*. Golden and Birchfield (1968) tested 30 cultivars and Manser (1968, 1971) tested 80 cultivars and no resistance was observed in both the cases.

Rao *et al.* (1969) reported that cultivars TKM 6 and Patna 6 were resistant while four others were moderately susceptible. Sampath *et al.* (1970) and Roy (1973) found two resistant cultivars and stated that resistance to *M. graminicola* had a chemical origin.

According to Jena and Rao (1974) the resistance to *M. graminicola* to cultivars Hamsa, IR5, IR47, IR2, Mena Larsali and Bharsia had no chemical origin but was due to the fact that they had a smaller number of roots, a denser rooting system, a sclerified exoderma, a thin cortex
and central cylinder, little phloem, much xylem and a high content of aspartic acid. Element penetration was more rapid and life cycle was shorter in susceptible than in resistant cultivars (Jena and Rao, 1975). Root systems of resistant varieties were characterized by low starch, protein and nitrogen contents, heavy lignification, and high contents of aspartic acid and alanine (Jena and Rao, 1976). Poor giant cell formation resulted in delayed nematode development in the tolerant variety, TKM 6 and the resistant variety, Hamsa showed further inhibition due to cell necrosis (Jena and Rao, 1977).

Rao (1978) reported that resistance to *M. graminicola* can be obtained by selecting hybrids for an increased accumulation of aspartic acid and alanine in the host.

Yik and Birchfield (1979) reported that among the 26 rice cultivars screened for resistance to *M. graminicola*, cultivars LA 110 and Bonnet 73 were resistant based on average root galling of less than 1 per cent. Toride 1, Lebonnet, Bellepatna, PI 338694, Dawn, Magnolia, Toride 2, 55 Starbonnet and I-Geo-T3e were moderately resistant with a range of about 1-10 per cent of galled roots. CL 9835 and Brazos were the most susceptible with 60-70 per cent of the root heavily galled.

The ability of different isolates of *M. graminicola* to infect rice cultivars and develop in the host tissue was examined by Sahu and Chawla (1988). IR 36, MW 10 and CR 190 were resistant to egg mass production by Cuttack and Kerala isolates. Low numbers of 2nd stage larva in the 2nd generation were produced by the Cuttack isolate on MW 10 and CR 190 and by the Kerala isolate on IR 36 and MW 10. Although the Agartala isolate penetrated the tissue of all 5 cultivars, the development of 2nd stage larvae in the 2nd generation in CR 190 was very low.

According to Swain and Prasad (1988) in resistant rice varieties, MW 10, IR 36 and Udaya, there was a significant increase in silica content with age and no such increase was observed in susceptible varieties, Annapurna and Parijat. Later in 1991, they reported that
application of nitrogen fertilizers did not alter the resistance to *M. graminicola*, in resistant varieties. However in the susceptible cultivars, root-knot infestation increased with nitrogen levels up to 80 kg ha\(^{-1}\) but decreased markedly at 120 kg ha\(^{-1}\).

Among the 45 rice varieties screened for their reaction against *M. graminicola*, RP 2542-1639-50, WGL 47978, WGL 47998, RP 2633-59-9-7, OR 621-20-1, TM 10222, RP 2362-110-54-27, MTU 7029 (B), Palghar 1, RP 2151-192-2-5 and RP 2151-178-1-2 were resistant (Mohanty et al., 1993).

Gergon and Prot (1993) reported that *M. graminicola* reproduced on all the wild rice species from Australia, Africa, South and Central America and Asia. *Oryza aurstraliensis* and *Oryza brachyantha* showed the greatest infestation (5855 and 10235 juveniles g\(^{-1}\) root respectively) compared with *Oryza officinalis* which was the lowest with 240 juveniles g\(^{-1}\) root. *Oryza latifolia*, *Oryza ridleyi* and *Oryza rufipogon* also had <500 juveniles g\(^{-1}\) root.

Under glass house conditions, a large prevalence of intermediate reaction (moderate resistance or moderate susceptibility) was observed for fourteen rice varieties namely Aragaria, Bhaiebelle, Guarani, IAC-25, IAC-47, IAC-238, IAC-242, IAC-4440, IAGA 409, IRGA 410, IRGA 413, IRGA 414, Jaguari and Rio Peranaiba to *M. graminicola* in Brazil (Ferraz, 1993).

Kalita and Phukan (1990) reported that of the 50 rice varieties screened against *M. graminicola*, Ilkorasali-2 was highly resistant, Jahinga resistant and Mugisali, Boga bordan, Pankaj and Bor Salpona moderately resistant.

Sahu et al. (1994) reported that the resistance to rice root-knot nematode *M. graminicola* was distributed in the AA and CC genome of the wild species.

The levels of tolerance of rice cultivars to *M. graminicola* vary with water management system. Among fifteen rice cultivars tested for their susceptibility to *M. graminicola* under
flooded conditions, IR 72 was the most resistant; IR 29 was the most susceptible and other cultivars such as IR 36 and IR 74 showed an intermediate response. Yield reductions by more than 20 per cent were observed under rainfed conditions with IR 29 and IR 74. The same cultivars were tolerant and their yield was not affected when they were grown in flooded soil. IR 36 and IR 72 were tolerant under both rain fed and flooded condition (Tandingan et al., 1996).

RAPD analysis conducted on 5 rice cultivars viz., 3 highly resistant (Ramakrishna, Rasi and Kalarata) and 2 highly susceptible (Annapurna and Kiran) to M. graminicola revealed highest number of loci amplification in Ramakrishna variety and lowest number of loci amplification in Annapurna. Kiran showed less polymorphism with resistant cultivars than Annapurna. The highest polymorphism occurred between Annapurna and Ramakrishna. It is suggested that the cross combination Annapurna / Ramakrishna could prove useful for mapping the M. graminicola resistance gene in rice (Bose et al., 1998).

Soriano et al. (1999) reported that all O. sativa entries were susceptible to the rice root-knot nematode. One accession of O. longistaminata and three accessions of O. glaberrima were resistant.

According to Debanand Das (1999) only one cultivar MTC 23/A showed resistance to M. graminicola out of 25 rice cultivars screened.

2.8. Alternate hosts of M. graminicola

Steiner (1934) observed root-knot nematodes on O. sativa and on two weeds associated with rice fields viz., Amaranthus spinosus L. and Echinochloa crusgalli L. Birchfield (1964) found M. graminicola to be primarily a parasite of rice and to prefer hosts such as Echinochloa colonum L., Avena sativa L., Poa annua L., Eleusina indica L. and Aleopecurus L. Phaseolus
*vulgaris* L. was the only reported dicotyledonous host. Many plants such as sweet potato, cucumber, tomato, watermelon and peppers grown in fields infested by *M. graminicola* were not hosts of this nematode.


Yik and Birchfield (1979) observed large terminal and root axil galls in abundance in *E. colonum*. The galled tissues contained mature females, egg masses, males and hatched second stage larvae. The rice root-knot nematode reproduced on two (*Cyperus corymbosus* Rottb. and *Ranunculus pusillus* L.) of the six weed species. Elliptical galls were observed on *R. pusillus* and *C. corymbosus* had spindle shaped galls containing females and egg masses. They also reported that few root galls were formed on five of the 21 crops and on ornamentals. None of the 21 crop plants and ornamental cultivars were considered as hosts because galls were not formed. The larvae were unable to develop or females were unable to reproduce in the swellings. In 1984, *M. graminicola* was found on purple nutsedge, *Cyperus rotundus* L. in Georgia (Minton, 1987).

*Meloidogyne graminicola* was recorded for the first time in Brazil and South America parasitizing roots of 2 *Cyperaceae* species. A preliminary study on its host range showed that lettuce, onion and eggplant were suitable hosts (Monteiro *et al.*, 1988).

The root growth of graminaceous plants *viz.*, *E. colonum*, sorghum and wheat was reduced by *M. graminicola*, with the degree of damage depending on the plant species. Soomro,
1988 suggested that plants with larger root systems and with faster root growth rates might be able to tolerate invasion by *M. graminicola* and thus limit damage.

*Meloidogyne graminicola* formed large number of galls on *E. crusgalli* in Punjab. The other plants sparsely infested by the nematodes were *Zea mays* L. and *Sesbania* (Daincha) (Kaul and Chabra, 1989). It was found on white clover (*Trifolium repens* L.) in a pasture in Oktibbeha County, Mississippi, USA. White clover was an excellent host and was severely galled by the nematode (Windham and Golden, 1990).

Siciliano *et al.* (1990) evaluated the reaction of 40 different plant species, both cultivated and uncultivated in relation to *M. graminicola*. A high prevalence of resistant plants was observed. Among the susceptible hosts were lettuce, eggplant and onion.

*Meloidogyne graminicola*, significantly suppressed root growth of graminaceous plants like wheat, sorghum and *E. colonum*. The largest number of galls and nematodes occurred on sorghum roots (Soomro and Hague, 1992a). Overall growth of wheat and sorghum was effectively reduced by nematode invasion but sorghum suffered greater loss than wheat. Some clumping of laterals around the invaded site occurred, but infested roots of wheat continued to elongate unlike infested sorghum roots, which twisted and ceased elongation (Soomro and Hague, 1992b).

The rice root-knot nematode was found to multiply on *Cyperus deformis* L. and galling mostly occurred near the root tips (Bajaj and Dabur, 2000). The most common banana cultivars in the Philippines (Saba, Lakatan and Latundan) were able to multiply and carry the rice root-knot nematode *M. graminicola* on their roots (Reversat and Soriano, 2002). Observations on plant samples collected from different districts of West Bengal revealed occurrence of *M. graminicola* on *Cyperus* spp., *E. colonum* and cabbage which was a new host record (Khan and Baburam Murmu, 2004).
2.9. **Management of *M. graminicola* in rice**

2.9.1. **Chemical control**

Soaking rice seeds for twelve hours in 500 ppm of oxamyl or phorate or for 24 hours in 1000 ppm of fensulfothion or carbofuran 3G was effective against *M. graminicola* (Prasad and Rao, 1976a). They also reported that soaking seedling roots in 100 ppm of oxamyl effectively controlled *M. graminicola* (Prasad and Rao, 1976b). Application of 50 ppm oxamyl or fensulfothion or 100 ppm of carbofuran 3G and DBCP significantly controlled *M. graminicola* in pots (Prasad and Rao, 1976c).

Oxamyl and propanil (Weedicide) at 50 ppm and above, fensulfothion at 100 ppm, chlorpyrifos, DBCP, phorate and quinalphos at 200 ppm, 2R - 777 and cycocyl (growth regulator) 500 ppm caused maximum death by direct contact. At sub lethal concentrations, these chemicals affected invasion of motile larvae into rice roots. Carbofuran 3G at 100 and 200 ppm exhibited maximum inhibition of larval penetration though it was ineffective as direct contact toxicant. Fensulfothion, oxamyl, propanil and phorate at 50 ppm and 2R-777 at 125 ppm were effective in reducing larval invasion. Exposure of larvae of *M. graminicola* to chemicals at sublethal concentrations affected the rate of invasion and development of the nematodes. Fensulthion, oxamyl, phorate and propanil at 50 ppm and above and carbofuran 3G at 200 ppm showed persistent toxicity (Krishnaprasad and Rao, 1980).

The studies of Krishnaprasad and Rao in 1984 indicated that oxamyl, phorate or carbofuran 3G could be effectively used as foliar sprays at 500 to 1000 ppm in the therapy of *M. graminicola* in rice roots. The inhibition of giant cell development exhibited basipetal movement of the pesticides.
Seedling root dip and soil drenching with phenamiphos, phosphomidon, isofenphos, carbosulfone, carbofuran 3G and chlorpyriphos at 0.02 per cent concentration significantly reduced adult females, egg masses and gall numbers in rice. There was also a significant increase in shoot height due to pesticide application (Prasad et al., 1984).

Carbofuran 3G reduced the number of second stage juveniles of *M. graminicola* in roots upto 12 days; oxamyl was more effective in preventing infective juveniles entering roots for 16 days. After juveniles had invaded the roots, carbofuran 3G and oxamyl were shown to delay development of the female. Carbofuran 3G gave better control at lower temperatures (Mohammad, 1987).

Rahman (1991) reported that carbofuran 3G, aldicarb, ethoprophos and fenamiphos at 1, 3 and 5 kg a.i. ha\(^{-1}\) as pre plant treatment reduced the population of *M. graminicola* in the soil and the percentage of plant infestation, number of galls and mature females in the roots of deep-water rice.

Soaking rice seeds in carbosulfan, monocrotophos, triazophos or phosalone at 0.1, 0.05 or 0.025 per cent reduced the number of galls and egg masses significantly with maximum reduction of 82.06 per cent occurring with carbosulfan at 0.1 per cent (Rahman and Das, 1994).

Panigrahi and Mishra (1995) reported that the pesticides such as carbofuran 3G, phorate, isazophos, cartap, carbosulfan or quinalphos reduced galling at 1 kg a.i. ha\(^{-1}\) or above. The greatest reduction in galling (82 per cent) occurred with carbosulfan at 2 kg a.i. ha\(^{-1}\). Use of carbosulfan 25 EC (0.1 per cent) was effective in reducing the population of *M. graminicola* in rice roots resulting in significant increase in yield (Mohanty et al., 2000).
2.9.2. **Organic amendments**

Amending soil with decaffeinated tea waste and water hyacinth compost reduced root-knot nematode (\textit{M. graminicola}) infestation on rice and increased rice plant growth. Greater efficacy of decaffeinated tea waste appeared to be due to evolution of ammonia, which inhibits hatching of larvae during decomposition (Roy, 1976).

Neem seed at 1 t ha\(^{-1}\) was highly effective in reducing \textit{M. graminicola} nematode infestation in rice cv. Jaya under field conditions resulting in significant increase in the plant growth (Debanand Das \textit{et al}., 1999). Neem oil cake significantly reduced the root-knot nematode severity reducing the population of infective developing larvae as well as males and females with and without eggs. The amendments increased the length and weight of shoot and roots and the growth of rice seedlings (Hossain \textit{et al}., 1999). Neem based products effectively reduced gall number, egg masses and soil nematode population of \textit{M. graminicola} in rice and improved plant growth (Das and Deka, 2002).

2.9.3. **Cultural control**

\textit{Meloidogyne graminicola} is an important parasite of rice and can cause heavy yield loss under upland conditions. It is well adapted to irrigated conditions and shallow flooding does not limit its development. Kinh \textit{et al}.
(1982) and Prot and Matias (1995) reported that flooding appears to favour the development of the root system of rice plants and limit the spread of \textit{M. graminicola} within the root system.

\textit{Meloidogyne graminicola} was poorly adapted to flooded conditions. Its penetration in roots was best when soil moisture is 32 per cent and its development was favoured at moisture of 20 to 30 per cent and by soil dryness at rice tillering and earring (Rao and Israel, 1971, 1972).

Garg \textit{et al}.
(1995) reported that puddling of soil reduced aeration and gave high moisture
levels for prolonged periods, which allowed poor respiration and movement of *M. graminicola*. Moisture regimes with submerged soil for 45 days after transplanting and alternate irrigation, therefore, in puddled and non-puddled fields was best for reducing *M. graminicola* densities and increasing crop performance.

The tolerance of rice cultivars to *M. graminicola* varies with water regime. Early flooding after sowing to limit invasion of roots by the nematode and to promote good establishment of the rice crop, appeared necessary to prevent or minimize yield loss due to *M. graminicola* in irrigated and in wet seeded rice. In addition, continuous flooding until the later stage of rice growth appeared to reduce the increase in nematode population. In areas where water supply was limited it could be more efficient to keep soil flooded at the beginning rather than later in the cropping season (Soriano *et al.*, 2000).

### 2.9.4. Cropping sequence

Rahman (1990) reported that the numbers of *M. graminicola* juveniles were reduced by 85 per cent when mustard and gutzil were mixed sown in winter followed by mixed sowing of deepwater rice with sesame. There was 65 per cent reduction when mustard was grown alone followed by deepwater rice mixed with millet.

Cropping sequences of rice mustard - rice followed by rice - fallow - rice and rice - maize - rice were effective in reducing galls, egg masses and *M. graminicola* population in the roots of rice plant and in soil (Kalita and Phukan, 1995).

### 2.9.5. Biological control

Rhizosphere bacteria, mainly fluorescent pseudomonads have been reported to be antagonistic to nematodes infecting rice (Oostendorp and Sikora, 1989; Spiegel *et al.*, 1991).
Soil application of *P. fluorescens* at 2.5 kg ha\(^{-1}\) along with chitin and neem cake was found to be very effective in reducing rice root nematode, *Hirschmaniella oryzae* and recorded 51 per cent decrease in root population (Swarnakumari, 1996; Swarnakumari and Lakshmanan, 1999). Seed treatment at 10 g kg\(^{-1}\) seed and seedling dip at 2.5 kg per 600 litre of water with *P. fluorescens* increased number of tillers in rice (Sreenivasan, 1998). Application of *P. fluorescens* as seed treatment at a dosage of 10 g kg\(^{-1}\) of seed resulted in maximum bacterial colonization and nematode suppression and increased yield by 13 per cent (Ramakrishnan *et al.*, 1998a).

Significant reduction in the population of *H. oryzae* and increased plant biomass were recorded under field conditions with *P. fluorescens* strain Pf1 isolated from rice rhizosphere. The effectiveness was attributed to intensive root colonizing ability and toxic metabolites produced during microbial decomposition (Sreenivasan *et al.*, 2000a, 2000b). Seed treatment with *P. fluorescens* followed by foliar application of the same at 45, 55 and 65 days after transplanting significantly reduced rice root nematode, *H. gracilis* population at harvest in addition to reduction in chaffiness due to white tip nematode *Aphelenchoides besseyi* (Ramakrishnan and Rajendran, 2002).
CHAPTER III
MATERIALS AND METHODS

3.1. Monoculture of rice root-knot nematode *M. graminicola*

The root-knot nematode isolated from rice roots was multiplied in glasshouse as pure culture on a susceptible rice cultivar, CO 43 and further used for all the glasshouse experiments.

3.1.1. Extraction of nematode eggs

Rice roots heavily infested with *M. graminicola* were collected from the Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore (Plate 1). The galled roots were thoroughly washed and cut into small bits of 1 cm and teased in a blender, shaken in 0.5 per cent sodium hypochlorite solution for two minutes. By passing the liquid suspension of eggs through a 200-mesh sieve rested upon a 500-mesh sieve the eggs were collected. The 500-mesh sieve with eggs was quickly placed under a stream of cold water to remove residual sodium hypochlorite (Barker, 1985). The eggs collected on the sieve were used for inoculation.

3.1.2. Inoculation of nematode eggs

Pre-soaked rice seeds (cv. CO43) were surface sterilized with 0.1 per cent streptomycin sulfate and sown in steam sterilized wetland clay soil (clay 43.2 per cent, silt 8.9 per cent and sand 45.6 per cent) in earthen pots of 5 kg capacity. After 7 days, eggs of *M. graminicola* were inoculated to the rhizosphere. The pots were regularly irrigated and fertilized with NPK. The nematodes thus multiplied were used for various experiments.

3.2. Identification of the root-knot nematode species infecting rice
The species of root-knot nematode infecting rice was identified based on morphological characters of infective juveniles, adult females, perineal pattern and males. The species identity was confirmed by studying the enzyme phenotypes.

3.2.1. *Extraction, processing and measurement of M. graminicola*

Root-knot nematode infected rice roots were collected from the pure culture maintained in the glasshouse. The roots were thoroughly washed free of adhering soil and stained using acid fuchsin lactophenol method (Mc Beth *et al.*, 1941). Adult females were dissected out of the roots and mounted on cavity slides.

Infective juveniles and males were extracted from the roots by modified Baerman funnel method (Schindler, 1961) after macerating the root bits for 15 seconds using a warring blender (Follis, 1943). The males and infective juveniles were fixed in 4 per cent formalin for 24 hours. Later they were processed by Seinhorst's slow method (Seinhorst, 1959), mounted on glass slides with dehydrated glycerol and permanent slides made.

Perineal patterns of 20 adult females collected from fresh rice roots were made as per the procedure given by Hartman and Sasser (1985).

The nematode body measurements and observations on other taxonomic characters were made with an Image Analyzer using Biovis software. Demanian ratios were worked out by following Deman's formula (de Man, 1880).

3.2.2. *Identification of the species using enzyme phenotypes*

The identity of *M. graminicola* was confirmed by studying the enzyme phenotypes as per the procedure given by Esbenshade and Triantaphyllou, 1985.

3.2.2.1. *Protein extraction and Electrophoresis*

Young egg laying females developing on infested roots were selected. The roots were
gently washed free of soil and other organic matter. Twenty female nematodes with medium sized, white egg masses were dissected out of the roots with forceps and placed in chilled extraction medium (Annexure-1). Later they were macerated using a small glass pestle. The crude sample made up to 10µl was transferred to tubes and stored at -10°C until electrophoresis.

Sample tubes containing the nematode extracts were removed from the freezer, placed into an ultra centrifuge and spun at 13000 g for 15 minutes. Centrifugation and electrophoresis were done at -4°C. After centrifugation the clear aqueous phase was drawn out and carefully loaded on to 7 per cent polyacrylamide gels (Sigma, USA). After electrophoresis, esterase and superoxide dismutase phenotypes were visualized by soaking the gels in staining solution.

3.2.2.2. Enzyme Staining
3.2.2.2.1. Esterase (Harris and Hopkinson, 1976)

The staining solution for esterase was prepared as follows:

Potassium phosphate buffer 0.1 m pH 7.2

100 ml EDTA - 30 mg

Fast Blue RR salt - 60 mg

□-Napthylacetate - 40 mg dissolved in 2 ml acetone, added dropwise to the buffer while stirring and vacuum filtered to remove the insoluble material.

The gel was covered with staining solution and incubated at 37°C for approximately one hour. The staining solution was agitated every 10 minutes by slow movement of the dish from side to side, to ensure even staining.

3.2.2.2.2. Superoxide dismutase (Ravindranath and Fridovich, 1975)

\textit{SOD stain solution}

\begin{align*}
\text{TRIS} & \quad - \quad 0.61 \text{ g}
\end{align*}
Water - 100 ml

pH adjusted to 8.2 with HCl

Sodium EDTA - 7.5 mg

Riboflavin - 4 mg

NBT - 10 mg

The gel was submerged in stain solution and incubated at 37°C for 20 minutes in the dark.

3.3. *Biology of root-knot nematode* M. graminicola *in rice*

The life cycle of rice root-knot nematode *M. graminicola* was studied under glass house conditions. Seeds of rice cv. CO 43 were surface sterilized with 0.1 per cent streptomycin sulfate and soaked overnight. Sprouted seeds were sown in plastic containers with steam sterilized wetland clay soil. The seeds were allowed to germinate for one week and later thinned to one seedling per cup at the time of nematode inoculation. Eggs of rice root-knot nematode *M. graminicola* were inoculated to one-week-old rice seedlings maintained in the glass house at the rate of 200 eggs per plant around the root zone and covered with sterilized soil.

After inoculation the seedlings were uprooted at daily intervals, roots stained with acid fuschin lactophenol and observation on the different life stages were made until the life cycle completed.

3.4. *Histopathology of rice root infected by* M. graminicola

Rice roots infected with root-knot nematode *M. graminicola* were collected from the culture maintained in the glass house and thoroughly washed under running tap water. The galled roots were cut into small bits of 1.0 to 2.0 cm length and fixed in formalin-aceto-alcohol (FAA)
for at least 10 days. The fixed root bits were dehydrated following the tertiary butyl-alcohol (TBA) dehydration schedule (Johansen, 1940). The dehydrated tissues were infiltrated with paraffin wax (58°C m.p.) and embedded in the wax blocks of same melting point. Sectioning of paraffin block containing the root tissues was done on a rotary microtome (Spencers, USA make) and ribbons of 15 µ thickness were mounted on glass slides coated with a small amount of Haupt’s adhesive (mixture of 1 g powdered gelatin, 100 ml distilled water, 2 g phenol crystals and 15 ml glycerin). The slides were flooded with 2 per cent formalin and placed on a warming tray held at 35 to 40°C. Staining of microtomed sections of tissues was carried out by following the sass safranin and fast green staining schedule (Sass, 1951) and mounted with DPX mountant (Jensen, 1962).

3.5. Assessment of avoidable yield loss in rice due to *M. graminicola*

A field experiment was conducted in a root-knot nematode, *M. graminicola* infested soil. Plots of size 2 x 2 m² were laid out as per the paired plot technique with 13 replications. The treatments were T₁ - soil application of carbofuran at the rate of 2 kg a.i. ha⁻¹ in the nursery and T₂ - untreated control. The seedlings from the treated and untreated plots were transplanted in the main field in plots of 4x3 m², without any mainfield treatment. Manures and other agronomic practices were applied as per the recommendations followed for the cultivar. Observations on plant growth and yield were recorded in the nursery and main field. The nematode population was assessed as per the standard procedure(Plate 3a).

3.6. Screening high yielding rice varieties for their reaction to *M. graminicola*

Seeds of commercial rice varieties were obtained from the varietal collection of the Paddy Breeding Station, TNAU, Coimbatore. Sprouted seeds were sown in 500g capacity pots filled with sterilized puddled soil at the rate of 5 seeds per pot. Seeds were allowed to germinate and
the seedlings were thinned to one per pot before inoculation. Eggs of root-knot nematode were extracted from infected rice roots and allowed to hatch. The infective juveniles were inoculated at the rate of 200 per plant (Prasad et al., 2000). Five replications were maintained for each variety. Plants were watered regularly and fertilized with recommended levels of NPK. The experiment was terminated 45 days after inoculation of nematodes. Plants were observed for root galling and the grading was done on a 1-5 scale (Heald et al., 1989)

3.7. Studies on the host range of M. graminicola among cultivated crops and weeds

Plants of seven cultivated crops viz., tomato, brinjal, onion, redgram, greengram, blackgram and cowpea were raised in pots with sterilized soil and inoculated with infective juveniles of M. graminicola at 200 per plant. The roots were examined for nematode infection and reproduction 45 days after sowing using standard techniques. Roots of weeds prevalent in rice fields infested with M. graminicola were examined under field conditions. After observing the root galling, the roots were stained with acid fuchsin lactophenol to observe the nematode development and egg laying.

The crops were classified as non-hosts or poor, good or excellent hosts based on the number of galls produced and the development and reproduction of the nematode. Plants having up to 10 galls and females with less than 50 eggs per egg sac were considered poor hosts; with 10 - 50 galls and females with more than 50 eggs per egg sac as good hosts and those with more than 50 galls and females with more than 50 eggs per egg sac were classified as excellent hosts (Gaur and Sharma, 1998).

In the case of weeds, since the population density of nematode varied from spot to spot, at least 20 weed plants of each species were examined and classified for host status based on general assessment of root galling and nematode development and reproduction.
3.8. Studies on the management of rice root-knot nematode *M. graminicola* in rice

3.8.1. Studies on the effect of different methods of application of *P. fluorescens* for the management of *M. graminicola* in rice

Susceptible cultivar CO43 and the population of *M. graminicola* obtained from the culture collection were used for the study. *P. fluorescens* strain Pf1 was obtained from the culture collections of the Department of Plant Pathology, TNAU, Coimbatore. The efficacy of different methods of application of *P. fluorescens* viz., seed treatment, soil application and a combination of both seed and soil treatment for the management of *M. graminicola* was compared with a chemical treatment and an untreated control.

3.8.1.1. Preparation of bacterial inoculum

The bacterial strain was grown in King’s medium B broth under constant shaking at 150 rpm for 48 hours at room temperature (25 ± 2°C). The culture at its stationary phase of growth was centrifuged at 6000 rpm for 10 minutes and bacterial cells were re-suspended in 10 mM phosphate buffer (pH 7.0). The concentration was adjusted to 9 X 10^8 cfu ml^{-1}. Then 2 per cent carboxy methyl cellosolve was mixed with the bacterial suspension as a sticking agent and used as bacterial inoculum (Thompson, 1996) (Plate 2).

3.8.1.2. *Pseudomonas* treatment in glass house and experimental design

For seed treatment, the rice seeds were surface sterilized with 2 per cent sodium hypochlorite solution and soaked in a double volume of bacterial suspension containing 9x10^8 cfu ml^{-1}. After 24 hours the bacterial suspension was drained and the seeds were shade dried for 30 minutes. The seeds were allowed to sprout for another 24 hours before sowing (Vidhyasekaran *et al.*, 1997a). For soil application 25 ml of bacterial suspension (9 x 10^8 cfu ml^{-1}) was poured per pot before sowing.
Eggs of rice root-knot nematode were extracted from root-knot nematode infected rice roots and allowed to hatch. Infective juveniles were inoculated at the rate of 200 per plant before sowing. All the treatments were replicated thrice in a Randomized Block Design.

3.8.1.3. Observations recorded

The experiment was terminated 30 days after planting and observations on shoot growth, root growth and nematode population in root and soil were recorded. Colonization of *P. fluorescens* in roots was also assessed.

3.8.2. Studies on the induction of systemic resistance in rice treated with *P. fluorescens* challenge inoculated with *M. graminicola*

3.8.2.1. *Pseudomonas* treatment and challenge inoculation in glass house

*Pseudomonas fluorescens* isolate Pf1 was used in the defense reaction in rice against root-knot nematode. The treatments included were as follows:

- **T<sub>1</sub>** - *P. fluorescens* - seed treatment + soil application @ 25 ml bacterial suspension containing 9 x 10<sup>8</sup> cfu ml<sup>-1</sup> without challenge inoculation
- **T<sub>2</sub>** - Nematode alone @ 200 IJ plant<sup>-1</sup>
- **T<sub>3</sub>** - *P. fluorescens* (seed treatment + soil application) challenge inoculated with nematode @ 200 IJ plant<sup>-1</sup>
- **T<sub>4</sub>** - Control plants without *P. fluorescens* treatment and nematode inoculation

The treatments were replicated five times in a Randomized Block Design

3.8.2.2. Sample collection for biochemical analysis

Plants were carefully uprooted without causing any damage to root tissues at different time intervals (7, 14 and 21 days after inoculation of nematode). Four plants were sampled from each replication of the treatment separately. Fresh roots were washed in running tap water and
homogenized with liquid nitrogen in a pre-chilled mortar and pestle. The homogenized root tissues were stored in deep freezer (-70°C) until used for biochemical analysis.

3.8.2.2.1. *Estimation of phenol*

Root samples (1 g) were homogenized in 10 ml of 80 per cent methanol and agitated for 15 min at 70°C (Zieslin and Ben-Zaken, 1993). One ml of the methanolic extract was added to 5 ml of distilled water and 250 µl of Folin-Ciocalteau reagent (1/V) and the solution was kept at 25°C. The absorbance of the developed blue colour was measured using a spectrophotometer at 725 nm. Catechol was used as the standard. The amount of phenolics was expressed as µg catechol mg per protein.

3.8.2.2.2. *Assay of peroxidase (PO)*

Root samples (1 g) were homogenized in 2 ml of 0.1 M phosphate buffer, pH 7.0 at 4°C. The homogenate was centrifuged at 16000 g at 4°C for 15 min and the supernatant was used as enzyme source. The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1 per cent H₂O₂. The reaction mixture was incubated at room temperature (28 ± 2°C). The changes in absorbance at 420 nm were recorded at 30s intervals for 3 min. The enzyme activity was expressed as changes in the absorbance min⁻¹ mg⁻¹ protein (Hammerschmidt et al., 1982).

3.8.2.2.3. *Assay of polyphenol oxidase (PPO)*

PPO activity was determined as per the procedure given by Mayer et al. (1965). Root samples (1 g) were homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 16000 g for 15 min at 4°C. The supernatant was used as the enzyme source. The reaction mixture consisted of 200 µl of the enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction 200 µl of 0.01 M catechol was added and the
activity was expressed as changes in absorbance at 495 nm min\(^{-1}\) mg\(^{-1}\) protein.

### 3.8.2.2.4. Estimation of phenylalanine ammonia lyase (PAL) activity

Root samples (1 g) were homogenized in 3 ml of ice cold 0.1 M sodium borate buffer, pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinyl pyrrolidone. The extract was filtered through cheesecloth and the filtrate was centrifuged at 16000 g for 15 min. The supernatant was used as enzyme source. PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nM as described by Dickerson et al. (1984). Sample containing 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1M borate buffer, pH 8.8 and 0.5 ml of 12 m M L-phenylalanine in the same buffer for 30 min at 30°C. The amount of trans-cinnamic acid synthesized was calculated using its extinction coefficient of 9630 m\(^{-1}\) (Dickerson et al., 1984). Enzyme activity was expressed as nmol trans-cinnamic acid min\(^{-1}\) mg\(^{-1}\) protein.

### 3.8.2.2.5. Assay of chitinase

Root samples (1 g) were homogenized in 2 ml of 0.1 M sodium citrate buffer (pH 5.0). The homogenate was centrifuged at 16000 g for 15 min at 4°C and the supernatant was used in the enzyme assay. The colorimetric assay of chitinase was carried out as per the method described by Boller and Mauch (1988). Colloidal chitin (Annexure-1) was prepared according to Berger and Reynolds (1958) from crab shell chitin (Sigma, USA). The reaction mixture consisted of 10 µl of 1 M sodium acetate buffer (pH 4.0), 0.4 ml of enzyme extract and 0.1 ml of colloidal chitin (10 mg). After 2 h incubation at 37°C, the reaction was stopped by centrifugation at 8000 g for 3 min. An aliquot of the supernatant (0.3 ml) was pipetted into a glass reagent tube containing 30 µl of 1 M potassium phosphate buffer (pH 7.1) and incubated with 20 µl desalted snail gut enzyme (Helicase). Finally, the mixture was incubated with 2 ml of dimethyl amino benzaldehyde (DMAB) for 20 min at 37°C and the absorbance was measured at 585 nm. The
enzyme activity was expressed as nmol GlcNAc min\(^{-1}\) mg\(^{-1}\) protein.

### 3.8.2.3. Native-PAGE analysis

The isoform profile of PO, PPO and SOD were examined by discontinuous native polyacrylamide gel electrophoresis (native-PAGE) (Laemmli, 1970). The protein extract was prepared by homogenizing 1 g of root samples in 2 ml of 0.1 M sodium phosphate buffer pH 7.0 and centrifuged at 16000 g for 20 min at 4\(^{\circ}\)C. The protein content of the sample was determined by the Bradford method (Bradford, 1976). Samples (50 µg protein) were loaded onto 8 per cent polyacrylamide gels (Sigma, USA). After electrophoresis, PO isoforms were visualized by soaking the gels in staining solution containing 0.05 per cent benzidine (Sigma, USA) and 0.03 per cent H\(_2\)O\(_2\) in acetate buffer (20 mM, pH 4.2) (Nadlony and Sequira, 1980). For assessing PPO isoforms profile, the gels were equilibrated for 30 min in 0.1 per cent p-phenylene diamine followed by addition of 10 mM catechol in the same buffer (Jayaraman et al., 1987). SOD isoforms were observed by placing the gel in the stain solution (TRIS - 0.61 g; Water - 100 ml; pH adjusted to 8.2 with HCl; Sodium EDTA - 7.5 mg; Riboflavin - 4 mg; NBT - 10 mg) incubated at 37\(^{\circ}\)C for 20 minutes in the dark (Ravindranath and Fridovich, 1975).

### 3.8.3. Nursery management of M. graminicola in rice

A field trial was carried out during kharif 2003 in a heavily infested field at the Paddy Breeding Station, TNAU, Coimbatore, to assess the efficacy of the plant growth promoting rhizobacterium, *P. fluorescens*, neem cake and nematicides for the management of *M. graminicola* in rice (cv.CO47) (Plate 3b).

### 3.8.3.1. Treatment details and Experimental Design

The experiment was conducted in a Randomized Block Design with six treatments and four replications. All the treatments were applied to the nursery at the time of sowing.
The talc-based formulation of *P. fluorescens* was prepared as per the method described by Vidhyasekaran and Muthamilan (1995). At the time of application, the propagule load of the bacterium in the formulation was $9 \times 10^8$ cfu g$^{-1}$ of talc powder. The experiment was terminated 30 days after sowing.

### 3.8.3.2. Observations recorded

Observations on plant growth parameters viz., shoot length, shoot weight, root length, root weight and nematode population in soil and root, root gall index and the number of females and eggs per gram root were recorded as per the standard procedure. Twenty seedlings were selected at random of which ten samples were assessed for plant growth and ten processed for estimation of nematode population in root. Soil samples were collected randomly from each replicated plot and processed for enumeration of nematode population. The mean was calculated for each treatment.

### 3.8.4. Integrated management of *M. graminicola* in rice

A replicated field experiment was conducted in a farmer's field at K.K.Chavadi with heavy incidence of root-knot nematode, *M. graminicola* during rabi 2003-2004 with medium duration rice variety ASD16. The influence of *P. fluorescens*, neem cake and carbofuran 3G and their combinations on the rice root-knot nematode was assessed (Plate 3c).
3.8.4.1. Treatment details and experimental design

\[ \begin{align*}
T_1 & - \quad P.\ fluosescens - \text{soil application @ 2.5 kg ha}^{-1} \\
T_2 & - \quad \text{Neem cake @ 1 ton ha}^{-1} \\
T_3 & - \quad \text{Carbofuran 3G @ 1 kg a.i. ha}^{-1} \\
T_4 & - \quad P.\ fluosescens @ 2.5 \text{ kg ha}^{-1} + \text{Neem cake @ 1 t ha}^{-1} \\
T_5 & - \quad P.\ fluosescens @ 2.5 \text{ kg ha}^{-1} + \text{Carbofuran 3G @ 1 kg a.i. ha}^{-1} \\
T_6 & - \quad \text{Neem cake @ 1 t ha}^{-1} + \text{Carbofuran 3G @ 1 kg a.i. ha}^{-1} \\
T_7 & - \quad P.\ fluosescens - \text{soil application @ 2.5 kg ha}^{-1} + \text{Neem cake @ 1 t ha}^{-1} + \text{Carbofuran 3G @ 1 kg a.i. ha}^{-1}. \\
T_8 & - \quad \text{Untreated control}
\end{align*} \]

The treatments given to nursery at sowing were replicated thrice in a randomized block design. The plot size of the nursery was 2 x 2 m\(^2\) and the main field plot size was 4 x 3 m\(^2\). Recommended dose of fertilizer was applied to the nursery and main field. The experiment was terminated 90 days after transplanting.

3.8.4.2. Observations recorded

Observations on plant growth parameters and nematode population in roots were made at transplanting. In the main field, plant height, tiller number and nematode population were recorded at monthly intervals. Grain yield, straw yield and nematode population in roots were recorded at harvest. Root colonization by \textit{P. fluorescens} was assessed at different growth stages in the nursery and main field. Twenty plants were selected at random of which ten samples were assessed for plant growth and ten processed for estimation of nematode population in root. Soil samples were collected at random from each replicated plot and processed for enumeration of
nematode population. The mean was worked for each treatment.

3.8.4.3. Assessment of bacterial colonization in roots

A direct assay of bacterial colonization of the root tissue was carried out by macerating 1 gm of root segment and through serial dilution. Colony forming units per gram of root was counted on King’s B medium after 48 hours. The composition of King’s B medium (King et al., 1954) is given in annexure.1

3.8.5. Statistical Analysis

The data generated from the glasshouse and field experiments were subjected to statistical analysis following the standard statistical procedures (Gomez and Gomez, 1984).
ANNEXURE I

Composition of King's B Medium

Peptone - 20 gm
Dipotassium hydrogen phosphate - 1.5 gm
Magnesium sulphate - 1.5 gm
Glycerol - 10 ml
Agar - 15 ml
Distilled water - 1000 ml

Extraction medium for nematode enzymes

Glycerol - 20% (w/v)
Triton X - 100 - 2% (w/v)
Distilled water
CHAPTER IV

RESULTS

4.1. Identification of the species of *Meloidogyne* infecting rice:

The root knot nematode species infecting rice in Tamil Nadu was identified as *M. graminicola* based on the morphological characters of adult female, male, infective juvenile and perineal pattern. The species identity was confirmed by studying the enzyme phenotype.

4.1.1. Description of the species:

**Females**: (n = 20) (Fig.1; Plate 4)

**Measurements**: Length = 0.651 mm (0.492 - 0.735); Width = 0.439 mm (0.362 - 0.488); a = 1.48 (1.3 - 1.8); stylet = 11.05 microns (µ) (10.54 - 11.50).

**Description**

Body pearly white, pear shaped with a relatively small neck. Body cuticle annulated. Head not distinctly set off from neck and without annules. Cephalic frame work present but not prominent. Stylet small and delicate with rounded knobs sloping backwards. Oesophagus well-developed with elongate cylindrical procorpus and large rounded metacarpus provided with heavily sclerotized valve. Dorsal oesophageal gland opening 3.5 µ (2.9 - 3.89) posterior to base of stylet. Excretory pore distinct, located one and a half stylet lengths from the base of stylet. Ovaries two prodelphic convoluted. Vulva and anus terminally located. Perineal pattern prominent and egg shaped with distinct striations.
Males : (n=20) (Fig.2; Plate 5b)

Measurements : Length = 1.322 mm (1.050 - 1.392) ; a = 107.4 (82.3 - 196.0) ; stylet = 16.7 µ (16.32 - 17.21).

Description

Body cylindroid, vermiform tapering gradually at both ends. Body width 30.8 µ (25-31.8). Head not clearly set off from the body. Cephalic frame work prominent. Cuticular annulation very distinct, 2.1 µ wide at mid-body. Lateral field consisting of 4 lines. Stylet stout with rounded knobs. Orifice of the dorsal oesophageal gland 3.30 µ (2.9 - 3.52) posterior to the base of the stylet.

Metacorpus elongate with well developed sclerotized valve. Length of oesophagus from anterior end is 212.0 µ (201.0 - 232.0).

Spicules arcuate, 27.91 µ (27.84 - 29.09) long. Testis single, gubernaculum 6.16 µ in length. Tail length 12.50 µ (9.16 - 13.56), phasmids small, post anal located about midway of tail.

Second stage juveniles : (n=20) (Fig. 3; Plate 5a)

Measurements : Length = 0.445 mm (0.423 - 0.456), a = 23.5 (22.5 - 26.7) ; b = 3.2 (2.9 - 3.5) ; c = 5.9 (5.6 - 6.3); stylet = 11.59 µ (11.40 - 12.22).

Description

Body cylindrical vermiform, tapering towards the posterior end. Average body width 11.5 µ. Head not off set from body. Cuticular annulations fine and distinct. Stylet small and delicate with rounded knobs sloping posteriorly. Dorsal oesophageal gland orifice 3.26µ (3.13 - 3.31) posterior to the base of the stylet. Median bulb spherical with prominent sclerotized valve.
Length of oesophagus (from anterior of base of oesophagus) 133 µ (122-138). Tail 70.3 µ (69 - 75.3) long. Hyaline part of tail terminus 18.6 µ (16.0 - 19.2) in length, without regular and distinct annulation. Tail terminus rounded and slightly clavate.

**Eggs**: (n = 20) (Plate 4)

*Measurements*: Length = 99.7 µ (97.6 - 100.5); width = 43.1 µ (42.2 - 46.3); length/width ratio = 2 : 3.

**4.1.2. Confirmation of species identity using enzyme phenotypes**

The root-knot nematode infecting rice in Tamil Nadu was differentiated from the commonly occurring root-knot nematode species, *M. incognita* by the variation in the esterase and superoxide dismutase phenotypes. The population of *M. incognita* exhibited one esterase phenotype while the population of *M. graminicola* had one slow band with a very large drawn out area of esterase activity (Plate 6).

Three phenotypes of superoxide dismutase (SOD) were detected in root knot nematode species *M. incognita* and *M. graminicola*. The banding pattern of the SOD phenotypes varied between *M. incognita* and *M. graminicola* (Plate 6).

**4.2. Biology of *M. graminicola* on rice**

*Biology of the root-knot nematode* *M. graminicola* *in rice cv. CO 43* *was studied under screen house condition.*

Eggs of *M. graminicola* hatched in soil and second stage juveniles were found to penetrate the roots within 48 hours of inoculation. The nematodes entered the root near the root tips. After penetration the juveniles entered into the cortex and oriented their bodies parallel to the stele. Minute swellings were formed on the root tip within 6 hours after penetration of juveniles. Giant cells were formed with hypertrophy and hyperplasia of cells. On the 5th day after
inoculation small galls were formed with juveniles slightly enlarged in size. The growth of the nematode continued and on the 12th day spike tail stage was observed. Young immature females and males were formed between 14-18 days. On the 20th day fully developed females and males were present within the root. Within two days after formation of adult females, eggs were laid within the roots (Plate 7).

The life cycle of the rice root-knot nematode from egg to egg stage was 22 days under glass house conditions at a temperature ranging between a maximum of 32 ± 1°C and a minimum of 25 ± 1°C (Table 3).

4.3. Histopathological changes in rice root caused by *M. graminicola*

Studies on histopathological changes in rice root infected by *M. graminicola* revealed the presence of all the developmental stages of the nematode including eggs, infective juveniles, immature females and adult females within the root. Eggs were laid in a gelatinous matrix with egg masses embedded in the cortical region (Plate 9 i,j,k). In old roots eggs were released from the roots by breaking the epidermal layer (Plate 8b). In young roots eggs hatched within the root (Plate 8a). The juveniles or immature females remained in the maternal gall or migrated intercellularly through the aerenchymatous tissues of the cortex to new feeding sides within the same root. Due to inter and intra cellular migration of larvae in root cortex, disruption and hypertrophy of cells occurred (Plate 8d). Juveniles gained access to the protophloem points by damaging the cells of pericycle. At points of larval establishment in stele, abnormal xylem proliferated around the giant cell causing swelling of vascular cylinder (Plate 8e).

Anaplasia originated in the cells adjacent to the head of the juvenile still in the second stage. Cell wall dissolution and coalescence of cells to form multinucleate giant cells occurred. Hyperplastic protoxylem cells all around the giant cell separated them from adjacent cells. Enlargement of giant cells was by intrusive growth (Plate 8f). As the endoparasites continued to
feed and develop, the giant cell became vacuolated until empty by which time, the endoparasite developed into ovipositing females (Plate 9 l).

Many adult nematodes were found to feed at a single point of infection, with their heads in the periphery of the vascular parenchyma tissue and the remaining portion of their bodies in the cortex (Plate 9 i). There was extreme hypertrophy of the cortical cells accompanied by hyperplasia leading to the formation of a group of giant cells with condensed granular cytoplasm (Plate 8 f). The number of giant cells ranged between 7 to 9. Hollow cavities confined to the cortex were formed around the giant cells. Cross sections of galled roots revealed the presence of 3 to 4 groups of young and old giant cells (Plate 9 j). The freshly formed giant cells were rich in cytoplasm and multinucleate. The old giant cells were vacuolated devoid of cytoplasmic contents (Plate 9 l). Thickening of cell wall was observed around the giant cells. Formation of giant cells caused the blockage of xylem vessels and sieve tubes.

4.4. **Assessment of avoidable yield loss in rice due to root-knot nematode, M. graminicola**

A trial was conducted in a field heavily infected with *M. graminicola* to assess the avoidable yield loss in rice cv. ASD 16 due to *M. graminicola*. The results indicated that the grain yield from plants treated with carbofuran 3G@ 2kg a.i. ha$^{-1}$ in the nursery was 5109.42 kg ha$^{-1}$ compared to 4803.48 kg ha$^{-1}$ from untreated plants leading to 5.99 per cent avoidable loss in yield due to *M. graminicola*. The rice root knot nematode caused significant reduction in seedling weight and plant height in the nursery and main field. The weight of seedlings from protected nursery plots was 7.11 gm (Plate 10a) compared to 3.48 gm in the case of unprotected nursery plots (Plate 10b) accounting for 51.06 per cent reduction due to the rice root knot nematode (Fig.4b). The plant height in the main field was also reduced by 45.57 per cent and 31.11 per cent respectively after 30 and 60 days of planting (Table 4; Fig.4 a, b)
Regarding the nematode infestation significantly higher population of *M. graminicola* was recorded in the root samples during seedling stage. The nematicidal treatment reduced the population of nematodes in roots by 44.49 per cent and 52.09 per cent respectively after 30 and 60 days of planting. The root-knot index at transplanting was 4 in untreated plants compared to 2 in treated plants. Similarly at harvest the gall index was significantly higher (4) in unprotected plants than the gall index in protected plants (2) (Table 5; Fig.4c).

4.5. **Screening of popular high yielding rice varieties for their reaction to *M. graminicola***

A total of 21 high yielding rice varieties were screened against the infection of *M. graminicola* and evaluated based on the percentage of root system galled.

All the 21 varieties screened were found either susceptible or highly susceptible to the rice root-knot nematode, *M. graminicola*, with gall index value ranging between 4 and 5 (Table 6).

More than 50 per cent of the root system was galled in all the varieties tested 30 days after inoculation of infective juveniles @ 200 per plant. Galls developed in a characteristic hook form with numerous hair-like lateral root growths. There was profuse proliferation of roots giving a puffy appearance.

4.6. **Studies on the host range of rice root-knot nematode, *M. graminicola* among cultivated crop and weeds**

The host range of the rice root-knot nematode, *M. graminicola* was studied under greenhouse and field conditions. Weeds commonly occurring in rice fields were observed under field conditions for their host status to *M. graminicola*. A total of twenty weed plants were
collected from a field with heavy incidence of *M. graminicola* and the roots were observed for
galls and nematodes (Table 7).

Among the 20 weed plants screened 11 were identified as hosts of *M. graminicola*. Roots
of the infected weeds exhibited hook like or spindle shaped elongate galls, characteristic to the
rice root knot nematode. The galled roots contained all stages of root-knot nematode including
eggs, juveniles and adults.

Most of the graminaceous weeds were good or excellent hosts. The most notorious weeds
in rice fields *viz.*, *E. colonum* and *C. dactylon* were identified as excellent alternate hosts of *M.
graminicola* (Plate 11). The roots exhibited severe galling symptoms with a very high population
of nematodes. The number of galls per plant was more than 50 with females having more than 50
eggs per egg sac. *Ammania baccifera* and *I. aquatica* were classified as good hosts with 10 to 50
galls per plant (Plate 11). Other weeds *viz.*, *P. repens, C. barbata* (Plate 12), *G. decumbens, E.
alba, E. prostrata* and *F. miliaceae* (Plate 13) were classified as poor hosts with less than 10 galls and
females with less than 50 eggs per egg sac.

The observation on root galling and reproduction revealed that the vegetable crops,
tomato and brinjal were highly preferred by *M. graminicola* while onion was not infected. None
of the pulse crops *viz.*, blackgram, greengram, redgram and cowpea screened were infected by
*M. graminicola*. The roots of tomato and brinjal exhibited characteristic hook shaped, elongated
galls with a high density of nematode population(Plate 14). All stages of *M. graminicola*
including eggs were present in the galled roots (Table 8).

### 4.6. Management of *M. graminicola* in rice

#### 4.6.1. Effect of different methods of application of *P. fluorescens* for the management of
*M. graminicola* in rice (Plate 15)
4.6.1.1. Plant growth parameters

4.6.1.1.1. Shoot length: Application of the plant growth promoting bacterium, *P. fluorescens* as seed treatment @ 25 ml bacterial suspension containing $9 \times 10^8$ cfu/ml or as soil treatment @ 25 ml bacterial suspension ($9 \times 10^8$ cfu/ml) or as a combination of both seed and soil treatment significantly enhanced the shoot growth by 116.49, 135.67 and 179.77 per cent respectively. Combined application of *P. fluorescens* (seed + soil) was the best in enhancing shoot growth which was followed by carbofuron 3G @ 1 kg a.i. ha$^{-1}$ (Table 9; Fig.5a).

4.6.1.1.2. Shoot weight: The highest shoot weight (0.34 g) was observed in plants treated with a combination of seed and soil application of *P. fluorescens*, whereas the untreated plants recorded a shoot weight of 0.11 g, accounting for 209.09 per cent increase. Pseudomonas treatment was equal to the nematicidal treatment with carbofuran 3G @ 1 kg a.i. ha$^{-1}$ in enhancing the weight of seedlings. However the combination treatment (seed + soil application) was on par with soil application alone (Table 9; Fig.5b).

4.6.1.1.3. Root length: Significant increase in root length was observed in *P. fluorescens* treated plants compared to untreated control. Combined application of *P. fluorescens* as seed treatment followed by soil treatment was the best recording an increase of 74.79 per cent in root length compared to untreated control. Application of carbofuran 3G @ 1 kg a.i. ha$^{-1}$ was the next best treatment with 52.23 per cent increase in root length. Soil application *P. fluorescens* was superior to seed treatment, recording 33.01 per cent increase in root length compared to untreated control (Table 9; Fig.5a).

4.6.1.1.4. Root weight: The mean fresh root weight of rice seedlings treated with *P. fluorescens* as seed treatment, soil application or as a combination of both was 0.27g, 0.30 g and 0.41 g respectively. Soil application of carbofuran 3G @ 1 kg a. i. ha$^{-1}$ increased the root weight by 140 per cent compared to untreated control plants. Dual application of the bacterium as seed
and soil treatment was the most effective treatment in enhancing the root weight followed by the nematicidal treatment (Table 9; Fig.5b).

### 4.6.1.2. Nematode population

#### 4.6.1.2.1. Root nematode population: In general all the treatments recorded reduction in the population of adult females and eggs in the root. The highest reduction of 60.18 per cent and 57.10 per cent was observed in the combination treatment with *P. fluorescens* applied through seed and soil. The next best treatment was soil application of *P. fluorescens* which registered 51.13 per cent and 48.47 per cent reduction in the number of females and eggs respectively. Root gall index ranged between 1 and 3 in the plants treated with *P. fluorescens* while untreated plants had a gall index of 5 (Table 10; Fig.5c,d).

#### 4.6.1.2.2. Soil nematode population: Soil application of *P. fluorescens* at the time of sowing resulted in significant reduction (53.03 per cent) in soil nematode population which was closely followed by the dual application of *P. fluorescens* of seed and soil treatment (51.97 per cent). Both these treatments were on par with the chemical carbofuron 3G @ 1 kg a.i. ha$^{-1}$ in reducing the nematode population in the soil. Seed treatment with *P. fluorescens* was least effective. However, it was found superior to untreated control (Table 10; Fig.5c).

### 4.6.1.3. Root colonization of *P. fluorescens*

Observation of rice roots treated with *P. fluorescens* for root colonization revealed significantly higher number of colony forming units ($36.75 \times 10^8$ g$^{-1}$) in plant roots applied with *P. fluorescens* as seed treatment and soil application simultaneously. Soil application of *P. fluorescens* was also found to increase the root colonization ($25.75 \times 10^8$ cfu g$^{-1}$). The least number of colonies ($16.00 \times 10^8$) were observed in roots which were applied with *P. fluorescens* as seed treatment alone (Table 10).
4.6.2. Induction of systemic resistance in rice treated with *P. fluorescens* challenge inoculated with *M. graminicola*

The results of the study on the induction of systemic resistance by *P. fluorescens* challenge inoculated with *M. graminicola* in rice cv. CO 47 under glass house condition are presented in Table 11.

**4.6.2.1 Phenols**

Studies on induction of defense mechanisms revealed higher accumulation of phenolics in bacterized rice roots challenge inoculated with *M. graminicola*. Accumulation of phenolics was observed seven days after challenge inoculation with nematode. The highest accumulation (2.23 µg catechol mg\(^{-1}\) protein) was observed on the 14\(^{th}\) day after challenge inoculation. Plants inoculated with the nematode alone also recorded increased accumulation of phenolics upto 7 days (1.79 µg catechol mg\(^{-1}\) protein) but declined 14 days after inoculation (1.77 µg catechol mg\(^{-1}\) protein). There was no marked change in plants treated with *P. fluorescens* alone during the time course of experimental period but the accumulation of phenolics was higher compared to untreated control (Fig.6a).

**4.6.2.2 Peroxidase (PO)**

Rice roots treated with *P. fluorescens* isolate Pf 1 upon challenge inoculation with *M. graminicola* expressed higher activity of peroxidase compared to either *P. fluorescens* alone or nematode alone. The highest activity of (5.85) expressed as change in absorbance min\(^{-1}\) mg\(^{-1}\) protein was observed 21 days after nematode inoculation. Bacterized plants without challenge inoculation with nematode also recorded increased peroxidase activity compared to untreated plants. The plants inoculated with the nematode alone expressed increased activity of peroxidase,
7 days after inoculation. The activity decreased after 14 days with the lowest activity recorded 21 days after inoculation (Fig. 6b)

4.6.2.3. Polyphenol oxidase (PPO)

Upon root-knot nematode, *M. graminicola* challenge in bacterized paddy root tissues, the activity of PPO gradually increased and was the highest, 21 days after inoculation. Plants inoculated with nematode alone had comparatively less PPO activity. In bacterized rice plants without nematode inoculation PPO activity was the highest 7 days after treatment. The activity declined gradually with the lowest activity observed 21 days after treatment. The untreated uninoculated control plants expressed the lowest activity of PPO (Fig. 6c).

4.6.2.4. Phenylalanine ammonialyase (PAL)

The highest activity of PAL was observed in bacterized root tissues challenged with the nematode on the 14th day after inoculation. Rice roots inoculated with the nematode alone recorded increased activity of PAL but the induction gradually declined after 7 days of inoculation. PAL activity also increased in *P. fluorescens* treated root tissues without challenge inoculation with *M. graminicola* but remained unchanged during the experimental period (Fig.7a).

4.6.2.5. Chitinase

The activity of chitinase was maximum in bacterized root tissues challenged with the nematode and the highest activity was recorded 21 days after inoculation. The root-knot nematode *M. graminicola* also increased the activity of chitinase in rice roots up to 7 days after inoculation and the activity was less on the 14th day and 21st day after inoculation. Bacterized plants without challenge inoculation with nematode also expressed higher activity of chitinase compared to untreated control (Fig.7b).
4.6.2.6. Isoforms of PO, PPO and SOD in rice root tissues treated with *P. fluorescens* challenge inoculated with *M. graminicola*

Native PAGE analysis revealed that peroxidase isoforms designated as PO1 to PO5 were observed in *P. fluorescens* treated root tissues challenged with the root-knot nematode *M. graminicola* and the expression of isoforms PO2, PO3, PO4 and PO5 were more prominent in bacterized plants challenge inoculated with the nematode compared to other treatments. The expression of PO4 and PO5 isoforms was more prominent in healthy plants and plants treated with *P. fluorescens* challenged with the nematode (Plate 16).

Five PPO isoforms, PPO1, PPO2, PPO3, PPO4 and PPO5 were observed in bacterized rice root tissues, inoculated with root-knot nematode. The induction of isoforms PPO1 and PPO2 were observed in all the treatments except in healthy plants. Isoforms, PPO3 and PPO4 were detected both in bacterized root tissues and root knot infected roots but it was more prominent in root tissues treated with *P. fluorescens* challenge inoculated with the nematode (Plate 17).

Seven isozymes of superoxide dismutase could be detected in *P. fluorescens* treated plants, inoculated with *M. graminicola*. Out of the 7 isozymes (SOD 1 - SOD 7) 4 isozymes viz., SOD 1, SOD 3, SOD 6 and SOD 7 were also detected in *M. graminicola* infected root tissues without treatment with *P. fluorescens*. However, the intensity was less, compared to the plants treated with *P. fluorescens*. All the seven isoforms of SOD appeared with a high intensity in bacterized plants challenge inoculated with the nematode. SOD 2 and SOD 4 were two newly induced isoforms expressed in rice root tissues by this treatment (Plate 18).

4.6.3. *Nursery management of M. graminicola in rice* (Plates 19 & 20)

The results of the nursery field trial on the management of *M. graminicola* in rice cv. CO 47 with *P. fluorescens* as seed treatment (10 g kg$^{-1}$ seed) and soil application (2.5 kg ha$^{-1}$), neem cake(1 t ha$^{-1}$), carbofuran 3G (1 kg a.i. ha$^{-1}$) and phorate 10 G (1 kg a.i. ha$^{-1}$) are presented in
4.6.3.1. **Plant growth**

**4.6.3.1.1. Shoot length**: All the treatments enhanced the shoot length of rice by 16.17 to 56.23 per cent, with the highest shoot length of 25.7 cm observed in seedlings treated with carbofuran 3G. This was followed by soil application of *P. fluorescens* or phorate 10 G with 39.70 and 39.51 per cent increase in shoot length respectively compared to untreated control. Seed treatment with *P. fluorescens* resulted in 30.75 per cent in shoot length followed by neem cake which recorded 16.17 per cent increase in shoot length (Fig. 8a).

**4.6.3.1.2. Shoot weight**: Soil application of chemical nematicides carbofuran 3G or phorate 10G increased the shoot weight by 53.85 per cent compared to untreated control. The next effective treatment was either soil application of *P. fluorescens* or neem cake, with 38.46 per cent increase in shoot weight (Fig.8b).

**4.6.3.1.3. Root length**: Application of *P. fluorescens* as soil treatment before sowing was on par with soil application of granular nematicides carbofuran 3G and phorate 10G in enhancing the root length of rice seedlings. The highest root length of 10.38 cm was recorded in carbofuran 3G treatment followed by *P. fluorescens* (9.9 cm) accounting for 106.36 and 96.8 per cent increase respectively, compared to untreated control (Fig.8a).

**4.6.3.1.4. Root weight**: All the treatments were found to increase the root weight by 50 to 130.67 per cent compared to untreated control. Application of *P. fluorescens* as soil treatment was on par with nematicides carbofuran 3G and phorate 10G recording the highest percentage increase (130.67 per cent) in root weight. Seed treatment with *P. fluorescens* recorded 50 per cent increase in root weight while neem cake recorded an increased root weight of 54 per cent compared to untreated control (Fig.8b).
4.6.3.2. Nematode population in roots

4.6.3.2.1. Number of females: The population of adult females of rice root knot nematode, *M. graminicola* in the roots of rice seedlings was reduced by 38.46 -61.54 per cent due to the treatments applied in the soil. The highest reduction in number of females (61.54 per cent) was recorded in roots treated with soil application of either *P. fluorescens* or carbofuran 3G. Seed treatment with *P. fluorescens* was on par with soil application of neem cake or phorate 10G, recording 38.46, 45.79 and 43.96 per cent reduction respectively in the number of females within the root system. The gall index ranged between 3 and 4 in treated plants compared to 5 in untreated plants (Fig.8c).

4.6.3.2.2. Number of eggs: Soil application of carbofuran 3G was the best treatment in decreasing the number of eggs (44.26 per cent) within roots of rice seedlings followed by *P. fluorescens* soil treatment with 35.12 per cent reduction. The lowest reduction (11.17 per cent) in egg number was recorded in seedlings applied with *P. fluorescens* as seed treatment. The roots of untreated seedlings had the highest number of eggs (908.3 egg g⁻¹ root) (Fig.8d).

4.6.3.3. Nematode population in soil

The number of infective juveniles in soil was highest (180/100 cc soil) in untreated nursery plots. There was 34.28 to 55.67 per cent reduction in soil population in soil treated with *P. fluorescens*, neem cake, phorate 10G and carbofuran 3G compared to untreated control, with the highest reduction observed in plots treated with carbofuran 3G (Fig.8c).

4.6.4. Integrated management of *M. graminicola* in rice cv. ASD 16

Observations recorded on the plant growth and nematode population in a field trial conducted on the integrated management of *M. graminicola* in rice cv. ASD 16 with *P. fluorescens* @ 2.5 kg ha⁻¹, neem cake @ 1 t ha⁻¹ and carbofuran 3G @ 1 kg a.i. ha⁻¹ either alone
or in different combinations are given in tables 13 to 18.

4.6.4.1. **Observations in nursery beds** (Plate 21)

4.6.4.1.1. **Shoot length**: There was a significant increase in shoot length (45.58 to 76.50 per cent) of rice seedlings treated with *P. fluorescens*, neem cake and carbofuran 3G either alone or in combination. Simultaneous application of *P. fluorescens*, neem cake and carbofuran 3G recorded the highest shoot length of 28.77 cm followed by 27.83 cm in seedlings treated with neem cake and *P. fluorescens* together (Table 13; Fig.9a).

4.6.4.1.2. **Shoot weight**: Rice seedlings which received the combination of all the three treatments viz., *P. fluorescens*, neem cake and carbofuran 3G registered the highest shoot weight of 0.2 g, accounting for 133.33 per cent increase compared to untreated control. Dual application of neem cake and carbofuran 3G recorded 111.11 per cent increase in shoot weight, which was on par with *P. fluorescens* either alone or *P. fluorescens* in combination with neem cake or carbofuran 3G (Table 13; Fig.9b).

4.6.4.1.3. **Root length**: Root length increased by 194.6 per cent when *P. fluorescens*, neem cake and carbofuran 3G were applied in combination. The next best treatment was the combined treatment with *P. fluorescens* and neem cake registering 170.85 per cent increase in root length compared to untreated control. All the treatments when applied either alone or in combination were found to significantly increase the length of the roots (Table 13; Fig.9a).

4.6.4.1.4. **Root weight**: All the treatments significantly increased the weight of roots by 30 to 90 per cent. Among the treatments, combined application of *P. fluorescens*, neem cake and carbofuran 3G was the best with the highest root weight of 0.19 g followed by the combination treatment with *P. fluorescens* and carbofuran 3G (0.17 g). Application of carbofuran 3G either
with P. fluorescens or neem cake was the best treatment registering 60 per cent increase in root weight which was on par with P. fluorescens soil application alone (Table 13; Fig.9b).

4.6.4.1.5. Number of females: Roots of seedlings treated with P. fluorescens, neem cake and carbofuran 3G in combination had the least population of M. graminicola females (49) compared to 115.67 females in roots of seedlings which did not receive any treatment. This was followed by application of P. fluorescens and neem cake together recording 55.04 per cent reduction. Neem cake when applied alone without P. fluorescens or carbofuran 3G registered only 14.13 per cent reduction in nematode population. However when it was applied together with P. fluorescens or carbofuran 3G there was 48.13 per cent and 49.28 per cent reduction in root population respectively compared to untreated plants (Table 14; Fig.9c).

4.6.4.1.6 Number of eggs: All the treatments viz., P. fluorescens, neem cake and carbofuran 3G when applied either alone or in combination were significantly different in decreasing the population of eggs in roots. Among the treatments highest reduction in the number of eggs (59.69 per cent) was observed in the combination treatment with P. fluorescens, neem cake and carbofuran 3G followed by 54.37 per cent reduction in roots of seedlings treated with P. fluorescens and carbofuran 3G. Application of carbofuran 3G alone without any combination recorded 53.3 per cent reduction in egg number compared to untreated control. The gall index ranged from 2 to 4 in treated plants whereas untreated plants recorded a gall index of 5 with more than 100 galls per plant (Table 14; Fig.9c).

4.6.4.1.7. Nematode population in soil: The highest reduction of 46.11 per cent in the population of infective juveniles was recorded when all the three treatments were combined. Application of neem cake along with carbofuran 3G or P. fluorescens decreased the soil nematode population by 40.22 and 38.33 per cent respectively. There was 33.5 to 34.05 per cent
reduction in the population of infective juveniles in soil when the treatments were applied separately (Table 14; Fig.9c).

4.6.4.2. Observations in the transplanted field (Plate 22)

4.6.4.2.1. Plant growth and tillering (30DAP)

Nursery application of *P. fluorescens*, neem cake and carbofuran 3G either alone or in combination significantly enhanced the plant height by 45.87 to 72.1 per cent compared to untreated control. The highest plant height (41.53 per cent) was recorded in the combination treatment with *P. fluorescens*, neem cake and carbofuran 3G, while untreated plants registered 24.13 cm height (Table 15; Fig.10a).

The number of tillers / hill was the highest (26.67) in the plants which received all the three treatments *viz.*, *P. fluorescens*, neem cake and carbofuran 3G compared to 8.33 in untreated plants, accounting for 220.17 per cent increase. The next best treatment was dual application of *P. fluorescens* and neem cake (26 tillers /hill) (Table 15; Fig.10a).

4.6.4.2.2. Nematode population in roots (30DAP)

The number of eggs and females in roots of treated plants (*P. fluorescens* + neem cake + carbofuran 3G) were reduced by 29.12 per cent and 33.46 per cent respectively compared to untreated control. Combined application of neem cake and carbofuran 3G was the next best treatment recording 28.46 per cent reduction in females. Combined application of all the three treatments was statistically on par with combinations involving any two treatments in reducing the nematode population in roots. Gall indices ranged between 1 and 2 in treated plants while untreated plants registered a gall index of 3 (Table 10; Fig.10c).
4.6.4.2.3 Plant growth and tillering (60 DAP)

Nursery application of *P. fluorescens*, neem cake and carbofuran 3G had a significant effect on the plant height 60 days after planting. The highest plant height of 73.63 cm was recorded in the combination treatment compared to 55.1 cm in untreated plants. The number of tillers was the highest (27) when *P. fluorescens* and neem cake were applied together compared to 10.33 in untreated plants (Table 16; Fig.10a).

4.6.4.2.4. Nematode population in roots (60 DAP)

The population of adult females and the number of eggs in the roots of rice plants which were treated with *P. fluorescens*, neem cake and carbofuran 3G as a combination was the lowest recording 25.39 and 14.15 per cent reduction respectively compared to plants which did not receive any treatment in the nursery. The next best nursery treatment was dual application of *P. fluorescens* and carbofuran 3G with a per cent reduction of 20.15 in the number of females in roots (Table 16; Fig.10c).

The gall index ranged between 1 and 2 in nursery treated plants and 3 in untreated plants. The number of galls were minimum in the combination treatment with *P. fluorescens*, neem cake and carbofuran 3G and with *P. fluorescens* and carbofuran 3G together.

4.6.4.2.5. Grain yield

Soil application of *P. fluorescens* along with neem cake and carbofuran 3G in the nursery at the time of sowing enhanced the yield of rice to the tune of 14.22 per cent compared to untreated control. The next best treatment was the combination of *P. fluorescens* and carbofuran 3G with 12.57 per cent increase in grain yield followed by *P. fluorescens* and neem cake application with 11.49 per cent increased yield. The lowest yield of 5250 kg ha$^{-1}$ was
recorded in plants treated with neem cake alone without *P. fluorescens* or carbofuran 3G (Table 17; Fig.10b).

Grain weight was significantly more (34.43 per cent) in the combination treatment with *P. fluorescens*, neem cake and carbofuran 3G followed by the combination of and *P. fluorescens* and carbofuran 3G with 33.81 per cent increase in grain weight. The other treatments recorded 12.09 to 27.47 per cent increase in grain weight.

4.2.4.2.6. *Straw yield*

The highest straw yield of 6108.33 kg ha\(^{-1}\) was recorded in plants treated with neem cake and carbofuran 3G together in combination followed by the integration of all the three treatments *viz.*, *P. fluorescens*, neem cake and carbofuran 3G (6025 kg ha\(^{-1}\)). Soil application of *P. fluorescens* along with neem cake in the nursery was the least effective treatment recording only 1.97 per cent increase in straw yield compared to untreated control (Table 17; Fig.10b).

4.6.4.2.7. Nematode population in roots (at harvest)

The population of females in the roots of nursery treated plants was reduced by 3.87 to 12.56 per cent compared to plants without any nursery treatment. However, there was no significant difference in the number of females in the root system between treated and untreated plants at the time of harvest.

There was a significant decrease in the number of eggs in the roots between treated and untreated plants. The number of eggs per gram root ranged between 172.33 to 202.00 in the treated plants while untreated plant roots had a population of 216.67 eggs per gram of root at the time of harvest (Table 17; Fig.10c). Root gall indices ranged between 2 and 3 in treated plants compared to 4 in untreated plants.
4.6.4.2.8. Root colonization by *P. fluorescens*

Colonies of *P. fluorescens* were observed in roots of plants treated with the talc based formulation of *P. fluorescens* either separately or along with carbofuran 3G and neem cake in the nursery. There was no significance difference in the number of colony forming units in the seedlings between the individual and combination treatment. Root colonization was the highest (35.67 x 10^{8} cfu g^{-1}) in plants treated with *P. fluorescens* in combination with neem cake, 30 days after planting, which was on par with *P. fluorescens* alone (35.33 x 10^{8} cfu g^{-1}). Combined application of *P. fluorescens*, neem cake and carbofuran 3G recorded the highest number of colonies of *P. fluorescens* (49.33 x 10^{8} cfu g^{-1}) 60 days after planting and during harvest (51.67 x 10^{8} cfu g^{-1}) (Table 18; Fig.11).
5.1. **Identification of the species of *Meloidogyne* infesting rice in Tamil Nadu**

The root-knot nematode infesting rice in Tamil Nadu was identified as *M. graminicola* based on morphological and taxonomic characters of adult female, male, infective juveniles, perineal pattern, enzyme phenotypes and the symptoms observed in roots.

The morphological characters and dimensions were within the range of variations given in the original descriptions by Golden and Birchfield, 1965. The root-knot nematode infecting rice in Tamil Nadu was diagnosed as *Meloidogyne graminicola* by the characteristic egg shaped perineal pattern with distinct striations; short delicate female stylet less than 12 μ; orifice of dorsal oesophageal gland closer (3.5 μ) to the base of the stylet; length of stylet in males less than 18 μm; dorsal oesophageal gland orifice to base of stylet less than 3-4 μm; thin and long stylet in second stage juvenile; second stage juvenile tail tapering to a long, narrow hyaline terminus.

The identity of the species was confirmed by studying the esterase and superoxide dismutase phenotypes. Enzyme phenotypes, especially those of esterases and superoxide dismutase are species specific and helpful in the taxonomic characterization for identification of most major and several minor *Meloidogyne* species (Esbenshade and Triantaphyllou, 1985; Carneiro et al., 2000. The present study is in confirmation with the previous report of presence of one slow band of esterase with a large drawn out area of enzymatic activity in *M. graminicola* (Esbenshade and Triantaphyllou, 1985). Presence of superoxide dismutase phenotypes in different locations differentiated the two species of *Meloidogone* viz., *M. graminicola* and *M. incognita*. 
The species identity was also confirmed by the root symptoms. The roots of rice infected by root-knot nematodes were severely galled with hooked terminal gall characteristic of infestation by *M. graminicola* (Plate 1c,d,e). As reported by Roy (1973) symptoms appeared as yellowing of the upper portion of the leaves, which gradually extended towards the base. The plants were stunted and there was profuse proliferation of roots, which were slender and fluffy. Galls developed in a characteristic ring form and there were numerous hair like lateral growth on the convex side of the curvature. Spindle, club or nodule-shaped galls were also observed in infected roots. The galled roots were relatively brighter, white in colour turning to light brown at a later stage. Galls were restricted to terminal root tips, which is accordance with the report on the occurrence of rice root-knot nematode in Haryana by Dabur et al. (2003).

Unlike other root-knot nematodes, *M. graminicola* adult female laid eggs inside galled root tissues. The eggs were either free or in egg sacs apparently consisting of modified host cells. No egg masses were observed on the roots. Egg laying within roots might be an adaptation to survive under anaerobic conditions in rice fields. Similar type of nematode behaviour and symptoms due to *M. graminicola* was reported by Kaul and Chabra (1989).

5.2. **Biology of root-knot nematode, *M. graminicola* in rice cv. CO 43**

The rice root-knot nematode *M. graminicola*, completed its life cycle within a period of 22 days in rice cv. CO 43 under screen house conditions. The isolate of *M. graminicola* from Tamil Nadu had a life cycle duration closer to an isolate from the USA which took 23-27 days to complete one life cycle at 26°C (Yik and Birchfield, 1979). A population of *M. graminicola* from Bangladesh also had a shorter life cycle on rice of less than 19 days at temperatures of 22 to 29°C (Bridge and Page, 1982). However, Patnaik (1969) reported that *M. graminicola* took 26 to 51 days to complete its life cycle according to the temperature prevailing in different seasons of the year. During summer the life cycle was completed in 24 to 27 days and it was extended by 10
to 12 days during winter. The present study revealed that the isolate from Tamil Nadu had a much shorter life cycle compared to that of other isolates from India.

Rao et al. (1984b) reported that eggs of *M. graminicola* remained viable in soil even up to 7 days. In the present experiment the eggs hatched and juveniles penetrated the roots within two days. Similar to the earlier reports by Patnaik (1969) and Pathi and Patnaik (1988) the juveniles invaded the roots and established feeding sites inside the roots within about 5 days after penetration. The juveniles entered into the cortex, oriented their bodies parallel to the stele, juveniles fixed themselves in the region of apical meristem and within 42-72 hours there was extreme hypertrophy of cortical cells accompanied by hyperplasia. Root galls were formed around the site of establishment of the nematode within 5 days.

### 5.3. Histopathology of rice root infected by *M. graminicola*

Root-knot nematodes (*Meloidogyne* spp.) induce a specific type of feeding structure classified as multinucleate giant cells (Huang, 1985; Ibrahim, 1991; Jones, 1981). Giant cells are essential for a successful host parasite relationship. Microtome sections of the rice roots infected by *M. graminicola* exhibited the presence of groups of multinucleate giant cells in the cortex. There was extreme hypertrophy of cortical cells accompanied by hyperplasia. As reported by Padhi and Patnaik (1988) nematodes fixed their heads around the nearest metaxylem vessel and giant cells were formed in the stelar region. Mechanical disruption of metaxylem vessels interfered with the uptake of nutrients and water in the seedlings.

The arrangement of the giant cells varied from typically hexaradiate to irregular shape. Such a variation in giant cell formation within a single gall might be due to the possible existence of races. *Meloidogyne graminicola* incited 5 to 8 giant cells causing swelling at steler region. In the roots hypertrophy and hyperplasia occurred in the meristem, cortex, endoderms
and xylem (Jairapuri and Baqri, 1991). The population of *M. graminicola* from Tamil Nadu was found to incite about 7 to 9 giant cells around the feeding site.

Subtle differences in histopathology of rice roots affected by *M. graminicola* were observed from that caused by *Meloidogyne* spp. in dicotyledonous plants. Hyperplasia occurred along the path of larval migration in dicots (Balasubramanian and Rangaswami, 1964). On the other hand, in monocots, mature parenchymatous cells had limited meristematic potential and as such, hyperplasia was reported to be less frequent (Sivaramakrishna, 1966).

Unlike other root-knot nematodes, *M. graminicola* females remained embedded within the root tissue and the eggs were deposited within the root tissue. Secondary infections by second stage juveniles migrating in the plant tissue without leaving the root system took place as reported by Eiesenback and Triantaphyllou (1991).

5.4. **Avoidable yield loss in rice due to *M. graminicola***

The rice root-knot nematode has been reported to cause more damage in rice growing in nurseries (Bunagswon *et al.*, 1971; Israel *et al.*, 1963; Manser, 1968; Rao and Israel 1971, 1972). It is clearly evident from the present study that *M. graminicola* causes considerable in reduction in plant vigour in the nursery and main field ultimately leading to loss in the grain yield.

The yield loss in rice is due to reduction in size of earhead and grain weight (Jayaprakash and Rao, 1982). The rice root-knot nematode, *M. graminicola* has established as a pest of rice and can cause yield reductions up to 32 per cent (Biswa and Rao, 1971). Yield losses up to 50 per cent have been reported under severe conditions. In the present study the avoidable yield loss due to *M. graminicola* in rice was 5.99 per cent. Significant reduction in plant height in nematode infested plants was reported by Prasad *et al.* (1990). Growth and development of rice seedlings were adversely affected by *M. graminicola* and major differences were observed 30
days after inoculation when shoot and root weights and the number of tillers were significantly lower (Soomro and Hague, 1993). A similar trend was noticed in *M. graminicola* infested plants in the present study. As reported by Mian and Khan (1995) there was significantly higher number of galls, developing larvae and mature females with eggs in rice roots when infected with *M. graminicola*.

### 5.5. Screening high yielding rice varieties for their reaction to *M. graminicola*

In a glass house trial conducted to study the reaction of high yielding rice varieties commercially cultivated in Tamil Nadu, all the varieties tested exhibited high degree of susceptibility to the rice root-knot nematode, *M. graminicola*. Eiesenback and Triantaphyllou (1991) reported that rice is the most important host and nearly all varieties and selections are susceptible to *M. graminicola*, which is in accordance with the present study. Similarly Golden and Birchfield (1968) tested 30 cultivars and Manser (1968, 1971) tested 80 cultivars and no resistant entry was observed in both cases. However a number of rice varieties / cultivars have been reported to be either resistant or moderately resistant to the rice root-knot nematode *M. graminicola* (Rao *et al*., 1969; Jena and Rao, 1974; Sahu and Chawla, 1988; Swain and Prasad, 1988; Mohanty *et al*., 1993; Kalita and Phukan, 1990; Debanand Das, 1999). The resistance / susceptibility of rice cultivars to *M. graminicola* may vary with root morphology, water and nutrient management system, cropping season and the inoculum of eggs and juveniles in soil, weed hosts and crop residues. It is inevitable that nematode resistant sources be identified and included in plant breeding programs, to evolve high yielding as well as root-knot nematode resistant rice varieties suitable for cultivation in nematode endemic areas.

### 5.6. Host range of rice root-knot nematode, *M. graminicola* among cultivated crops and weeds

The success of any cropping system in achieving reduction in nematode population
depends not only upon the poor or non host status of the substitute crop but also upon the absence of alternative host plants

in the field, which usually are provided by the weeds and volunteer plants (Gaur and Sharma, 1998).

It is evident from the present investigation that the *M. graminicola* infects a number of weed hosts which serve as a source for their survival in the absence of rice crop. The commonly occurring wetland weed *E. colonum* was the most preferred host of *M. graminicola* as reported by a number of workers (Birchfield, 1964; Golden and Birchfield, 1965; Rao et al., 1970; Yik and Birchfield, 1979; Soomro, 1988; Kaul and Chabra, 1989; Soomro and Hague, 1992).

*M. graminicola* was recorded parasiting roots of *Cyperus* species and *P. repens* in Brazil (Sperandia and Amaral, 1994; Monteiro et al., 1988). Bajaj and Dabur (2000) reported that *M. graminicola* completed its life cycle on paddy and also multiplied on *C. deformis*. It was also found, to infect *C. rotundus* in Georgia (Minton, 1987). The present investigation also revealed that *C. deformis* was a good host as it was found to support the development and reproduction of *M. graminicola*.

In the present investigation, *E. alba*, *F. miliacea* and *C. dactylon* were recorded as hosts of *M. graminicola* which is confirmation with the report by Rao et al. (1970).

Other weeds viz., *A. baccifera*, *I. aquatica*, *G. decumbens*, *C. barbata* and *E. prostrata* have been recorded as hosts of the rice root-knot nematode for the first time in Tamil Nadu. Based on the observations it could be concluded that the commonly occurring weeds including grasses and broad leaved plants may serve as a source of inoculum in the absence of the main crop.
In the present investigation on possible rotation crops for use in *M. graminicola* infested land, it was observed that vegetable crops tomato and brinjal were good hosts favouring nematode multiplication and development. The roots were heavily galled exhibiting typical root-knot symptoms. Unlike the root galls produced by *M. incognita* in tomato and brinjal, *M. graminicola* infected root galls were elongate and spindle shaped. Tomato was reported as a host of *M. graminicola* by Rao *et al.* (1970). Egg plant was found to be infected by *M. graminicola* (Monteiro *et al.*, 1988; Siciliano *et al.*, 1990). According to Gregon and Davide (2002), the rice root-knot nematode, *M. graminicola* can cause damage to onion in continuous rice - onion rotation. To the contrary the present investigation revealed that onion did not support the development of *M. graminicola*. The reason may be attributed to variation among the populations of *M. graminicola* or the possibility of existence of races. None of the pulse crops tested were hosts of *M. graminicola*. Similarly cowpea has been reported to be a non-host of *M. graminicola* (Roy, 1977). In crop rotation experiments conducted by Alberto *et al.* (1997) *M. graminicola* infection was not observed in mungbean.

The results of this study provide the necessary clues for designing alternative cropping systems for fields infested with *M. graminicola* and also cautions about the weed hosts of the nematode. Proper control of weeds is therefore necessary for ensuring the protection of rice crop from the root-knot nematodes. The present results indicated that pulses are non-hosts of *M. graminicola*. This seems to be an important and useful finding as pulses are grown in rice fallows after rice in many regions of Tamil Nadu.

5.7. **Management of rice root-knot nematode *M. graminicola* in rice**

5.7.1. Effect of different methods of application of *P. fluorescens* for the management of *M. graminicola* in rice
Any bio-control agent having the ability to suppress the disease needs to be applied through a reliable established method for its consistent performance. Combination of different methods of application could be more effective in disease management than a single method of application (Vidhyasekaran and Muthamilan, 1999) The combined application of *P. fluorescens* as seed treatment and soil application proved to be effective in reducing the nematode severity, though seed treatment alone or soil application alone could manage the nematode and it may be due to increased inoculum potential in inducing resistance. The inoculum level may determine the efficacy of the antagonist in controlling the nematode. Combined application of *P. fluorescens* as seed treatment and nursery soil application was more effective in reducing *H. gracilis* population in rice both in nursery and main field (Ramakrishnan and Sivakumar, 1999; Ramakrishnan *et al*., 1998b) than seed treatment alone. Seed bacterization of tomato with *P. fluorescens* improved germination percent and reduced *Rotylenchulus reniformis* 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). Earlier reports indicate a positive relationship between population size of *P. fluorescens* and the degree of suppression of various diseases (Johnson 1994; Chatterjee *et al*., 1996; Vidhyasekaran *et al*., 1997b).

Colonization of *P. fluorescens* was the highest in the roots of seedlings receiving dual application of the bacterium both through seed treatment and soil application. Combined application of *P. fluorescens* as seed treatment and soil application was found effective in enhancing the growth of black gram. Significant reduction in cyst nematode population was observed. Root colonization by *P. fluorescens* was also significantly more in the combined treatment (Senthamizh and Rajendran, 2003).
5.7.2. Induction of defense mechanism by *P. fluorescens* against *M. graminicola*

Plants have endogenous defense mechanisms that can be induced in response to attack by plant parasitic nematodes. It is well known that the defense genes are inducible genes and appropriate stimuli or signals are needed to activate them. Inducing the plant own defense mechanisms by prior application of a biological inducer is thought to be a novel plant protection strategy (Ramamoorthy *et al.*, 2001). The use of fluorescent pseudomonads for inducing systemic resistance against phytonematodes has been well documented (Oostendorp and Sikora, 1990; Sikora, 1992; Sikora and Hoffman-Hergarten, 1992; Anita *et al.*, 2002).

Phenolic compounds are known to play a major role in the defense mechanism of plants against various external infectious agents. Distinct correlation between the degree of plant resistance and phenols has been reported by Pitcher *et al.* (1960) and Brueska and Dropkin (1973). *P. fluorescens* releases antimicrobial factors including 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). Accumulation of phenolics by prior application of *P. fluorescens* has been reported in peas (Benhamou *et al.*, 1996), tomato (Mipiga *et al.*, 1997), maize (Shabaev *et al.*, 1999), chickpea (Srivastava, 2001) and rice (Meena *et al.*, 1999, 2000).

In rice, seed treatment with PGPR strains increased the chitinase enzyme activity and phenolic content, which was correlated with reduced nematode infestation (Swarnakumari, 1996). Results of the present study revealed that *P. fluorescens* enhanced the accumulation of phenols in rice roots in response to attack by the nematode *M. graminicola*.

Oxidative enzymes play a decisive role in plant resistance to biotic stress. They are considered to be scavengers of hydrogen peroxide and involved in several plant defense
responses including lignification (Walter, 1992), cross linking of cell wall proteins, wound healing and production of anti-microbial radicals (Lamb and Dixon, 1997). Various studies reported that P. fluorescens induced peroxidase in response to pathogen attack (Van Loon et al., 1998; Kloeper et al., 1999; Chen et al., 2000). The present study also indicated that P. fluorescens induced higher accumulation of peroxidase upon invasion by M. graminicola.

Similar to PO, enhanced activity of PPO was observed in the root tissues treated with P. fluorescens upon challenge with the nematode. Enhanced activity of peroxidase and polyphenol oxidase was observed in tomato roots treated with P. fluorescens and Fusarium oxysporum f. sp. lycopersici (Ramamoorthy et al., 2001) and in rice against sheath blight (Nandakumar et al., 2001).

PAL in the first enzyme in phenylpropanoid metabolism involved in the production of phenolics and phytoalexins that prevent establishment of the pathogen (Daayf et al., 1997). The present investigation revealed increased activity of PAL due to P. fluorescens treatment, which might have prevented the establishment of the nematode within the roots. Enhanced activity of PAL by P. fluorescens has been reported in tomato (Kandan et al., 2003) and pearl millet (Niranjan Raj et al., 2003) against pathogens.

Production and secretion of chitinase by non-pathogenic microorganisms may be important in the biological control of plant parasitic nematodes as it degrades the chitin which is the major component of the egg shell. Chitinases may also play a secondary function as signal molecules, which elicit the induction of other pathogenesis related proteins or metabolites, which are involved in plant-defense reactions (Rahmi et al., 1998). The present study demonstrated increased expression of chitinase in P. fluorescens upon challenge inoculation with the nematode. Enhanced activity of chitinase has been suggested to have direct or indirect role in the induction of systemic resistance against pathogens (Dalisay and Kuc, 1995). Many of the P.
*Pseudomonas* treatment of rice cv. IR 50 led to the induction of systemic resistance against *Rhizoctonia solani*, as a result of increase in chitinase activity which directly or indirectly involved in the reduction of sheath blight disease development (Nandakumar *et al.*, 2001).

Enhanced activities of defense enzymes have been suggested to have a direct or indirect role in the induction of systemic resistance in plants against pathogens (Dalisay and Kuc, 1995). The strong induction of various isoforms of PO, PPO and SOD in bacterized plants challenge inoculated with the root-knot nematode, *M. graminicola* would have induced systemic resistance, which alternately reduced the development of the nematode. Detection of greater activity but similar banding pattern for peroxidase and polyphenol oxidase in treated and untreated plants suggests that differences in the isoforms associated with induced resistance are quantitative but not qualitative. A similar quantitative type of ISR was observed by Nandakumar *et al.* (2000) in rice against, sheath blight.

Radjacommare *et al.* (2003) detected unique PO and PPO isoforms in rice treated with *P. fluorescens* against *Rhizoctonia solani*. PO and PPO isoforms were expressed at higher levels in bacterized tomato root tissues challenge inoculated with *M. incognita* (Anita *et al.*, 2002) and *Fusarium oxysporum* f sp. *lycopersici* (Ramamoorthy *et al.*, 2001).

Similar to other enzymes, SOD activity was induced by *P. fluorescens* against the inoculated nematode. Induction of two new SOD isoforms and a higher level of expression of other isoforms of SOD might have also been implicated in induced defense responses against the nematode invasion. Root colonizing *P. fluorescens* of the saprophytic fluorescent pseudomonads group expressed increased levels of SOD and catalase. SOD activity was also present in extract of cells obtained from roots colonized from inoculum applied to seed. One isozyme of SOD was
also detected during the growth of the bacterium on root surface components (Katswon and Anderson, 1990).

In conclusion, the present study implies that earlier and higher accumulation of phenols and defense enzymes viz., peroxidase, polyphenoloxidase, phenylalanine ammonia lyase and chitinase in rice root tissue treated with _P. fluorescens_ in response to invasion by _M. graminicola_, might have collectively contributed to induced systemic resistance.

5.7.3. Nursery management of _M. graminicola_ in rice

Efficient nursery management is one of the integrated nematode management modules of effective crop management. Effectiveness of _P. fluorescens_, neem cake and nematicides, carbofuran 3G and phorate 10G, for the management of _M. graminicola_ in rice nursery was evaluated under field conditions. All the treatments applied were found to enhance the growth of seedlings in terms of shoot length, shoot weight, root length and root weight. Among them soil application of the plant growth promoting bacterium, _P. fluorescens_ at 2.5 kg ha^{-1} at the time of sowing was as effective as carbofuran 3G @ 1 kg a.i. ha^{-1} in enhancing of the plant growth. Similarly both the treatments were equally capable of reducing the population and reproduction of nematodes in roots and soil. Among the different methods of application of _P. fluorescens_, soil application was comparatively more effective than seed treatment in enhancing plant growth of rice seedlings and among the nematicides carbofuran 3G performed better than phorate 10G. Neem cake was the least effective treatment.

It has been established that fluorescent pseudomonads enhance plant growth in several ways mainly producing plant growth regulators, such as gibberellins, cytokinins and indole acetic acid which can either directly or indirectly modulate the plant growth and development (Mew and Rosales, 1986; Dubeikovsky _et al._, 1993; Glic, 1994). Further more it is evident that
the increased productivity of the plant results in large part from the suppression of plant parasitic nematodes by *P. fluorescens*.

Earlier reports have proved the effectiveness of *P. fluorescens* in suppression of economically important plant parasitic nematodes in rice ecosystem. Significant reduction in root and soil population of the rice root nematode, *H. gracilis* was obtained with *P. fluorescens* (Ramakrishnan *et al.*, 1998a,b; Ramakrishnan and Sivakumar, 1999). Another important species of rice root nematode, *H. oryzae* was also successfully managed by the application of *P. fluorescens* (Swarnakumari, 1996; Sreenivasan *et al.*, 2000a).

Several mechanisms were attributed to the suppression of phytonematodes by the application of *P. fluorescens*. They induced resistance by altering root exudates or inducing the host to produce repellents that effect nematode attraction or recognition of the host (Oosterdorp and Sikora, 1990; Racke and Sikora, 1992). They are also reported to alter the syncytial development or sex ratio in the root tissue (Wyss, 1989) and to reduce hatching and invasion of phytonematodes by the production of toxic metabolites like protease and collagenase (Spiegel *et al.*, 1991). Secondary metabolites such as 2,4-diacetyl phloroglucinol (PHL) and lytic enzymes produced by *P. fluorescens* 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* (Elsherif and Grossmann, 1996; Dunn *et al.*, 1998). Mutants of *P. fluorescens* were found to be involved in the ovicidal activity of the ring nematode (Wechter *et al.*, 2001).

Intensive rice cropping increases the nematode problem, and hence evolving an effective method of nematode controlled with nematicides is of importance in rice plant protection (Israel and Rao, 1971). The chemical nematicides carbofuron 3G and phorate 10G are being successfully used for the management of a wide range plant parasitic nematodes. In the present
study soil application of carbofuran 3G are phorate 10G proved to be highly effective in reducing the rice root nematode severity resulting in enhanced plant growth. In accordance with the present study there are a number of reports on the successful control of rice root-knot nematode *M. graminicola* in rice (Prasad and Rao, 1976 a, b and c; Krishnaprasad and Rao, 1980, 1984; Mohammed, 1987; Rahman, 1991) with carbofuran 3G either as seed treatment, foliar spray or soil application. The reduction in the population of *M. graminicola* by soil application of phorate may be due to the reduction in larval invasion and development in rice roots as reported by Krishnaprasad and Rao (1980).

Although application of neem cake was the least effective among the different treatments, there was increased growth and decreased nematode population in treated plants. Neem cake was highly effective in reducing *M. graminicola* infestation in rice var. Jaya under field conditions resulting in significant increase in the plant growth (Debanand Das *et al.*, 1999; Hossain *et al.*, 1999). Neem based formulations were used successfully for controlling *M. graminicola* in rice resulting in improved plant growth (Das and Deka, 2002). Antinemic effect of neem cake may be attributed to the phenolic compounds released during its degradation (Alam *et al.*, 1979), apart from its stimulatory effect on root growth and predaceous fungi and also to small amount of azadirachtin present in it.

5.7.4. Integrated Management of *M. graminicola* in rice using *P. fluorescens*, neem cake and carbofuran

An integrated management strategy prevents the excessive build up of any single nematode and minimizes the development of pest resistance (Sethi and Gaur, 1986; Wani and Alam, 1988). The present investigation on the integration of three components *viz.*, a bio-control agent (*P. fluorescens*), an organic amendment (neem cake) and a nematicide (carbofuran 3G) for
the management of *M. graminicola* in rice nursery revealed that all the three were compatible with each other in reducing the root-knot nematode population and in enhancing rice plant growth and yield. The combination treatments were more effective than individual treatments perhaps due to the additive effect of the bio-control agent, organic amendment and nematicide.

The effect of individual treatments on the rice root-knot nematode *M. graminicola* has been discussed earlier in this chapter and several reports indicate the compatibility of bio-control agents, organic amendments and nematicides for the management of plant parasitic nematodes. Use of organic amendments along with nematicides proved to be effective for managing *H. oryzae* in rice (Prasad *et al.*, 1986). Combination of chitin amendments, neem cake and *P. fluorescens* was effective in reducing *H. oryzae* population both in soil and root (Swarakumari *et al.*, 1999). *P. fluorescens* seed treatment along with soil application of neem cake and farmyard manure significantly reduced root rot incidence in mungbean (Vinita and Suresh, 2001) and cowpea (Purushothaman *et al.*, 2003).

The plant growth promoting bacterium *P. fluorescens* has been reported to be compatible with the nematicide carbofuran 3G for the management of phytonematodes in potato (Mani *et al.*, 1998) and papaya (Shanthi *et al.*, 2002) and root-knot nematode in brinjal (Mahapatra and Mohanty, 2002) and okra (Senthilkumar and Ramakrishnan, 2003). According to Latha *et al.* (2002) combination of fungicide (carbendazim) asseed treatment with carbofuran 3G as soil application and *P. fluorescens* as seed treatment was the most effective treatment for the management of root-rot incidence due to *Macrophomina phaseolina* and cyst nematode *Heterodera cajani*. Combination of *P. fluorescens* with fungicides was found to effectively suppress the fruit rot of ber (Nallathambi and Thakore, 2003). The bacterium was also highly compatible with mancozeb, carbendazim (fungicides), imidacloprid, etofenprox, chlorpyrophos
and triazophos (insecticides) at the recommended dosage for field use in the management of fungal diseases of rice (Mathew, 2003).

5.7.5. **Root colonization by *P. fluorescens* and induced systemic resistance under field conditions**

The effectiveness of *P. fluorescens* in the control of root-knot nematode is attributed to the intensive root colonization by the bacterium. Shanthi and Sivakumar (1995) also viewed that nematode suppressing ability of the pseudomonad bacterial strains was related to their root colonizing ability. Earlier reports indicate positive relationship between population size of *P. fluorescens* and the degree of suppression of various diseases (Johnson, 1994; Chatterjee *et al*., 1996; Vidhyasekaran *et al*., 1997a). It is evident from the present study that *P. fluorescens* promoted plant growth and protected rice crop from *M. graminicola*, which is attributed to aggressive root colonization by the bacterium which induced systemic resistance under field conditions. Induction of resistance is dependent on colonization of the root system by the inducing rhizobacteria in sufficient numbers to trigger systemic resistance (Van Loon *et al*., 1998). This is usually accomplished by adding bacterial suspensions to the soil before sowing or at transplanting or by coating seeds with high numbers of bacteria (Kloepper, 1996). The present study also indicated sufficient root colonization by *P. fluorescens* in rice plants that were applied with a talc based formulation of *P. fluorescens* as soil application before sowing induced systemic resistance against *M. graminicola*. Once activated the resistance mechanisms of the host maintain an enhanced defensive mechanism for prolonged periods. Four PGPR strains applied as seed treatments followed by a soil drench at transplanting (Wei *et al*., 1996) suppressed angular leaf spot and anthracnose of cucumber over a period of two years. Under similar conditions naturally occurring bacterial wilt (Kloepper *et al*., 1993) was also significantly reduced. In rice seed treatment with *P. fluorescens* strain Pf-1 induced resistance which was
observed up to 45 days after sowing (Vidhyasekaran et al., 1997b). A similar experiment conducted by Nayar (1996) indicated that induction of defense mechanisms by *P. fluorescens* persisted up to 60 days when applied as seed treatment. In sugarcane induction of resistance by PGPR persisted for 90 days of crop growth (Viswanathan and Samiyappan, 1999).

In light of the result obtained in the present study, it appeared that effective and rational management of the rice root-knot nematode *M. graminicola* is possible through *P. fluorescens* mediated induced systemic resistance which is a valuable addition to other options available *viz.*, organic amendments and nematicides, as it is ecofriendly and cost effective.
CHAPTER VI

SUMMARY

The root-knot nematode, *M. graminicola* is recognized as one of the most important pests of rice. The deviation from the usual cropping pattern due to decrease in water supply for irrigation has drastically increased the economic significance of rice root knot nematode in Tamil Nadu. Investigations were carried out to identify the species of the nematode, assess the yield loss and formulate suitable management strategies in rice. The inference from the results of the investigations is summarized hereunder.

1. The root knot nematode infecting rice in Tamil Nadu was identified as *Meloidogyne graminicola* based on the morphological characters of adult females, males, infective juveniles and perineal pattern.

2. The identity of the species was confirmed by the variation in esterase and superoxide dismutase phenotypes.

3. The life cycle of *M. graminicola* in rice from egg to egg stage was 22 days at a temperature ranging between a maximum of $32 \pm 1^\circ C$ and a minimum of $25 \pm 1^\circ C$.

4. Histopathological studies of infected roots revealed presence of groups of 7 to 9 multinucleate giant cells with granular cytoplasm around the site of infection. Abnormal xylem proliferated around the giant cells causing swelling of vascular cylinder.

5. All the developmental stages including eggs, infective juveniles, immature females and adult females were present within the root.

6. Inter and intra cellular migration of larvae within the root cortex caused disruption and
hypertrophy of cells.

7. The avoidable yield loss due to *M. graminicola* was 5.99 per cent in rice cv. ASD 16 under field conditions. The seedling weight was reduced by 51.06 per cent and the plant height was reduced by 45.51 and 31.11 per cent respectively, after 30 and 60 days of planting due to infestation by *M. graminicola*.

8. All the 21 high yielding rice varieties were either susceptible or highly susceptible to the rice root knot nematode *M. graminicola* with gall indices ranging between 4 and 5.

9. Commonly occurring weeds in rice fields *viz.*, *E. colonum* and *C. dacyton* were classified as excellent hosts of *M. graminicola*. Other weeds hosts identified were *A. baccifera*, *J. aquatica*, *P. repens*, *F. miliacea*, *C. barbata*, *E. alba*, *E. prostrata* and *G. decumbens*.

10. Among crop hosts tomato and brinjal were highly susceptible to *M. graminicola* while onion was not infected. None of the major pulse crops like blackgram, greengram, red gram and cowpea were hosts for the rice root-knot nematode.

11. Dual application of *P. fluorescens* both as seed treatment and soil application @ 25 ml bacterial suspension proved to be effective in reducing the root knot nematode population in root and soil and enhanced growth of rice seedlings. Colonization of *P. fluorescens* was the highest in roots receiving the bacterium both through seed treatment and soil application.

12. Increased accumulation of phenols and defense enzymes *viz.*, peroxidase, polyphenoloxidase, phenylalanine ammonialyase and chitinase in response to nematode invasion in roots treated with *P. fluorescens* collectively contributed to induced systemic resistance.

13. Native PAGE analysis revealed strong and unique induction of various isoforms of
peroxidase, polyphenoloxidase and superoxidase dismutase in bacterized plants challenge inoculated with *M. graminicola*.

14. Under field conditions soil application of *P. fluorescens* @ 2.5 kg ha\(^{-1}\) at the time of sowing in the nursery was as effective as application of carbofuran 3 G @ 1 kg a.i. ha\(^{-1}\) in reducing the nematode population and reproduction and in enhancing growth of seedlings.

15. Among the different methods of application of *P. fluorescens*, soil application @ 2.5 kg ha\(^{-1}\) was more effective than seed treatment @ 10 g kg\(^{-1}\) seed, resulting in significant increase in plant growth and decrease in nematode population.

16. Simultaneous application of *P. fluorescens* @ 2.5 kg ha\(^{-1}\), neem cake @ 1 t ha\(^{-1}\) and carbofuran 3G @ 1 kg a.i. ha\(^{-1}\) in the nursery before sowing significantly enhanced plant growth in the nursery and mainfield in terms of plant growth and tillering.

17. The grain and straw yield increased by 12.57 per cent and 9.55 per cent respectively in plants treated with a combination of *P. fluorescens*, neem cake and carbofuran 3G in the nursery. There was 36.7 per cent increase in grain weight due to the integration of all the three treatments.

18. Significant reduction in the population and reproduction of *M. graminicola* was observed both in the nursery and mainfield as a result of application of *P. fluorescens* along with neem cake and carbofuran 3G. Root colonization in rice roots treated with a talc based formulation of *P. fluorescens* as soil application before sowing induced systemic resistance against *M. graminicola* under field conditions. The bacterium was found to multiply and persist in the roots upto 120 days of crop growth.
RESEARCH FINDINGS


The root knot nematode infecting rice in Tamil Nadu was identified as Meloidogyne graminicola Golden and Birchfield, 1965. The life cycle from egg to egg stage was 22 days at a temperature ranging between a maximum of 32 ± 1°C and a minimum of 25 ± 1°C. All stages of the nematode including eggs, juveniles, immature females and mature females were present within the root. Eggs hatched within the root and the juveniles remained in the maternal gall. Inter and intra cellular migration of juveniles within the root cortex caused disruption and hypertrophy of cells. At the site of larval establishment groups of 7-9 multinucleate giant cells with granular cytoplasm were found. Abnormal xylem proliferated around the giant cells causing swelling of vascular cylinder, limiting the uptake of nutrients and water.

The rice root knot nematode caused significant reduction in seedling weight and plant height resulting in 5.99 per cent avoidable yield loss. Infected seedlings turned chlorotic and remained stunted. The roots of infected rice were severely galled with hooked characteristic terminal gall. There was profuse proliferation of roots with numerous hair like lateral growth on the galls. Spindle, club or nodule shaped galls were also observed in infected roots. Commonly occurring wetland weeds, Echinochloa colonum and Cyanodon dactylon were excellent hosts of M. graminicola. The other weed hosts identified were Panicum repens, Fimbristylis miliaceae, Ammania baccifera, Ipomea aquatica, Eclipta alba, E. prostrata and Chloris barbata. Among crop hosts, tomato and brinjal favoured the development of M. graminicola, while onion and pulses were not infected.

Dual application of P. fluorescens as seed treatment and soil application significantly enhanced plant growth and reduced nematode population. Increased accumulation of phenols and defense enzymes viz., peroxidase, polyphenol oxidase, phenylalanine ammonialyase and chitinase in P. fluorescens treated plants collectively contributed to induced systemic resistance against M. graminicola. Under field conditions soil application of P. fluorescens @ 2.5 kg ha⁻¹ in
the nursery before sowing was as effective as carbofuran 3G @ 1 kg a.i. ha\(^{-1}\) in reducing the nematode population and reproduction and increasing the seedling vigour. The plant growth promoting bacterium \textit{P. fluorescens} was compatible with neem cake and carbofuran 3G in increasing the grain yield and reducing the rice root knot nematode population leading to 12.57 per cent increased grain yield.
Table 3. Biology of *M. graminicola* in rice cv. CO 43 under screen house conditions

<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Penetration and stages of development in roots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Penetration initiated near the root tip</td>
</tr>
<tr>
<td>3</td>
<td>Infective juveniles completely within the root</td>
</tr>
<tr>
<td>4</td>
<td>Hypertrophy and hyperplasia of cells</td>
</tr>
<tr>
<td>5</td>
<td>Minute galls with infective juveniles</td>
</tr>
<tr>
<td>12</td>
<td>Spike tail stage</td>
</tr>
<tr>
<td>14-18</td>
<td>Young females and males</td>
</tr>
<tr>
<td>20</td>
<td>Mature females and males</td>
</tr>
<tr>
<td>22</td>
<td>Adult females with eggs within roots</td>
</tr>
</tbody>
</table>
Table 9. Effect of different methods of application of *P. fluorescens* on growth of rice cv CO 43 infected with *M. graminicola* under screen house conditions

(Mean of four replications)

<table>
<thead>
<tr>
<th>T.No.</th>
<th>Treatments</th>
<th>Shoot</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Length (cm)</td>
<td>Per cent increase</td>
</tr>
<tr>
<td>T1</td>
<td><em>P. fluorescens</em> - Seed treatment @ 25 ml bacterial suspension</td>
<td>22.58&lt;sup&gt;d&lt;/sup&gt;</td>
<td>116.49</td>
</tr>
<tr>
<td>T2</td>
<td><em>P. fluorescens</em> - Soil application @ 25 ml bacterial suspension</td>
<td>24.58&lt;sup&gt;c&lt;/sup&gt;</td>
<td>135.67</td>
</tr>
<tr>
<td>T3</td>
<td><em>P. fluorescens</em> - Seed treatment + Soil application @ 25 ml bacterial suspension</td>
<td>29.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>179.77</td>
</tr>
<tr>
<td>T4</td>
<td>Carbofuran 3G @ 1 kg a.i./ha</td>
<td>27.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>162.70</td>
</tr>
<tr>
<td>T5</td>
<td>Untreated control</td>
<td>10.43&lt;sup&gt;e&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CD (0.05)</td>
<td>1.49</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Figures followed by the same alphabets are statistically not significant.
Table 10. Effect of different methods of application of *P. fluorescens* on the population of *M. graminicola* infecting rice cv. CO 43 under screen house conditions

(Mean of four replications)

<table>
<thead>
<tr>
<th>T.No.</th>
<th>Treatments</th>
<th>No. of females/g root</th>
<th>Per cent decrease</th>
<th>Nematodes / 100 cc soil</th>
<th>Per cent decrease</th>
<th>No. of eggs/g root</th>
<th>Per cent decrease</th>
<th>Gall index</th>
<th><em>P. fluorescens</em> (x10^8 cfu/g soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td><em>P. fluorescens</em> - Seed treatment @ 25 ml bacterial suspension</td>
<td>35.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35.29</td>
<td>48.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.82</td>
<td>706.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>20.56</td>
<td>3</td>
<td>16.00 (4.06)</td>
</tr>
<tr>
<td>T2</td>
<td><em>P. fluorescens</em> - Soil application @ 25 ml bacterial suspension</td>
<td>27.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.13</td>
<td>32.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.05</td>
<td>458.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.47</td>
<td>2</td>
<td>25.75 (5.12)</td>
</tr>
<tr>
<td>T3</td>
<td><em>P. fluorescens</em> - Seed treatment + Soil application @ 25 ml bacterial suspension</td>
<td>22.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.18</td>
<td>33.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.97</td>
<td>380.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.10</td>
<td>1</td>
<td>36.75 (6.10)</td>
</tr>
<tr>
<td>T4</td>
<td>Carbofuran 3G @ 1 kg a.i./ha</td>
<td>29.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.06</td>
<td>33.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.61</td>
<td>526.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40.82</td>
<td>3</td>
<td>0.0 (0.71)</td>
</tr>
<tr>
<td>T5</td>
<td>Untreated control</td>
<td>55.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-</td>
<td>69.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>888.75&lt;sup&gt;e&lt;/sup&gt;</td>
<td>-</td>
<td>5</td>
<td>0.0 (0.71)</td>
</tr>
</tbody>
</table>

CD (0.05) 3.93 - 4.12 - 58.00 -

Figures within parentheses indicate log transformed values
Table 5. Infestation of *M. graminicola* during different growth phases of rice cv. ASD 16

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Nematode population / g root at</th>
<th>Gall index at*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transplanting</td>
<td>30 DAP</td>
</tr>
<tr>
<td>Carbofuran 3G @ 2 kg a.i./ha*</td>
<td>21.15</td>
<td>27.46</td>
</tr>
<tr>
<td>Untreated*</td>
<td>43.62</td>
<td>49.46</td>
</tr>
</tbody>
</table>

(t = 0.05)*

* 1 = No galls; 2 = 1 - 25% of root system galled; 3 = 26 - 50% of root system galled; 4 = 50 - 75% of the root system galled; 5 = 76 - 100% of root system galled.
Table 4. Influence of *M. graminicola* on plant growth and yield of rice cv. ASD 16

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Seedling weight (g)</th>
<th>Plant height (cm)</th>
<th>Yield (kg / ha)</th>
<th>Per cent avoidable yield loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 DAP</td>
<td>60 DAP</td>
<td></td>
</tr>
<tr>
<td>Carbofuran 3G @ 2 kg a.i./ha*</td>
<td>7.11</td>
<td>44.99</td>
<td>75.04</td>
<td>5109.42</td>
</tr>
<tr>
<td>Untreated*</td>
<td>3.48</td>
<td>24.49</td>
<td>51.69</td>
<td>4803.58</td>
</tr>
</tbody>
</table>

(t = 0.05)*
Table 13. Plant growth of rice cv. ASD 16 treated with *P. fluorescens*, neem cake and carbofuran at transplanting (mean of 3 replications)

<table>
<thead>
<tr>
<th>T.No.</th>
<th>Treatments</th>
<th>Shoot</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Length (cm)</td>
<td>Per cent increase</td>
</tr>
<tr>
<td>T1</td>
<td><em>P. fluorescens</em> - Soil application @ 2.5 kg/ha</td>
<td>27.23&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>67.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T2</td>
<td>Neem cake @ 1 t/ha</td>
<td>23.73&lt;sup&gt;e&lt;/sup&gt;</td>
<td>45.58&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>T3</td>
<td>Carbofuran 3G @ 1 kg a.i./ha</td>
<td>24.80&lt;sup&gt;de&lt;/sup&gt;</td>
<td>52.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T4</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Neem cake @ 1 t/ha</td>
<td>26.00&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>59.51&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T5</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Carbofuran 3G @ 1 kg a.i./ha</td>
<td>27.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.74&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T6</td>
<td>Neem cake @ 1 t/ha + Carbofuran 3G @ 1 kg a.i./ha</td>
<td>26.63&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>63.37&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T7</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Neem cake @ 1 t/ha + Carbofuran 3G @ 1 kg a.i./ha</td>
<td>28.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>T8</td>
<td>Untreated control</td>
<td>16.30&lt;sup&gt;f&lt;/sup&gt;</td>
<td>-</td>
</tr>
</tbody>
</table>

Figures followed by the same alphabets are statistically not significant.
Table 14. Population of *M. graminicola* in rice cv. ASD 16 treated with *P. fluorescens*, neem cake and carbofuran at transplanting

(mean of 3 replications)

<table>
<thead>
<tr>
<th>T.No.</th>
<th>Treatments</th>
<th>No. of females/g root</th>
<th>Per cent reduction</th>
<th>No. of eggs/g root</th>
<th>Per cent reduction</th>
<th>No. of juveniles / 100 cc soil</th>
<th>Per cent reduction</th>
<th>Gall index</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td><em>P. fluorescens</em> - Soil application @ 2.5 kg/ha</td>
<td>66.67&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>42.36</td>
<td>1256.00&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>82.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>46.48</td>
<td>34.05</td>
<td>3</td>
</tr>
<tr>
<td>T2</td>
<td>Neem cake @ 1 t/ha</td>
<td>99.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.13</td>
<td>1386.00&lt;sup&gt;de&lt;/sup&gt;</td>
<td>82.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.95</td>
<td>33.78</td>
<td>4</td>
</tr>
<tr>
<td>T3</td>
<td>Carbofuran 3G @ 1 kg a.i./ha</td>
<td>59.67&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>48.41</td>
<td>1096.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.30</td>
<td>33.51</td>
<td>2</td>
</tr>
<tr>
<td>T4</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Neem cake @ 1 t/ha</td>
<td>52.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.04</td>
<td>1118.00&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>76.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>52.37</td>
<td>38.87</td>
<td>2</td>
</tr>
<tr>
<td>T5</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Carbofuran @ 1 kg a.i./ha</td>
<td>60.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>48.13</td>
<td>1071.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>76.67&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>54.37</td>
<td>38.33</td>
<td>2</td>
</tr>
<tr>
<td>T6</td>
<td>Neem cake @ 1 t/ha + Carbofuran @ 1 kg a.i./ha</td>
<td>58.67&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>49.28</td>
<td>1119.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>74.33&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>52.32</td>
<td>40.22</td>
<td>2</td>
</tr>
<tr>
<td>T7</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Neem cake @ 1 t/ha + Carbofuran @ 1 kg a.i./ha</td>
<td>49.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.64</td>
<td>946.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.69</td>
<td>46.11</td>
<td>2</td>
</tr>
<tr>
<td>T8</td>
<td>Untreated control</td>
<td>115.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-</td>
<td>2347&lt;sup&gt;f&lt;/sup&gt;</td>
<td>124.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
</tbody>
</table>

CD (0.05) | 13.42 | - | 141.12 | - | 12.00 | - | - |

Figures followed by the same alphabets are statistically not significant.
Table 15.  Plant growth and nematode population in rice cv. ASD 16 treated with *P. fluorescens*, neem cake and carbofuran 30 days after transplanting

(mean of 3 replications)

<table>
<thead>
<tr>
<th>T.No.</th>
<th>Treatments</th>
<th>Plant growth</th>
<th>Nematode population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plant height (cm)</td>
<td>Per cent increase</td>
</tr>
<tr>
<td>T1</td>
<td><em>P. fluorescens</em> - Soil application @ 2.5 kg/ha</td>
<td>36.86&lt;sup&gt;d&lt;/sup&gt;</td>
<td>57.76</td>
</tr>
<tr>
<td>T2</td>
<td>Neem cake @ 1 t/ha</td>
<td>39.70&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>64.53</td>
</tr>
<tr>
<td>T3</td>
<td>Carbofuran 3G @ 1 kg a.i./ha</td>
<td>35.20&lt;sup&gt;e&lt;/sup&gt;</td>
<td>45.87</td>
</tr>
<tr>
<td>T4</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Neem cake @ 1 t/ha</td>
<td>40.60&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>68.26</td>
</tr>
<tr>
<td>T5</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Carbofuran 3G @ 1 kg a.i./ha</td>
<td>38.90&lt;sup&gt;c&lt;/sup&gt;</td>
<td>61.21</td>
</tr>
<tr>
<td>T6</td>
<td>Neem cake @ 1 t/ha + Carbofuran 3G @ 1 kg a.i./ha</td>
<td>41.20&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>70.74</td>
</tr>
<tr>
<td>T7</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Neem cake @ 1 t/ha + Carbofuran 3G @ 1 kg a.i./ha</td>
<td>41.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.11</td>
</tr>
<tr>
<td>T8</td>
<td>Untreated control</td>
<td>24.13&lt;sup&gt;f&lt;/sup&gt;</td>
<td>-</td>
</tr>
</tbody>
</table>

Figures followed by the same alphabets are statistically not significant.
Table 16. Plant growth and nematode population in rice cv. ASD 16 treated with *P. fluorescens*, neem cake and carbofuran 60 days after transplanting

(Mean of 3 replications)

<table>
<thead>
<tr>
<th>T.No.</th>
<th>Treatments</th>
<th>Plant growth</th>
<th>Nematode population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plant height (cm)</td>
<td>Per cent increase</td>
</tr>
<tr>
<td>T1</td>
<td><em>P. fluorescens</em> - Soil application @ 2.5 kg/ha</td>
<td>68.77</td>
<td>24.81</td>
</tr>
<tr>
<td>T2</td>
<td>Neem cake @ 1 t/ha</td>
<td>63.33</td>
<td>14.94</td>
</tr>
<tr>
<td>T3</td>
<td>Carbofuran 3G @ 1 kg a.i./ha</td>
<td>66.83</td>
<td>21.29</td>
</tr>
<tr>
<td>T4</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Neem cake @ 1 t/ha</td>
<td>71.77</td>
<td>30.25</td>
</tr>
<tr>
<td>T5</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Carbofuran 3G @ 1 kg a.i./ha</td>
<td>70.00</td>
<td>27.04</td>
</tr>
<tr>
<td>T6</td>
<td>Neem cake @ 1 t/ha + Carbofuran 3G @ 1 kg a.i./ha</td>
<td>72.37</td>
<td>31.34</td>
</tr>
<tr>
<td>T7</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Neem cake @ 1 t/ha + Carbofuran 3G @ 1 kg a.i./ha</td>
<td>73.63</td>
<td>37.26</td>
</tr>
<tr>
<td>T8</td>
<td>Untreated control</td>
<td>55.1</td>
<td>-</td>
</tr>
</tbody>
</table>

 Figures followed by the same alphabets are statistically not significant.
Table 17.  Yield and nematode population in rice cv. ASD 16 treated with *P. fluorescens*, neem cake and carbofuran at harvest  
(Mean of 3 replications)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatments</th>
<th>Grain yield (kg/ha)</th>
<th>Per cent increase</th>
<th>Straw yield (kg/ha)</th>
<th>Per cent increase</th>
<th>1000 grain weight (g)</th>
<th>Per cent increase</th>
<th>No. of females /g root</th>
<th>Per cent reduction</th>
<th>No. of eggs/g root</th>
<th>Per cent reduction</th>
<th>Gall index</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td><em>P. fluorescens</em> - Soil application @ 2.5 kg/ha</td>
<td>5475&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.71</td>
<td>6942&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.03</td>
<td>34.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.47</td>
<td>32.67</td>
<td>7.53</td>
<td>182.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.85</td>
<td>2</td>
</tr>
<tr>
<td>T2</td>
<td>Neem cake @ 1 t/ha</td>
<td>5250&lt;sup&gt;de&lt;/sup&gt;</td>
<td>3.29</td>
<td>5892&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.12</td>
<td>30.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.09</td>
<td>33.33</td>
<td>5.66</td>
<td>186.00&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>14.16</td>
<td>3</td>
</tr>
<tr>
<td>T3</td>
<td>Carbofuran @ 1 kg a.i./ha</td>
<td>5392&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>6.08</td>
<td>5642&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.59</td>
<td>34.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.11</td>
<td>31.00</td>
<td>12.26</td>
<td>185.67&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>14.31</td>
<td>2</td>
</tr>
<tr>
<td>T4</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Neem cake @ 1 t/ha</td>
<td>5667&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>11.49</td>
<td>5608&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.97</td>
<td>34.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.99</td>
<td>34.00</td>
<td>3.87</td>
<td>195.67&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>9.69</td>
<td>2</td>
</tr>
<tr>
<td>T5</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Carbofuran 3G @ 1 kg a.i./ha</td>
<td>5722&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.57</td>
<td>5942&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.02</td>
<td>36.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.81</td>
<td>31.33</td>
<td>11.32</td>
<td>188.00&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>13.03</td>
<td>2</td>
</tr>
<tr>
<td>T6</td>
<td>Neem cake @ 1 t/ha + Carbofuran 3G @ 1 kg a.i./ha</td>
<td>5500&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>8.20</td>
<td>6108&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.06</td>
<td>34.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.74</td>
<td>32.67</td>
<td>7.53</td>
<td>202.00&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>6.77</td>
<td>2</td>
</tr>
<tr>
<td>T7</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Neem cake @ 1 t/ha + Carbofuran 3G @ 1 kg a.i./ha</td>
<td>5806&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.22</td>
<td>6025&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.55</td>
<td>36.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.43</td>
<td>31.00</td>
<td>12.56</td>
<td>172.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.46</td>
<td>2</td>
</tr>
<tr>
<td>T8</td>
<td>Untreated control</td>
<td>5083&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td>5500&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


| CD (0.05) | 183.36 | 428.63 | 2.51 | NS | 10.75 |

Figures followed by the same alphabets are statistically not significant.
Table 11. Activity of phenols and defense enzymes in rice roots treated with *P. fluorescens* challenge inoculated with *M. graminicola* (Mean of 5 replications)

<table>
<thead>
<tr>
<th>T. No.</th>
<th>Treatments</th>
<th>Phenols (mg g⁻¹ fresh tissue)</th>
<th>Peroxidase (change in absorbance m⁻¹ mg⁻¹ protein)</th>
<th>Polyphenoloxidase (change in absorbance m⁻¹ mg⁻¹ protein)</th>
<th>Phenylalanine ammonialyase (nmol transcinnamic acid m⁻¹ mg⁻¹ protein)</th>
<th>Chitinase (nmol GlcNAc m⁻¹ mg⁻¹ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>1.59</td>
<td>4.32</td>
<td>2.31</td>
<td>21.1</td>
<td>18.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.66</td>
<td>4.61</td>
<td>1.4</td>
<td>20.72</td>
<td>14.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.51</td>
<td>3.78</td>
<td>1.29</td>
<td>20.23</td>
<td>14.79</td>
</tr>
<tr>
<td>T₂</td>
<td><em>Meloidogyne graminicola</em></td>
<td>1.79</td>
<td>3.82</td>
<td>1.86</td>
<td>25.48</td>
<td>20.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.77</td>
<td>2.95</td>
<td>1.69</td>
<td>24.49</td>
<td>19.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.52</td>
<td>2.56</td>
<td>1.68</td>
<td>21.32</td>
<td>13.82</td>
</tr>
<tr>
<td>T₃</td>
<td><em>P. fluorescens + M. graminicola</em></td>
<td>1.81</td>
<td>5.26</td>
<td>2.85</td>
<td>32.9</td>
<td>28.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.23</td>
<td>5.81</td>
<td>3.06</td>
<td>33.96</td>
<td>34.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.17</td>
<td>5.85</td>
<td>3.28</td>
<td>33.41</td>
<td>34.8</td>
</tr>
<tr>
<td>T₄</td>
<td>Untreated uninoculated control</td>
<td>1.03</td>
<td>2.40</td>
<td>1.25</td>
<td>19.46</td>
<td>17.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.44</td>
<td>2.79</td>
<td>1.19</td>
<td>20.56</td>
<td>13.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.09</td>
<td>2.01</td>
<td>1.17</td>
<td>14.99</td>
<td>12.22</td>
</tr>
</tbody>
</table>

CD (0.05) Treatment: 0.049 0.153 0.129 1.161 0.770
CD (0.05) Days: 0.043 0.132 0.112 1.001 0.670
CD (0.05) Treatment x Days: 0.086 0.264 0.225 2.01 1.341
Table 12. Nursery management of *M. graminicola* in rice var. CO47  
(Mean of 4 replications)

<table>
<thead>
<tr>
<th>T.No.</th>
<th>Treatments</th>
<th>Plant growth</th>
<th>Nematode population</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shoot length (cm)</td>
<td>Shoot weight (g)</td>
<td>Root length (cm)</td>
</tr>
<tr>
<td>T1</td>
<td><em>P. fluorescens</em> – Seed treatment @ 10 g/kg seed</td>
<td>21.51&lt;sup&gt;b&lt;/sup&gt; (30.75)</td>
<td>0.16&lt;sup&gt;b&lt;/sup&gt; (23.08)</td>
<td>6.75&lt;sup&gt;b&lt;/sup&gt; (50.1)</td>
</tr>
<tr>
<td>T2</td>
<td><em>P. fluorescens</em> – Soil application @ 2.5 kg/ha</td>
<td>22.98&lt;sup&gt;b&lt;/sup&gt; (39.70)</td>
<td>0.18&lt;sup&gt;b&lt;/sup&gt; (38.46)</td>
<td>9.89&lt;sup&gt;a&lt;/sup&gt; (119.78)</td>
</tr>
<tr>
<td>T3</td>
<td>Neem cake @ 1 t/ha</td>
<td>19.11&lt;sup&gt;c&lt;/sup&gt; (16.17)</td>
<td>0.18&lt;sup&gt;b&lt;/sup&gt; (38.46)</td>
<td>6.93&lt;sup&gt;b&lt;/sup&gt; (54.00)</td>
</tr>
<tr>
<td>T4</td>
<td>Phorate @ 1 kg a.i./ha</td>
<td>22.95&lt;sup&gt;d&lt;/sup&gt; (39.51)</td>
<td>0.16&lt;sup&gt;a&lt;/sup&gt; (53.85)</td>
<td>9.69&lt;sup&gt;a&lt;/sup&gt; (115.33)</td>
</tr>
<tr>
<td>T5</td>
<td>Carbofuran @ 1 kg a.i./ha</td>
<td>25.70&lt;sup&gt;a&lt;/sup&gt; (56.23)</td>
<td>0.20&lt;sup&gt;b&lt;/sup&gt; (53.85)</td>
<td>10.38&lt;sup&gt;a&lt;/sup&gt; (130.67)</td>
</tr>
<tr>
<td>T6</td>
<td>Untreated control</td>
<td>16.45&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.50&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

CD (0.05) | 3.24 | 0.08 | 0.96 | 0.04 | 5.4 | 65.8 | 24.4 |

Figures within parentheses indicate per cent increase / decrease over control.  
Figures followed by the same alphabets are statistically not significant.
Table 6. Response of high yielding rice varieties to *M. graminicola*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Varieties</th>
<th>Gall index*</th>
<th>Host reaction**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CO 43</td>
<td>5</td>
<td>HS</td>
</tr>
<tr>
<td>2</td>
<td>CO 45</td>
<td>4</td>
<td>S</td>
</tr>
<tr>
<td>3</td>
<td>CO 46</td>
<td>4</td>
<td>S</td>
</tr>
<tr>
<td>4</td>
<td>CO 47</td>
<td>5</td>
<td>HS</td>
</tr>
<tr>
<td>5</td>
<td>ADT 43</td>
<td>4</td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>ADT 44</td>
<td>4</td>
<td>S</td>
</tr>
<tr>
<td>7</td>
<td>ADT 45</td>
<td>5</td>
<td>HS</td>
</tr>
<tr>
<td>8</td>
<td>ADT 46</td>
<td>5</td>
<td>HS</td>
</tr>
<tr>
<td>9</td>
<td>ASD 16</td>
<td>5</td>
<td>HS</td>
</tr>
<tr>
<td>10</td>
<td>ASD 17</td>
<td>4</td>
<td>S</td>
</tr>
<tr>
<td>11</td>
<td>ASD 18</td>
<td>4</td>
<td>S</td>
</tr>
<tr>
<td>12</td>
<td>ASD 19</td>
<td>5</td>
<td>HS</td>
</tr>
<tr>
<td>13</td>
<td>ASD 20</td>
<td>5</td>
<td>HS</td>
</tr>
<tr>
<td>14</td>
<td>TKM 11</td>
<td>4</td>
<td>S</td>
</tr>
<tr>
<td>15</td>
<td>TKM 12</td>
<td>4</td>
<td>S</td>
</tr>
<tr>
<td>16</td>
<td>TPS 3</td>
<td>4</td>
<td>S</td>
</tr>
<tr>
<td>17</td>
<td>CR 1009</td>
<td>5</td>
<td>HS</td>
</tr>
<tr>
<td>18</td>
<td>MDU 5</td>
<td>5</td>
<td>HS</td>
</tr>
<tr>
<td>19</td>
<td>White ponni</td>
<td>5</td>
<td>HS</td>
</tr>
<tr>
<td>20</td>
<td>TRY-1</td>
<td>5</td>
<td>HS</td>
</tr>
<tr>
<td>21</td>
<td>TRY 2</td>
<td>5</td>
<td>HS</td>
</tr>
</tbody>
</table>

* Gall index:  
  1 = No galls  
  2 = 1 - 25% of root system galled  
  3 = 26 - 50% of root system galled
4 = 51 - 75% of the root system galled
5 = 76 - 100% of root system galled
** S - Susceptible          HS - Highly susceptible

**Table 7. Host status of major weeds prevalent in rice fields to M. graminicola**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Weed</th>
<th>*Root gall</th>
<th>*Reproduction</th>
<th>**Host status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Echinochloa colonum L.</em></td>
<td>+++</td>
<td>+++</td>
<td>E</td>
</tr>
<tr>
<td>2.</td>
<td><em>Panicum repens</em> L.</td>
<td>+</td>
<td>+</td>
<td>P</td>
</tr>
<tr>
<td>3.</td>
<td><em>Cyperus deformis</em> L.</td>
<td>++</td>
<td>++</td>
<td>G</td>
</tr>
<tr>
<td>4.</td>
<td><em>Fimbristylis mileacea</em> Vahl.</td>
<td>++</td>
<td>++</td>
<td>G</td>
</tr>
<tr>
<td>5.</td>
<td><em>Ammania baccifera</em> L.</td>
<td>++</td>
<td>++</td>
<td>G</td>
</tr>
<tr>
<td>7.</td>
<td><em>Astercantha longifolia</em> Nees.</td>
<td>-</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>8.</td>
<td><em>Chloris barbata</em> Sw.</td>
<td>+</td>
<td>+</td>
<td>P</td>
</tr>
<tr>
<td>9.</td>
<td><em>Centella asiatica</em> Urban.</td>
<td>-</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>10.</td>
<td><em>Eclipta prostrata</em> L.</td>
<td>+</td>
<td>+</td>
<td>P</td>
</tr>
<tr>
<td>12.</td>
<td><em>Cyanotis axillaris</em> Roem and Schult</td>
<td>-</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>13.</td>
<td><em>Marselia quadrifolia</em> L.</td>
<td>-</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>14.</td>
<td><em>Ipomea aquatica</em> Forsk.</td>
<td>++</td>
<td>++</td>
<td>G</td>
</tr>
<tr>
<td>15.</td>
<td><em>Monochoria vaginalis</em> Presl.</td>
<td>-</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>16.</td>
<td><em>Alternanthera triandra</em> Lank.</td>
<td>-</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>17.</td>
<td><em>Cynodon dactylon</em> L.</td>
<td>+++</td>
<td>+++</td>
<td>E</td>
</tr>
<tr>
<td>18.</td>
<td><em>Lippia nodiflora</em> Rich.</td>
<td>-</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>19.</td>
<td><em>Rungia repens</em> Nees.</td>
<td>-</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>S.No.</td>
<td>Name of the crop</td>
<td>*Root galling</td>
<td>*Reproduction</td>
<td>**Host status</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------</td>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td>1.</td>
<td>Tomato</td>
<td>+++</td>
<td>+++</td>
<td>E</td>
</tr>
<tr>
<td>2.</td>
<td>Brinjal</td>
<td>+++</td>
<td>+++</td>
<td>E</td>
</tr>
<tr>
<td>3.</td>
<td>Onion</td>
<td>-</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>4.</td>
<td>Redgram</td>
<td>-</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>5.</td>
<td>Greengram</td>
<td>-</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>6.</td>
<td>Blackgram</td>
<td>-</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>7.</td>
<td>Cowpea</td>
<td>-</td>
<td>-</td>
<td>N</td>
</tr>
</tbody>
</table>

*+++ : > 50 galls and females with > 50 eggs / egg sac  
**N - non-host   P - Poor   G - Good   E - Excellent
Table 18. Colonization of *P. fluorescens* (strain Pf 1) in rice roots under field conditions

(Mean of three replications)

<table>
<thead>
<tr>
<th>T. No.</th>
<th>Treatments</th>
<th>x 10^8 cfu g^-1 root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Transplanting</td>
</tr>
<tr>
<td>T1</td>
<td><em>P. fluorescens</em> - Soil application @ 2.5 kg/ha</td>
<td>25.33&lt;sup&gt;a&lt;/sup&gt; (5.08)</td>
</tr>
<tr>
<td>T2</td>
<td>Neem cake @ 1 t/ha</td>
<td>0&lt;sup&gt;b&lt;/sup&gt; (0.71)</td>
</tr>
<tr>
<td>T3</td>
<td>Carbofuran 3G @ 1 kg a.i./ha</td>
<td>0&lt;sup&gt;b&lt;/sup&gt; (0.71)</td>
</tr>
<tr>
<td>T4</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Neem cake @ 1 t/ha</td>
<td>24.67&lt;sup&gt;a&lt;/sup&gt; (5.02)</td>
</tr>
<tr>
<td>T5</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Carbofuran 3G @ 1 kg a.i./ha</td>
<td>25.00&lt;sup&gt;a&lt;/sup&gt; (5.05)</td>
</tr>
<tr>
<td>T6</td>
<td>Neem cake @ 1 t/ha + Carbofuran 3G @ 1 kg a.i./ha</td>
<td>0&lt;sup&gt;b&lt;/sup&gt; (0.71)</td>
</tr>
<tr>
<td>T7</td>
<td><em>P. fluorescens</em> @ 2.5 kg/ha + Neem cake @ 1 t/ha + Carbofuran 3G @ 1 kg a.i./ha</td>
<td>24.33&lt;sup&gt;a&lt;/sup&gt; (4.98)</td>
</tr>
<tr>
<td>T8</td>
<td>Untreated control</td>
<td>0&lt;sup&gt;b&lt;/sup&gt; (0.71)</td>
</tr>
</tbody>
</table>

CD (0.05) | 0.47 | 0.18 | 0.17 | 0.11

Figures within parentheses indicate log transformed values.
Figures followed by the same alphabets are statistically not significant.
Fig. 2. *Meloidogyne graminicola* male

Anterior portion

Tail

25 μ
Fig. 1. *Meloidogyne graminicola* adult female

Anterior portion

Perineal pattern
Fig. 3. *Meloidogyne graminicola* infective juvenile

Anterior portion

Tail

25 μm
**Fig. 11. Root colonization by *P. fluorescens* (strain - Pf 1) in rice cv. ASD 16**

![Bar chart showing root colonization by *P. fluorescens* at different stages of growth and treatments.](chart.png)

- **Y-axis:** $\times 10^8$ cfu/g root
- **X-axis:** Treatments (T1, T2, T3, T4, T5, T6, T7, T8)
- **Legend:**
  - Nursery
  - 30 DAP
  - 60 DAP
  - Harvest
Fig. 5. Effect of different methods of application of P. fluorescens on the management of M. graminicola in rice (cv. Co 43)
Fig. 4. Infestation of *M. graminicola* during different growth phases of rice cv. ASD 16
Fig. 7. Accumulation of PAL and chitinase in rice treated with *P. fluorescens* against *M. graminicola*

a. PAL (n mol transcinnamic acid m⁻¹ mg⁻¹)

![Graph showing PAL activity over days for different treatments.]

b. Chitinase (n mol Glu Nac m⁻¹ mg⁻¹)

![Graph showing chitinase activity over days for different treatments.]

Fig. 6. Accumulation of phenolics and induction of PO and PPO in rice treated with *P. fluorescens* against *M. graminicola*.

b. PO (change in absorbance m$^{-1}$mg$^{-1}$)
c. PPO (change in absorbance m⁻¹mg⁻¹)
Fig. 9. Effect of *P. fluorescens*, neemcake and carbofuran 3G and their combination on plant growth and nematode population in rice (cv.ASD 16) nursery.
Fig. 8. Nursery management of *M. graminicola* in rice cv. CO 47

![Graph a](image)

- **Length (cm)**
- Treatments: T1, T2, T3, T4, T5, T6
- Graph a: Shoot (red) and Root (blue)

![Graph b](image)

- **Weight (g)**
- Treatments: T1, T2, T3, T4, T5, T6
- Graph b: Shoot (red) and Root (blue)

![Graph c](image)

- **Nematode population in root & soil**
- Treatments: T1, T2, T3, T4, T5, T6
- Graph c: Soil (red) and Root (blue)

![Graph d](image)

- **No. of eggs/g root**
- Treatments: T1, T2, T3, T4, T5, T6
- Graph d: (Bar chart)

---

*Note:* The figures represent the yield and nematode population in different treatments of rice cv. CO 47, showing the impact of nursery management on *M. graminicola*.
Fig. 10. Effect of *P. fluorescens*, neemcake and carbofuran 3G and their combination on plant growth, yield and nematode population in rice (cv. ASD 16) mainfield

**Graph a:**
- No. of tillers (30 DAP)
- No. of tillers (60 DAP)
- Plant height (30 DAP)
- Plant height (60 DAP)

**Graph b:**
- Grain
- Straw

**Graph c:**
- Females (30 DAP)
- Females (60 DAP)
- Eggs (30 DAP)
- Eggs (60 DAP)
Plate 21
Integrated management of *M. graminicola*
on rice cv. ASD 16

a. Treated nursery

*Pseudomonas fluorescens* + neem cake + carbofuran 3G

b. Untreated nursery
Plate 20

Nursery management of *M. graminicola* on rice

- **T₁** - *P. fluorescens*- ST
- **T₂** - Neem cake
- **T₃** - Carbofuran 3G
- **T₄** - Phorate 10G
- **T₅** - Control
- **T₆** - Control
Plate 19
Nursery management of *M. graminicola* in rice

*P. fluorescens*

Carbofuran 3G

Untreated control
Plate 18

Native - PAGE analysis for SOD isoform profile induced by *P. fluorescens* isolate Pf1 in rice challenged with or without *M. graminicola*

Lane 1. Nematode
Lane 2. Control (Healthy)
Lane 3. *Pseudomonas*
Lane 4. *Pseudomonas* + Nematode
Plate 17

Native - PAGE analysis for PPO isoform profile induced by *P. fluorescens* isolate Pf1 in rice challenged with or without *M. graminicola*

Lane 1. Nematode
Lane 2. Control (Healthy)
Lane 3. *Pseudomonas*
Lane 4. *Pseudomonas + nematode*
Plate 16

Native - PAGE analysis for PO isoform profile induced by *P. fluorescens* isolate Pf1 in rice challenged with or without *M. graminicola*

Lane 1. Nematode
Lane 2. Control (Healthy)
Lane 3. *Pseudomonas*
Lane 4. *Pseudomonas + nematode*
Plate 15

Effect of different methods of application of *P. fluorescens* for the management of *M. graminicola* on rice
Plate 14

Alternate crop hosts of *M. graminicola*

a. Tomato

Infected plant  
Infected root

b. Brinjal

Infected plant  
Infected root
Plate 13

Contd...

Eclipta alba

Eclipta alba root

Eclipta prostrata  Gomphrena decumbens  Fimbristylis mileacea
Plate 12

Contd.....
Plate 11

Alternate weed hosts of *M. graminicola*

![Echinochloa colonum](image1)
![Ammania baccifera](image2)

![Cyanodon dactylon](image3)
![Ipomea aquatica](image4)
Plate 10

Yield loss in rice due to *M. graminicola*

a. Carbofuran 3G - treated nursery

b. Untreated nursery
Plate 9

Contd....
Plate 8

Histopathology of rice root infected by *M. graminicola*
Plate 7
Developmental stages of *M. granincola* in rice

- Egg
- Adult female
- Immature stage
- Spike tail stage
- Second stage juvenile

*Images at the bottom show details of the developmental stages with 'N' indicating the nucleus.*
Plate 6

Enzyme phenotypes of *Meloidogyne* spp.

Lane 1 - *M. graminicola*
Lane 2 - *M. incognita*
Plate 5
Parts of *M. graminicola*

a. Infective juvenile

Anterior portion
Tail

b. Male

Anterior portion
Tail
Plate 4

*Meloidogyne graminicola* - Adult female

Anterior portion

Perineal pattern

Egg
Plate 3
Field trials

a. Assessment of avoidable yield loss in rice due to *M. graminicola*

b. Nursery management of *M. graminicola*

c. Integrated management of *M. graminicola*
Plate 2

*Pseudomonas fluorescens*

a. Culture filtrate

b. Colony forming units
Plate 1
Root knot nematode incidence in rice

A - Infected nursery   B - Infected seedling
C, D, E - Root knot symptoms
Plate 22

Integrated management of *M. graminicola* on rice (main field)

a. *P. fluorescens* + neem cake + cabofuran 3G

b. Untreated