BIOCHEMICAL AND HISTOPATHOLOGICAL ALTERATIONS DUE TO ROOT- KNOT NEMATODE, *Meloidogyne graminicola* IN RICE (*Oryza sativa* L.) AND VARIETAL REACTIONS.

DARSANA. V. S. LAL

(2013-11-160)

Thesis Submitted in partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

2015

Department of Agricultural Entomology

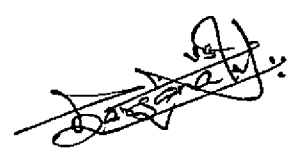
COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM- 695 522

KERALA, INDIA

DECLARATION

I, hereby declare that this thesis entitled **"Biochemical and histopathological alterations due to root-knot nematode, *Meloidogyne graminicola* in rice (*Oryza sativa* L.) and varietal reactions."** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.



Vellayani
Date : 18-08-15

Darsana. V. S. Lal
(2013-11-160)

CERTIFICATE

Certified that this thesis entitled **"Biochemical and histopathological alterations due to root-knot nematode, *Meloidogyne graminicola* in rice (*Oryza sativa* L.) and varietal reactions."** is a record of bonafide research work done independently by Ms. Darsana. V. S. Lal (2013-11-160) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellayani
Date: 18-08-15

Dr. R. Narayana
(Major Advisor), Assistant Professor
Department of Agricultural Entomology
College of Agriculture, Vellayani,
Thiruvananthapuram- 695 522

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Darsana. V. S. Lal (2013-11-160), a candidate for the degree of **Master of Science in Agriculture** with major in Agricultural Entomology agree that this thesis entitled **"Biochemical and histopathological alterations due to root-knot nematode, *Meloidogyne graminicola* in rice (*Oryza sativa* L.) and varietal reactions."** may be submitted by Ms. Darsana. V. S. Lal in partial fulfilment of the requirement for the degree.

Dr. R. Narayana
(Chairman, Advisory Committee)
Assistant Professor
Department of Agrl. Entomology
College of Agriculture, Vellayani,
Thiruvananthapuram – 695 522

Dr. Sheela M. S
(Member, Advisory Committee)
Associate Director of
Research and Head
Department of Agrl. Entomology
College of Agriculture, Vellayani,
Thiruvananthapuram – 695 522

Dr. M. S. Nisha
(Member, Advisory Committee)
Assistant Professor
Department of Agrl. Entomology
College of Agriculture, Vellayani,
Thiruvananthapuram – 695 522

Dr. Aparna. B
(Member, Advisory Committee)
Assistant Professor
Department of Soil science and
Agrl. Chemistry
College of Agriculture, Vellayani,
Thiruvananthapuram – 695 522

18/8/2015
EXTERNAL EXAMINER

(Name and Address)
M. SIVAKUMAR
Professor (Nematology)
TNAU, Coimbatore

Acknowledgement

I feel immense pleasure to express my profound and heartfelt thankfulness to Dr. R. Narayana, Assistant Professor, Department of Agricultural Entomology and Chairman of the advisory committee, for his guidance, suggestions, constant encouragement, support, unfailing patience and above all the kind of understanding throughout the course of this research work and preparation of the thesis.

I wish to express my sincere gratitude to Dr. Sheela M. S. Associate Director of Research and Head, Department of Agricultural Entomology and member of advisory committee, for the help rendered for the smooth conduct of research work, co-operation and critical evaluation of thesis.

I am grateful to Dr. M. S. Nisha Assistant Professor, Department of Agricultural Entomology and member of advisory committee for her valuable suggestions, timely support and critical evaluation during the course of this work.

I am thankful to Dr. Aparna.B, Assistant Professor, Department of Soil science and Agrl. Chemistry, for her valuable suggestion, encouragement and co-operation rendered throughout the study, as a member of advisory committee.

I wish to express my heartfelt thanks to Dr. Sverup John, Dean, College of Agriculture, Vellayani for providing me all the necessary facilities from the University during the whole course of study.

I express my sincere thanks to all the teachers of the Department of Agrl. Entomology for their entire well wishes help and support.

I greatly acknowledge the help rendered by Dr. K. Umamaheswaran, Professor of Plant Pathology in the biochemical analysis of my research work.

I am obliged to Professor and Head, RRS, Moncompu for providing the seeds of different rice varieties to carry out the research work in proper time.

I earnestly express my profound gratitude to my dear friend Anju for her valuable guidance, moral support, encouragement during the entire period of study.

My loving and wholehearted thanks to my classmates Akhila, Aswathy, Divya, Nithya and Murali for their help, good company, positive criticism and moral support throughout my P.G. programme.

I also express my sincere thanks to my friends Anila, Arya, Litty, soumya, Aswathy and Krishna priya. I am deeply obliged to Narmada chechi for her advice and support. I wish to express my gratefulness to my seniors and juniors for their timely help and support. I take this opportunity to acknowledge them for all the inspiring words, unconditional love, care and support they offered to me.

I am most indebted to Amma, Achan, Jhanciammachi, Akkama, Achamma, Kashi, archechi and Chettan for their affection, constant encouragement, moral support and blessings that have enabled me to finish this work, without which I would not have completed this research.

Above all, I believe it is the unbound love and blessings of God, which is the prime factor that led to the successful completion of my entire work. I humbly bow my head before that almighty for the numberless blessings that he shower upon my life.

Darsana. V. S. Lal

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
nm	Nano meter
µl	Micro litre
@	At the rate of
°C	Degree Celsius
CD	Critical difference
cm	Centimeter
J_2	Second stage juvenile
et al.	And other co workers
Fig.	Figure
g	Gram
Pi	Initial population density
h.	Hours
i.e.	that is
l.	Litre
ml	Milli litre
WAI	Week After Inoculation
GI	Gall Index
EMI	Egg Mass Index
Kg	Kilo gram
min	Minutes
mg	Milli gram
Sec	Seconds
sp. or spp.	Species (Singular and plural)
viz.	Namely
Rr	Reproduction rate

pH	Negative logarithm of hydrogen ions
ppm	Parts per million
Min	Minutes
rpm	Revolution per minute
OD	Optical density
No.	Number

LIST OF APPENDICES

INTRODUCTION

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. It is used as staple food for majority of the world populations, predominantly in Asia where more than 90 per cent of world rice is grown and consumed. Out of the total rice area under cultivation, 53 per cent is irrigated, 31per cent is low land rain fed and 13 per cent is upland and 3 per cent is under deep water. Rice is grown extensively in India and area under rice cultivation has increased to a greater extent during the last 10 years. India ranked second largest producer of rice on the world map. Rice crop is affected by several abiotic and biotic stresses, of which plant parasitic nematodes constitute an important component. Jain *et al.* (2011) reported that the ecological conditions suitable for the cultivation of rice crop are congenial for the multiplication of nematodes infesting rice.

Nematodes constitute an important group of animals on earth whose existence is intricately woven with man and his activities. In an undisturbed ecosystem, they remain in harmony with the environment dynamics and attain equilibrium in population density. Prot (1994) reported that over 200 species of plant parasitic nematodes were associated with rice. Coyne and Plowright (2000) considered that nematodes are important pests in the rapidly changing production system of rice. Rice root-knot nematode, *Meloidogyne graminicola* is an important pest of rice around the world (MacGowan and Langdon, 1989) and it is one of the great concerns for yield loss in rice and wheat crops under rice-wheat cropping system.

In rice growing areas, among various economically important nematode problems, rice root-knot nematode, *M. graminicola* happens to be a key nematode pest. Golden and Birchfield (1965) for the first time recorded and described *M. graminicola* from the roots of barnyard grass, *Echinochloa colonum* in Louisiana. In India, the distribution of *M. graminicola* in rice growing areas of different states

has been documented in nematode distribution atlas prepared by All India Coordinated Research Project (Nematodes) and published by Indian Council of Agricultural Research, New Delhi, India during 2010. In Kerala, Sheela *et al.* (2005) reported that Paddy crop grown in high ranges of Idukki district and water scarce area in Palakkad belt was heavily infested with root- knot nematode.

Second stage juveniles (J_2) penetrate the roots closely behind the root tip region and they migrate to vascular cylinder turning it into multinucleated giant cells, by endomitosis and cell hypertrophy characterized by hook shaped galls. This nematode is remarkably well adapted to flooded condition as it can survive in the aerenchymatous tissue of the graminaceous plant in flooded condition. *M. graminicola* affected rice plants show stunting and chlorosis, characteristic terminal swellings or galls on the roots which ultimately result in severe reduction in growth and yield.

The changes in the physiological, growth and yield parameters and biochemical processes of infested host decide whether the host becomes susceptible or resistant to nematode attack. In this context an intimate knowledge of nematode physiology and biochemistry along with its host is absolutely essential for developing plant resistance against the nematodes. In recent past some progress has also been made in this direction to understand, the basic biochemical mechanism of plant nematode interactions by several workers (Ganguly and Dasgupta, 1983; Mohanty, *et al.,* 1995).

Histopathological studies revealed that the penetration of J_2 into the root tips and later migration into the root tissue causing cortical hypertrophy, giant cell formation and increase in size of epidermal, cortical and stellar cells. The infected roots shows increased amount of insoluble polysaccharides, proteins and nucleic acid compared to healthy roots.

The defense enzymes *viz.*, peroxidase, phenylalanine ammonia lyase, polyphenol oxidase activities and phenol content were found to be higher in *M. graminicola* infested resistant rice varieties. The peroxidase activity decreased in nematode infested susceptible roots compared to nematode resistant roots. Sequential development of polyphenol oxidase increased in nematode infested resistant rice varieties (Kumar *et al.*, 2007). The growth and yield parameters decreased in nematode infested susceptible plants compared to nematode resistant plants.

No such works have been made in the crop in Kerala.

This backdrop necessitated the present study and the investigation was taken up with the following objectives.

1. To determine the biochemical changes in rice due to the infestation of root knot nematode, *M. graminicola.*
2. To assess the histopathological alterations in rice due to the attack of *M. graminicola*
3. To assess the changes in growth and yield parameters of rice due to *M. graminicola* infestation.
4. To screen 10 important rice varieties against root knot nematode, *M. graminicola.*

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Rice is the world's most important staple food and around 162 mha area is cultivated annually with an annual global production of 464 mmt (FAOSTAT, 2013). Irrigated, deep water, upland, lowland and rainfed are the five major ecosystems where the rice is cultivated. In India, rice is grown in all five major ecosystems (Jairajpuri and Baqri, 1991). Bridge *et al.* (2005) reported that about 53% of the world's rice is grown under irrigated conditions that provide 75% of total global production. Gaur and Pankaj (2010) studied the common nematode pest of rice and reported that rice is quite susceptible to root-knot nematode and is attacked by *M. graminicola, M. triticoryzae, M. incognita, M. javanica, M. oryzae* and *M. arenaria.*

Sasser (1989) studied the root knot nematode infestation in agricultural crops and reported that *Meloidogyne* spp. is one of the most devastating and widespread nematode pests of agricultural crops. Similarly, Bridge *et al.* (2005) studied the host range and intensity of attack of root knot nematode in different crops and reported that the root knot nematode has exceedingly wide host range and attacks almost all cereal, vegetable, pulse, fiber, fruit and beverage crops. *M. graminicola* causes terminal, hook shaped or spiral galls which are characteristic symptoms of the infection of this nematode species. In India, about 16-32% yield loss occurred due to the infestation of this nematode in rainfed and upland rice (Prasad *et al.*, 2010). Dutta *et al.* (2012) reported that *M. graminicola* is a primary pest of rice and poses a substantial threat to rice cultivation in Southeast Asia.

2.1 RICE ROOT KNOT NEMATODE (*Meloidogyne graminicola*)

2.1.1 Economic importance and yield loss

Prasad *et al.* (1986) reported that in India, 17-30% yield loss occurred due to poorly filled kernel in upland rice. Jain *et al.* (2007) studied the combined yield loss due to rice root-knot nematode (*M. graminicola*), white tip nematode (*A. besseyi*), stem nematode (*D. angustus*) and cyst nematode *(H. oryzicola),* and

reported that combined yield loss was 10.50% and in monetary terms, it accounted for losses of 779.30 million rupees.

Golden and Birchfield (1965) first recorded and described *M. graminicola* from the roots of barnyard grass, *Echinochloa colonum* in Louisiana. Buangsuwon *et al.* (1971) found that the root knot nematode causing typical root gall in entire rice growing areas of Thailand and creates problems in nursery seedbed conditions. Prot (1994) studied the relation between the status of nematode problems and the changes in agricultural policy and adoption of new rice production technologies in the South-East Asian countries. Prasad *et al.* (2001) reported that in India, outbreak of *M. graminicola* infestation in kharif rice has been witnessed in around 800 ha in Mandya district of Karnataka and many parts of India like West Bengal, Orissa, Assam, UP, Himachal Pradesh etc.

2.1.2 Distribution

Rice root-knot nematode (*M. graminicola*) has wide distribution particularly in the rice growing areas of the world. This nematode species has been reported from India, Nepal, Burma, Bangladesh, US, Brazil, Colombia, France, Laos, Georgia, Libya, Thailand, Philippines, Indonesia, Singapore, Louisiana, Pakistan, Vietnam and South Africa. *M. graminicola* has also been reported to damage the rice crop in Hanian Island, China. Pokharel (2007) and Dangal *et al.* (2008) studied the rice root-knot nematode problems in different parts of Nepal.

In India, *M. graminicola* which were found in West Bengal (Mukhopadhyay and Khan, 2000), Orissa, Assam, Kerala have been spread to newer areas of Eastern Uttar Pradesh, Delhi, Haryana, Punjab, Himachal Pradesh, Tamil Nadu, Karnataka, Orissa and recently to Gujarat as per the reports from various cooperating centres of All India Coordinated Research Project (AICRP) on nematodes. Khan and Jairajpuri (2010) studied the distribution of *M. graminicola* and identified major hot spot areas in different parts of India and well documented in a nematode distribution atlas.

2.1.3 Host range

M. graminicola has a wide host range that includes many of the common weeds of rice fields and agricultural crops that are grown in rotation with rice, such as onion, cabbage and tomato. MacGowan and Langdon (1989) reported the important host plants of *M. graminicola*, which includes food, fodder, fruits, ornamentals and weeds. Bajaj and Dabur (2000) demonstrated that the *M. graminicola* could multiply on the *Cyperus difformis* weed plant under rice-wheat crop sequence. Khan *et al.* (2004) recorded 17 weeds associated with rice grown in Nadia district of West Bengal and they reported that all these were supporting *M. graminicola* for their survival and multiplication in field situations. *Echinochloa colonum, Cyperus compressus, Brachiaria ramosa, Cyperus rotundus and Ranunculus pusillus* are the important alternate/collateral hosts of rice root knot nematode.

2.1.4 Life cycle

M. graminicola moults four times throughout its life cycle. The first moult takes place inside the egg, and newly hatched second-stage juveniles (J2) accumulate around the roots in the zone of elongation. Mulk (1976) found that the juveniles hatch inside the roots and re-infect the same root by migrating inside the root to a new feeding site. Females of *M. graminicola* remain within the galled roots, and eggs are deposited in egg masses inside the root cortex. Bridge *et al.* (2005) studied the number of egg laying females in a single gall and they reported that up to 50 egg-laying females can be found in a single gall, indicating that infection can be extremely high. Jaiswal and Singh (2010) reported that the duration of life cycle of rice root knot nematode varies considerably in different environments, ranging from a very short life cycle of only 15 days at 27-37°C.

Rao and Israel (1973) found that root knot nematode showed long life cycle of up to 51 days in some regions in India. Yik and Birchfield (1979) studied a rice root knot nematode population from the USA and reported that the nematode population completed its life cycle in 23-27 days at 26°C.

Kumar *et al.* (2007) reported that the life cycle of *M. graminicola* was completed in 25-30 days at 30°C.

2.2 BIOCHEMICAL CHANGES

2.2.1 Phenol Content

Mishra and Mohanty (2007) studied that root knot nematode infected roots of rice varieties Annapurna, Manika and Ramakrishna produced greater amount of phenolics, by 27.7, 47.6 and 104.2 per cent respectively as compared to their healthy ones. Varanasi and Thalati (2014) reported that the induction of total phenols was more in chickpea roots than in leaves, as roots are the primary sites of infection for root-knot nematodes. The increase in total phenol was higher at 10,000 J_2/plant.

The initial amount of phenolic compounds present in the resistant varieties was greater than that of the susceptible variety as observed earlier by Narayan and Reddy (1980) in tomato. Ganguly and Dasgupta (1982) observed that there is greater enhancement of phenolics in the resistant rice variety than the susceptible one. Mote *et al.* (1990); Chakrabarti and Mishra (2002) and Swain *et al.* (2004) observed that in nematode infected resistant plants phenolics content increased much faster than that of a susceptible one. Chakrabarti and Mishra (2002) studied the negative correlation between the amount of phenolics in the plant roots and the gall number.

Farkas and Kiraly (1962) reported that the accumulation of phenol may be due to the excess production of hydrogen peroxide by increased respiration. Goodman *et al.* (1967) recorded that the phenol accumulation was due to the activation of hexose monophosphate shunt pathway, acetate pathway and release of bound phenols by hydrolytic enzymes. Ganguly and Dasgupta (1982) reported that the increase in the phenolics was occurred due to liberation of phenols from their glycosides by the action of hydrolytic enzymes secreted by the nematode or *de novo* synthesis of phenols through host-parasite interaction. Increase in free phenols following infection with nematodes was also reported by Ganguly and Dasgupta (1984) in tomato, Wuyts *et al.* (2005) in banana,

Bajaj *et al.* (1985) in tomato, Shukla and Chakraborthy (1988) in tobacco and Muzzafera *et al.* (1989) in coffee. Fogain (1996) and Valette *et al.* (1997) reported higher amount of phenolics in the resistant genotype Yangambi Km 5 of banana whereas Wuyts *et al.* (2005) had given a contradictory report.

Sempio *et al.* (1975) observed that the phenolic compounds were possibly converted by increased peroxidase activity to quinines in resistant cultivars and quinines were reported to be more toxic to microorganism. Bajaj and Mahajan (1977) studied the correlation between increased level of phenolic compounds and the resistance of plants to various nematode diseases. They also reported that the oxidized forms of phenolic compounds occurring in high concentration in roots of resistant tomato plants, might contribute to the *M. incognita* resistance by creating a toxic environment for nematode penetration and multiplication. Alam *et al.* (1991) reported that phenolic compounds were considered as non-specific defense metabolites against the pathogen and resistant plants accumulated those metabolites in higher amounts than in susceptible ones. Devi *et al.* (2007) observed that total phenol estimated in roots of banana genotypes showed that these compounds were higher in resistant accessions than susceptible ones. Rani *et al.* (2008) reported that the increased phenolic content in roots indicated the degree of resistance to root knot nematode. Farahat *et al.* (2012) reported that phenol and ortho-hydroxyphenol levels increased in tomato roots infected with *M. incognita* but the increase was greatest in the resistant cultivar. Choudhary *et al.* (2013) reported that the phenolic compounds showed an increase in the resistant tomato varieties after inoculation with root knot nematode, whereas susceptible varieties showed a gradual decrease in phenolic compounds after inoculation.

Mayer and Harel (1979) observed that lower levels of phenols during the later stages was linked to the oxidation of phenols by polyphenoloxidase (PPO) and this enzyme was widely distributed in plants and catalysed the hydroxylation of monophenols to O-diphenols and their oxidation to O-diquinones. Taiz and Zeiger (2002) reported that early increases in phenol caused by pathogen invasion triggered the transcription of messenger RNA that codes for

Phenylalanine Ammonia Lyase (PAL); increasing amounts of PAL in the plant brought about the synthesis of phenolic compounds. Melo *et al.* (2006) reported that the quinones were highly reactive molecules that spontaneously aggregated into large structures of various types of molecules, including proteins, lipids, nucleic acids and carbohydrates.

2.2.2. Peroxidase, Polyphenol Oxidase and Phenylalanine Ammonia Lyase Activity

Alterations of plant enzymes mainly peroxidase, polyphenol oxidase, catalase and phenylalanine ammonia lyase in the tissues of nematode infected, susceptible and resistant varieties were extensively studied. Such alterations differed in susceptible and resistant cultivars (Molinari, 1995; Sharma, 1993). The root-knot nematode infection increased peroxidase activity, phenol content, polyphenol oxidase activities, phenylalanine ammonia lyase in roots of cotton, coffee, banana and rice (Mishra and Mohanty, 2007; Sundararaju and Suba, 2006; Xu *et al.*, 2008). Silva *et al.* (2010) reported that Peroxidase (PO), Polyphenol Oxidase (PPO) and Phenylalanine Ammonia Lyase (PAL) activities significantly increased in roots of *M. exigua* inoculated coffee plants compared with roots of uninoculated plants. PO activity in roots of nematode-inoculated plants of cvs Catuai 44 and IAPAR 59 increased by 39.9 and 31.3 per cent, respectively; PPO increased by 54.9 and 56.1%; and PAL activity was also higher at 26.6 and 62.9 per cent.

Shukla and Chakraborty (1988) found that peroxidase activity increased in tomato resistant cultivars up to 5 times than that in healthy plants as measured 10 days after inoculation by *M. incognita* and decreased thereafter to normal levels within few days. Also, *M. incognita* resistant varieties of tomato had significantly higher peroxidase enzyme specific activity than the susceptible varieties. Patel *et al.* (2001) reported that *Meloidogyne* spp. have ability to induce synthesis of peroxidase, polyphenol oxidase and total phenol in roots of chickpea.

Giebel (1973) found that in the healthy plants, activity of PAL was higher in the resistant potato variety than the susceptible one. Furthermore, their rate of increase was greater in the roots of infected potato plant than in the healthy. Mohanty *et al.* (1997) reported that the enhancement of enzyme activity in resistant varieties was associated with the reduction of aromatic aminoacids like phenyl alanine and tyrosine and those amino acids play greater role in lignification process.

The biochemical alterations in resistance banana accessions showed relatively higher PAL activity than the susceptible ones indicating the inherent higher content of PAL in resistant accessions (Devi *et al.*, 2007).

Salicylic acid (SA) activates host resistance to pathogens by inducing the synthesis of PR proteins and defense-related phenolic substances and enzymes (Vlot *et al.*, 2009).

2.2.2.1 Peroxidase

Arrigoni *et al.* (1981) correlated increased peroxidase activity with enhanced resistant response against *M. incognita.* Langrimini and Rothstain (1987) stated that these two responses occurred in plants due to accumulation of peroxidase following nematode infestation. Kumar *et al.* (2007) reported that the peroxidase activity decreased in nematode infested susceptible rice roots compared to nematode resistant roots. Varanasi and Talati (2014) observed that PO activity increased with infection level and was found to be significantly higher at 10,000 J_2/plant than at 1000 J_2/plant.

2.2.2.2 Phenylalanine Ammonia Lyase

Phenylalanine ammonia lyase is the most important enzyme in the synthesis of phenolics, phytoalexin and lignin. Hence it is considered as the most important enzyme in disease resistance. PAL is the first enzyme in the phenylpropanoid pathway and thus PAL is involved in the defense mechanism of the plant. Increased activity of peroxidase in tomato and phenylalanine ammonia lyase in brinjal was positively correlated with nematode resistance

(Rajasekar *et al.*, 1997; Sirohi and Dasgupta, 1993). Devrajan and Seenivasan (2002) observed that inoculation of *M. incognita* increased the polyphenol oxidase activity in banana. Kumar *et al.* (2007) found that the degree of PAL activity was comparatively lesser in susceptible rice than resistant cultivars.

2.2.2.3 Polyphenol Oxidase

Ganguly and Dasgupta (1984) confirmed the increased PPO activity in response to nematode infestation in resistant varieties of crops. Kumar *et al.* (2007) reported that sequential development of polyphenol oxidase increased in nematode infested resistant rice varieties.

2.2.3 Chlorophyll content

Rao *et al.* (1988) reported that the total chlorophyll in leaves of healthy rice plants was 3.7 mg and of a and b fractions were 2.1 and 1.6 mg per g. Chlorophyll was reduced, owing to root-not nematode incidence, by 30.2 % of total and 39.5 and 32 % of the a and b fractions. Mishra and Mohanty (2008) reported that due to the nematode infection chlorophyll a was reduced from 2.367 mg per g to 0.812 mg per g in Annapurna; 2.349 to 1.719 mg per g in Manika and 2.117 to 2.097 mg per g in Ramakrishna, which accounted for reductions of 65.7, 26.8 and 1 % respectively in these varieties. After computing chlorophyll b fraction it was observed that the total chlorophyll in the leaves got reduced by 60.7% in Annapurna, 24.9% in Manika and 1% in Ramakrishna. This indicates that nematode infection causes nutrient shortage, which triggers depletion of chlorophyll, which in turn reduces carbon assimilation and successive metabolic processes.

Melakeberhan *et al.* (1986) reported that a reduction in total chlorophyll has also been reported in French bean and rice infected with *M. javanica*. Patel *et al.* (2001) reported that the chlorophyll content of leaf decreased in root knot nematode infected chickpea. Reduction in chlorophyll content of the infected plant has been reported by Poornima and Vadivelu (1998); Ramakrishnan and Rajendran (1998) and Praveen *et al.*, (2006). Takamiya *et al.* (2000) found

that the chlorophyll released from damaged chloroplasts has to be degraded rapidly to avoid cellular damage owing to its high reactivity. Various forms of abiotic and biotic stresses damage plant leaf tissue and the chloroplasts (Karpinski *et al.*, 2003). Abbasi and Hissamuddin (2014) reported that the chlorophyll (a+b) content decreased significantly at higher inoculum levels *ie.* Pi= $800J_2$ and Pi= 1600 J_2, when compared with control.

Swain and Prasad (1988) found that in a few cases, particularly in resistant varieties, chlorophyll content was found to increase after nematode infection.

2.2.4 N, P, K and Micro Nutrients

Patil *et al.* (2013) have demonstrated reduction in uptake of macronutrients, Nitrogen (N), Phosphorus (P), Potassium (K) and micronutrients, Iron (Fe) and Zinc (Zn) due to *M. graminicola* infection of the roots. Venkatesan *et al.* (2013) reported that the content of major plant nutrients N, P and K was much reduced in the shoot of rice plants inoculated with *M. graminicola*. The reduction was more at higher inoculum level. This would indicate lesser uptake or translocation of these nutrients.

Bergeson (1966) studied the mobilization of several minerals in tomato infested with *M. incognita* and concluded that the nematode caused an increase in their concentration in the roots but not in the leaves. Devrajan *et al.* (2003) showed that *M. incognita* infestation reduced the N, P, and K levels in the leaves. The ability to absorb boron, copper and zinc by roots of ginger was decreased due to *Meloidogyne* spp. infection (Gauo and Wang, 2004). The degree of uptake of nitrogen, phosphorus, potassium and total sugar content were significantly reduced in Cotton var. H777 infected with *M. incognita* (Verma and Jain, 2006). Patil and Gaur (2014) reported that in both pot and field experiment, in general Fe and Zn content in leaves of a non- basmati Pusa- 44 and a basmati sugandh- 5 cultivars of rice showed significantly lower with increasing nematode population.

2.2.4.1 N, P and K Content

Patil and Gaur (2014) reported that the N, P and K contents in the rice leaves were significantly reduced in a non-basmati Pusa-44 and a basmati Pusa Sugandh-5 cultivars of rice infestated with *M. graminicola* under both pot and field conditions. The residual contents of N, P, K, in soil were more with increasing nematode population. Thus, the nutrients remained unutilized in the soil while the plant suffered severe nutrient deficiencies and growth reduction in the presence of nematode infestation.

In healthy tomato plants, the N, P and K content of stem and leaves is much higher than the root knot nematode infected plants. This may be probably due to the fact that the metabolic activities are higher in the galled roots than in healthy roots. Consequently, there is a greater accumulation of these elements in addition to free amino acids, amide, protein etc (Haseeb and Srivastava, 1992). Qudassi *et al.* (2014) reported that the infection caused by root-knot nematodes in tomato results in an accumulation of N, P and K in the roots of the infected plants and consequently they decline in stems and leaves.

N in the leaves is broken down to simpler proteins and gets translocated. Furthermore, chlorophyll itself is broken down, thereby reducing the shoot protein content (Jena and Rao, 1977; Ganguly *et al.*, 1991). Mishra and Mohanty (2008) found that the shoot N content was 1.73, 1.64 and 1.72% in the varieties Annapurna, Manika and Ramakrishna, respectively in healthy plants and they decreased by 22, 16.6 and 2.4% respectively in the root knot nematode infected rice plants.

Reduction in P content indicates general reduction in cell activity, both in root and shoot, excepting the galls (Rao *et al.*, 1986). Phosphorus content decreased in the rice varieties Annapurna, Manika and Ramakrishna due to root knot infection. The reductions were 34.8, 9.8 and 10.6%, respectively in the varieties annapurna, Manika and Ramakrishna. Due to nematode infection P content of shoot decreased by 25.2, 6.7 and 1% in these varieties (Mishra and Mohanty, 2008).

Chakrabarti and Mishra (2002) recorded decrease in K content of the root due to root knot infection in chickpea, but conversely the extent of reduction of K was less in the resistant variety. In the healthy rice plants K content of the shoot was found to be 4.225% in Annapurna, 3.517% in Manika and 2.465% in Ramakrishna which reduced by 59.6, 18.9 and 22.7% respectively in the infected plants (Mishra and Mohanty, 2008).

Dropkin and King (1956) concluded that phosphorus translocation was not affected in tomato. Hunter (1958) observed an increase in phosphorus content in tomato roots infected by *M. incognita*. Since there was no alteration in the concentration in shoot it was concluded that the transport of phosphorus was not affected. No alteration was observed with respect to calcium. Oteifa and Elgindi (1962) found that in roots of tomato infected with *M. javanica*, there was accumulation of 32P and reduction in phosphorus translocation to the shoot. The root-knot nematode, *M. konaensis* on coffee reduced nitrate and ammonia uptake rates by 63% and 54%, respectively and the lesion nematode, *P. coffeae* reduced nitrate uptake rate by 56% and ammonia uptake rate by 24% (Vast *et al.*, 1998).

The concentration and uptake of nitrogen, phosphorus, potassium and total sugars and phenol contents were significantly reduced in cotton var. H 777 infected with *M. incognita* (Verma and Jain, 2006).

2.2.4.2 Micro nutrients

Patil (2014) reported that the Fe and Zn contents in the rice leaves were significantly reduced in a non-basmati Pusa-44 and a basmati Pusa Sugandh-5 cultivars of rice infestated with *M. graminicola* under both pot and field conditions. The degree of reduction did not significantly differ in the two cultivars. The residual contents of Fe and Zn in soil were more with increasing nematode population. Thus, the nutrients remained unutilized in the soil while the plant suffered severe nutrient deficiencies and growth reduction in the presence of nematode infestation.

Iron content in the shoots of healthy rice plants was 1.7 mg/g, while in the nematode infested plants, there was a reduction by 19.0 per cent (Rao *et al.*, 1988). Gauo and Wang (2004) reported that the ability to absorb boron, copper and zinc by roots of ginger was decreased due to *Meloidogyne* spp. infection. Gauo and Wang (2004) opined that the decrease in total boron, iron, copper and zinc contents in ginger indicated that the ability of the roots to absorb nutrients was weakened by root-knot nematode, *M. incognita* infection. The concentration of Fe and Zn were significantly reduced in leaves of nematode infected plants as compared to the leaves of non-infected plants whereas in soil in the rhizosphere of nematode infected plants remained high. Hence, nematodes reduced the uptake of nutrients from soil to shoot part of the plant (Patil and Gaur, 2014).

2.2.5 Starch and Protein in Seed

Patil and Guar (2014) studied the effect of root-knot nematode, *M. graminicola* on the quality of grain under pot and field conditions using two cultivars of rice, a basmati, cv. Pusa Sugandh-5 and a non-basmati, cv. Pusa-44. They found that there was a significant decrease in the seedling vigour indices of both the rice cultivars. These results showed that the rice grains produced on plants infected with the nematode *M. graminicola* were lighter in weight and had poorer nutrient qualities, such as amylose and protein content.

Mohanty and Pradhan (1989) found that the susceptible green gram plant showed low protein content and high level of amino acids after inoculation of nematodes. They also reported that protein contents decreased and amount of free aminoacid and amides increased after root knot nematode inoculating susceptible as well as resistant cultivars. Abbasi and Hissamuddin (2014) reported that the seed protein content of *V. radiata* exhibited reduction at all the initial inoculum levels when compared with the protein content of uninoculated control. These findings were in agreement with Korayem *et al.* (2013) who found that crude protein and fat contents decreased in the peanut seeds influenced by *M. arenaria*.

2.2.6 Total and reducing sugar

Rao *et al.* (1988) reported that the rice grains showed reduction of reducing sugars due to infestation by *H. oryzicola* and *M. graminicola.* Charles and Venkitesan, (1993) reported that the percentage of reducing sugar content of fruit was lower in banana plants without the nematode inoculum (*H. oryzicola*) than in the nematode inoculated plants. The percentage of non reducing sugar was significantly reduced in the fruit of inoculated plants. This was found to decrease with the amount of initial inoculum per plant. The total sugar content of fruit was also less in inoculated plants compared to healthy plants.

In rice, total and reducing sugar contents were increased in nematode infested roots o f resistant cultivars, as reported by Ganguly and Dasgupta (1983). Mohanty *et al.* (1997) reported increased total sugar content in resistant (Manika) compared to susceptible (Annapurna) variety to *M. graminicola* in rice. Silva *et al.* (2010) reported that the sugar content of bitter gourd roots increased 50.33 per cent in *M. incognita* inoculated plants compared to healthy plants. Hofmann *et al.* (2007) and Nayak and Mohanty (2010) agreed with increase sugar levels in roots are due to high metabolic activities in diseased tissues.

2.2.7 Plant growth and yield parameters

Patil and Gaur (2014) reported that the rice cultivars Pusa- 44 and sugandh- 5 showed significantly lower plant height, shoot and root dry weight with increasing nematode population.

The plant response to nematode parasitism causes morphological and physiological changes that affect photosynthetic processes (Melakeberhan *et al.,* 1986; Hussey and Williamson, 1997). Infection caused by *M. incognita* leads to reduction in height and weight as has been reported by by Ramakrishnan and Rajendran (1998) and Jonathan and Rajendran (2002).

Mhatre *et al.* (2015) reported that about 50-75% reduction in root system of rice was observed with swelling along the roots which contributed to poor root system.

Poudyal *et al.* (2005) conducted an experiment to examine the effect of initial population density (Pi) of *M. graminicola* on rice cv. Masuri and nematode population development. They reported that plant height and tiller number were unaffected, but root length, panicle number, total panicle length, grain number and weight declined significantly with increasing nematode density.

Khan *et al.* (2012) reported that rice grown in nematode infested soil exhibited considerable degree of reduction in plant growth which varied with cultivars. Greatest decrease in the shoot length was recorded in cvs. Sugandh (37.8%) and R-Dhan (21.5%) over respective controls. The rice cvs. Swarna (34.5%) and Sugandh (27.9%) exhibited greatest decrease in root length whereas greatest decrease of dry weight of shoot was recorded in the cvs. Samba Mehsuri (46.4%) and Sugandh (29.2%). The dry weight of root was decreased by 28 % (cv. Sugandh) and 23% (cv. R- Dhan).

Devi (2014) reported that the pathogenicity of *M. graminicola* on rice varieties *viz.*, Dharam, Tampha, RCM-9, SK, Ayangleima, Jatra, Maminthondabi, Thangjing, Priya and Lamyanba. Rice variety Lamyanba showed maxnimum root length 10 cm, shoot length 45 cm, fresh shoot weight 22 g, fresh root weight 3.4 g, number of seeds 100 and number of galls 45 followed by rice variety Priya and Thangjing. Rice varity Dharum showed maximum root length 15 cm, shoot length 60 cm, fresh shoot weight 44 g, fresh root weight 5.3 g, number of seeds 375, which was followed by Tampha and RCM-9.

Reduction in rice yield may depend upon the initial population density (Pi) of *M. graminicola* (Prasad *et al.*, 1990; Prot *et al.*, 1994; Main and Khan, 1995). High population density of *M. graminicola* second stage juveniles (J_2) can cause seedling mortality, reduction in plant height, tiller number and grain yield losses up to 98% (Plowright and Bridge 1990; Prasad *et al.*, 1990).

Prot and Matias (1995) also reported the adverse effect of Pi on plant growth parameters and grain yield of rice cv. UPL Ri5.

Win *et al.* (2013) studied the host response of low land and upland rice varieties from Myanmar to the rice root- knot nematode, *M. graminicola* and reported that at 8 WAI fresh root weight and dry shoot weight were significantly reduced as a result of inoculation with *M. graminicola.*

Win *et al.* (2013) reported that at 8 WAI the plant height and root length were significantly reduced in the inoculated rice varieties Yatanartoe, MR 9 and Saytanar 1 compared with the uninoculated plants.

Plowright and Bridge (1990) reported that high initial population density of *M. graminicola* caused wilting of seedlings along with severe reduction in growth parameters while low population caused only reduction in growth parameters. The differences in host response to *M. graminicola* infection among rice varieties have been reported by several workers (Poudyal *et al.,* 2004; Bridge *et al.*, 2005; Prasad *et al.*, 2006).

Patil and Guar (2014) studied the effects of a series of infestation levels of *M. graminicola* on the nutritional and seed quality parameters of the grain under pot and field conditions using two cultivars of rice cvs. Pusa Sugandh-5 and Pusa-44. They reported that 1000 grain weight was significantly reduced by as much as 44.5% in cv. Pusa Sugandh-5 and 50.7% in cv. Pusa-44 seed when the parent plants were grown in soil with very high levels of *M. graminicola* infestation.

Many researchers have shown that the nematode may cause high yield loss to rice and it may be up to 30-80% depending upon nematode population, soil ondition and cultivar (Bridge *et al.*, 2005; Padgham *et al.*, 2004; Gaur and Pankaj, 2010).

Poudyal *et al.* (2002) reported that root knot nematode infested rice plants had lower number of total and effective tillers, filled grains per panicle and grain

yield. Yield reduction in diseased plants was 40.5% as compared to healthy plants in the variety 'Masuli'.

2.3 HISTOPATHOLOGICAL CHANGES

The second-stage juveniles of *M. graminicola* made their point of entry at the zone of elongation just behind the root cap in both susceptible (TN1) and resistant cultivars (TKM 9) and occasionally at the root cap at 24 h after inoculation. After penetration, the juveniles entered into cortex, oriented their bodies parallel to the stele and moved along cortical layer of cells directed towards apical meristem and started feeding between 24 - 48 h after inoculation. Disruption and hypertrophy of cortical cells due to migration and movement of *M. graminicola* larvae partly contributed to the development of knots in rice roots. stele at the sites of nematode attack and their establishment. In resistant variety (TKM 9) poor giant cell formation caused delay in nematode development while in susceptible cultivar nematodes were developed and reproduced without disruption and produced egg mass which can be seen in matured healthy females (Kumar *et al.*, 2007).

Kumar *et al.* (2008) reported that the rice varieties *viz.*,Jaya, Rasi, Jyothi and IR-64 under healthy condition, showed normal organization of tissue in the root section and the cells in the epidermal, cortical and stelar region were also normal. However, under infected conditions, the cultivar IR-64 and Jyothi recorded maximum post infection and increased size of epidermis, cortex and stelar region compared to Jaya followed by Rasi, and cells were irregularly shaped, enlarged cells were found and the cells in stellar region were irregularly arranged.

The formation of giant cells had caused aberration of the vascular region and resulted curved swollen root tips. It was observed that the females developed within the root and eggs were laid mainly in the cortex. Bridge *et al.* (1990) reported that juveniles of *M. graminicola* can either remain in the gall or migrate through the parenchyma tissue of the cortex to new feeding sites within the same root.

Siddiqui and Taylor (1970) observed that thick walled giant cells in the vascular region of wheat and oat roots infected by *M. nassi*. Taylor (1976) observed giant cells mostly in the phloem and a few in the xylem and cortical tissue and observed thin walls in giant cells. Tandon and Kumar (1979) and Ekanayake (1985) reported that clusters of giant cells in the vascular region of tomato roots infected by *M. lucknowica* and *M. incognita* respectively.

Gopinath (2001) found higher concentration of the insoluble polysaccharides, nucleic acids and proteins in the infected roots compared to healthy roots. Similarly, Praveen (2002) reported that increased concentration of total insoluble polysaccharides, total proteins and nucleic acids was also observed in root knot nematode infected roots when compared to healthy roots. Kumar *et al.* (2008) reported that there was increase in the overall growth of cells in epidermal, cortical and stelar region in the root infected with *M. graminicola* when compare to healthy plants of each cultivar. Similarly, all the cultivars recorded post infection increase in concentration of total insoluble polysaccharides, total proteins and nucleic acids in infected roots of paddy cultivars viz., IR-64, Jaya, Jyothi and Rasi.

Shah and Raju (1977) reported that the giant cell nuclei and cytoplasm were rich in nucleic acids, minute protein granules were present in the giant cells and on the outer side of the egg sac a thick layer of insoluble polysaccharide was present.

Siddiqui *et al.* (2014) reported that the histopathology of the galled roots of centaurea, calendula, and papaver during later stages of root knot nematode infection showed that nematodes were localized entirely within the cortex and generally oriented horizontally to the vascular cylinder. Most of the females were mature, and a few of them were associated with egg masses. Giant cells with a variation in cell sizes were observed in the galled roots of all three of the plant species and exhibited a granular cytoplasm and hypertrophied nuclei as a typical reaction to nematode feeding.

2.3.1 Root knot nematode and vascular tissues of host plant

Robab *et al.* (2010) found that a large portion of ground tissue of the stele was malformed into abnormal xylem, which was the most noticeable feature of galled roots. The host parasite interaction of root-knot nematode infection causes disintegration of the xylem and phloem tissues, causing an interruption in the transport of water and mineral nutrients and the translocation of food materials in the host plant.

2.3.2 Root knot nematode and giant cells

Yasmeen (2002) studied that the giant cells were generally transformed from undifferentiated vessel elements or from xylem parenchyma or from provascular strand or from protophloem cells. Azam and Hisamuddin (2008) and Azam *et al.* (2011) reported that *Meloidogyne* infections accompanied cortical and stellar proliferations, hypertrophy and hyperplasia in the cortex, pericycle and stele of the roots.

Singh *et al.* (2013) studied the structural variation in the provascular region of the root knot nematode infected root system and reported that the cells near the head of the nematode were severely hypertrophied. These incipient giant cells contained a large central vacuole and parietally distributed granular cytoplasm.

Parveen (2006) and Niyaz and Hisamuddin (2009) reported that enlargement and fragmentation of the nucleoli of the affected cells occurred in various plants due to root knot infection.

Chandel *et al.* (2001) and Sayed *et al.* (2010) studied the various abnormalities in the cortical and stellar region of the root knot nematode infected lentil root. Singh *et al.* (2007) reported that the parenchyma cells adjacent to the giant cells transformed into vessel like elements and these changes were responsible for transport of water towards the giant cells. Singh *et al.* (2013) reported that the multinucleate giant cells require more amount of water for enhanced rate of synthesis of cytoplasmic materials which was fulfilled by the modification of the xylem strand in the galled portion.

Khan *et al.* (2007) studied the modification of giant cells for the nutrition of nematode and reported that giant cells act as sink for the photosynthetic metabolites. Kumar *et al.* (2007), Azam (2009) and Niyaz *et al.* (2011) reported that in case of severe infection a large number of galls on the roots were formed in each gall, several giant cell complexes become functional towards which mineral nutrients, water and metabolites were diverted. Singh *et al.* (2013) reported that a large amount of metabolites were translocated towards the giant cells, formation of gall and differentiation of abnormal xylem elements contributed in sustaining giant cells on which the survival of nematode is depended.

2.4 VARIETAL REACTION

Mohanty *et al.* (1992) and Prasad *et al.* (2000) screened certain locally important rice varieties against *M. graminicola* and reported that TKM 9 was moderately resistant to *M. graminicola.* Kumar *et al.* (2007) screened a total of 52 rice varieties, comprising of short (19), medium (30) and long (3) duration, against *M. graminicola* under greenhouse conditions and reported that the short duration varieties *viz.,* TKM3, TKM7, TKM8, TKM9 and MDU1 and medium duration varieties *viz.,* MDU2, TKM11 and PY1 were found resistant to *M. graminicola.* The rest of short, medium and all long duration varieties were graded as susceptible or highly susceptible.

Myint (1981)tested the reaction of five rice hybrids, Indo- American hybrid No. 300-001, Indo- American hybrid No. 200- 003, Indo- American hybrid No. 200-004, IR 64616 H and IR 75584 H against the infection of rice- root knot nematode, *M. graminicola.* They reported that all the 5 hybrids fell into the root knot index value of 5 and none of these was resistant to rice root knot nematode, *M. graminicola.* Among the test hybrids, infected root percentage and root-knot number per plant of Indo- American hybrid No. 200- 003 and Indo- American hybrid No. 200- 004 were significantly less than those of Indo- American hybrid No. 300- 001 and IR 75584 H.

Devi *et al.* (2014) reported that out of the 10 rice varieties, Dharam was recorded as moderately resistant variety against root-knot nematode followed by rice variety Tampha were recorded. SRCM-9, SK, Ayangleima, Jatra were recorded as susceptible whereas Mamingthondabi, Thangjing, Priya, Lamyanba were recorded as highly susceptible.

Arayarungsarit *et al.* (1985) studied the resistance action of three selections from IR36/RD25 crosses to *Meloidogyne* spp. in Thailand. Poudyal *et al.* (2002) reported that rice cvs. Masuli and Chaite-6 were moderately resistant to *M. graminicola.* Hassan *et al.* (2004) found that the rice cvs. Loknath 505 and M-36 were resistant to root-knot nematode, *M. graminicola.* Kumar *et al.* (2007) observed resistance to this nematode in rice cvs. TKM3, TKM7, TKM8, TKM9, MDU1, MDU2, TKM11. Devi and Thakur (2007) screened 8 rice varieties, screening rice varieties for resistance against *M. graminicola.*

Srivastava *et al.* (2011) evaluated eighty seven cultivars of rice and fifty nine cultures of wheat against root-knot nematode infection in the field during *kharif* 2007, 2008 and 2009 and *rabi* 2008 and 2009 revealed two rice cultivars Achhoo and Naggardhan and two wheat cultivars HS 295 and VL 829 as resistant with 2 score. Rice lines Ranbir Basmati, Hasan Sarai, and Purple cultures and wheat cultivar HS 240 were rated as susceptible.

Soriano *et al.* (1999) reported that one accession of *O. longistaminata* represented by two individuals (WL02-2 and WL02-15) and three accessions of *O. glaberrima* (TOG7235, TOG5674 and TOG5675) were resistant to the rice root-knot nematode. Poudyal *et al.* (2004) evaluated 12 Nepalese rice varieties under green house condition and found that Masuli and Chaite- 6, were moderately resistant to the root knot nematode. Evaluation of advanced backcross populations developed for water stress environment revealed that Teqing and the donors cvs Type 3, Zihui 100, Shwe Thwe Yin Hyv were resistant to the nematode (Prasad *et al.*, 2006). Das *et al.* (2011) reported that *O. glaberrima* accessions CG 14 and TOG 5674, traditional cultivars WAB 638-1 and IRAT 216

and aerobic rice genotypes IR 81426-B-B-186-4 and IR81449-B-B-51-4 to be resistant to *M. graminicola.*

Poudyal *et al.* (2004) evaluated twelve commonly cultivated rice cultivars viz. Radha-7, Ghaiya-2, Rampur Masuli, Bindeshwari, Makawanpur-1, Sabitri, Janaki, Chaite-2, Radha-9, Radha-4, Chaite-6 and Masuli against rice root knot nematode in sick soil under greenhouse condition and reported that all the cultivars were susceptible *to M. graminicola* except Masuli and Chaite-6, which were moderately resistant.

Research conducted in many countries revealed that the most of rice cultivars were susceptible to *M. graminicola* (Bridge *et al.,* 1990) however, Soriano *et al.* (1998) reported that limited number of rice cultivars and breeding lines were resistant to *M. graminicola.*

Berliner *et al.* (2014) reported that two rice germplasm lines from NBPGR collection and 4 aerobic rice cultivars were tolerant to root-knot nematode leaving all other in susceptible and highly susceptible category.

2.4.1 Gall Index and Egg Mass Index

Root galls, which are terminal and spiral or hooked are characteristic symptom of the root knot nematode (Khan and Anwer, 2011). Devi (2014) reported that maximum numbers of root galls (45) were recorded in rice variety Lamyanba whereas minimum root galls (2) were recorded in rice variety Dharam. A large variation in number of galls caused by *M. gramainicola* per root system in different rice cvs./landraces was observed by Mhatre *et al.* (2015) and reported that Out of total 64 rice genotypes, seven cvs., Abhishek, Khaja, Super Sugandhamati, Kishori Dehraduni, Gaudeshwari, Tuniaslet, Chima Kamin showed less galling (<10 galls/plant). Among the tested cvs./landraces, Abhishek exhibited the best resistance response (2 galls/plant). On the contrary, >100 galls/plant were observed in cv. Bangla Patni (highly susceptible) having typical hook shaped terminal galls, characteristic of *M. graminicola* infection.

Khan *et al.* (2012) reported that the severity of root-knot in terms of number of galls/root system varied considerably. A maximum of 57 and 51 galls/root system were recorded on the rice cv. Sugandh and R-Dhan, respectively. The cv. Sadabhar, Samba Mehsuri, Sharbati and Hazari developed 35-38 galls which were significantly less than the cvs. Sugandh and R-Dhan. On the cv. Abhishek, only 8 galls and 3 egg masses/root system were formed. Egg mass production in the rice cultivars was more or less similar to gall formation. Greatest number of egg masses/root system was developed on cv. Sugandh. In cv. R-Dhan, egg masses were significantly less than cv. Sharbati. Lowest numbers of egg masses were recorded on the cv. Abhishek.

Khan *et al.* (2014) reported that the plants of rice cv PS-5 grown in the nematode-infested field showed extensive terminal galls which varied in number from 73 to 81 galls per root system, while 61 to 69 egg masses were recorded in the two years. Berliner *et al.* (2014) reported that the rice cultivars were categorized as resistant or susceptible types on the basis of Gall Index (GI),Out of 414 rice genotypes screened, only two entries from breeding lines, 127-28-1-1-1 and 183-6- 1-1-3 were found resistant with score 2.

2.4.2 Nematode population

Khan *et al.* (2012) reported that soil population of *M. graminicola* showed considerable degree of variation with regard to cultivar. Highest soil population of *M. graminicola* was recorded in the root zone of rice cv. R- Dhan and Sugandh. Population in cultivars Samba Mehsuri and Sharbati were significantly less than the cv. R-Dhan or Sharbati. Lowest population of *M. graminicola* was recorded from the pots in which cv. Abhishek was grown.

Poudyal (2001) reported that final (150 days after inoculation) soil and root J_2 population egg density and root knot index differed significantly with initial nematode population levels. Reproduction rates (Rr) decreased with increased initial population levels. Khan *et al.* (2014) found that one month after planting, the *M. graminicola* population in the soil was almost double the initial

population and further increased to approximately four times the initial population at harvest (4 months).

Devi (2014) Screened 10 common rice varieties of Manipur against rice root-knot nematode (*M. graminicola*) and reported that the variety Lamyanba showed maximum soil population (7500), root population (3500) and total population (11000).

The rice cvs. Anjali, Swarna, Vandana and Virendra showed 28-38 galls/root system. These cultivars also supported reproduction of *M. graminicola* which produced 20-38 egg masses leading to 60-90% increase in the soil population of the nematode (Khan *et al.*, 2012). They also reported that the cvs. Sadabhar, Samba Mehsuri, Sharbati and Hazari were found to be moderately susceptible as the soil population, number of galls (35-38) and egg masses (23-32) were lesser than cvs. R-Dhan and Sugandh.

Jena and Rao (1976) have suggested that the criteria for evaluation of rice cultivars against *M. graminicola* should be based on the reproduction in terms of egg masses and eggs.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Pot culture experiments were conducted at College of Agriculture, Vellayani during 2014-2015 to determine the biochemical changes, histopathological alterations and changes in growth and yield parameters of rice (*Oryza sativa* L.) due to the infestation of root knot nematode, *Meloidogyne graminicola* and to determine the varietal reaction of 10 important varieties of rice in Kerala against root knot nematode.

Sieved field soil, sand and well decomposed farmyard manure were mixed in the ratio 2:1:1 and the denematized potting mixture was used for pot culture studies. Pure culture of *M. graminicola* was raised from egg masses collected from infested rice roots and multiplied on rice maintained in sterilized soil. Subculturing was done periodically to ensure availability of sufficient larval population for inoculation following standard procedures.

Viable egg masses were hand picked from the infested roots of culture plants and kept on two layers of tissue paper supported by aluminium wire gauge in 10 cm sized petri dishes filled with fresh water for obtaining required number of J_2 (second stage juveniles). The population of J_2 of *M. graminicola* in the suspension was determined by counting J_2 in 5 ml aliquot. Three such counts were made and the average of 3 counts was taken as population in 5 ml suspension. The larval concentration was adjusted to required number of juveniles per ml of suspension by adding required quantity of sterile water.

3.1. BIOCHEMICAL STUDIES

Biochemical analysis of rice plants with different nematode inoculums were carried out. Seeds of rice cv. Uma were surface sterilized with 0.1 per cent mercuric chloride and soaked overnight for sprouting. The sprouted paddy seeds were sown in pots filled with steam sterilized soil. The seeds were allowed to germinate and after

Plate 1. Root knot nematode infested rice field

Plate 2. Patchy yellowing in rice field due to infestation of *M. graminicola*

seven days seedlings were inoculated with freshly hatched juveniles at the rate of 0, 100, 500, 1000, 5000 and 10,000 J_2 per pot through the holes around the plant within the radius of two centimeters and plugged with the sterilized soil soon after inoculation. To maintain soil moisture in the pot, regular watering was done. Each treatment was replicated five times and the pots were arrainged in Complete Randomized Block design. Uninoculated set of plants served as control. There were six sets of pots as given below:

- T1: Uninoculated plant
- T2: 100 J_2 / pot
- T3: 500 J_2 / pot
- T4: 1000 J_2 / pot
- T5: 5000 J_2 / pot
- T6: 10,000 J_2/ pot

After 45 days of inoculation, the following parameters were taken into account for describing the results. Biochemical analysis was conducted to estimate the changes in the total phenols, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, pH of the leaf extract, chlorophyll a and b, NPK contents in leaf samples, Micro nutrients in leaf samples. The starch, protein and total sugar content in grain was estimated after harvesting the grain.

3. 1.1 Estimation of phenol

The phenol content was estimated following the procedure described by Bray and Thorpe (1954). One gram leaf sample was homogenized in 10 ml of 80 per cent ethanol. The homogenate was then centrifuged at 10000 rpm for 20 min, supernatant was saved and residue was extracted with five times the volume of 80 per cent ethanol and centrifuged as above. The supernatant was saved and evaporated to dryness in a boiling water bath. The residue was dissolved in 5 ml distilled water. Folin- Ciocalteau reagent (0.5 ml) was added and 2 ml of 20 per cent sodium

carbonate solution was added to each tube after three min. This was mixed thoroughly and kept in boiling water for one minute. The reaction mixture was cooled and absorbance was measured at 650 nm against a reagent blank. Standard curve was prepared using different concentrations of catechol equivalents as milligram per gram of leaf tissue on fresh weight basis.

3.1.2 Peroxidase (PO)

Peroxidase activity was assayed by a spectrophotometric method as described by Srivastava (1987). Leaf sample of 1 g was homogenized in 5 ml of 0.1 M sodium phosphate buffer (pH 6.5) to which a pinch of polyvinyl pyrrolidone (PVP) was added. The homogenization was done at 4^0C using a pre- chilled mortar and pestle. The homogenate was filtered through a muslin cloth and centrifuged at 5000 rpm for 15 min at 4^0C. The supernatant was used as the enzyme extract for the assay of PO activity.

The reaction mixture consisting of one ml of 0.05 µl pyrogallol and 50 µl of enzyme extract was taken in both reference and sample cuvettes, mixed and kept in a spectrophotometer (systronics UV- Vis spectrophotometer 118) and the reading was adjusted to zero at 420 nm. To initiate the reaction, one ml of one percent hydrogen peroxide (H_2O_2) was added to the sample cuvettes and the changes in absorbance were recorded at 30 seconds interval upto 180 sec. The PO activity was expressed as changes in absorbance min^{-1} g^{-1} fresh weight of tissue.

3.1.3 Polyphenol Oxidase (PPO)

Polyphenol Oxidase activity was determined as per the procedure given by Mayer *et al.*, (1965). The enzyme extract was prepared as per the procedure given for the estimation of PO.

The reaction mixture contained one ml of 0.1 M sodium phosphate buffer (pH 6.5) and 50 µl of enzyme extract. The reaction was initiated after adding one ml

of 0.01 M catechol. The observations were recorded in a spectrophotometer (Systronics UV-VIS spectrophotometer 118). The change in absorbance was recorded at 495 nm at 30 seconds interval up to 180 sec. PPO activity was expressed as change in the absorbance of the reaction mixture per minute per gram of leaf tissue on fresh weight basis.

3.1.4 Phenylalanine Ammonia Lyase (PAL)

PAL activity was assayed spectrophotometrically by assaying the rate of conversion of L-phenyl alanine to trans- cinnamic acid at 290 nm as described by Dickerson *et al.* (1984). The enzyme extract was prepared by homogenizing one gram leaf sample in 5 ml of 0.1 M borate buffer (pH 8.8) containing a pinch of PVP using chilled mortar and pestle.

The homogenate was centrifuged at 10,000 rpm for 10 min at 4^0C. The supernatant was used for the assay of PAL activity. The reaction mixture contained 3 ml of 0.1 M sodium borate buffer (pH 8.8) 0.2 ml enzyme extract and 0.1 ml of 12 Mm L- phenyl alanine prepared in the same buffer. The blank contained 3 ml of 0.1 M sodium borate buffer (pH 8.8) and 0.2 ml enzyme extract. The reaction mixture and blank were incubated at 40^0C for 30 minutes and reaction was read at 290 nm in a spectrophotometer (Systronics UV- VIS spectrophotometer 118). PAL activity was expressed as micrograms of cinnamic acid produced per minute per gram of leaf tissue on fresh weight basis.

3.1.5 pH of the leaf extract

Distilled or demineralised water was added to the ground leaf sample, to give water to leaf sample volume ratio of 8:1. The samples were shaked in a laboratory rotary shaker for 1 h, and centrifuged until there was a clear separation of the sediment and the supernatant. The supernatant was measured for pH by a wide range

of laboratory pH meter, as long as calibration is adequate (using buffer solutions of pH 4 and pH 7).

3.1.6 Estimation of Chlorophyll

Chlorophyll was estimated by the method described by Arnon (1949). One gram of leaf sample was finely cut and ground in a mortar with 20 ml of 80 percent acetone. The homogenate was centrifuged at 5000 rpm for five minutes and the supernatant was transferred to a 100 ml volumetric flask. The above procedure was continued till the residue become colourless. The final volume in volumetric flask was made up to 100 ml with 80 percent acetone. Absorbance of the solution at 645 and 663 nm was read in a spectrophotometer against the solvent (80 per cent acetone) as blank. The chlorophyll content was calculated using the following equations and expressed as milligrams of chlorophyll per gram of leaf tissue on fresh weight basis.

Chlorophyll a $= [12.7\ (A_{663}) - 2.69\ (A_{645})]\ V/1000W$

Chlorophyll b $= [22.9\ (A_{645}) - 4.68\ (A_{663})]\ V/1000W$

Total Chlorophyll $= [20.2 = (A_{645}) + 8.02\ (A_{663})]\ V/1000W$

Where A= absorbance at specific wave lengths, V= final volume of chlorophyll extract in 80 percent acetone and W = fresh weight of tissue extracted.

3. 2 PLANT ANALYSIS

3.2.1 Nutrient Content

The shoots after 45 days were used for analysis of N, P and K contents. The samples were oven dried at 60^0C to a constant weight in a hot air oven and the samples were ground and passed through 0.5 mm sieve, digested and used for analysis of nutrient content. The required quantity of sample was weighed out accurately in an electronic balance.

3.2.1.1 Total Nitrogen Content

Total nitrogen content was estimated by modified Microkjeldals method (Jackson, 1973) and expressed as percentage.

3.2.1.2 Total Phosphorus Content

Total phosphorus content was found out by Nitric- perchloric acid (9:4) digested and spectrophotometry using vanado molybdo phosphoric yellow colour method and expressed as percentage (Jackson, 1973).

3.2.1.3 Total Potassium Content

Total potassium content in plant was determined by using Niric- perchloric acid (9:4) digestion and flame photometer (Jackson, 1973) and expressed as percentage.

3.2.1.4 Micronutrients

Iron, Manganese, Zinc and copper contents were found out by Nitric-perchloric acid (9:4) digestion and AAS (Jackson, 1973).

3.3 GRAIN ANALYSIS

3.3.1 Estimation of Reducing Sugars- Glucose

The reducing sugar content in seeds was estimated by Fehlings method (Sadasivam and Manickam, 2008).

Five ml of Fehling solution A and B was taken in a clean conical flask and diluted with 10 ml of water. Few porcelain pieces or glass beads were added in to the conical flask and it was heated on wire gauze. When the solution started boiling, the digested sample grain solution was added to it from a burette. When the colour of the Fehling solution nearly faded, two drops of methylene blue indicator was added. The

titration was continued while adding digested grain sample solution in drops. The end point was indicated by the disappearance of blue colour of the indicator and appearance of bright red colour of cuprous oxide. The titration was carried out with another sample till concordant values were obtained.

$$\text{Percentage of glucose} = \frac{100}{V} \times 0.05$$

3.3.2 Estimation of Non Reducing Sugars - Sucrose

The non reducing sugar content in seeds was estimated by Fehlings method (Sadasivam and Manickam, 2008).

Two millilitre of digested grain sample solution was taken in a 250 ml beaker and diluted with 100 ml water. Five ml concenterated hydrochloric acid was added in to the solution with constant stirring and contents were kept in thermostat maintained at 60-70^{0}C for about 10 min. The beaker was then removed from the thermostat and a piece of blue litmus was dipped into it. Sodium hydroxide solution was added until the acid was nearly neutralized. The neutralization was completed by the addition of sodium carbonate. The solution was filtrated in to a 250 ml measuring flask and made up using distilled water. Ten ml of Fehling solution was titrated against this solution from the burette, until brick red colour appeared.

$$\text{Weight of sucrose in 100 cc of the sample solution} = \frac{250 \times 0.05 \times 5 \times 0.95}{V} \text{ g}$$

3.3.3 Estimation of Starch

The starch content in seeds was estimated by Anthrone method (Sadasivam and Manickam, 2008).

0.5 g of the sample was homogenized in hot 80% ethanol to remove sugars and centrifuged and retained the residue. The residue was repeatedly washed with hot 80% ethanol till the washing does not give colour with anthrone reagent. The residue was dried over a water bath. To that residue 5 ml of water and 6.5 ml of 52% perchloric acid were added and extracted at 0^0C for 20 min. Again centrifuged and saved the supernatant. The extraction was repeated using fresh perchloric acid, centrifuged and pooled the supernatant and made up to 100 ml. 0.1 or 0.2 ml of the supernatant was pipeted and made up the volume to one ml with water. The standard were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and made up the volume to 1 ml in each tube with water. 4 ml of anthrone reagent was added to each tube and heated for eight minutes in a boiling water bath cooled it and the intensity of green to dark green colour was measured at 630 nm.

3.3.4 Estimation of Protein

Protein content of grain samples was estimated by using the biuret method (Sadasivam and Manickam, 2008).

To find out the standard protein, make up the volume of 50 mg bovine serum albumin in to 50 ml water. Pipetted out 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml of standard protein in to a series of test tubes. Then making up volume in each to 4 ml of water and add 6 ml of biuret reagent and mix well. Biuret reagent was prepared by the addition of 3g of $CuSO_4$. $2H_2O$ and 9 g of sodium potassium tartarate in 500 ml of 0.2 N NaOH solution and made up the volume in to 1 litre with 0.2 N NaOH after the addition of 5 g of KI.

To find out the protein content in rice grain, make up 3 ml of digested grain sample to 1 ml with water followed by 6 ml of biuret reagent. This mixture mix well and incubate it at 37°C for 10 min. This solution was further cooled and read the absorbance at 520 nm against a reagent blank. Bovine serum albumin was used as calibration curve. The protein content was calculated and expressed as percentage.

3.4 GROWTH PARAMETERS

3.4.1 Fresh Weight of Plant

The fresh weight of plant after proper washing was weighed at 45 days after nematode inoculation and the mean per plant was ascertained.

3.4.2 Shoot Dry Weight

Shoot weight of the plants at 45 days after the nematode inoculation was assessed by cutting off the roots and weighing the oven dried shoot separately for each plant and mean shoot dry weight per plant was determined.

3.4.3 Root Dry Weight

The root system of each plant after proper washing and oven drying was weighed at 45 days after nematode inoculation and the mean per plant was ascertained.

3.4.4 Height of Plant

The height of plant was assessed at 45 days after nematode inoculation. Height was measured from the base of the stem to the tip of the largest leaf and the average height was calculated.

3.5 YIELD PARAMETERS

3.5.1 Thousand Grain weight

One thousand seeds were counted and their weight recorded and expressed in g.

3.5.2 Number of Spikelets per panicle

All the spikelets from each panicle were separated out and counted. The mean value for single panicle was also calculated.

3.6 HISTOPATHOLOGICAL STUDIES

Seeds of paddy (*Oryza sativa*) cv. Uma, were used for histopathological studies and histochemical localization. Seeds were surface sterilized in 0.1% mercuric chloride for one minute and washed thrice with autoclaved distilled water. Seeds were soaked overnight for sprouting. The sprouted seeds were sown in plastic pots (10 cm diam) filled with steam sterilized clay soil collected from paddy field and arranged under net house conditions. The seeds were allowed to germinate and thinned to one seedling per pot followed by inoculation of juveniles of *M. graminicola* obtained from pure culture @ 0, 100, 500, 1000, 5000 and 10,000 juveniles per pot at 7 days after sowing (DAS). Each concentrations were replicated five times. After 45 days the plants were uprooted and roots were used for histopathological studies and histochemical localization.

3.6.1 Histopathology of roots inoculated with *M. graminicola*

The root bits (1cm) were fixed in Carney's B fixative prepared with 6: 3: 1 ratio of ethyl alcohol: chloroform: acetic acid (v/v) for two hours to arrest any cytological changes in the plant tissues. The fixed roots were then processed for microtomy. The sections were examined under microscope and micro photographs were taken to study the changes. Root from uninoculated pots served as control.

3.6.1.1 Total insoluble Polysaccharides

Localization and assessment of total insoluble polysaccharides were done by employing periodic acid Schiffs (PAS) method (Hatchkis, 1948). The slides were deparaffinised and dehydrated following the procedure given earlier. They were

placed in 0.05 per cent periodic acid for 5 minutes, Washed thoroughly in water, Stained in Schiffs reagent for 5 minutes, rinsed in water for differentiation and removing excess stain and mounting in DPX.

Preparation of Schiffs reagent (Longley, 1952)

One gram of basic fuchsine powder was dissolved in 90 ml of boiling water. The mixture was cooled and 15 ml of 1N HCL was added with shaking at frequent intervals. To this, 1.8 g of potassium metabisulphate was added. The contents were transferred to an air-tight container and kept overnight in dark, with agitation of the contents of frequent intervals. Later on, 1.0 g of activated charcoal was added and mixture was shaken and filtered immediately. The colourless or straw yellow coloured filtrate was stored in amber coloured bottle and used as the reagent.

3.6.1.2 Total Proteins

In the present study, assessment for total proteins was done by mercuric bromophenol blue (MBB) method (Mazia *et al.*, 1953).

The sections were deparaffinized and dehydrated as mentioned earlier. The slides were immersed in MBB stain for 5 minutes. The slides were dipped in water to remove the superfluous stain. The differentiation produced final greenish blue colour at the sites of proteins. The sections were dehydrated in n- butanol grades, cleaned with xylene and mounted in DPX.

The sites of proteins had taken blue colour. The intensity of the colour was taken as a measure of amount of proteins in the tissue.

Preparation of mercuric bromophenol blue

The stain was prepared by dissolving 10 g of mercuric chloride and 100 mg of bromophenol blue in 100 ml of absolute alcohol.

3.6.1.3 Nucleic acid (RNA and DNA)

The dye toludine blue was used for the purpose of detecting sites containing RNA and DNA. This being a metachromatic stain, offers an excellent method for staining both RNA and DNA with clear contrast. The sites containing RNA takes blue colour and those having DNA stain green colour. The sections were deparaffinised and hydrated as mentioned earlier, Stained for 45 to 60 seconds by immersing the slides in toludine dye solution, rinsed in water by vigorous shaking until the slides were nearly free from the superfluous stain. The excess was cleared in xylene and mounted in DPX and the sites of RNA take blue colour and sites of DNA take green colour.

Preparation of toludine blue

In the present study, blue stain was prepared by dissolving 0.5 g of Toludine blue powder in 100 ml of Benzoate buffer solution of pH 4.0.

3.7 VARIETAL REACTION

Seeds of rice varieties *viz.*, Bhadra, Pavizham, Karthika, Kanakom, Uma, Revathy, Karishma, Prathyasa, Jyothi and Aiswarya were collected from the Rice Research Station, moncompu, Kerala. The variety TN1 had taken as the susceptible check variety. The seeds were sown at 5 seeds/ pot in 500 g earthen pots filled with steam sterilized paddy field soil. Seeds were allowed to germinate and thinned to two per pot at 7 DAS. The active infective second-stage juveniles (J_2) of *M. graminicola* obtained from pure culture were inoculated at 500 per seedling at 7 DAS and the plants were maintained with ten replications for each variety in completely randomized design under net house condition.

A similar set of pots was maintained simultaneously for subjecting the plant sample to study the biochemical alterations induced by *M. graminicola* in rice resistant/susceptible cultivars. The experiment was terminated at 45 days after

inoculation (DAI) and the plants were observed for Egg Mass Index and Root Knot Index.

Ten popular rice varieties of Kerala screened for comparing the relative susceptibility/tolerance to root-knot nematode, studying the biochemical changes and growth parameters. The trial was laid out in Completely Randomised Design with five replications.

3.7.1 Nematode population characters in varieties

3.7.1.1 No. of egg mass per plant

The number of egg mass per plant was counted and the mean egg mass per plant was ascertained.

3.7.1.2 Egg Mass Index in Varietal Screening

The number of egg mass per plant was counted and the Egg Mass Indexing was done as detailed below.

$$\text{Egg Mass Index} = \frac{\text{Number of Egg mass in test entry} * 4}{\text{No. of egg mass in susceptible check}}$$

The Egg Mass Indexing in varietal screening was done as follows:

Egg Mass Index	Host Reaction
0	Highly Resistant
0.1-1.0	Resistant
1.1-2.0	Moderately Resistance
2.1-3.0	Susceptible
3.1 and above	Highly Susceptible

3.7.1.3 No. of galls per plant

The number of galls per plant was counted and the mean gall per plant was ascertained.

3.7.1.4 Root Knot Index in Varietal Screening

The number of galls per plant was counted and the root- knot indexing was done as detailed below (Heald *et al.*, 1989).

Observation	Root Knot Index	Host Reaction
0 galls/ Plant	1	Highly Resistant
1-10 galls/ Plant	2	Resistant
11-30 galls/ Plant	3	Moderately Resistance
31-100 galls/ Plant	4	Susceptible
Above 100 galls/ Plant	5	Highly Susceptible

3.7.2 Biochemical Studies of Varieties

3.7.2.1 Peroxidase (PO)

Peroxidase activity was assayed by a spectrophotometric method as described by Srivastava (1987). Leaf sample of 1 g was homogenized in 5 ml of 0.1 M sodium phosphate buffer (pH 6.5) to which a pinch of polyvinyl pyrrolidone (PVP) was added. The homogenization was done at 4 ^{0}C using a pre- chilled mortar and pestle. The homogenate was filtered through a muslin cloth and centrifuged at 5000 rpm for 15 min at 4^{0}C. The supernatant was used as the enzyme extract for the assay of PO activity.

The reaction mixture consisting of 1ml of 0.05 µl pyrogallol and 50 µl of enzyme extract was taken in both reference and sample cuvettes, mixed and kept in a

spectrophotometer (systronics UV- Vis spectrophotometer 118) and the reading was adjusted to zero at 420 nm. To initiate the reaction, one ml of one percent hydrogen peroxide (H_2O_2) was added to the sample cuvettes and the changes in absorbance were recorded at 30 seconds interval upto 180 sec. The PO activity was expressed as changes in absorbance min^{-1} g^{-1} fresh weight of tissue.

3.7.2.2 Polyphenol oxidase (PPO)

Polyphenol activity was determined as per the procedure given by Mayer *et al.* (1965). The enzyme extract was prepared as per the procedure given for the estimation of PO.

The reaction mixture contained one ml of 0.1 M sodium phosphate buffer (pH 6.5) and 50 µl of enzyme extract. The reaction was initiated after adding one ml of 0.01 M catechol. The observations were recorded in a spectrophotometer (Systronics UV-VIS spectrophotometer 118). The change in absorbance was recorded at 495 nm at 30 seconds interval up to 180 sec. PPO activity was expressed as change in the absorbance of the reaction mixture per minute per gram of leaf tissue on fresh weight basis.

3.7.2.3 Phenyl alanine ammonia- lyase (PAL)

PAL activity was assayed spectrophotometrically by assaying the rate of conversion of L-phenyl alanine to trans- cinnamic acid at 290 nm as described by Dickerson *et al.* (1984). The enzyme extract was prepared by homogenizing one gram leaf sample in 5ml of 0.1 M borate buffer (pH 8.8) containing a pinch of PVP using chilled mortar and pestle.

The homogenate was centrifuged at 10,000 rpm for 10 min at 4^0C. The supernatant was used for the assay of PAL activity. The reaction mixture contained 3 ml of 0.1 M sodium borate buffer (pH 8.8) 0.2 ml enzyme extract and 0.1 ml of 12 Mm L- phenyl alanine prepared in the same buffer. The blank contained 3 ml of

0.1 M sodium borate buffer (pH 8.8) and 0.2 ml enzyme extract. The reaction mixture and blank were incubated at 40 ^{0}C for 30 minutes and reaction was read at 290 nm in a spectrophotometer (Systronics UV- VIS spectrophotometer 118). PAL activity was expressed as micrograms of cinnamic acid produced per minute per gram of leaf tissue on fresh weight basis.

3.7.3. Growth parameters

3.7.3.1. Fresh Weight of Plant

The fresh weight of plant after proper washing was weighed at 45 days after nematode inoculation and the mean per plant was ascertained.

3.7.3.2 Dry Weight of Shoot

Shoot weight of the plants at 45 days after the nematode inoculation was assessed by cutting off the roots and weighing the oven dried shoot separately for each plant and mean shoot dry weight per plant was determined.

3.7.3.3 Dry Weight of Root

The root system of each plant after proper washing and oven drying was weighed at 45 days after nematode inoculation and the mean per plant was ascertained.

3.7.3.4 Height of Plant

The height of plant was assessed at 45 days after nematode inoculation. Height was measured from the base of the stem to the tip of the largest leaf and the average height was calculated.

3.7.4 Nematode Population

3.7.4.1 Nematode Population in Soil

Soil samples were collected from each pot 45 days after nematode inoculation and nematode was extracted from the respective soil samples following the method of Cobb's sieving and decanting technique (Cobb, 1918) and modified Baermann's method (Schindler, 1961). The population of the nematode thus extracted was counted under a stereoscopic microscope.

3.7.4.2 Nematode Population in Roots

Root samples collected were washed thoroughly in water under a tap. Five gram of the root was weighed and cut into small bits and placed on tissue paper supported by the wire net placed on a petri plate. Emerging nematodes were collected at regular intervals of 24 hours up to four days. The nematode suspension thus collected were pooled and counted under a stereoscopic microscope

3.8 STATISTICAL ANALYSIS

Data generated from the experiment were subjected to statistical analysis applaying ANOVA technique and significance was tested by 'F' test. In the cases where the effects were found to be significant, CD was calculated using standard procedure.

RESULTS

4. RESULTS

An experiment entitled "Biochemical and histopathological alterations due to root- knot nematode, *Meloidogyne graminicola* in rice (*Oryza sativa* L.) and varietal reactions" was undertaken at Department of Agricultural Entomology, College of Agriculture, Vellayani, during 2013-14. The biochemical and histopathological variation in rice due to the infestation of *M. graminicola* and the varietal reaction of ten important rice varieties in Kerala were evaluated. The results of the experiments are presented in this chapter.

4.1 BIOCHEMICAL CHANGES

4.1.1 Phenol Content in Root and Leaf Samples

Changes in phenol content in root and leaf samples of rice at different inoculum levels of *M. graminicola* was shown in table 1.

4.1.1.1 Phenol Content in Root Samples

Population of *M. graminicola* at different densities tested, resulted in a progressive increase in phenol content of root in plant at 45 DAI compared to that of uninoculated plants (1.39 mg per g tissue). The maximum phenol content was seen in plants inoculated with 10,000 J_2/ pot (2.60 mg per g tissue) and was on par with that of plants inoculated with 5000 J_2 (2.55 mg per g tissue). The phenol content of root in plants inoculated with 100, 500 and 1000 J_2/ pot was 1.56, 1.87 and 2.21 mg per g tissue respectively. Significant difference was seen between all the treatment except plants inoculated with 5000 and 10,000 J_2/ pot. The root phenol content in plants inoculated with 1000 J_2/ pot (2.21 mg per g tissue) increased significantly from that of the plants inoculated with 500 J_2/ pot (1.87 mg per g tissue). The percentage increase in the root phenol content of plants inoculated with 10,000 J_2 / pot was

Table 1. Effect of different inoculm levels of *M. graminicola* on phenol content in root and leaf samples (mean of five replications)

Treatments (No. of J_2/ pot)	Phenol content (mg per g tissue)	
	Root samples	Leaf samples
Uninoculated Plant	1.39	0.76
100	1.56	0.79
500	1.87	0:8
1000	2.21	0.88
5000	2.55	0.85
10000	2.60	0.93
CD (0.05)	0.064	0.043

46.37% and plants inoculated with 5000 J_2 was 45.28% when compared with that of the uninoculated plants.

4.1.1.2 Phenol Content in Leaf Samples

Inoculation of *M. graminicola* in varying inoculum levels resulted in a corresponding increase in phenol content of leaf at 45 days after inoculation. However, there was no significant difference between the phenol content of leaf in plants inoculated with 1000 and 5000 J_2/ pot. The phenol content of plants inoculated with100 J_2/ pot was on par with that of the uninoculated plants (0.76 mg per g tissue). The phenol content of the plants inoculated with 100 (0.79 mg per g tissue) and 500 (0.80 mg per g tissue) J_2/ pot were on par with each other. The percentage increase of the phenol content of leaf in plants inoculated with 10,000 J_2 was 18.36% and the plants inoculated with 5000 J_2 was 13.79% when compared with that of the uninoculated plants.

4.1.2 Peroxidase, Polyphenol Oxidase and Phenylalanine Ammonia Lyase Activity in Root Samples

Changes in peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activity in root at different inoculum levels of *M. graminicola* was shown in table 2.

4.1.2.1 Peroxidase (PO) Activity in Root Samples

The various levels of nematode population showed progressive increase in the peroxidase activity of rice plants compared to uninoculated plants. The peroxidase activity of root in plants inoculated with 10,000 J_2/ pot was significantly different from all other treatments. The peroxidase activity of plants inoculated with 1000 J_2/ pot was 5.38 OD per g per min and which was on par with that of the plants inoculated with 500 J_2/ pot (5.36 OD per g per min). The PO activity of root was highest in plants inoculated with 10,000 J_2/pot. The root PO activity of plants inoculated with 100 J_2/pot was on par with that of the uninoculated plants. The root

Table 2. Effect of different inoculum levels of *M. graminicola* on peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activities in root samples (mean of five replications)

Treatments (No. of J_2/ pot)	Enzymatic activity in root samples (OD per g/min)		
	Peroxidase (PO)	Phenylalanine Ammonia Lyase (PAL)	Polyphenol Oxidase (PPO)
Uninoculated plant	4.45	2.14	1.32
100	4.51	2.37	1.41
500	5.36	2.67	1.52
1000	5.38	3.00	1.72
5000	7.37	3.37	1.76
10000	7.60	3.44	2.57
CD (0.05)	0.078	0.402	0.132

PO activity of plants inoculated with 10,000 J_2 increased 70.79% compared to that of the uninoculated plants.

4.1.2.2 Polyphenol Oxidase (PPO) Activity in Root Samples

The polyphenol oxidase content increased with increasing nematode population levels. The maximum activity of enzyme was observed at 10,000 J_2/ pot (2.98 OD per g per min) and was increased 94.69% over the uninoculated plant. The PPO content of root at different inoculum level was significantly different from that of uninoculated plant. The plants inoculated with 10,000, 5000 and 1000 J_2/ pot were significantly different. However, the PPO content in roots of plants inoculated with 100 and 500 J_2/ pot was on par with each other. The percentage increase of PPO content in roots of plants inoculated with 5000 J_2 was 33.33% which was significantly different from all other treatments.

4.1.2.3 Phenylalanine Ammonia Lyase (PAL) activity in Root Samples

Phenylalanine ammonia lyase activity increased with increase in nematode inoculum levels. The PAL activity of roots in plants inoculated with different inoculum levels of nematodes were significantly different from uninoculated plant (2.14 OD per g per min) except the plants inoculated with 100 J_2/ pot (2.37 OD per g per min). The maximum PAL activity was observed in plants inoculated with 10,000 J_2/ pot (3.436 OD per g per min) and was on par with plants inoculated with 5000 J_2/ pot (3.37 OD per g per min). The percentage increase of PAL activity of root in plants inoculated with 5000 and 10,000 J_2/ pot were 57.47% and 60.75% respectively. The PAL content of root in plants inoculated with 500 J_2 (2.673 OD per g per min) was on par with that of the plants inoculated with 1000 J_2/pot, the percentage increase over the uninoculated plants were 24.77% and 40.19% respectively.

4.1.3 Peroxidase, Polyphenol Oxidase and Phenylalanine Ammonia Lyase Activity in Leaf Samples

Changes in peroxidase, phenylalanine ammonia lyase and polyphenol oxidase activity in leaf samples at different inoculum levels of *M. graminicola* was shown in table 3.

4.1.3.1 Peroxidase (PO) Activity in Leaf Sample (OD per g per min)

The PO activity in leaf samples increased with increase in nematode inoculum levels. The maximum increase was observed in plants inoculated with 10,000 J_2/pot (0.60 OD per g per min) which was on par with that of plants inoculated with 5000 and 1000 J_2. The PO activity of plants inoculated with 100 and 500 J_2/ pot was on par with that of the uninoculated plant. The percentage increase in PO activity of plants inoculated with 10,000 J_2 was 62.16% over uninoculated plants.

4.1.3.2 Polyphenol Oxidase Activity in Leaf Samples

The PPO activity in leaf samples also increased with increase in nematode inoculum levels. The plants inoculated with 5000 J_2/ pot showed maximum PPO activity (1.76 OD per g per min) and which was on par with that of plants inoculated with 10,000 J_2/ pot (1.75 OD per g per min). The PPO activity of plants inoculated with 100 and 500 J_2 were significantly different from all other treatments. The percentage increase in PPO activity in plants inoculated with 5000 J_2 was 33.33% over uninoculated plants.

4.1.3.3 Phenylalanine Ammonia Lyase Activity in Leaf Samples

The PAL activity also increased with increase in nematode population. The plants inoculated with 10.000 and 5000 J_2/ pot showed maximum PAL activity (1.42 OD per g per min) and was on par with that of the plants inoculated with 500 and 1000 J_2/ pot. The plants inoculated with 500 J_2/ pot was on par with that of the plants

Table 3. Effect of different inoculum levels of *M. graminicola* on peroxidase, phenylalanine ammonia lyase and polyphenol oxidase activities in leaf samples (mean of five replications)

Treatments (No. of J2/pot)	Enzymatic activity in leaf sample (OD per g/min)		
	PO	PAL	PPO
Uninoculated plant	0.37	0.89	1.32
100	0.43	1.06	1.41
500	0.43	1.29	1.52
1000	0.52	1.41	1.72
5000	0.60	1.42	1.76
10000	0.60	1.42	1.75
CD (0.05)	0.091	0.305	0.033

inoculated with100 J_2/ pot. The percentage increase in the PAL activity in plants inoculated with 10,000 J_2/ pot was 61.36% compared with that of uninoculated plant.

4.1.4 pH, Chlorophyll A, Chlorophyll B and Total Chlorophyll Content of Leaf Samples

Changes in pH, Chlorophyll A, chlorophyll B and total chlorophyll content of leaf samples at different inoculum levels of *M. graminicola* was shown in table 4.

4.1.4.1 pH of Leaf Samples

A progressive decrease in pH of leaf sample was observed with increase in nematode inoculum levels. The minimum pH was observed in plants inoculated with 10,000 J_2/ pot, being 5.68 per plants. The pH of the leaf sample of plants inoculated with 1000 J_2/ pot was on par with that of the plants inoculated with 100 J_2. The pH of leaf in plants inoculated with 5000 J_2/ pot was significantly different from all other treatments and the percentage reduction was only 4.78% with that of uninoculated plant. The pH of the plants inoculated with 10,000 J_2 decreased 9.91% when compared with the uninoculated plants.

4.1.4.2 Chlorophyll A Content of Leaf Samples

The various levels of nematode population showed a progressive reduction in the chlorophyll A content of leaves compared to uninoculated plants. The chlorophyll A content of plants inoculated with 100 J_2/ pot reduced significantly from that of the uninoculated plants. Minimum chlorophyll A content was observed in 10,000 J_2 inoculated plants (0.194 mg per g of tissues) which significantly differed from all other treatments. The chlorophyll A content of the plants inoculated with 500, 1000 and 5000 J_2/ pot were 0.24, 0.23 and 0.227 mg per g tissue respectively. These treatments were on par with each other. The chlorophyll A content of the plants inoculated with 10,000 J_2 reduced 40.67% compared with that of the uninoculated plants.

Table 4. Effect of different inoculum levels of *M. graminicola* on pH, chlorophyll A, chlorophyll B and total chlorophyll content in leaf samples (mean of five replications)

Treatments (No. of J_2/ pot)	pH of the leaf samples	Chlorophyll content of leaf samples (mg per gm of tissue)		
		Chlorophyll A	Chlorophyll B	Total Chlorophyll
Uninoculated plant	6.31	0.33	0.53	0.93
100	6.20	0.29	0.48	0.79
500	6.25	0.24	0.45	0.74
1000	6.13	0.23	0.41	0.71
5000	6.01	0.23	0.41	0.71
10000	5.69	0.19	0.38	0.68
CD (0.05)	0.111	0.014	0.023	0.011

4.1.4.3 Chlorophyll B Content of Leaf Samples

The different levels of nematode population showed progressive decrease in the chlorophyll B content of leaf samples compared to uninoculated plant. The chlorophyll B content of leaf in nematode uninoculated plant was o.53 mg per g tissue, it was 0.48 mg per g tissue in plants inoculated with 100 J_2/ pot. The treatment differed statistically from other treatments. The chlorophyll B content of 5000 J_2/ pot inoculated plants was 0.41 mg per g tissue which was on par with that of the plants inoculated with 1000 J_2 (0.411 mg per g leaf tissue). The minimum chlorophyll B content was observed in 10,000 J_2 inoculated plants (0.38 mg per g tissue) and was significantly differed from all other treatments. The chlorophyll B content of 10,000 J_2 inoculated plants reduced 27.55 % when compared to that of the uninoculated plants.

4.1.4.4 Total Chlorophyll Content of leaf samples

A progressive decrease in the total chlorophyll content was also noted with increase in the inoculum levels of *M. graminicola*. The total chlorophyll content of the uninoculated plant was 0.93 mg per g tissue; it was 0.78 mg per g tissue in plants inoculated with 100 J_2/ pot. The total chlorophyll content of plants inoculated with 100, 500 J_2/ pot and uninoculated plants were significantly different from each other. The total chlorophyll content of the plants inoculated with 1000 J_2/ pot was on par with that of the plants inoculated with 5000 J_2/ pot. The maximum reduction was observed in plants inoculated with 10,000 J_2 (0.686 mg per g tissue). The percentage reduction in the total chlorophyll content of plants inoculated with 10,000 J_2/ pot was 20.24% and 5000 J_2/ pot was 23.44% when compared with that of the uninoculated plants.

4.1.5. N, P and K content in leaf samples

Changes in N, P and K content in leaf samples at different inoculum levels of *M. graminicola* were shown in table 5.

4.1.5.1 Nitrogen Content in Leaf Samples

A progressive decrease in nitrogen content of leaf sample was noted with increase in the inoculum levels of *M. graminicola*. The maximum reduction was observed in plants inoculated with 10,000 J_2/ pot (1.14%) which was on par with that of plants inoculated with 5000 J_2 (1.15%) and 1000 J_2 (1.18%) per pot. The nitrogen content of leaf in plants inoculated with 100 J_2/ pot (1.22%) was significantly different from that of the plants inoculated with 500 J_2/ pot (1.17%). The nitrogen content of plants inoculated with 500, 1000 and 5000 J_2/ pot were on par with each other. The percentage reduction of nitrogen content in plants inoculated with 10,000 and 5000 were 13.64 and 12.80% respectively over the uninoculated plant.

4.1.5.2 Phosphorus Content in Leaf Samples

The different levels of nematode population showed progressive reduction in phosphorus content in leaf samples compared to uninoculated plants. The minimum phosphorus content was observed in plants inoculated with 10,000 J_2 (0.18%) which was significantly different from all other treatments. The percentage reduction in phosphorus content in plants inoculated with 10,000 J_2/ pot was 48.57% over uninoculated plant. The phosphorus content of leaf samples in plants inoculated with 1000 (0.26%) and 5000 (0.23%) were on par with each other and the percentage reduction was 25.71 and 34.28% respectively over uninoculated plant. The phosphorus content of leaf sample in plants inoculated with 500 and 1000 were on par with each other. The plants inoculated with 100 J_2/ pot was significantly different from all other treatments.

Table 5. Effect of different inoculum levels of *M. graminicola* on N, P and K content in leaf samples (mean of five replications)

Treatments (No. of J_2/ pot)	Nitrogen (%)	Phosphorus (%)	Potash (%)
Uninoculated Plant	1.32	0.35	0.49
100	1.22	0.30	0.45
500	1.17	0.27	0.42
1000	1.18	0.26	0.39
5000	1.15	0.23	0.37
10000	1.14	0.18	0.29
CD (0.05)	0.030	0.027	0.055

4.1.5.3 Potash Content in Leaf Samples

The potash content decreased with increase in nematode inoculum levels. Maximum reduction was observed in plants inoculated with10,000 J_2/ pot (0.29%) and the percentage reduction was 40.82% over uninoculated plant. Potash content of leaf samples in plants inoculated with 10,000 J_2 was significantly different from all other treatments. The potash content of leaf samples in plants inoculated with 500 (0.42%), 1000 (0.39%) and 5000 (0.37%) were on par with each other and the percentage reduction were 14.28, 20.41 and 24.49% respectively. The plants inoculated with 100 J_2/ pot was on par with that of the uninoculated plant and the percentage reduction was only 8.16% over uninoculated plant.

4.1.6 Micronutrient Content in Leaf Sample (ppm)

Changes in Fe, Zn, Cu and Mn content in leaf samples at different inoculum levels of *M. graminicola* were shown in table 6.

4.1.6.1 Iron (Fe) Content

A progressive decrease in Fe content of leaf sample was noted with increase in the inoculum levels of *M. graminicola.* Plants inoculated with 10,000 J_2/ pot showed the lowest Fe content (82.79 ppm) and was significantly different from all other treatments. It was followed by 5000, 1000 and 500 J_2/ pot (95.78 ppm, 109.07 ppm and 120. 34 ppm respectively) which were statistically different from each other. Plants inoculated with 100 J_2/ pot with the Fe content of 128.20 ppm differed statistically from all other treatments and uninoculated control plants (149.99 ppm).

4.1.6.2 Zinc (Zn) Content

Different levels of nematode population showed reduction in Zn content of leaf samples compared to uninoculated plants. Maximum reduction in Zn content was observed in plants inoculated with 10,000 J_2/ pot and was significantly different

Table 6. Effect of different inoculum levels of *M. graminicola* on Fe, Zn, Cu and Mn content of leaf samples (mean of five replications)

Treatments (No. of J_2/ pot)	Micro nutrients (ppm)			
	Fe	Zn	Cu	Mn
Uninoculated plant	149.99	47.17	63.92	59.15
100	128.20	43.06	54.96	45.86
500	120.34	32.88	37.84	40.42
1000	109.07	31.77	34.64	36.81
5000	95.78	30.69	31.28	34.11
10000	82.79	27.34	30.92	29.34
CD (0.05)	7.250	2.393	4.579	3.315

from all other treatments. The Zn content of leaf samples in plants inoculated with 500 (32.88 ppm), 1000 (31.77 ppm) and 5000 (30.69 ppm) were on par with each other and differed statistically from the uninoculated plant (47.17 ppm). Plants inoculated with 100 J_2/ pot with Zn content of leaf sample (43.06 ppm) differed statistically from all other treatments and uninoculated control plant.

4.1.6.3 Copper (Cu) Content

A progressive decrease in the Cu content of leaf samples was also noted with increase in the inoculums levels of *M. graminicola*. Plants inoculated with 10,000 J_2/ pot recorded the lowest Cu content (30.92 ppm) and was on par with that of the plants inoculated with 5000 J_2 (31.28 ppm) and 1000 J_2 (34.64 ppm) per pot respectively. The Cu content of leaf sample in plants inoculated with 500 and 100 J_2 was significantly different from all other treatments and uninoculated control plant (63.92 ppm).

4.1.6.4 Manganese (Mn) Content

Mn content of leaf samples decreased with increase in nematode inoculum levels. Maximum reduction was seen in plants inoculated with 10,000 J_2/ pot (29.34 ppm) and was significantly different from all other treatments. It was followed by 5000 (34.11 ppm) and 1000 (36.81 ppm) respectively. The Mn content of leaf samples in plants inoculated with 5000 and 1000 J_2/ pot were on par with each other. The plants inoculated with 100 and 500 J_2 recorded 45.86 and 40.42 ppm Mn content and were statistically differed from all other treatments and uninoculated control plant (56.15 ppm).

4.1.7 Starch and Protein Content of Grain Samples

Effect of different levels of *M. graminicola* on starch and protein content in grain sample was shown in table 7.

4.1.7.1 Starch Content of Grain Samples

The starch content of grains exhibited reduction at different nematode inoculum levels when compared to uninoculated plants. The starch content of grain in the plants inoculated with 1000 J_2/ pot (59.83%) was on par with that of the plants inoculated with 500 J_2 (68.18%). The starch content of grains in plants inoculated with 10,000 and 5000 J_2/ pot was statistically on par. Highest decrease of starch content (41.43%) was observed in plants inoculated with 10,000 J_2/ pot.

4.1.7.2 Protein Content of Grain Samples

The different inoculum levels of *M. graminicola* resulted in a corresponding decrease in the percentage of protein in the grain compared to uninoculated plant. However, there was no significant difference between protein present in plants inoculated with 100, 500 and 1000 J_2/ pot, the percentage protein content of the grain being 2.37%, 2.21% and 2.16% respectively. The maximum reduction in protein content of grain was observed in plants inoculated with 10.000 J_2/ pot. In 5000 and 10,000 J_2 inoculated plants, the protein content of the grain was decreased by 38.84% and 42.09% significantly over uninoculated plant.

4.1.8 Reducing Sugar, Non Reducing Sugar and Total Sugar Content in Grain Samples

Changes in reducing sugar, non reducing sugar and total sugar content in grain sample due to infestation of *M. graminicola* was given in table 8.

Table 7. Effect of different inoculum levels of *M. graminicola* on starch and protein content in grain samples (mean of five replications)

Treatments (No. of J_2/ pot)	Starch content of grains (%)	Protein content of grains (%)
Uninoculated Plant	86.78	2.65
100	65.18	2.38
500	68.19	2.22
1000	59.83	2.15
5000	55.12	1.62
10000	50.82	1.54
CD (0.05)	9.023	0.246

4.1.8.1 Reducing Sugar

Different levels of nematode population showed reduction in reducing sugar content of grain samples compared to uninoculated plants. Minimum content of reducing sugar in grain sample was observed in plants inoculated with10,000 J_2/ pot (38.57%) and was significantly different from all other treatments. The percentage decrease in reducing sugar content of grain sample in plants inoculated with 5000 and 10,000 was 29.54 and 44.45% respectively over the uninoculated control plant. The reducing sugar content of grain in plants inoculated with 1000 J_2/ pot was 55.35% and was on par with that of the plants inoculated with 500 J_2 (58.67%) and 100 J_2 (57.67%) per pot and differed significantly from uninoculated plant (69.44%).

4.1.8.2 Non Reducing Sugar

Non reducing sugar content of grain samples decreased with increase in nematode inoculum levels. Maximum reduction was observed in plants inoculated with 10,000 J_2/ pot (1.57 %) and was on par with that of the plants inoculated with 5000 J_2 (1.67%). It was followed by 1000 (2.06%) and 5000 (2.46%) J_2 inoculating plants respectively. The non reducing sugar content of grain sample in plants inoculated with 100 and 500 J_2/ pot was on par with each other. The percentage reduction in non reducing sugar content of grain samples in plants inoculated with 10,000 and 5000 were 55.01 and 52.15% respectively.

4.1.8.3 Total Sugar Content

A progressive decrease in total sugar content of grain was seen with increase in nematode population levels. Maximum reduction was observed in plants inoculated with 10,000 J_2 (40.15%) and was statistically different from all other treatments. The total sugar content of grain samples in plants inoculated with 100 (60.34%), 500 (61.14%) and 1000 (57.41%) were statistically on par with each other. The total sugar content of grain samples in plants inoculated with 5000 J_2/ pot

Table 8. Effect of different inoculum levels of *M. graminicola* on reducing sugar, non reducing sugar and total sugar content in grain samples (mean of five replications)

Treatments (No. of J_2/ pot)	Sugar content (Per cent wt)		
	Reducing sugar	Non reducing sugar	Total Sugar
Uninoculated Plant	69.44	3.49	72.94
100	57.67	2.67	60.34
500	58.67	2.46	61.14
1000	55.35	2.06	57.41
5000	48.93	1.67	50.60
10000	38.57	1.57	40.15
CD (0.05)	4.794	0.219	4.739

Plate 3. Effect of different inoculum levels of root knot nematode in rice

Plate 4. *M. graminicola* infested rice roots

Plate 5. Healthy rice roots

(50.60%) was significantly different from all other treatments and uninoculated control plant (72.94%). The percentage reduction in total sugar content of grain sample in plant inoculated with 10,000 J_2/ pot was 44.95% over uninoculated plant.

4.2 GROWTH PARAMETERS OF RICE

The effect of varying population densities of *M. graminicola* on growth parameters of rice are present in table 9.

4.2.1 Fresh Weight of Plant

The fresh weight of plants decreased with increase in inoculum levels of *M. graminicola* at 45 DAI. While the fresh weight of uninoculated plants was 7.032 g, it was 6.53 g in plants inoculated with 100 J_2/ pot and the treatment differed significantly from all other treatments. At 500 and 1000 J_2/ pot, the fresh weight of the plants was 5.41 g and 5.08 g respectively and the treatments were on par. Maximum reduction in fresh weight was seen in plants inoculated with 10,000 J_2, the fresh weight of the plants being 4.53 g. The treatment differed significantly from all other treatments except the plants inoculated with 5000 J_2/ pot. The treatment two (100 J_2/ pot) and treatment three (500 J_2/ pot) were significantly different from each other. At 1000 and 5000 J_2, the fresh weight of the plants was 5.08 and 4.81 g respectively and the treatments were on par. The fresh weight of uninoculated plants, plants inoculated with 100 J_2 and plants inoculated with 500 J_2 were differ significantly to each other.

4.2.2 Dry Weight of Root

A progressive decrease in the dry weight of roots was also noted with increase in the inoculum levels of *M. graminicola*. Plants inoculated with 10,000 J_2/ pot recorded the lowest dry weight (0.74 g). It was followed by 5000, 1000 and 500 J_2/ pot levels (0.80 g, 0.84 g and 0.91 g respectively) which were statistically different from the un inoculated plants but on par with each other. The dry weight of root in

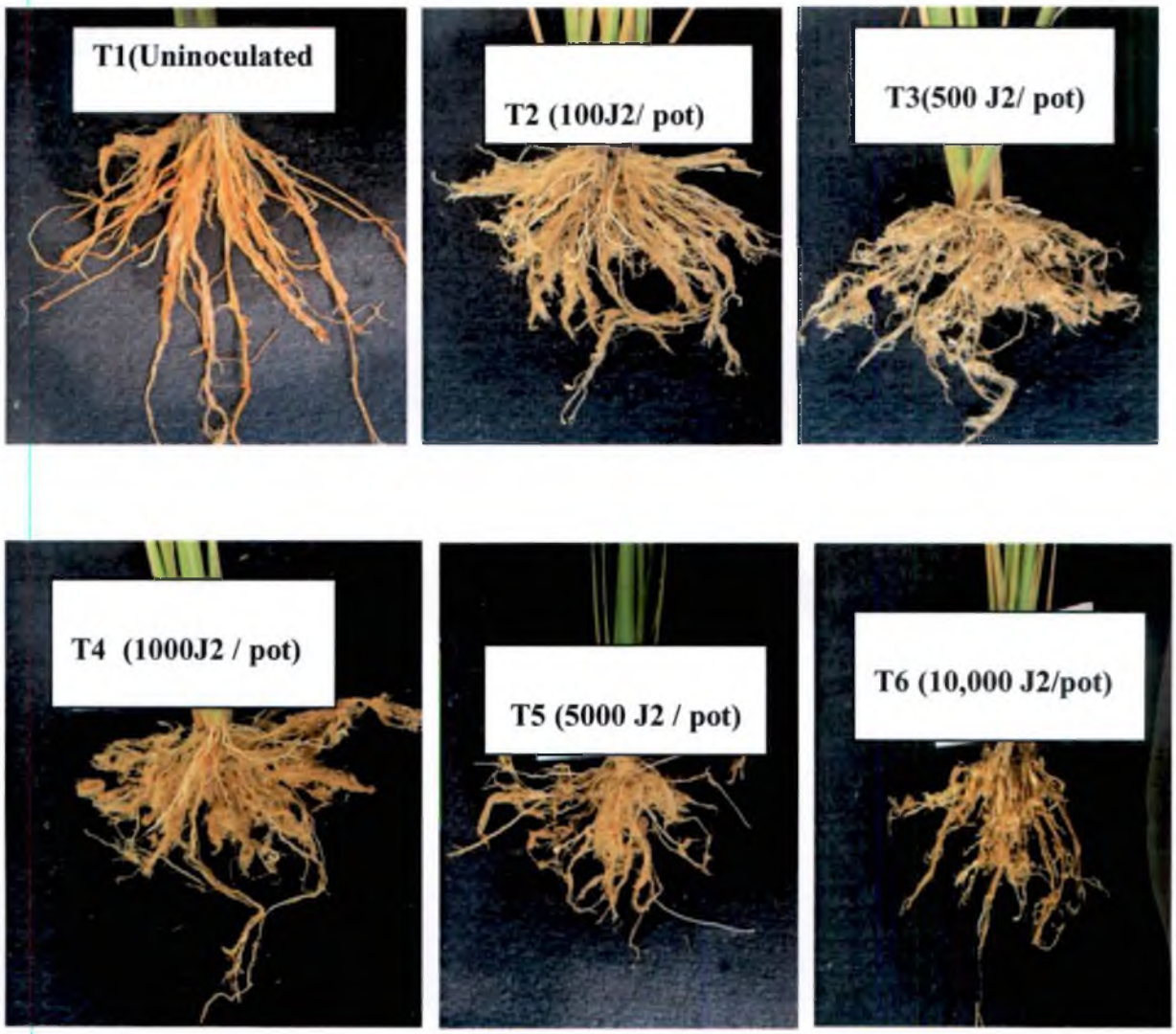


Plate 6. Symptoms on roots of different root knot nematode inoculum levels in rice

Table 9. Effect of different inoculum levels of *M. graminicola* on growth parameters of rice (mean of five replications)

Treatments (No. of J_2/ pot)	Plant growth parameters (45 days after nematode inoculation)			
	Plant height (cm)	Fresh weight of plant (g)	Dry weight of root (g)	Dry weight of shoot (g)
Uninoculated Plant	56.52	7.03	1.67	2.25
100	51.46	6.53	1.09	2.09
500	46.80	5.41	0.91	1.84
1000	46.10	5.08	0.84	1.76
5000	46.94	4.81	0.80	1.73
10000	44.76	4.53	0.74	1.47
CD (0.05)	5.660	0.438	0.219	0.118

uninoculated plant was 1.67 g, which was significantly different from all other treatments. The plants inoculated with 100 J_2/ pot with the dry weight of root 1.09 g differed significantly from all other treatments except the dry weight of root in plants inoculated with 500 J_2/ pot

4.2.3 Dry Weight of Shoot

Dry weight of shoot decreased with increase in inoculum levels of root knot nematode, *M. graminicola*. The dry weight of shoot in nematode uninfested plants was 2.25 g, it was 2.09 g in plants inoculated with 100 J_2/ pot. The treatment differed statistically from other treatments. The dry weight of shoot in plants inoculated with 500 J_2 was 1.84 g which was on par with the plants inoculated with 1000 J_2/ pot (1.76 g) and 5000 J_2/ pot (1.73 g). The minimum weight was observed in 10,000 J_2 inoculated plants (1.47g) which was greatly significant with all other treatments. The shoot weight of 10,000 J_2 inoculated plants reduced 34.60% when compared with that of the uninoculated plants.

4.2.4 Plant Height

Population of *M. graminicola* at all densities tested resulted in progressive reduction in the height of plants at 45 DAI compared to the uninoculated plants (56.52cm). The height of uninoculated plant was on par with that of plants inoculated with 100 J_2/ pot. The height of plants inoculated with 100, 500, 1000 and 5000 J_2 were on par with each other. A progressive reduction was seen in the height of plants with an increase in the inoculum densities. Maximum reduction was seen in plants inoculated with 10,000 J_2/ pot, the height of the plants being 44.76 cm and which was on par with the height of the plants inoculated with 5000, 1000 and 500 J_2/ pot. The height of plants inoculated with 500 J_2/ pot reduced 16.95% and 10,000 J_2/ pot reduced 20.81% compared with that of the uninoculated plant. The height of plants inoculated with 1000 J_2 was reduced 17.19% with that of the uninoculated plant.

4.3 YIELD PARAMETERS OF RICE

Changes in yield parameters of rice at different inoculum levels of root knot nematode, *M. graminicola* was shown in table 10.

4.3.1 Thousand Seed Weight

A progressive reduction was seen in the seed weight of rice plants with increasing inoculum levels of *M. graminicola* at 45 DAI. The seed weight of plants inoculated with 100 J_2/ pot was 22.17 g and was on par with that of the seed weight of the uninoculated plant (23.61 g). The plants inoculated with 500 J_2/ pot was on par with that of the seed weight of the plants inoculated with 100 J_2/ pot. Maximum reduction in 1000 seed weight was observed in plants treated with 10,000 J_2/ pot, it was reduced 16.77% with that of the uninoculated plants. The seed weight of plant inoculated with 500, 1000, 5000 and 10,000 J_2/ pot was 20.62 g, 20.25 g, 20.23 g and 19.65 g respectively and these were on par with each other.

4.3.2 Number of Seeds per Panicle

Inoculation of *M. graminicola* in varying inoculum levels resulted in a corresponding decrease in the number of seeds per panicle. There was significant difference between the number of seeds per panicles in plants inoculated with 100 J_2/ pot and nematode uninfected plants the number being 64.80 and 71.60 per plant respectively. At 100 J_2/ pot, the number of seeds per panicle was on par with plants inoculated with 500 J_2. The number of seeds per panicle of plants inoculated with 1000 J_2/ pot was statistically on par with the number of seeds per panicle of plants inoculated with 5000 J_2. Maximum reduction in the number of seeds per panicle (51.00) was observed in plants inoculated with 10,000 J_2 and was significantly different from all other treatments. The seeds per panicle of the plants inoculated with 10,000 J_2/ pot reduced 28.77% when compared with that of the uninoculated plant.

Table 10. Effect of different inoculum levels of *M. graminicola* on yield parameters of rice (mean of five replications)

Treatments (No. J_2/pot)	1000 seed weight (g)	No. of seeds per panicle
Uninoculated Plant	23.61	71.60
100	22.17	64.80
500	20.62	62.80
1000	20.25	57.20
5000	20.23	57.80
10000	19.65	51.00
CD (0.05)	1.643	5.411

4.4 HISTOPATHOLOGICAL AND HISTOCHEMICAL CHANGES

4.4.1 Accumulation of Polysaccharide Granules in Root Section

Effect of accumulation of polysaccharide granules in epidermal, cortical and stelar region of root section at different inoculum levels of *M. graminicola* was shown in table 11.

The accumulation of polysaccharides in root was increased with increase in population levels of nematode. The maximum accumulation was observed in plants inoculated with 10,000 J_2/ pot.

The maximum number of accumulation of polysaccharide granules in epidermal cells was observed in plants inoculated with 10,000 J_2 (2.68) and was on par with that of the plants inoculated with 5000 (2.48) and 1000 (2.38) respectively. The percentage increase in accumulation of polysaccharide in epidermal cells in plants inoculated with 10, 000 J_2 was 71.42 % over uninoculated control plant. The polysaccharide accumulation in plants inoculated 100 and 500 was 0.86 and 1.57 respectively and was significantly different from all other treatments and uninoculated plant (0.26)

The maximum accumulation of polysaccharide granules in cortical cells of root section was observed in plants inoculated with 10, 000 J_2/ pot (3.97) and was statistically on par with that of the plants inoculated with 5000 J_2 (3.27). The polysaccharide granule accumulation in cortical cells of root section in plants inoculated with 100 J_2 (1.03) and 500 J_2 (1.47) per pot were on par with each other and statistically different from uninoculated control plant (0.26)

A progressive increase in accumulation of polysaccharide granules in stelar cells was observed with increase in nematode population levels. Maximum accumulation was seen in plants inoculated with 10,000 J_2/ pot (1.87) and was on par

4.4 HISTOPATHOLOGICAL AND HISTOCHEMICAL CHANGES

4.4.1 Accumulation of Polysaccharide Granules in Root Section

Effect of accumulation of polysaccharide granules in epidermal, cortical and stelar region of root section at different inoculum levels of *M. graminicola* was shown in table 11.

The accumulation of polysaccharides in root was increased with increase in population levels of nematode. The maximum accumulation was observed in plants inoculated with 10,000 J_2/ pot.

The maximum number of accumulation of polysaccharide granules in epidermal cells was observed in plants inoculated with 10,000 J_2 (2.68) and was on par with that of the plants inoculated with 5000 (2.48) and 1000 (2.38) respectively. The percentage increase in accumulation of polysaccharide in epidermal cells in plants inoculated with 10, 000 J_2 was 71.42 % over uninoculated control plant. The polysaccharide accumulation in plants inoculated 100 and 500 was 0.86 and 1.57 respectively and was significantly different from all other treatments and uninoculated plant (0.26)

The maximum accumulation of polysaccharide granules in cortical cells of root section was observed in plants inoculated with 10, 000 J_2/ pot (3.97) and was statistically on par with that of the plants inoculated with 5000 J_2 (3.27). The polysaccharide granule accumulation in cortical cells of root section in plants inoculated with 100 J_2 (1.03) and 500 J_2 (1.47) per pot were on par with each other and statistically different from uninoculated control plant (0.26)

A progressive increase in accumulation of polysaccharide granules in stelar cells was observed with increase in nematode population levels. Maximum accumulation was seen in plants inoculated with 10,000 J_2/ pot (1.87) and was on par

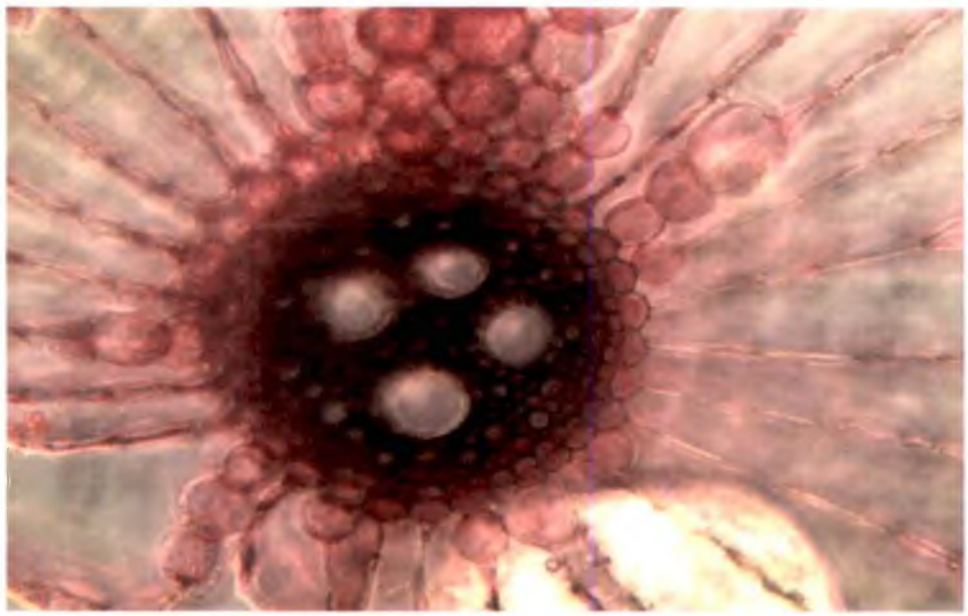


Plate 7. Healthy root section (no accumulation of polysaccharides)

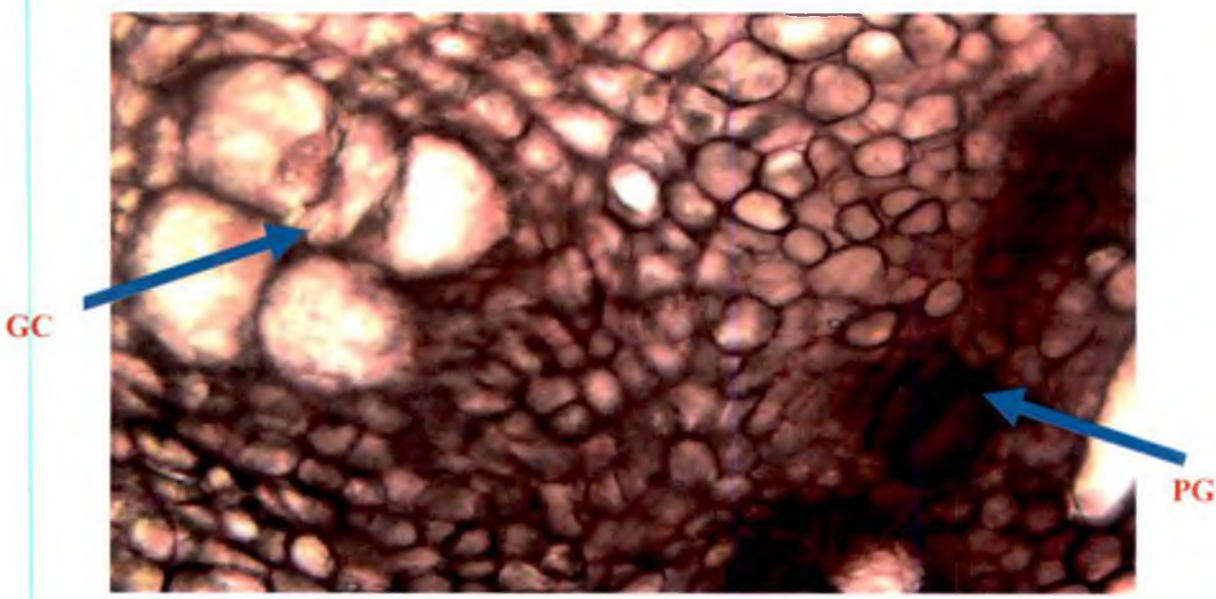


Plate 8. Polysaccharide accumulation and giant cells in root knot nematode infested root section.

(GC: Giant cell; PG: Polysaccharide granules)

Table 11. Effect of accumulation of polysaccharide in epidermal, cortical and stelar region of root at different inoculum levels of root knot nematode (mean of five replications)

Treatments (No. of J_2/ pot)	Accumulation of polysaccharide granules (no. per root section)		
	Epidermal cells	Cortical cells	Stelar cells
Uninoculated Plant	0.26 (1.12)	0.26 (1.12)	0.36 (1.16)
100	0.86 (1.36)	1.03 (1.43)	0.66 (1.29)
500	1.57 (1.60)	1.47 (1.57)	1.03 (1.43)
1000	2.38 (1.84)	2.58 (1.89)	1.47 (1.57)
5000	2.48 (1.87)	3.27 (2.07)	1.87 (1.69)
10000	2.68 (1.92)	3.97 (2.23)	1.87 (1.69)
CD (0.05)	0.150	0.171	0.180

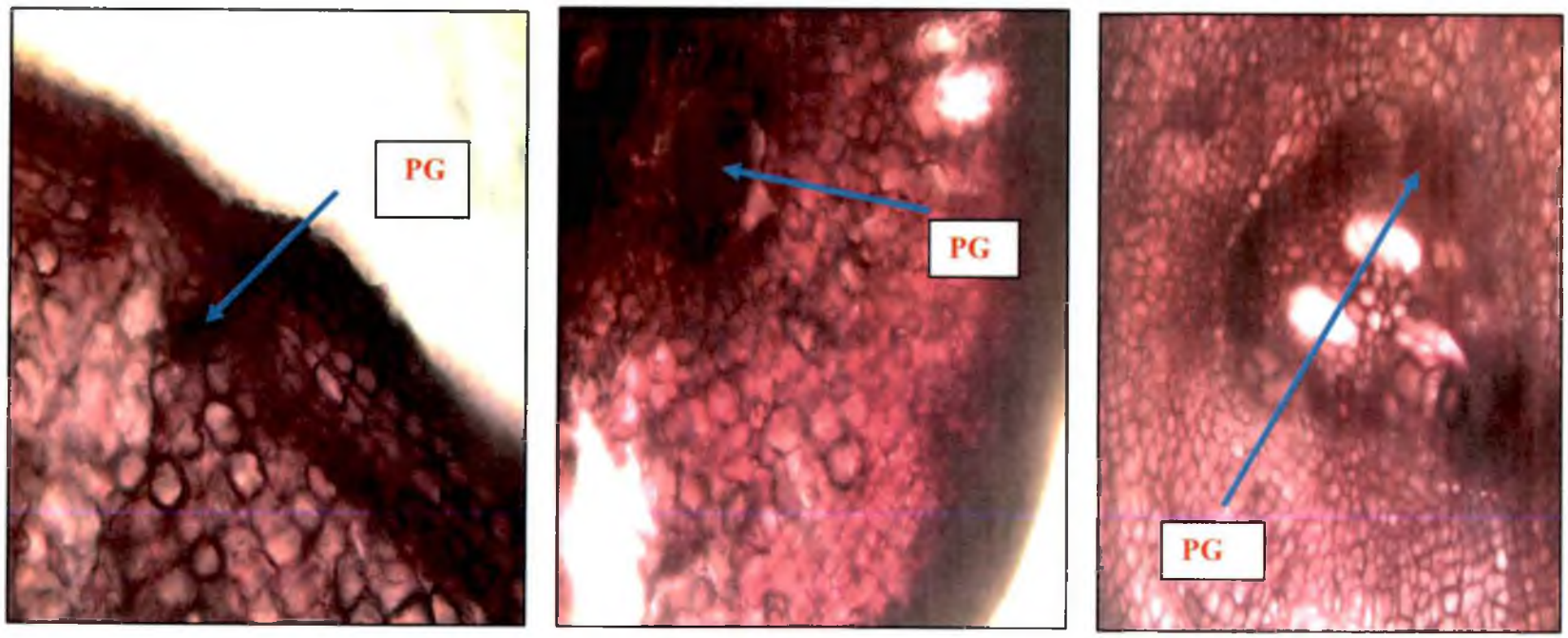


Plate 9. Accumulation of polysaccharides in epidermal (a), cortical (b) and stelar region of root section of root knot nematode inoculated plant

(PG: Polysaccharide granules)

with that of the plants inoculated with 5000 (1.87) and 1000 (1.47) J_2. The stelar accumulation of polysaccharides in plants inoculated with 100 J_2/ pot (0.66) was statistically on par with the uninoculated control plant (0.36)

Among the epidermal, cortical and stelar cells more accumulation of polysaccharide granules was seen in cortical cells. The healthy root section showed normal organization of tissue and the cells in the epidermal, cortical and stelar region were also normal. In *M. graminicola* infected root section recorded increased size of epidermis, cortex and stelar region and the cells in stelar region were irregularly arrainged.

4.4.2 Accumulation of Protein in Root Section [area of accumulation of protein/ total area (taken as 1)]

Effect of accumulation of protein in epidermal, cortical and stelar region of root section at different inoculum levels of *M. graminicola* was shown in table 12.

The accumulation of proteins in epidermal cells increased with increase in inoculum levels of nematode. Maximum accumulation of protein was seen in plants inoculated with 10, 000 J_2/ pot (0.49) and was on par with that of the plant inoculated with 5000 (0.42) and 1000 (0.42) J_2. The maximum percentage increase in protein accumulation was seen in plants inoculated with 10, 000 J_2/ pot (18.45%).

Accumulation of protein in cortical cells was increased with increase in inoculum levels. Maximum accumulation was seen in plants inoculated with 10,000 J_2 and was significantly different from all other treatments. The cortical accumulation of protein in plants inoculated with 100 and 500 J_2 were 0.42 and 0.45 and were on par with each other.

The stelar accumulation of protein was increased with increase in nematode inoculum levels. The maximum accumulation was seen in plants inoculated with

Plate 10. Healthy root section (without accumulation of proteins)

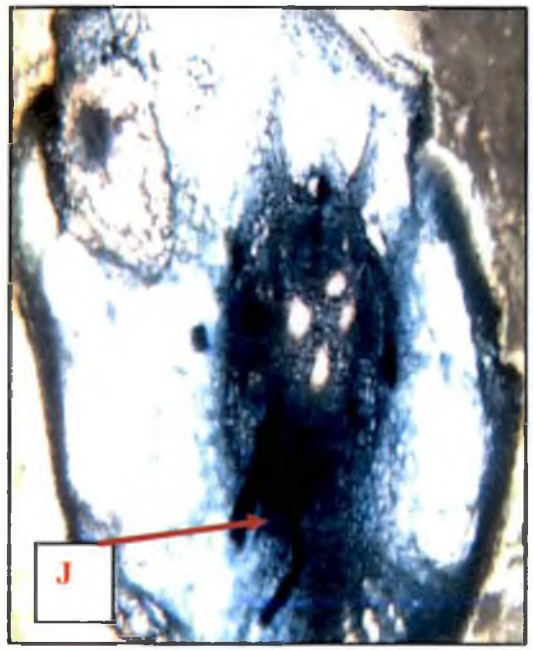


Plate 11. Protein accumulation and juveniles of root knot nematode in nematode infested root section

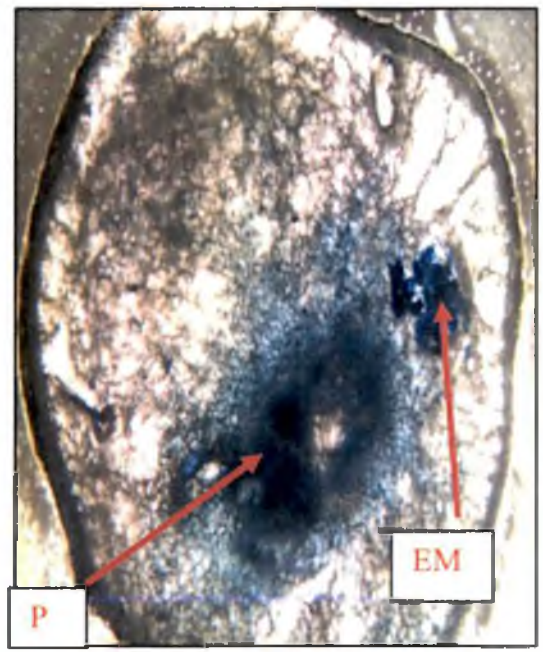


Plate 12. Protein accumulation and egg mass in root section

(J: Juvenile; EM: Egg mass; P: Protein accumulation)

Table 12. Effect of accumulation of proteins in epidermal, cortical and stelar region of root at different inoculum levels of root knot nematode (mean of five replications)

Treatments (No. of J_2/ pot)	Accumulation of Proteins (Area per root section)		
	Epidermal cells	Cortical cells	Stelar cells
Uninoculated Plant	0.07 (1.03)	0.12 (1.06)	0.07 (1.03)
100	0.35 (1.16)	0.42 (1.19)	0.32 (1.15)
500	0.35 (1.16)	0.45 (1.20)	0.37 (1.17)
1000	0.42(1.19)	0.57 (1.25)	0.37 (1.17)
5000	0.42 (1.19)	0.75 (1.32)	0.35 (1.16)
10000	0.49 (1.22)	0.85 (1.36)	0.39 (1.18)
CD (0.05)	0.054	0.048	0.049

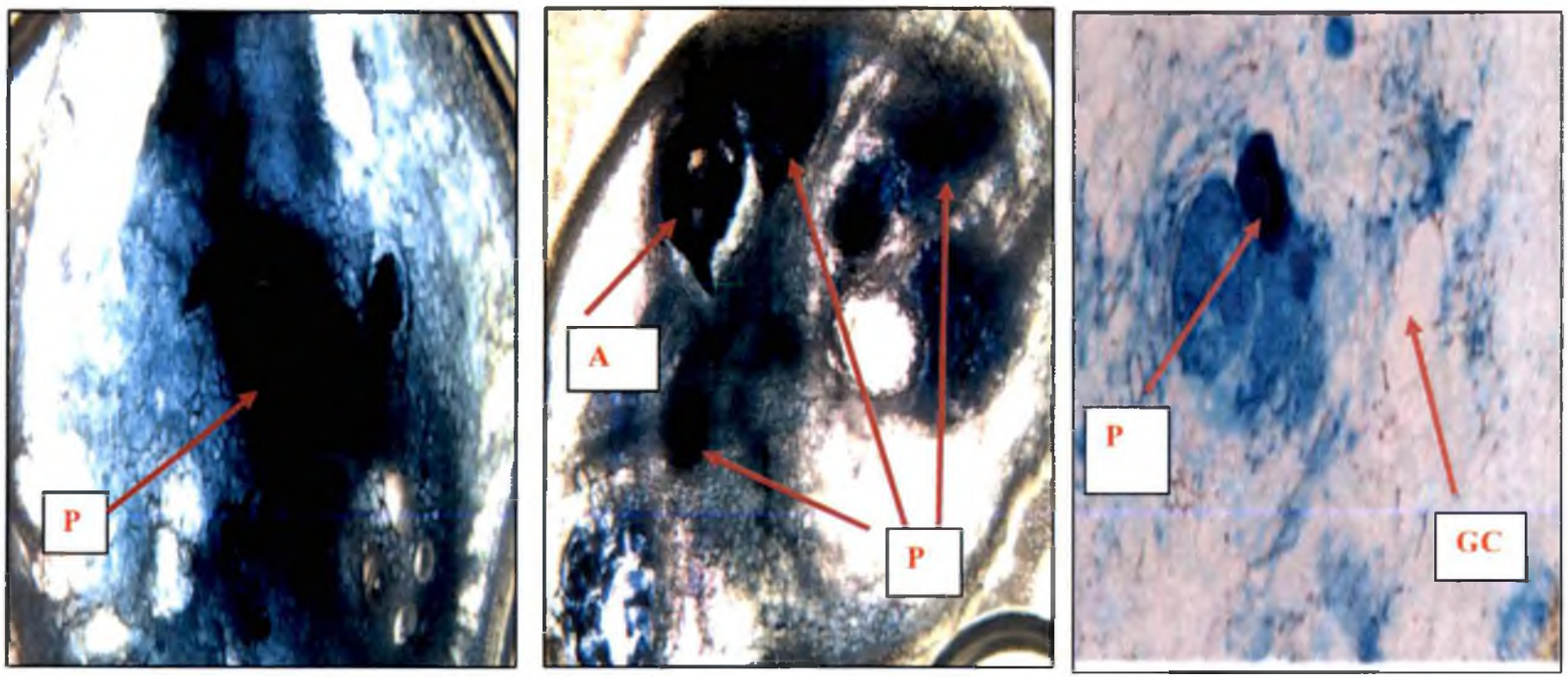


Plate 13. Feeding adult and accumulation of protein in root knot nematode infested root sections

(P: Protein accumulation; A: Adult nematode; GC: Giant cell)

10, 000 J_2/ pot (0.39) and was on par with that of the plants inoculated with 5000 (0.35), 1000 (0.37) and 500 (0.37) J_2.

The root knot nematode infested root sections recorded disorganized xylem and phloem cells and increased size of epidermis, cortex and stelar regions.

4.4.3 Accumulation of Nucleic Acid (RNA and DNA) in Root Section [area of accumulation of nucleic acid / total area (taken as 1)]

Effect of accumulation of nucleic acid (RNA and DNA) in epidermal, cortical and stelar region of root at different inoculum levels of *M. graminicola* was shown in table 13.

Accumulation of RNA and DNA in epidermal cells increased with increase in nematode population levels. The nucleic acid accumulation in epidermal cells of root section in plants inoculated with 100, 500, 1000, 5000 and 10,000 J_2/ pot were on par with each other and were statistically different from uninoculated control plant (0.07). The maximum percentage increase in accumulation of nucleic acid (RNA and DNA) in epidermal cells was seen in plants inoculated with 10,000 J_2 (12.5%).

A progressive increase in accumulation of nucleic acid (RNA and DNA) in cortical cells was observed with increase in nematode inoculum levels. Maximum accumulation was seen in plants inoculated 5000 J_2 (0.49). The cortical accumulation of nucleic acid (RNA and DNA) in plants inoculated with 500 and 1000 J_2/ pot were on par with each other and were significantly different from uninoculated control plant (0.09).

Accumulation of RNA and DNA in stelar cells increased with increase in nematode population levels. Maximum accumulation was seen in plants inoculated with 10,000 J_2/ pot (0.67) and was significantly different from all other treatments.

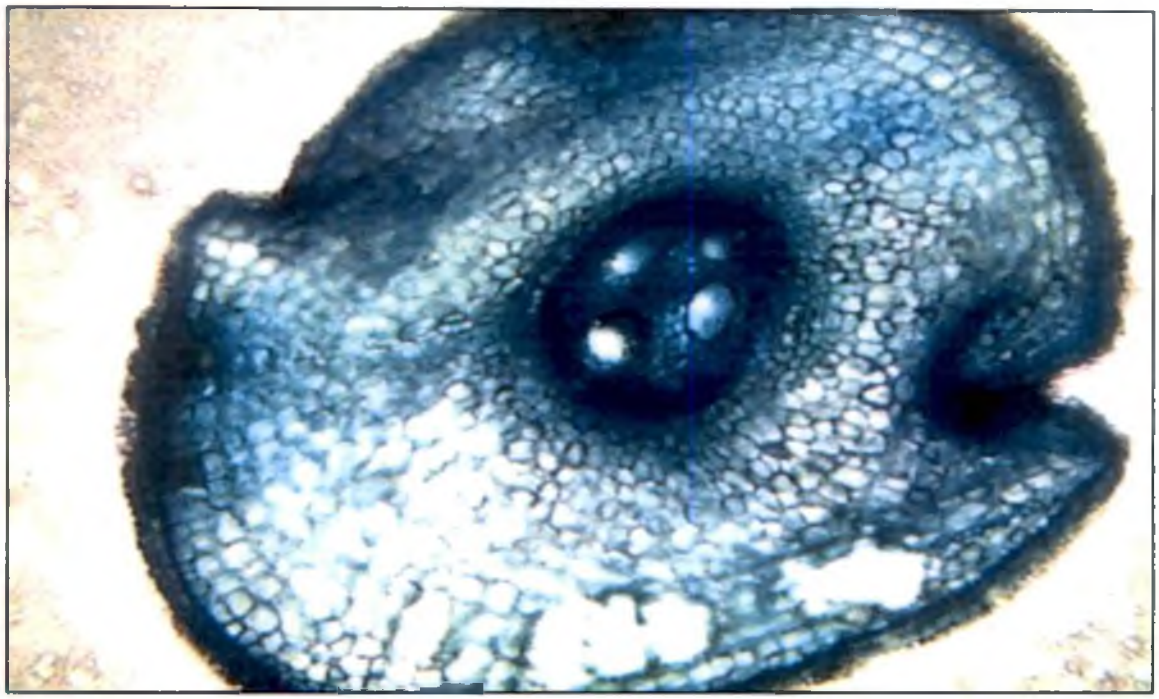


Plate 14. Healthy root (without accumulation of nucleic acid)

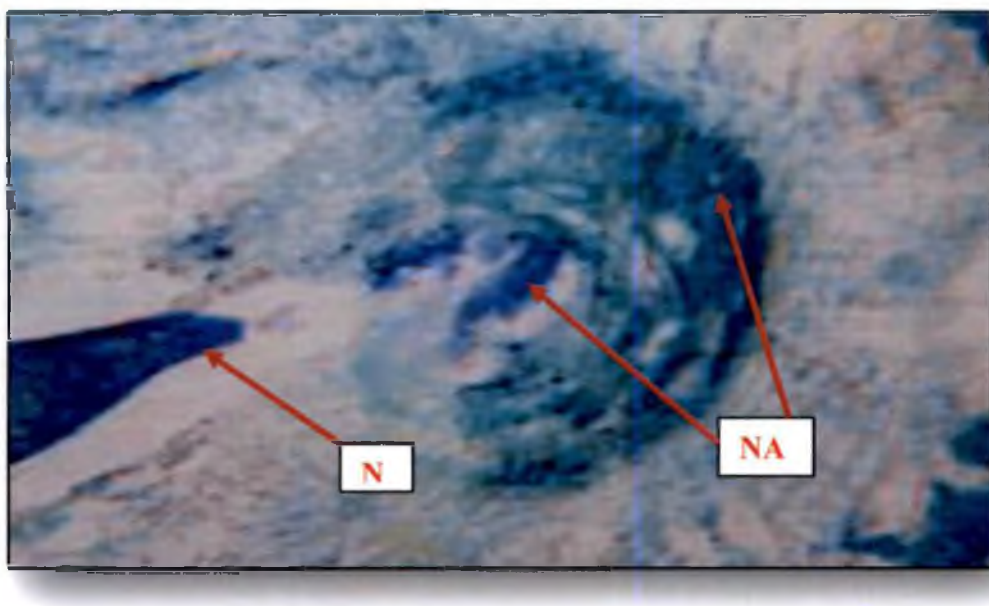


Plate 15. Infested root section showing presence of nematode and accumulation of nucleic acid (RNA and DNA)

(N: Nematode; NA: Nucleic acid)

Table 13. Effect of accumulation of nucleic acid in epidermal, cortical and stelar region of root at different inoculum levels of root knot nematode (mean of five replications)

Treatments (No. of J_2/ pot)	Accumulation of Nucleic acid (Area/root section)		
	Epidermal cells	Cortical cells	Stelar cells
Uninoculated Plant	0.07 (1.04)	0.09 (1.05)	0.12 (1.06)
100	0.29 (1.14)	0.32 (1.15)	0.37 (1.17)
500	0.32 (1.15)	0.35 (1.16)	0.45 (1.20)
1000	0.35 (1.16)	0.39 (1.18)	0.49 (1.22)
5000	0.35 (1.16)	0.49 (1.22)	0.52 (1.23)
10000	0.37 (1.17)	0.61 (1.27)	0.67 (1.29)
CD (0.05)	0.048	0.064	0.058

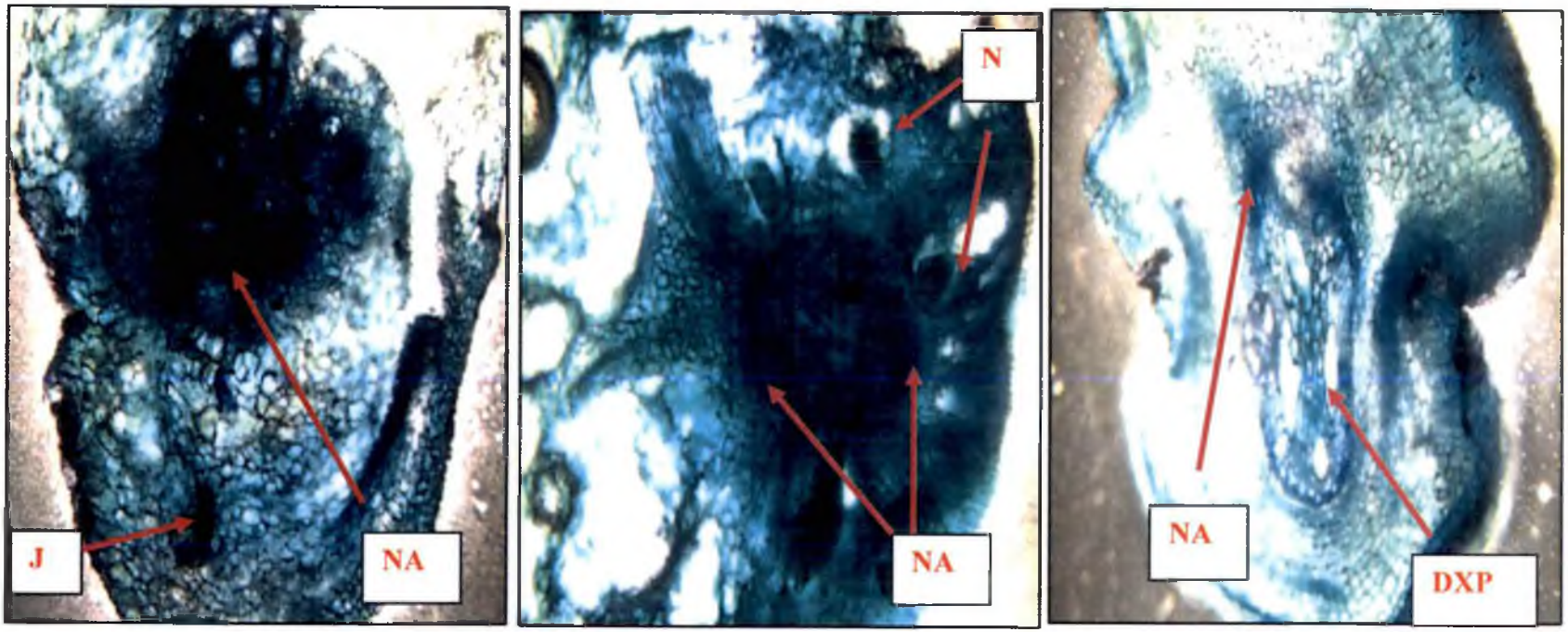


Plate 16. Accumulation of nucleic acids in different region of root section

(N: Nematode; NA: Nucleic acid; J: Juvenile; DXP: Dislocated xylem and phloem tissues)

The accumulation of RNA and DNA in root section of plants inoculated with 500, 1000 and 5000 were on par with each other.

The more accumulation of nucleic acid (RNA and DNA) was seen in stelar cells of the root sections and minimum accumulation of nucleic acid was seen in epidermal cells. The root section of infested plants recorded increased size of epidermis, cortex and stelar region and the cells in stelar region were irregularly arraigned. The root section of uninoculated plants showed normal organization of tissues and cells.

4.5 VARIETAL REACTION

4.5.1 Galls and Gall Index

Number of galls and gall index in roots of different rice varieties due to infestation of root knot nematode, *M. graminicola* was shown in tables 14 and 15.

Maximum number of galls per plant was observed in the variety Karishma (83.2) and was on par with that of Pratyasa (81.2) and Aiswarya (79.6). Lowest number of galls per plant was observed in variety Uma (51.80) and was on par with that of the variety Pavizham (57.4). The number of galls in the variety Kanakom (72.2) and Revathy (76.4) were on par with that of the susceptible check variety, TN1 (71). The number of galls per plant in varieties Karishma, Prathyasa, Jyothi, Aiswarya and Kanakom were more than that of the susceptible check variety (TN1). In varieties Uma, Karthika, Pavizham and Bhadra, the number of galls per plant was less than that of the susceptible check variety. The gall index of all the varieties was recorded as 4. According to the gall index, all the varieties were rated as susceptible (S) to root knot nematode.

Table 14. Effect of *M. graminicola* on number of galls and egg mass in roots of different rice varieties (mean of five replications)

Varieties	Number of galls per Plants	No. of egg mass per plant
Bhadra	65	73.00
Pavizham	57.4	67.20
Karthika	61.2	79.00
Kanakom	72.2	87.20
Uma	51.8	64.60
Revathy	76.4	92.60
Karishma	83.2	94.40
Prathyasa	81.2	94.00
Jyothi	77	91.20
Aishwarya	79.6	90.40
TN1 (Susceptible check)	71	88.00
CD (0.05)	5.976	7.243

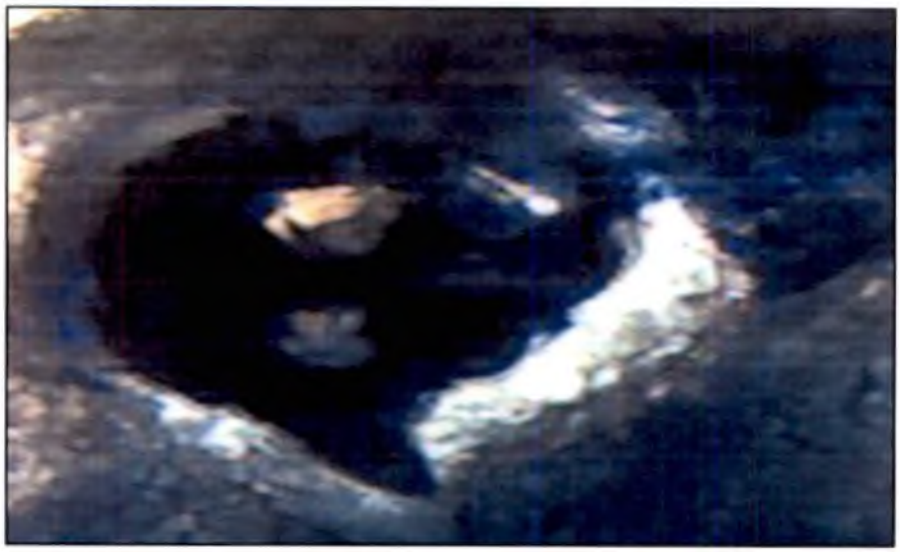


Plate 17. Adult female inside the root section

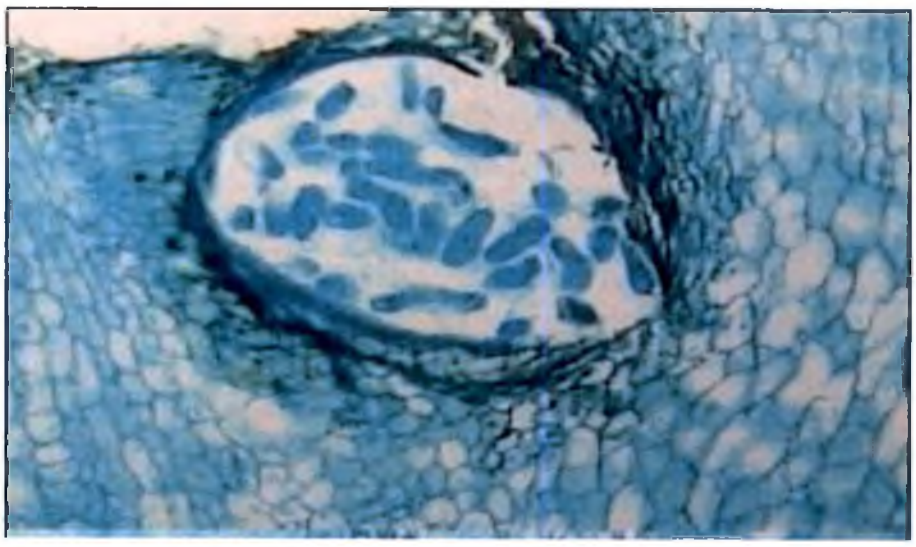


Plate 18. Egg mass inside root section

Table 15. Root knot Index, Egg mass Index and host reaction of various rice varieties against *M. graminicola*

Varieties	Root knot Index	Host reaction	Egg mass Index	Host reaction
Bhadra	4	S	3.31	HS
Pavizham	4	S	3.05	HS
Karthika	4	S	3.59	HS
Kanakom	4	S	3.96	HS
Uma	4	S	2.94	S
Revathy	4	S	4.21	HS
Karishma	4	S	4.29	HS
Prathyasa	4	S	4.27	HS
Jyothi	4	S	4.14	HS
Aishwarya	4	S	4.11	HS
TN1 (Susceptible check)	4	S	4.00	HS

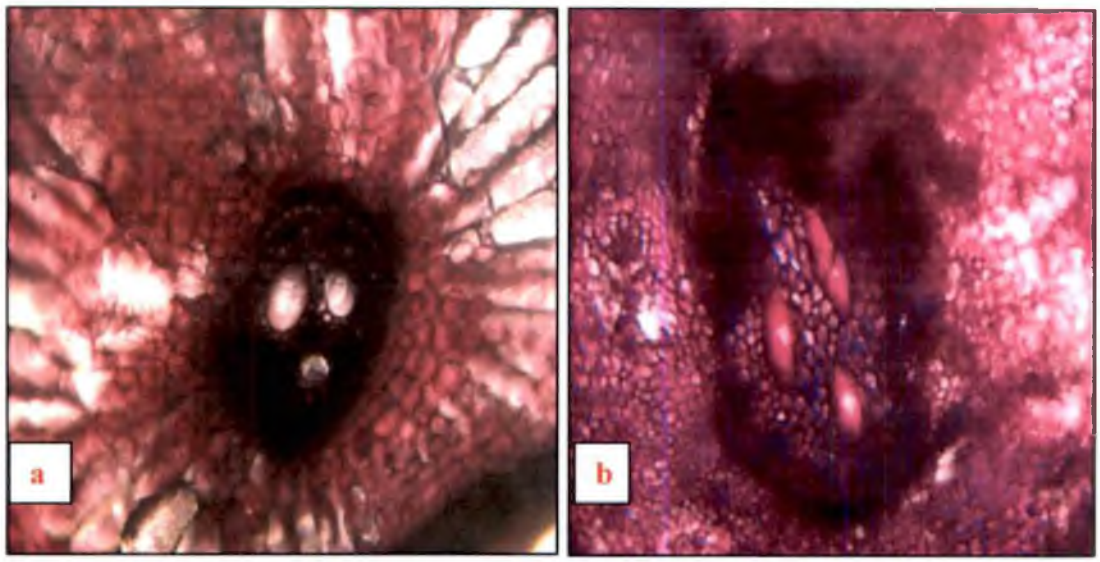


Plate 19. a. Healthy root section with intact xylem and phloem cells,
b. Disorganized xylem and phloem cells in nematode infested root section

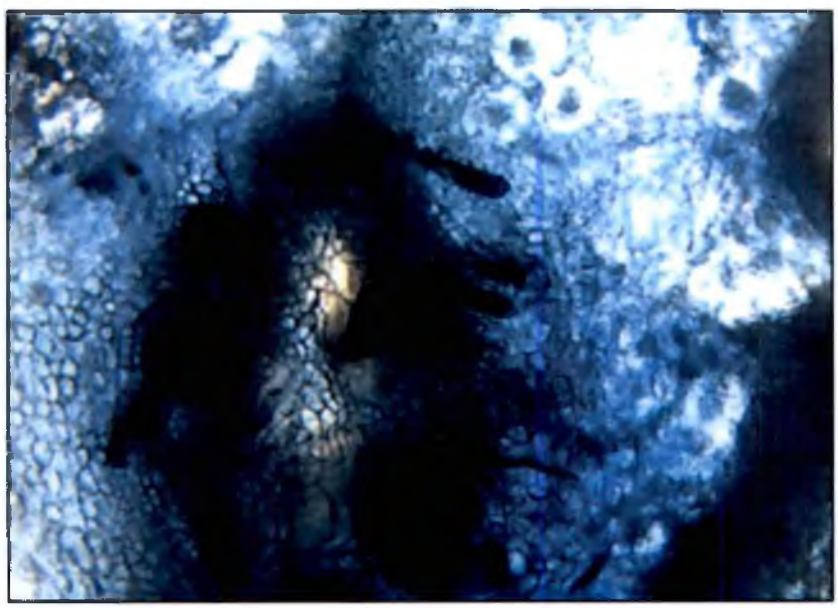


Plate 20. Juveniles of root knot nematode in root section

4.5.2 Egg mass and Egg mass index

Number of egg mass and egg mass index in roots of different rice varieties due to infestation of *M. graminicola* was shown in tables 14 and 15.

Highest number of egg mass per plant was observed in variety Karishma (94.40) and was on par with that of Revathy (92.60), Prathyasa (94.00), Jyothi (91.20), Aiswarya (90.40) and Kanakom (87.20). Lowest number of egg mass per plant was observed in variety Uma (64.60) and was on par with that of the variety Pavizham (67.20). The number of egg mass per plant in varieties Bhadra, Pavizham, Karthika and Uma were more than that of the susceptible check variety (TN1). The egg mass index was highest in variety Karishma (4.29) and lowest in variety Uma (2.94). On the basis of egg mass index, all other varieties were highly susceptible except Uma.

4.6 ENZYMATIC AND GROWTH ACTIVITY OF VARIETIES

4.6.1 Peroxidase (PO), Polyphenol Oxidase (PPO) and Phenylalanine Ammonia Lyase (PAL) Activity in Root

Changes in PO, PPO and PAL activity in root of different rice varieties due to infestation of *M. graminicola* was shown in table 16.

Highest PO activity was observed in variety Uma (6.82 OD per g/min) and was on par with that of Karthika (6.75 OD per g/min), Pavizham (6.70 OD per g/min) and Bhadra (6.52 OD per g/min). Lowest PO activity was observed in variety Karishma (4.49 OD per g/min) and was on par with that of variety Prathyasa (4.90 OD per g/min). The varieties Uma, Karthika, Pavizham and Bhadra were showed more PO activity than the susceptible check variety (TN1).

The PPO activity was highest in Variety Uma (1.77 OD per g/min) and was on par with that of the variety Bhadra (1.48 OD per g/min). Lowest PPO activity was observed in variety Karishma (0.64 OD per g/min) and was on par with that of

Table 16. Effect of *M. graminicola* on peroxidase, polyphenol oxidase and phenyl alanine ammonia lyase activities in roots of various rice varieties (mean of five replications)

Varieties	PO (OD per gm/min)	PPO (OD per gm/ min)	PAL (OD per gm/min)
Bhadra	6.52	1.48	3.27
Pavizham	6.70	0.96	3.54
Karthika	6.75	1.13	3.44
Kanakom	6.07	1.26	3.20
Uma	6.82	1.77	3.55
Revathy	5.01	0.86	3.10
Karishma	4.49	0.64	2.87
Prathyasa	4.90	0.69	2.37
Jyothi	5.85	0.88	2.89
Aishwarya	4.96	0.88	2.25
TN1 (Susceptible check)	6.15	0.89	1.49
CD (0.05)	0.462	0.397	0.595

Revathy (0.86 OD per g/min), Prathyasa (0.69 OD per g/min), Jyothi (0.88 OD per g/min) and Aiswarya (0.88 OD per g/min).. The PPO activity of Bhadra, Pavizham, Karthika, Kanakom and Uma was more than that of TN1.

Highest PAL activity was observed in variety Uma (3.55 OD per g/min) and was on par with that of Pavizham (3.54 OD per g/min), Bhadra (3.27 OD per g/min) Karthika (3.44 OD per g/min), Kanakom (3.20 OD per g/min) and Revathy (3.10 OD per g/min). Lowest PAL activity was observed in variety Aiswarya (2.25 OD per g/min) and was on par with that of variety Prathyasa (2.37 OD per g/min). The PAL activity of all the varieties was more than that of the susceptible check variety. PO, PPO and PAL activity was highest in variety Uma and that showed more defense action against root knot nematode, *M graminicola*.

4.6.2 Growth Parameters

The changes in plant growth parameters of different rice varieties due to *M. graminicola* was shown in table 17.

Observable variation in fresh weight of plant, dry weight of shoot and root were observed in selected rice varieties. The highest fresh weight of plant was observed in variety Uma (5.69 g) and was on par with that of Pavizham (5.55 g) and Karthika (5.52 g). Lowest fresh weight was observed in variety Aiswarya (3.65 g) and was on par with Karishma (3.82 g). The variety Revathy had fresh weight of 4.47 g and was on par with that of variety Prathyasa (4.12 g).

Maximum dry weight of root was observed in variety Uma (1.22 g) and was on par with that of variety Karthika (1.14 g). Dry weight of root was minimum in variety Karishma (0.39 g) and was on par with that of Jyothi (0.48 g). In variety Bhadra, the dry weight of root was 0.53 g and was on par with Jyothi.

Dry weight of shoot was maximum in variety Uma (2.22 g) and was on par with that of variety Pavizham (2.14 g). Minimum was observed in variety Karishma

Table 17. Effect of *M. graminicola* on the plant growth parameters of various rice varieties (mean of five replications)

Varieties	Plant growth parameters (45 days after nematode inoculation)			
	Fresh weight of plant (g)	Dry weight of root (g)	Dry weight of shoot (g)	Plant height(cm)
Bhadra	5.05	0.53	1.99	41.28
Pavizham	5.55	0.81	2.14	45.96
Karthika	5.52	1.14	2.02	42.14
Kanakom	4.52	0.96	1.95	41.74
Uma	5.69	1.22	2.22	47.76
Revathy	4.47	0.93	1.85	39.52
Karishma	3.82	0.39	1.69	39.46
Prathyasa	4.12	0.82	1.79	40.08
Jyothi	4.53	0.48	1.76	40.00
Aishwarya	3.65	0.74	1.75	40.20
CD (0.05)	0.352	0.114	0.132	2.098

Plate 21. ***M. graminicola*** **infested roots of different rice varieties of Kerala**

(1.69 g) and was on par with that of Prathyasa (1.79 g) Jyothi (1.76 g) and Aiswarya (1.75 g).

The maximum plant height was seen in variety Uma (47.76 cm) and was on par with that of the variety Pavizham (45.96 cm). No much variation was observed in plant height due to the infestation of *M. graminicola*. The plant height was seen lowest in variety Karishma (39.46 cm).

4.7 NEMATODE POPULATION

Results on the preference of *M. graminicola* for different rice varieties of rice in Kerala assessed in terms of nematode population characteristics are presented in table 18.

4.7.1 Nematode Population in Soil

Significant difference was observed in population of the second stage juveniles recorded from the rhizosphere of the varieties tested 45 days after inoculation. Among the varieties screened lowest population of *M. graminicola* was recorded from the rhizosphere of Uma (1885.20). It differed significantly from all other varieties except Karthika (1940.00). The rice varieties viz., Kanakom, Revathy and Jyothi supported a soil population of 2125.20, 2187.60 and 2148.40 were on par with that of the susceptible check variety TN1 (2091.60). A significantly higher population of nematode was observed in Karishma (2362.60) and its effect was statistically differed from the susceptible check and all other varieties. Prathyasa (2243.60) and Aiswarya (2213.20) supported a large population of the nematode and were on par with each other.

4.7.2 Nematode Population in Root

Observable variation was also seen in the nematode population obtained from the roots of the different rice varieties. The variety Uma had the lowest population

Table 18. Effect of *M. graminicola* on nematode population in root and soil and reproduction rate of nematode in various rice varieties (mean of five replications)

Varieties	Nematode Population (45 DAI)		Reproduction rate (Rf= Pf/Pi)
	Soil (100 g)	Root (5 g)	
Bhadra	2034.20	40.00	20.74
Pavizham	2021.20	41.80	20.63
Karthika	1940.00	36.60	19.76
Kanakom	2125.20	47.20	21.72
Uma	1885.20	35.00	19.20
Revathy	2187.60	42.40	22.30
Karishma	2362.60	53.20	24.15
Prathyasa	2243.60	42.60	22.86
Jyothi	2148.40	48.20	21.96
Aishwarya	2213.20	52.80	22.66
TN1(Susceptible check)	2091.60	51.40	21.43
CD (0.05)	111.425	6.483	1.124

(35.00) and was on par with that of Karthika (36.60) and Bhadra (40.00). The root population obtained from the varieties Bhadra, Pavizham, Karthika, Revathy Prathyasa and Jyothi were 40.00, 41.80, 36.60, 42.40, 42.60 and 48.20 respectively which were on par, but differed significantly from the susceptible check variety (TN1). A significantly higher population was recorded from the root samples of variety Karishma (53.20) which was on par with that of variety Aiswarya (52.80) and susceptible check TN1(51.40).

4.7.3 Reproduction Rate

Variation was seen in the reproduction rate of *M. graminicola* in different rice varieties. Reproduction rate of nematode was significantly low in Uma, being (19.20) and was on par with Karthika where the reproduction rate was 19.76. In Bhadra the nematode had a reproduction rate of 20.74 and was on par with that of Pavizham (20.63) and Karthika (19.76). Reproduction rate of *M. graminicola* was highest in Karishma (24.15) and was significantly different from all other varieties and susceptible check variety TN1. The varieties Kanakom and Revathy recorded reproduction rate of 21.72 and 22.30 were on par with the susceptible check TN1 (21.43) in their effect on the reproduction rate of the nematode.

DISCUSSION

5. DISCUSSION

The root knot nematode, *Meloidogyne graminicola* is an internationally important pest of rice found throughout the world where ever rice is cultivated. In India it is a well established pest of upland and well drained soils and causes enormous losses in yield due to poor filling of kernels. Though occurrence of the nematode in rice is known, its pathogenic potential has not adequately documented in Kerala. Information on the biochemical and histopathological alterations, yield and growth characters of rice at different population density of nematode and the varietal reaction studies are essential for the cost effective management of the pest. The varietal reaction studies also help to identify the best varieties.

The results on the evaluation of biochemical and histopathological alterations, changes in growth and yield characters due to different inoculum levels of nematode and the varietal screening are discussed in this chapter under different headings.

5.1 BIOCHEMICAL CHANGES

5.1.1 Phenol Content

Inoculation of *M. graminicola* in varying population levels resulted in a corresponding increase in phenol content of leaves and roots at 45 DAI. The maximum phenol content was observed in plants inoculated with 10,000 J_2/ pot. The per cent increase of the phenol content of leaf and root in plants inoculated with 10,000 J_2/pot was 18.36 and 46.37 per cent respectively. Higher phenol production was seen in roots than in leaves. Phenol production in plants helps to reduce the infestation of *M. graminicola.* Higher the infestation of nematode leads to the increased production of phenolic substances in both root and leaf samples. The plants itself have the capacity to reduce the infestation of the nematode by producing the phenols.

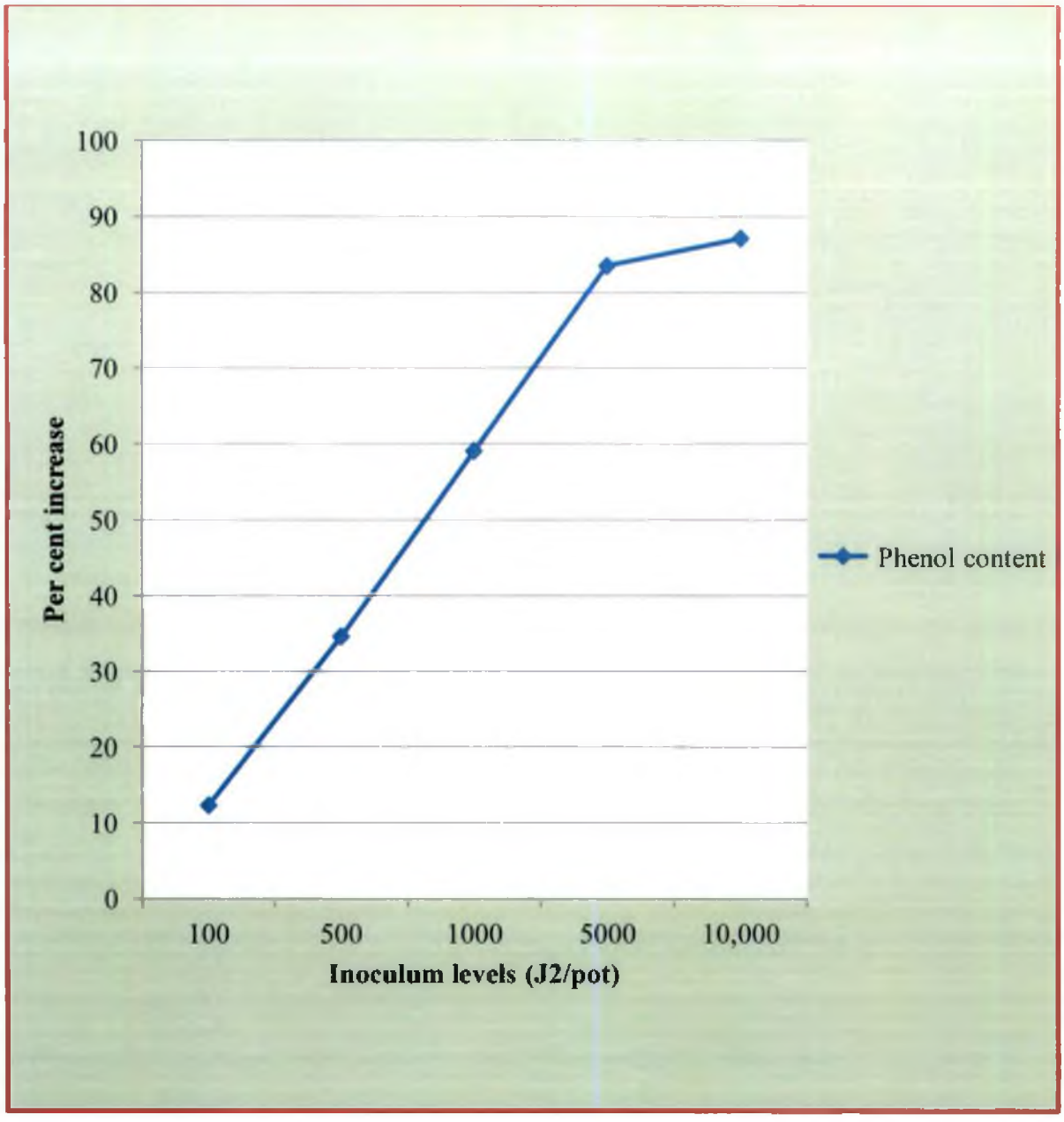


Fig. 1. Per cent increase in phenol content of root at different inoculums levels of *M. graminicola*

Similarly, the root knot nematode infected rice varieties Annapurna, Manika and Ramakrishna produced greater amount of phenolics compared to the healthy plants (Mishra and Mohanty, 2007). Taiz and Zeiger (2002) reported that the early increases in phenol caused by pathogen invasion triggered the transcription of messenger RNA that codes for phenylalanine ammonia lyase; increasing amounts of PAL in the plant brought about the synthesis of phenolic compounds.

The phenolic accumulation was due to the activation of hexose monophosphate shunt pathway, acetate pathway and release of bound phenols by hydrolic enzymes (Goodman *et al.*, 1967). Sempio *et al.* (1975) reported that the phenolic compounds were possibly to quinines in resistant cultivars. The increased phenolic content in roots indicated the degree of resistance to root knot nematode (Rani *et al.*, 2008).

5.1.2 Peroxide (PO), PolyPhenol Oxidase (PPO) and Phenylalanine Ammonia Lyase (PAL) Activity

PO, PPO and PAL are the defense enzymes in plants and they synthesis when the plant is infected by any pathogen.

The PO, PPO and PAL activity in leaf and root samples increased with increasing nematode inoculum levels. The maximum activity of these enzymes was seen in plants inoculated with 10,000 J_2/pot. The PO activity of roots in plants inoculated with 100 J_2 was on par with that of the uninoculated plants. The PO activity of root and leaf samples in plants inoculated with 10,000 J_2 increased 70.79 and 62.16 per cent compared to that of the uninoculated plants. The PPO activity of roots increased 94.69 per cent in plants inoculated with 10,000 J_2 over uninoculated plants. The PAL activity of root and leaf samples in plants inoculated with 10, 000 J_2 increased 60.75 and 61.36 per cent over the uninoculated control plants.

Similarly, Xu *et al.* (2008) found that *M. graminicola* infection increased the activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in roots of rice. The PO activity increased in *M. incognita* infected resistant tomato cultivars

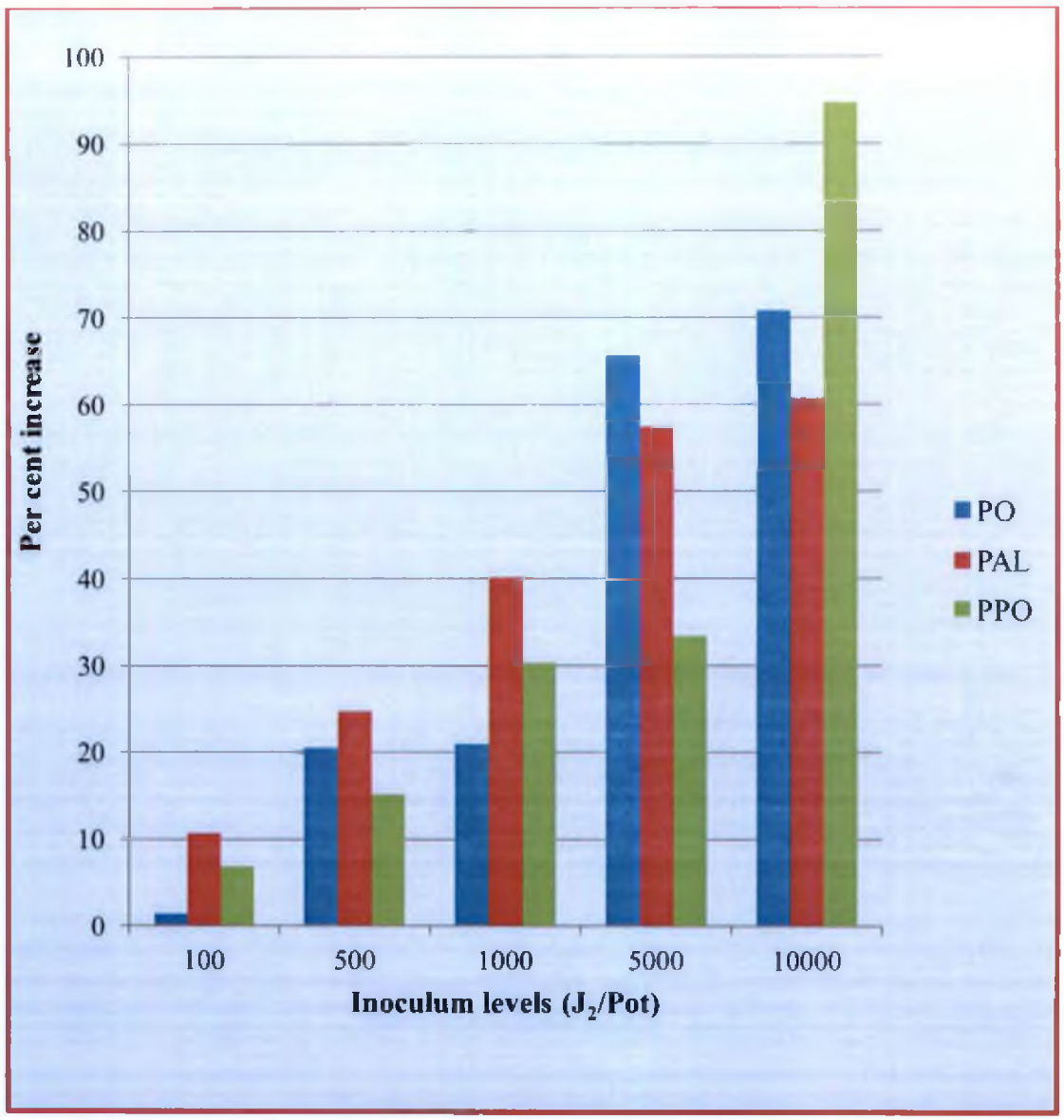


Fig 2. Per cent increase in PO, PAL, PPO in root samples at different inoculum levels of *M. graminicola*

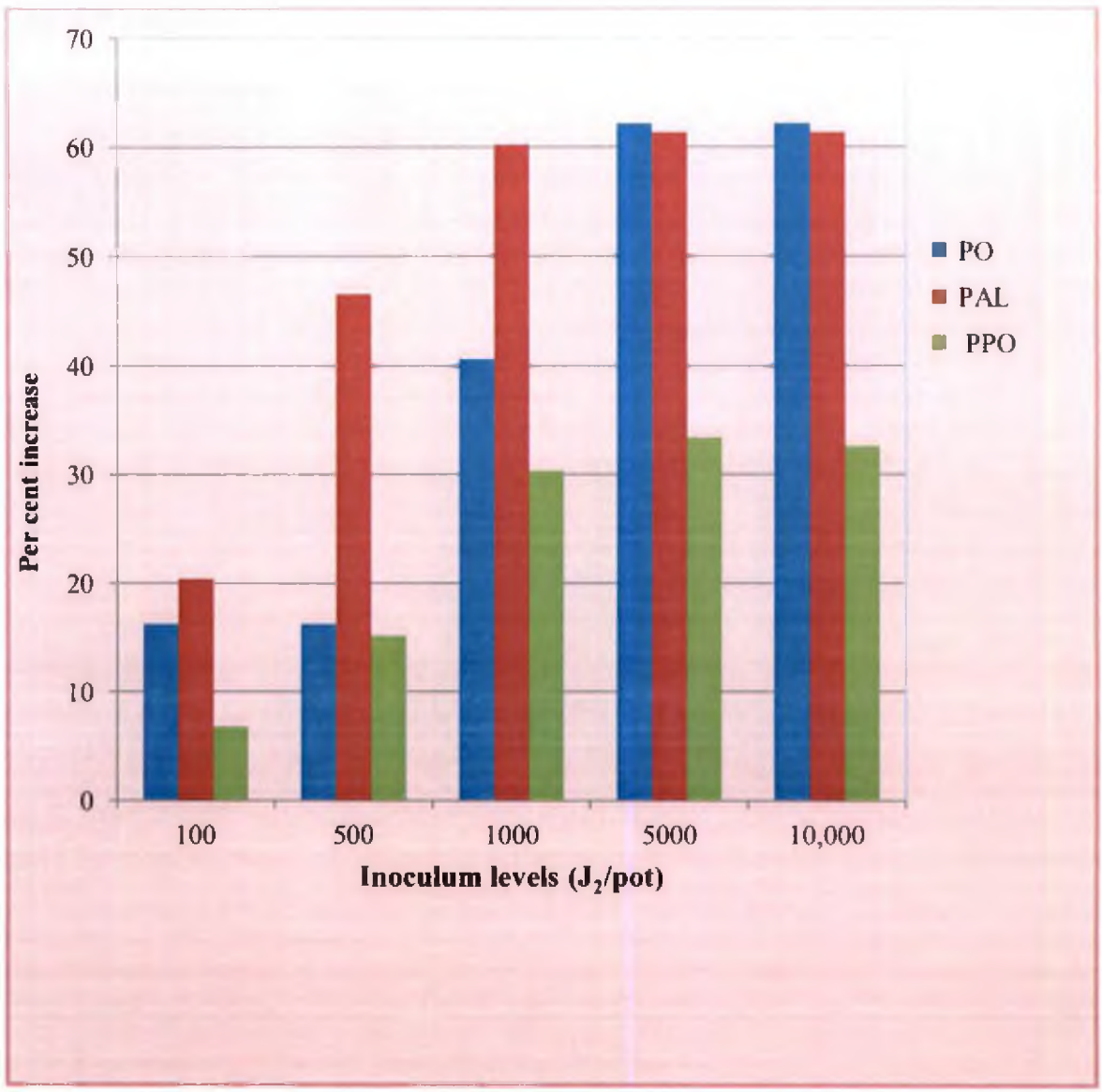


Fig 3. Per cent increase in PO, PAL, PPO in leaf at different inoculum levels of *M. graminicola*

up to 5 times than the healthy plants (Shukla and Chakraborthy, 1988). Devrajan and Seenivasan (2002) observed that the inoculation of *M. incognita* increased the PPO activity in banana. Kumar *et al.* (2007) found that the degree of PAL activity was comparatively lesser in susceptible rice than resistant cultivars and also reported that sequential development of PPO increased in nematode infested resistant rice varieties.

5.1.3 Chlorophyll Content of Leaf Samples

Chlorophyll pigments are essential for photosynthesis in plants. Changes in this pigment in leaf will affect the photosynthetic rate of the crop plants.

Chlorophyll a, b and total chlorophyll content of leaf samples decreased with increasing nematode inoculum levels, minimum content was observed in 10,000 J_2 inoculated plants (0.194, 0.38 and 0.686 mg per g tissue respectively). The chlorophyll a content of the plants inoculated with 10,000 J_2 reduced 40.67 per cent compared to that of the uninoculated plants. The chlorophyll b and total chlorophyll content of 10,000 J_2 inoculated plants reduced 27.55 and 20.24 per cent respectively when compared to that of the uninoculated plants.

The total chlorophyll content of the uninoculated plant was 0.93 mg per g tissue and it was 0.78 mg per g tissue in plants inoculated with 100 J_2/pot. This indicates that nematode infection causes nutrient shortage, which triggers depletion of chlorophyll pigments. Rao *et al.* (1988) reported that the total chlorophyll in leaves of healthy rice plants was 3.7 mg and of a and b fractions were 2.1 and 1.6 mg per g and chlorophyll was reduced, owing to root knot nematode incidence by 30.20 per cent of the total and 39.5 and 32 per cent of the a and b fractions. Similarly, Mishra and Mohanty (2008) reported that the per cent reduction in chlorophyll a, b and total chlorophyll content were 65.7, 48.6 and 60.7 per cent respectively in rice variety Annapurna. There were similar results in rice, tomato and French bean due to root knot nematode (Ramakrishnan and Rajendran, 1998; Loveys and Bird, 1973; Melakeberhan *et al.*, 1986; Swain and Prasad, 1988). Abbasi and Hisamuddin (2014)

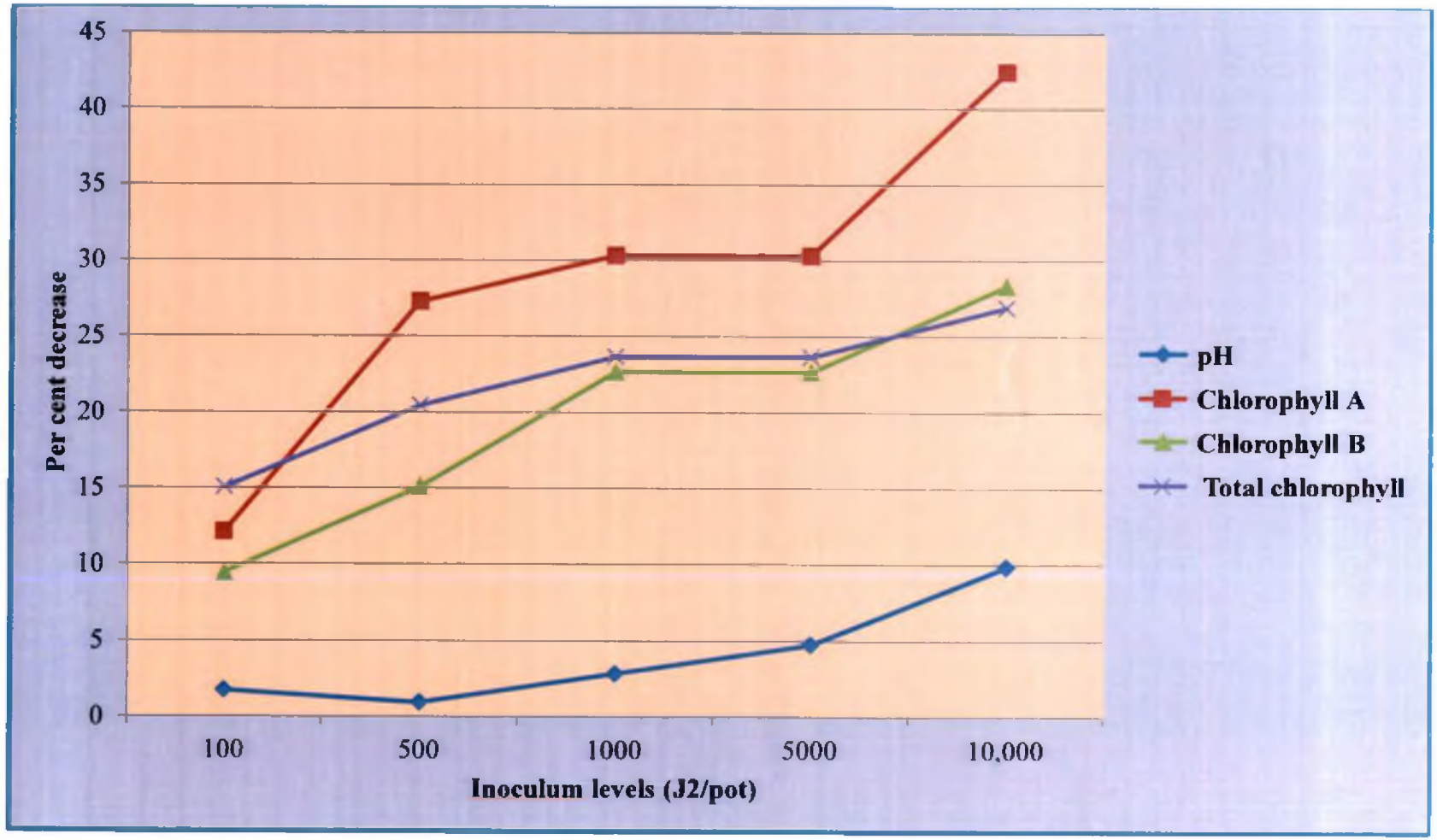


Fig 4. Per cent decrease in pH, chlorophyll A, chlorophyll B and total Chlorophyll in leaf samples at different inoculum levels of *M. graminicola*

reported that the chlorophyll (a+b) content of green gram decreased significantly at higher inoculum levels compared with the control. A contradictory report was given by Swain and Prasad (1988). They found that the chlorophyll content increased after nematode infestation.

5.1.4 N, P and K Content in Leaf Samples

The content of major plant nutrients N, P and K was much reduced in the leaf samples of rice plants inoculated with *M. graminicola.* The reduction was more at higher inoculum level (10,000 J_2/pot). This would indicate lesser uptake or translocation of these nutrients, which confirms the finding of Patil *et al.* (2013). Venkatesan *et al.* (2013) reported that the content of major plant nutrients N, P and K was reduced in the shoot of rice plants inoculated with *M. graminicola.* The cotton var. H777 infected with *M. incognita* showed a significant reduction in the degree of uptake of nitrogen, phosphorus and potassium (Verma and Jain, 2006).

The percentage reduction of nitrogen content in plants inoculated with 10,000 and 5000 J_2/pot was 13.64 and 12.80 per cent respectively over the uninoculated plants. The N uptake in plants reduced due to decreased fine root growth caused by the presence of the sedentary nematode (Miller and Cramer, 2004). Patil and Gaur (2014) found that the nutrient remained unutilized in the soil while the plant suffered severe nutrient deficiencies and growth reduction in the presence of nematode infection. Vast *et al.* (1988) reported that the rate of uptake of nitrate and ammonia in coffee plants reduced 63 per cent and 54 per cent due to the infection of root knot nematode, *M. konaensis.* Ganguly *et al.* (1991) reported that N in the leaves is broken down to simpler proteins and get translocated. The shoot nitrogen content of rice varieties Annapurna, Manika and Ramakrishna decreased by 22, 16.6 and 2.4 per cent respectively in the root knot nematode infected plants compared to healthy rice plants (Mishra and Mohanty, 2008).

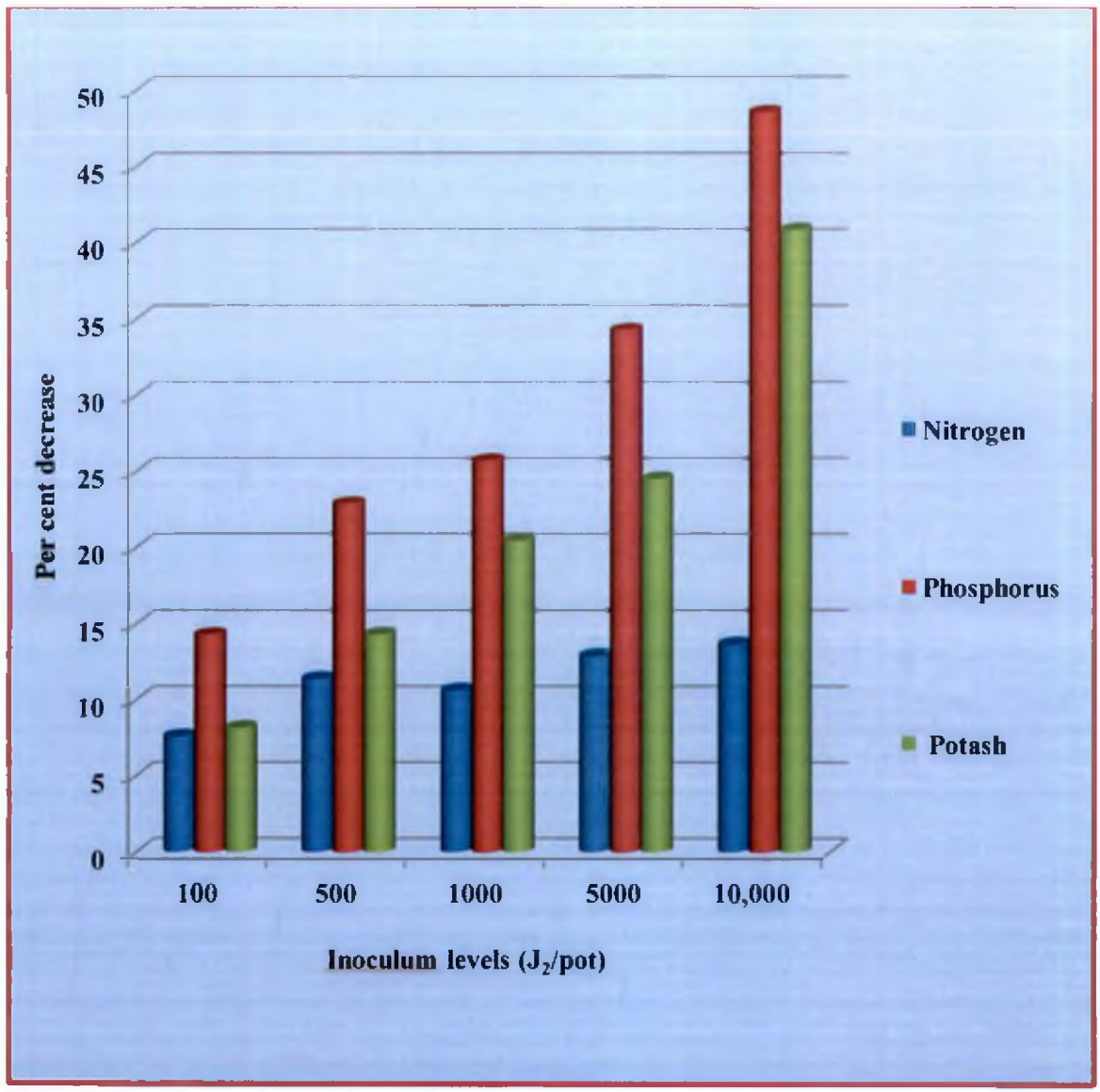


Fig 5. Per cent reduction in N, P and K content in leaf due to infestation of *M. graminicola*

A progressive decrease in P content of leaf samples was noted with increase in the nematode inoculum levels. The minimum P content was observed in plants inoculated with 10,000 J_2 (0.18 per cent), which was significantly different from all other treatments. The general reduction in cell activity in root and shoot was due to the low P content in plant (Rao *et al.*, 1986). The percentage reduction in P content of leaf samples in plants inoculated with 1000 and 5000 was 25.71 and 34.28 per cent respectively over uninoculated plant. The finding was in line with Mishra and Mohanty, (2008), who reported that the P content of shoots in root knot infected rice varieties Annapurna, Manika and Ramakrishna reduced 25.2, 6.7 and 1 per cent respectively over uninoculated plants.

The potash content of leaf samples also decreased with increase in nematode inoculum levels. Maximum reduction was observed in plants inoculated with 10,000 J_2/ pot (0.29 per cent) and the percentage reduction was 40.82 per cent over uninoculated plants. Mishra and Mohanty (2008) reported that in *M. graminicola* infected rice, the K content of the shoot reduced by 59.6, 18.9 and 22.7 per cent in varieties Annapurna, Manika and Ramakrishna respectively. Chakrabarti and Mishra (2002) have also recorded decrease in K content of the shoot due to root knot nematode infection in chickpea, but conversely there the extent of reduction of K was less in the resistant variety.

5.1.5 Micronutrient Content in Leaf Samples

The present investigation shows that the concentration of micronutrients *viz.*, Fe, Cu, Zn and Mn in rice leaves were significantly reduced with increasing nematode population. Plants inoculated with 10,000 J_2/pot showed the lowest Fe content and was significantly different from all other treatments. The Zn content of leaf samples in plants inoculated with 500, 1000 and 5000 were 32.88, 31.77 and 30.69 ppm respectively. Similarly Patil and Gaur (2014) reported that Fe and Zn content were significantly reduced in leaves of nematode infected plants as compared to the leaves of uninfected plants. Rao *et al.* (1988) reported that the Fe content in

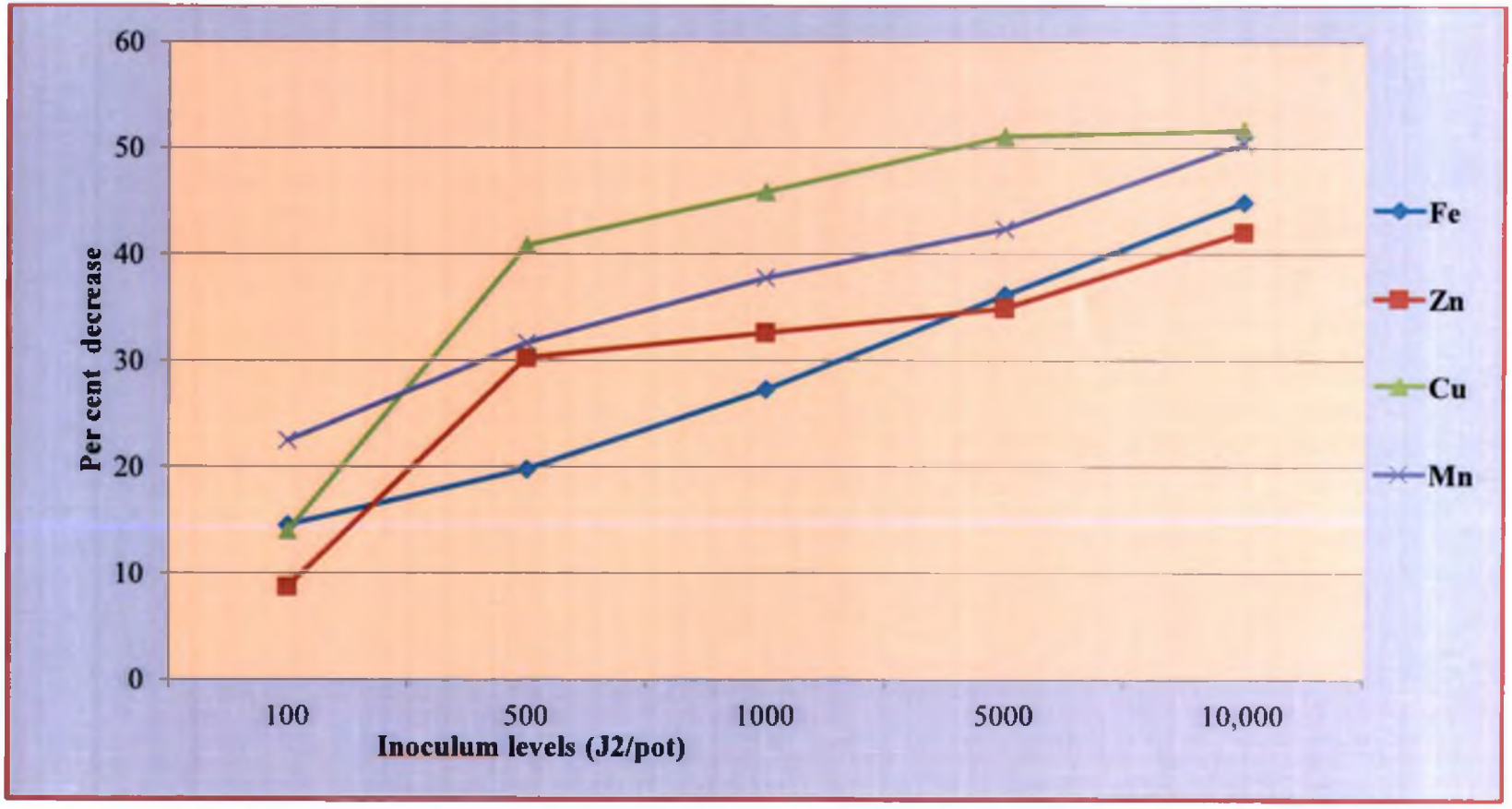


Fig 6. Per cent reduction in Fe, Zn, Cu and Mn content in leaf at different inoculum levels of *M. graminicola*

the shoots of healthy plants was 1.7 mg per g, while in the nematode infested plants there was a reduction by 19.00 per cent.

A progressive decrease in the Cu and Mn content of leaf samples were also noted with increasing inoculum levels of *M. graminicola*. The plants inoculated with 10,000 J_2/ pot recorded the lowest Cu (30.92 ppm) and Mn (29.34 ppm) content. In ginger, the ability of the roots to absorb nutrients was weakened by root knot nematode *M. incognita* and it leads to the reduction in total iron, copper, zinc and boron conent in ginger plant (Gauo and Wang, 2004). Patil and Gaur (2014) reported that the symptoms of nutrient deficiency become apparent on the leaves at higher levels of nematode infection.

5.1.6 Starch and Protein content of Grain Samples

The starch content of grains exhibited reduction at different nematode inoculum levels when compared to uninoculated plants. Highest decrease of starch content (41.43 per cent) was observed in plants inoculated with 10,000 J_2/ pot. The varying nematode inoculum levels of *M. graminicola* resulted in a corresponding decrease in the percentage of protein in the grain compared to uninoculated plant. The maximum reduction in protein content of the grain was observed in plants inoculated with 10,000 J_2/pot. In 5000 and 10,000 J_2 inoculated plants, the protein content of the grain was decreased by 38.84 and 42.09 per cent significantly over uninoculated plant. Patil and Gaur (2014) reported that the rice grains produced on plants inoculated with the nematode *M. graminicola* were lighter in weight and had poorer nutrient qualities, such as amylase and protein content. Similarly, Abbasi and Hisamuddin (2014) reported that the protein content of green gram exhibited reduction at all the initial nematode inoculum levels when compared with the protein content of uninoculated control. The protein content decreased and amount of free amino acid amides increased after root knot nematode inoculum in susceptible as well as resistant cultivars of green gram (Mohanty and Pradhan, 1989).

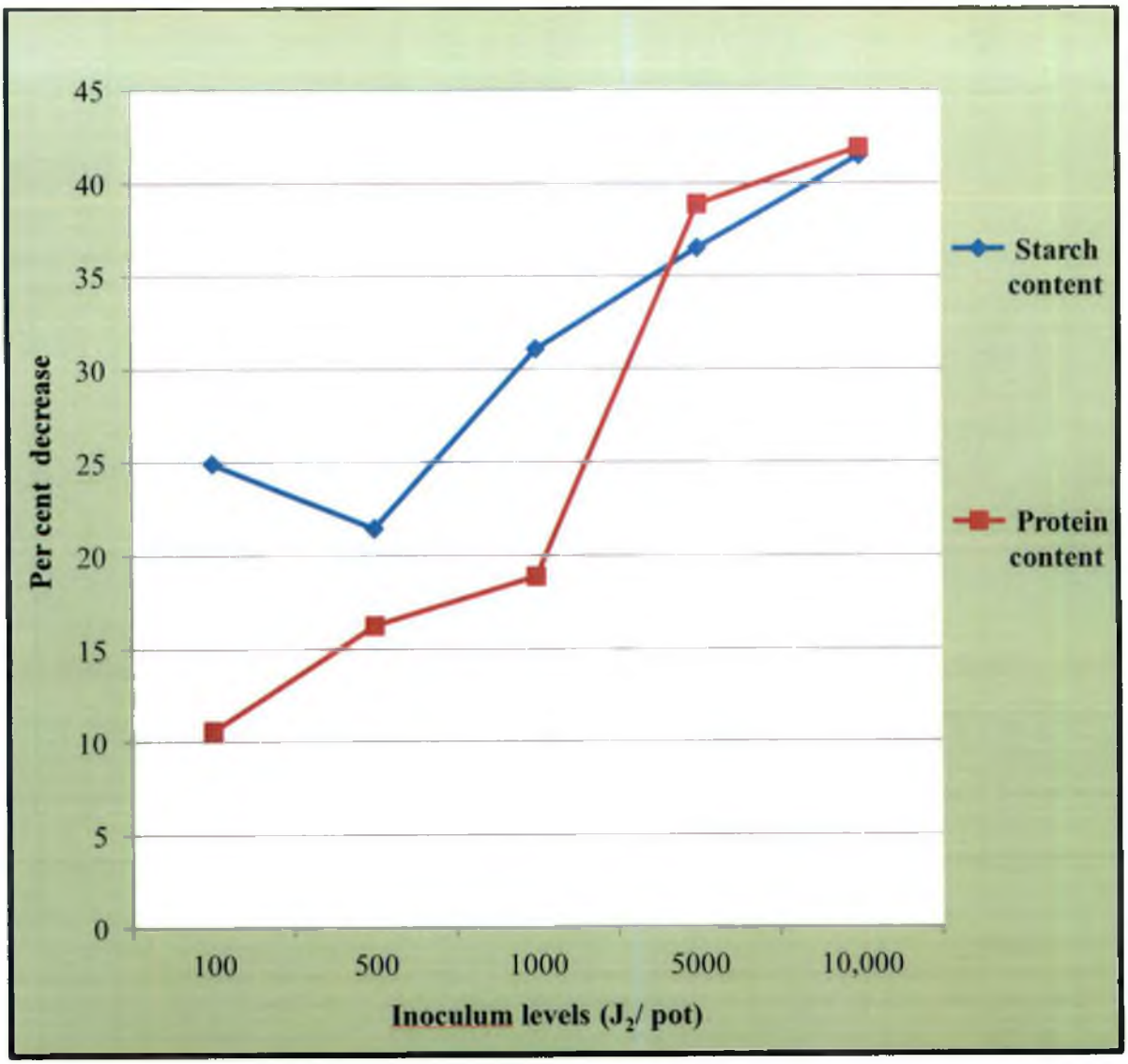


Fig 7. Per cent reduction in starch and protein content of grain at different inoculum levels of *M. graminicola*

Korayem *et al.* (2013) found that crude protein and fat contents decreased in peanut seeds influenced by *M. arenaria.*

5.1.7 Reducing, Non reducing and Total Sugar Content in Grain Samples

A progressive reduction in reducing, non reducing and total sugar content of grains were observed with increasing nematode inoculum levels. The plants inoculated with 10,000 J_2 showed maximum reduction of sugar content, the percentage reduction of reducing non reducing and total sugar content was 44.45, 55.01 and 44.95 per cent respectively. Thus the quality of the grain was affected by the infestation of the nematodes. The translocation of sugars to shoot and grains was affected in infected plants and the sugars accumulated in the root system of the plant.

Rao *et al.* (1988) reported that the rice grains showed reduction of reducing sugar content due to the infestation of *H. oryzicola* and *M. graminicola.* Mohanty *et al.* (1997) reported that in rice, the total sugar content of root was increased in resistant variety (Manika) compared to susceptible variety (Annapurna). In banana, *H. oryzicola* infestation increased reducing sugars in fruits, while non reducing sugars decreased. Total sugar content was less than in fruit of infested plants (Charles and Venkitesan, 1993).

5.2 GROWTH PARAMETERS

The plant growth parameters *viz.*, plant height, fresh weight of plant, dry weight of root and shoot were decreased with increasing nematode inoculum levels. Maximum reduction was observed in plants inoculated with 10,000 J_2/pot. The rice cultivars non-basmati Pusa-44 and basmati Sugandh-5 showed significantly lower plant height, shoot and root dry weight with increasing nematode population (Patil and Gaur, 2014). Ramakrishnan and Rajendran (1998) reported that the infection caused by *M. incognita* leads to reduction in height and weight of the plant. Poorer root system about 50-75 per cent reduction in root system was observed in rice plants inoculated with root knot nematode (Mhatre *et al.*, 2015).

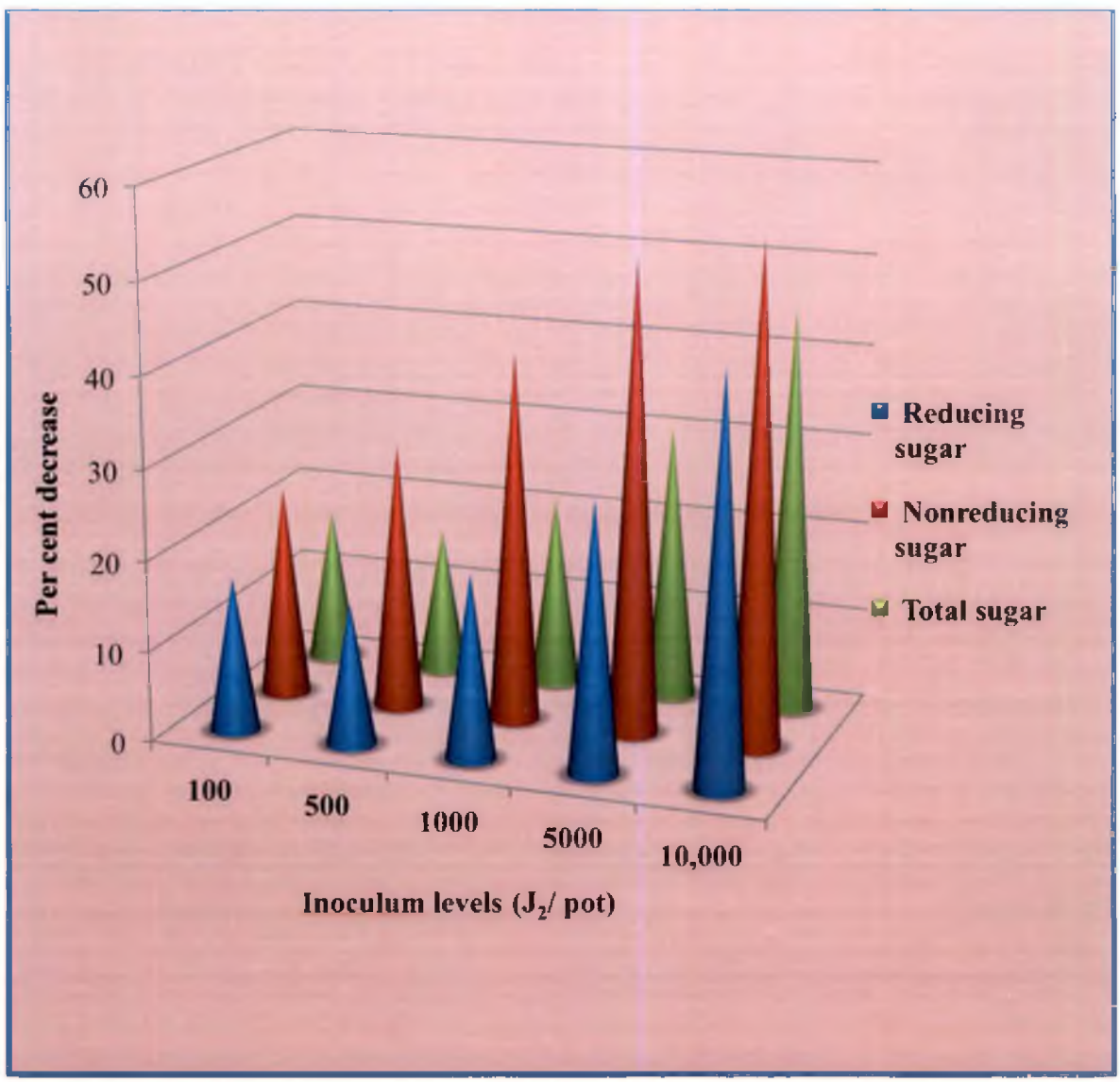


Fig 8. Per cent reduction of reducing, non reducing and total sugar content in grain at different inoculum levels of *M. graminicola*

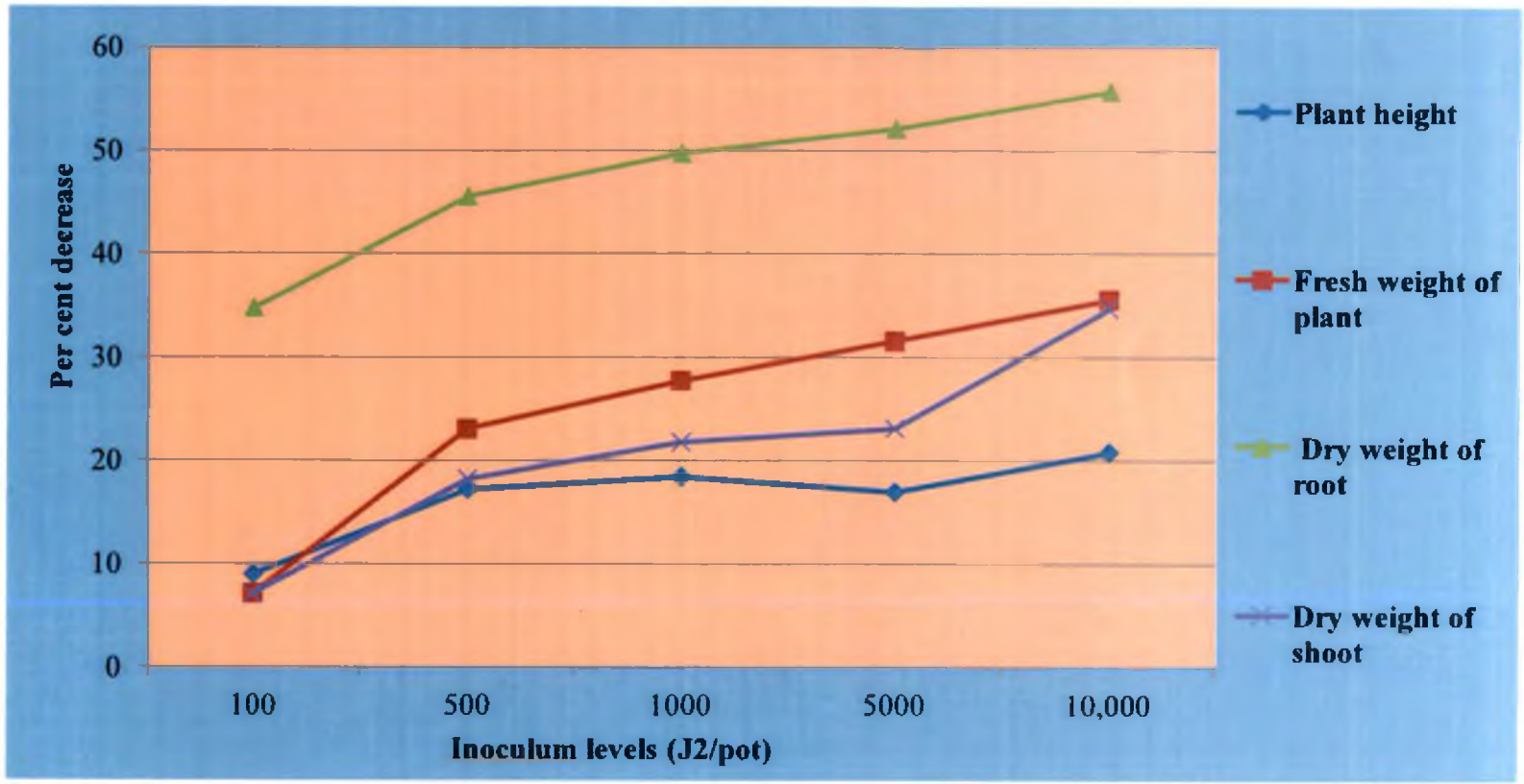


Fig 9. Per cent reduction in plant growth parameters of rice at different inoculum levels of *M. graminicola*

The fresh weight of uninoculated and 10,000 J_2 infected rice plant was 7.032 and 4.53 g respectively. The plants inoculated with 10,000 J_2/pot recorded the lowest dry weight (0.74g). The dry weight of root in uninoculated plant was1.67g and was significantly different from all other treatments. The shoot weight of 10,000 J_2 inoculated plants reduced 34.60 per cent when compared with that of the uninoculated plants. Maximum reduction in plant height was seen in plants inoculated with 10,000 J_2/pot, the height of the plants being 44.76 cm and which was on par with the height of the plants inoculated with 5000, 1000 and 500 J_2/pot.

Khan *et al.* (2012) reported that rice grown in nematode infested soil exhibited considerable degree of reduction in plant growth which varied with cultivars. Greatest reduction in shoot length was recorded in cvs. Sugandh (37.8 per cent) over uninoculated plants.

The dry weight of shoot decreased 46.4 and 29.2 per cent in rice varieties Samba Mahsuri and Sugandh respectively. Abbasi and Hisamuddin (2014) reported that the fresh weight of the whole plant decreased with an increase in nematode inoculum levels. The plant height of green gram inoculated with 800 J_2/pot and 1600 J_2/pot decreased by 32.02 and 38.05 per cent significantly over the uninoculated plants.

5.3 YIELD PARAMETERS

In comparison to uninoculated plants the number of grains per panicle and thousand grain weight were decreased with an increase in nematode inoculum levels. Maximum reduction in the number of seeds per panicle (51.00) was observed in plants inoculated with 10,000 J_2 and was significantly different from all other treatments. The seeds per panicle of the plants inoculated with 10,000 J_2/pot reduced 28.77 per cent when compared with that of the uninoculated plant. Poudyal *et al.* (2002) found that root knot nematode infested rice plants had lower number of total and effective tillers, filled grains per panicle and grain yield and they also reported that yield reduction in diseased rice plants (cv. Masuri) was

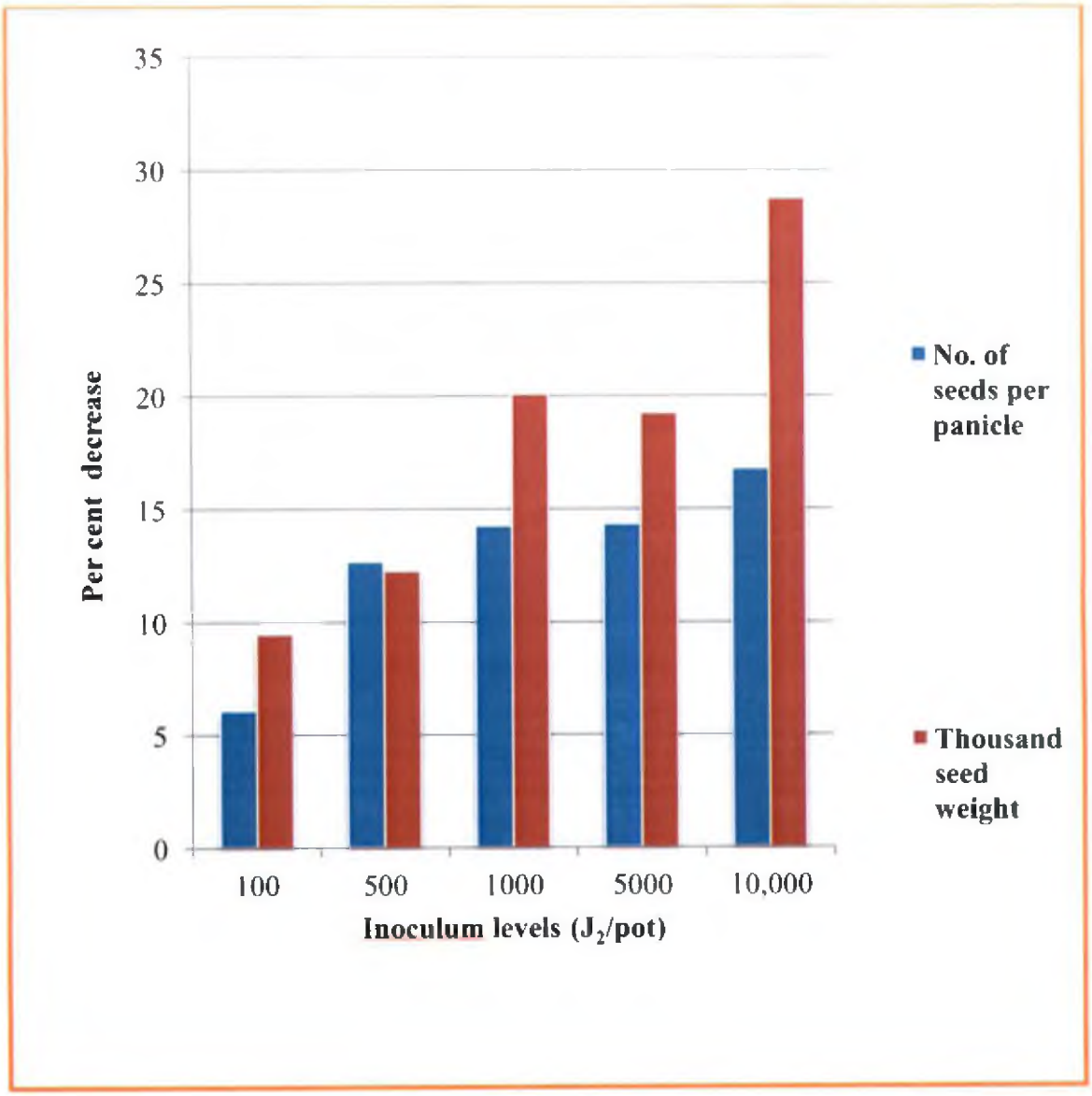


Fig 10. Per cent reduction in no. of seeds per panicle and thousand seed weight at different inoculum levels of *M. graminicola*

40.5 per cent as compared to healthy plants. The grain yield loss up to 98 per cent was observed at high population density of *M. graminicola* (Prasad *et al.*, 1990).

A progressive reduction was seen in the seed weight of rice plants with increasing inoculum levels of *M. graminicola.* Maximum reduction in thousand seed weight was observed in plants treated with 10,000 J_2/pot, it was reduced 16.77 per cent with that of the uninoculated plants. Similarly Patil and Gaur (2014) reported that thousand seed weight of rice was significantly reduced by as much as 44.5 per cent in cv. Pusa Sugandh-5 and 50.7 per cent in cv. Pusa-44 seed when the parent plants were grown in soil with very high levels of *M. graminicola* infestation.

5.4 HISTOCHEMICAL AND HISTOPATHOLOGICAL STUDIES

5.4.1 Histochemical Studies

The accumulation of polysaccharide granules in root increased with increase in nematode population levels. The maximum accumulation was observed in plants inoculated with 10,000 J_2/pot. The percentage increase in accumulation of polysaccharide in epidermal cells in plants inoculated with 10,000 J_2 was 71.42 per cent over uninoculated plant. Among the epidermal, cortical and stelar cells, higher accumulation of polysaccharide granules was seen in cortical cells. The accumulation of proteins in epidermal cells increased with increase in inoculum levels of nematode. Maximum accumulation of protein was seen in plants inoculated with 10, 000 J_2/pot. The maximum percentage increase in protein accumulation was 18.45 per cent in plants inoculated with 10,000 J_2/pot.

Accumulation of RNA and DNA in epidermal, cortical and stelar region of root increased with increase in nematode population levels. Maximum accumulation was seen in plants inoculated with 10,000 J_2/ pot. The more accumulation of nucleic acid (RNA and DNA) was seen in stelar cells of the root sections and minimum accumulation of nucleic acid was seen in epidermal cells. Gopinath (2001) found that higher concentration of the insoluble polysaccharides, nucleic acids and proteins

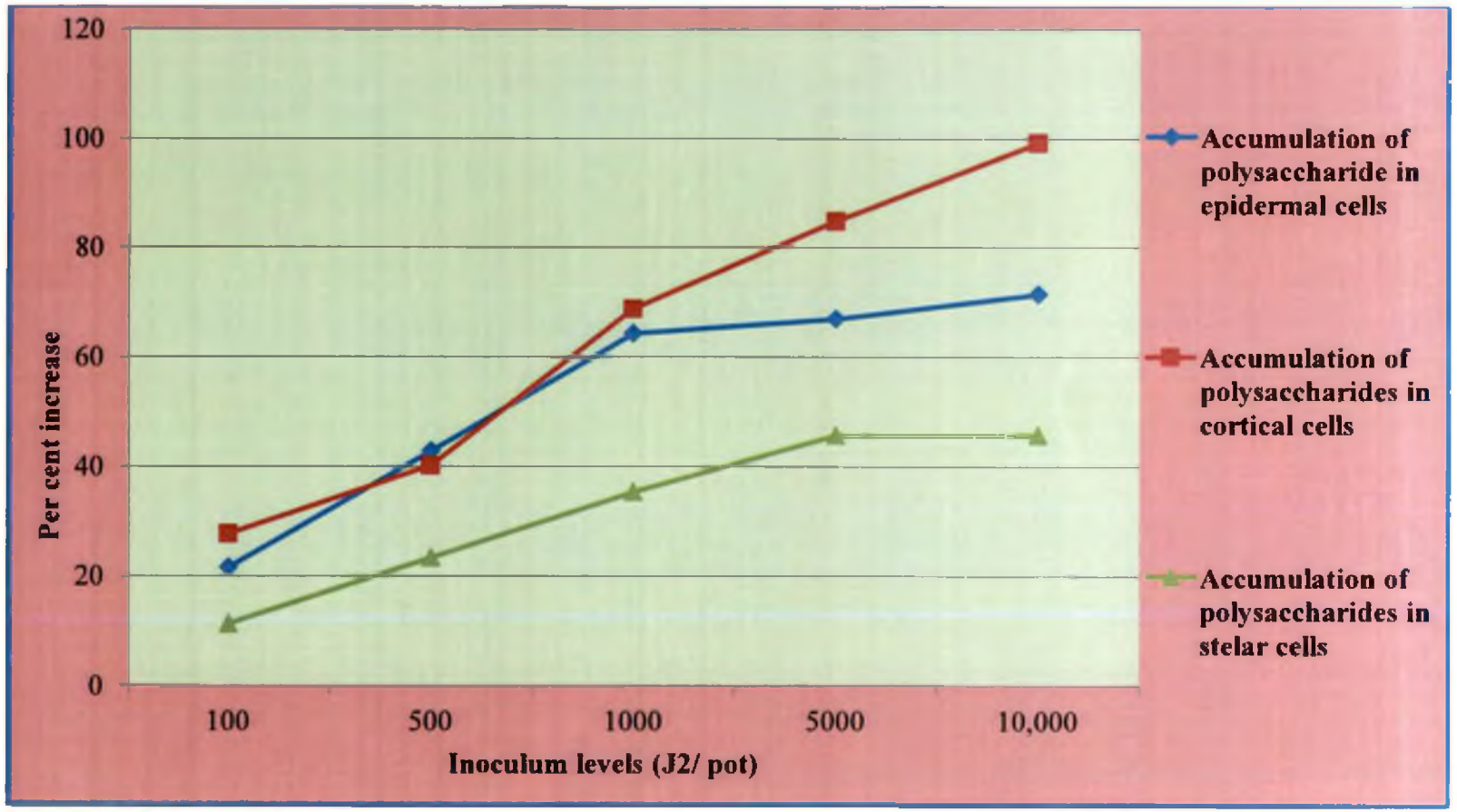


Fig 11. Per cent increase in accumulation of polysaccharide in epidermal, cortical and stelar region of root at different inoculum levels of *M. graminicola*

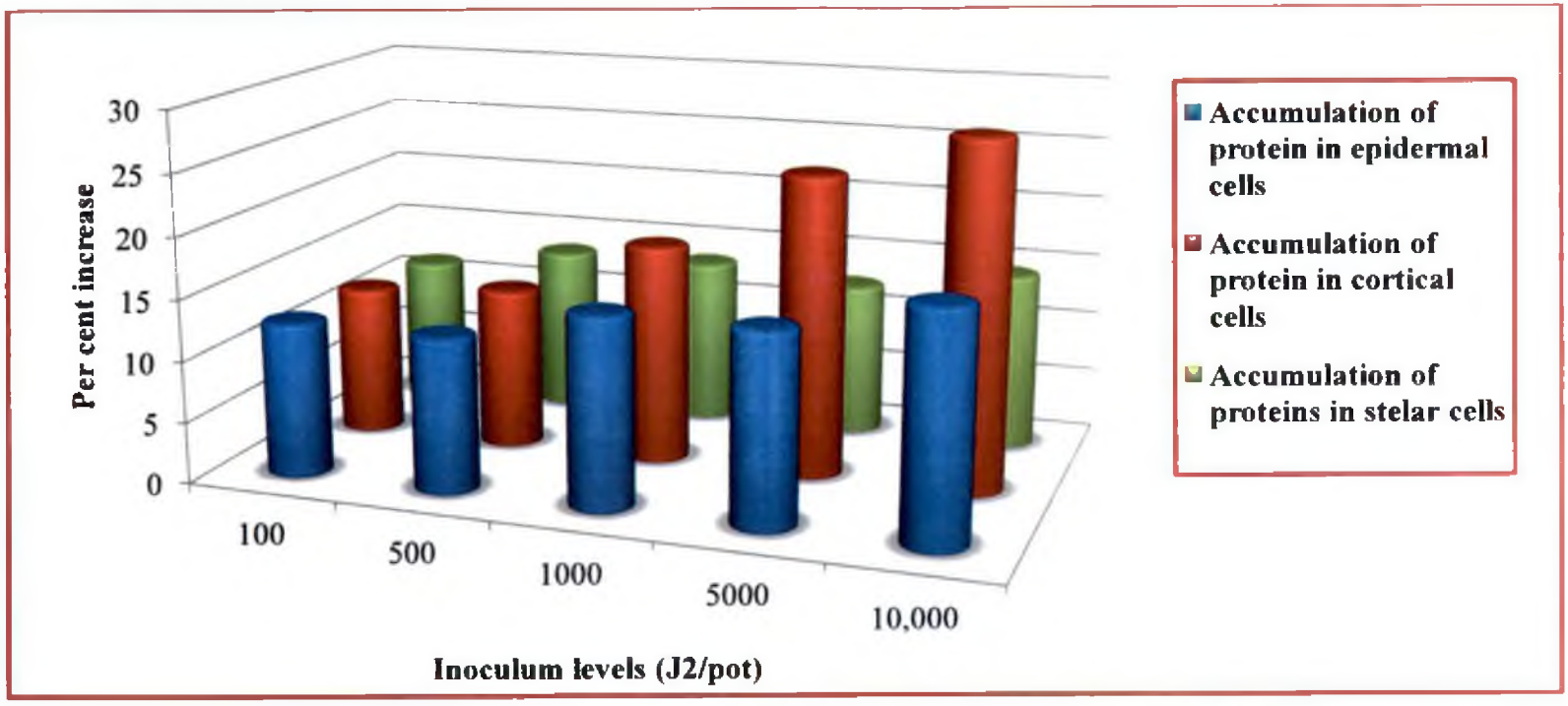


Fig 12. Per cent increase in accumulation of protein in epidermal, cortical and stelar region of root at different inoculum levels of root knot nematode

in the infected roots compared to healthy roots. Similarly, Praveen (2002) reported that increased concentration of total insoluble polysaccharides, total proteins and nucleic acids was also observed in healthy roots when compared to infected plants. Kumar *et al.* (2008) reported that the concentration of total insoluble polysaccharides, total proteins and nucleic acids was increased in root knot nematode infected root of paddy cultivars *viz.*, IR-64, Jaya, Jyothi and Rasi.

5.4.2 Histopathological Studies

The healthy root section showed normal organization of tissue and the cells in the epidermal, cortical and stelar region were also normal. In *M. graminicola* infected root section recorded increase in size of epidermal, cortical and stelar cells and the cells in stelar region were irregularly arranged. The root knot nematode infected root sections recorded disorganized xylem and phloem cells. Giant cells were formed near the stelar region of the root section. Root knot nematode, *M. graminicola* infected root showed an overall growth of cells in epidermal, cortical and stelar region of root section (Kumar *et al.*, 2008). Tandon and Kumar (1979) reported that clusters of giant cells in the vascular region of tomato roots infected with *M. lucknowica* and *M. incognita* respectively. The root knot nematode infection caused disintegration of the xylem and phloem tissues causing an interruption in the transport of water and mineral nutrients in the host plant (Robab *et al.*, 2010). Azam *et al.* (2011) reported that *Meloidogyne* infection accompanied cortical and stelar proliferation, hypertrophy and hyperplasia in the cortex, pericycle and stele of the roots. Chandel *et al.* (2001) studied the various abnormalities in the cortical and stelar region of the root knot nematode infected lentil root. The giant cells were modified for the nutrition of nematode and act as sink for the photosynthetic metabolites (Khan *et al.*, 2007).

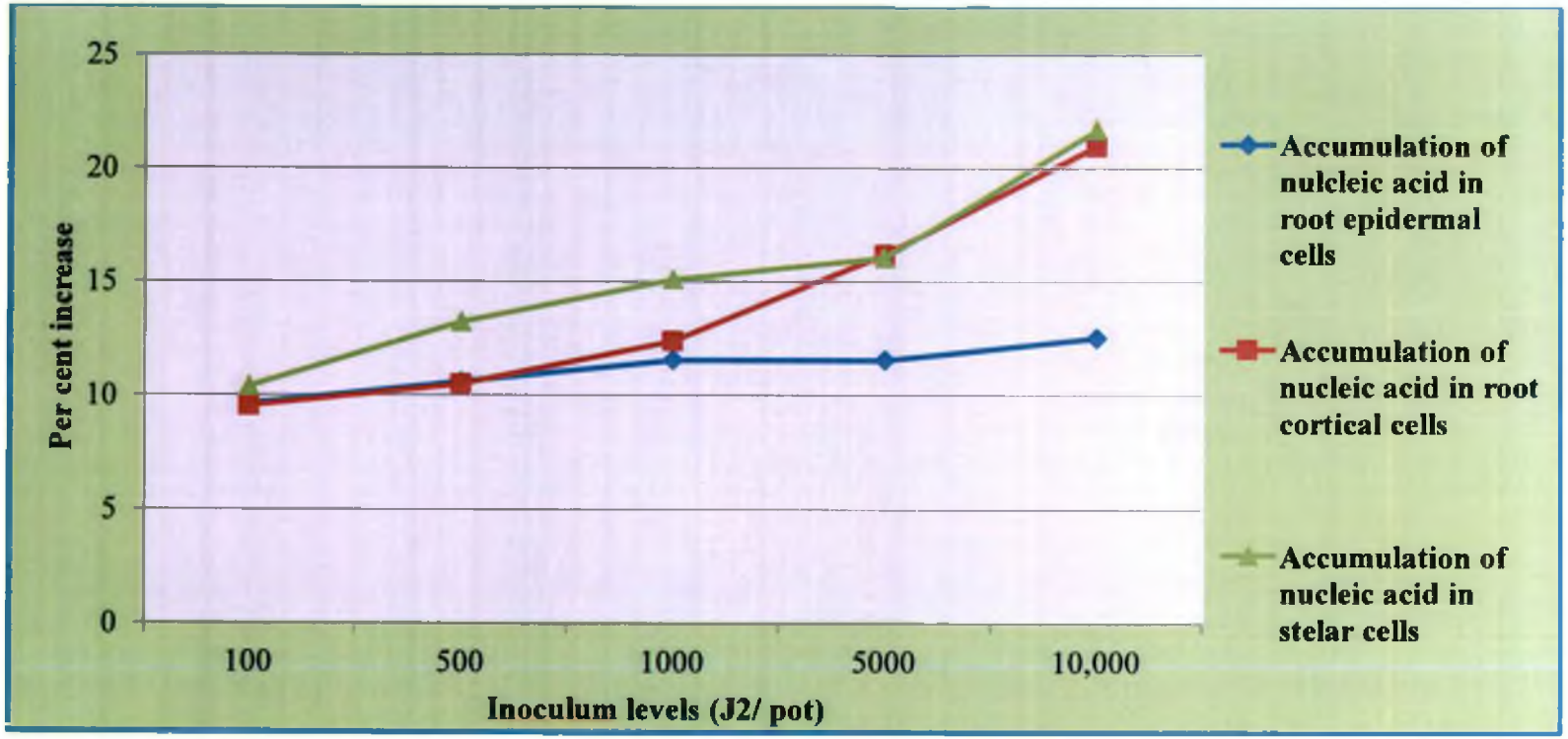


Fig 13. Per cent increase in accumulation of nucleic acid in epidermal, cortical and stelar region of root at different inoculum levels of root knot nematode

5.5 VARIETAL REACTION

The important rice varieties in Kerala *viz.*, Bhadra, Pavizham, Karthika, Kanakom, Uma, Revathy, Karishma, Prathyasa, Jyothi and Aiswarya were recorded as susceptible to *M. graminicola* infection. Maximum number of galls and egg mass per plant was observed in the variety Karishma (83.2, 94.4) and lowest number was observed in variety Uma (51.80, 64.60). In varieties Uma, Karthika, Pavizham and Bhadra, the number of galls per plant was less than that of the susceptible check variety (TN1). The gall index of all the varieties was recorded as 4. According to the gall index, all the varieties were rated as susceptible to root knot nematode. The egg mass index was highest in Karishma (4.29) and lowest in variety Uma (2.94). On the basis of egg mass index, all the varieties were highly susceptible except Uma.

Berliner *et al.* (2014) reported that the rice cultivars were categorized as resistant or susceptible types on the basis of Root Knot Index (RKI), Out of 414 rice genotypes screened, only two entries from breeding lines, 127-28-1-1-1 and 183-6- 1-1-3 were found resistant. Khan *et al.* (2012) reported that the rice cvs. Sadabhar, Samba Mehsuri, Sharbati and Hazari developed 35-38 galls which were significantly less than the cvs. Sugandh and R-Dhan. On the cv. Abhishek, only 8 galls and 3 egg masses/root system were formed. Greatest number of egg masses/root system were developed on cv. Sugandh. In cv. R-Dhan, egg masses were significantly less than cv. Sharbati. Jena and Rao (1976) have suggested that the criteria for evaluation of rice cultivars against *M. graminicola* should be based on the reproduction in terms of egg masses and eggs.

Similarly, Kumar *et al.* (2007) reported that out of 52 rice varieties screened against *M. graminicola* under greenhouse conditions the short duration varieties *viz.*, TKM3, TKM7, TKM8, TKM9 and MDU1 and medium duration varieties *viz.*, MDU2, TKM11 and PY1 were found resistant to *M. graminicola.*

The varied reaction of rice cultivars to *M. graminicola* has been reported earlier (Ramakrishnan *et al.*, 1984; Bridge *et al.*, 1990; Bose *et al.*, 1998). Rice lines Ranbir Basmati, Hasan Sarai, and Purple cultures were rated as susceptible

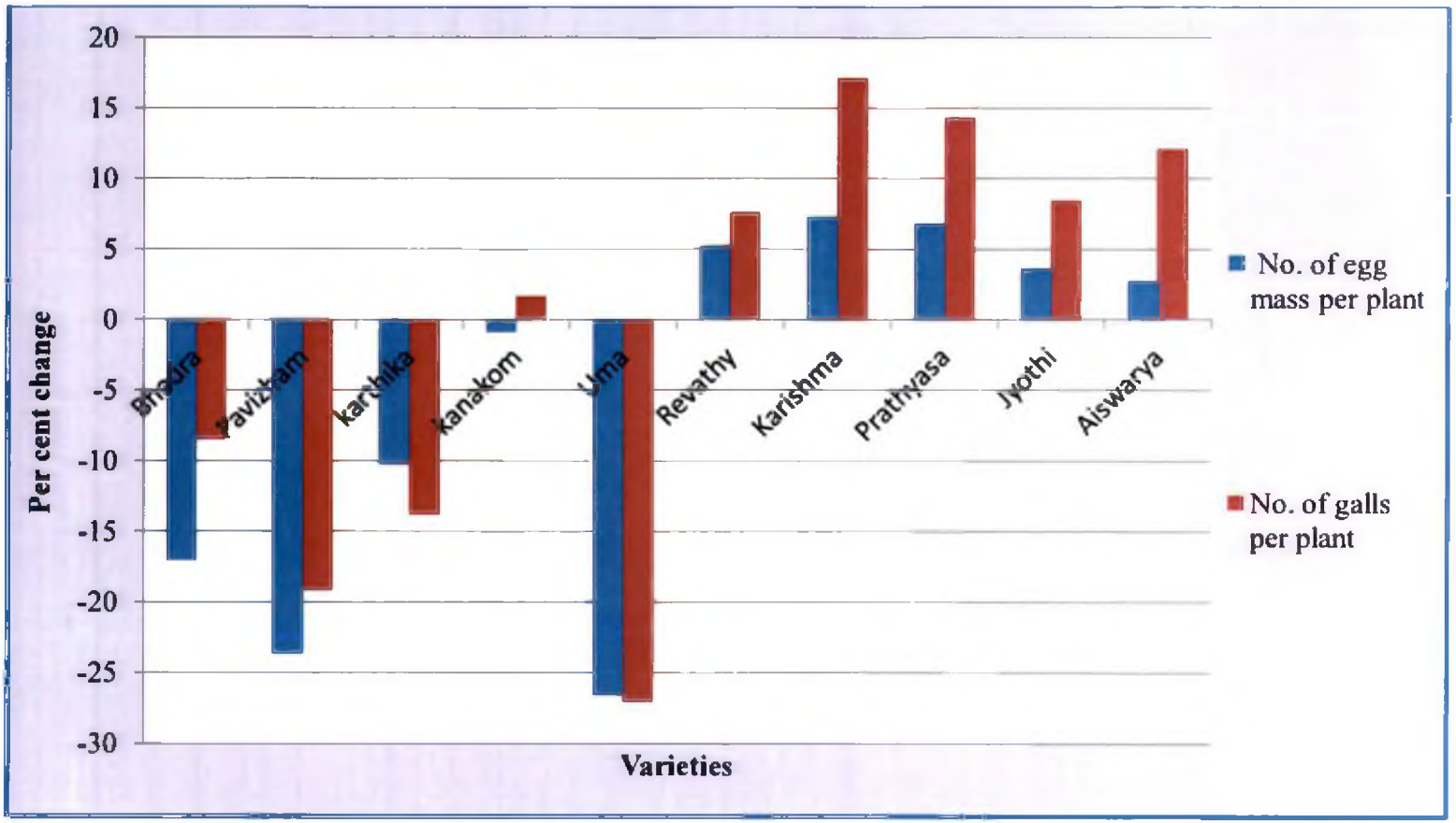


Fig 14. Per cent change in no. of galls, egg mass per plant of different rice varieties infested by *M. graminicola*

(Srivastava *et al.*, 2011). A large variation in number of galls caused by *M. gramainicola* per root system in different rice cvs./ land races was observed by Mhatre *et al.* (2015). Research conducted in many countries revealed that the most of rice cultivars were susceptible to *M. graminicola* (Bridge *et al.*, 1990).

5.6 ENZYMATIC ACTIVITY OF VARIETIES

Variation was observed in peroxidase, polyphenol oxidase, and phenyl alanine ammonia lyase activity of different rice varieties. Highest PO, PPO and PAL content was observed in the variety Uma (6.82, 1.77 and 3.55 OD per g per min respectively) and lowest PO and PPO activity was seen in variety Karishma (4.49 and 0.64 OD per g per min). The lowest PAL activity was observed in variety Aiswarya (2.25 OD per g per min). The varieties Uma, Karthika, Pavizham and Bhadra were showed more PO activity than the susceptible check variety (TN1). The PPO activity of Bhadra, Pavizham, Karthika, Kanakom and Uma was more than that of TN1. The PAL activity of all the varieties was more than that of the susceptible check variety. Compared to all other varieties, the PO, PPO and PAL activity was highest in variety Uma and that showed more defense action against *M. graminicola.*

Root knot nematode infection increased PO, PPO and PAL in roots of cotton, coffee, chickpea, banana and rice (Mishra and Mohanty, 2007; Patel *et al.*, 2001; Sundararaju and Suba, 2006; Xu *et al.*, 2008). The peroxidase activity was more in resistant rice varieties than the susceptible varieties (Kumar *et al.*, 2007). Similar findings were reported by several workers (Arrigoni *et al.*, 1981; Langrimini and Rothstain, 1987; Shukla and Chakraborthy, 1988). Kumar *et al.* (2007) found that the degree of PAL activity was comparatively lesser in susceptible rice than resistant cultivars. They also reported that the sequential development of PPO increased in nematode infested rice varieties. The enhancement of enzyme activity in resistant varieties was associated with the reduction of aromatic amino acids like phenyl alanine and tyrosine and those amino acids play greater role in lignifications process (Kumar *et al.*, 2007).

Fig 15. Per cent change in peroxidase content in roots of various rice varieties inoculated with *M. graminicola*

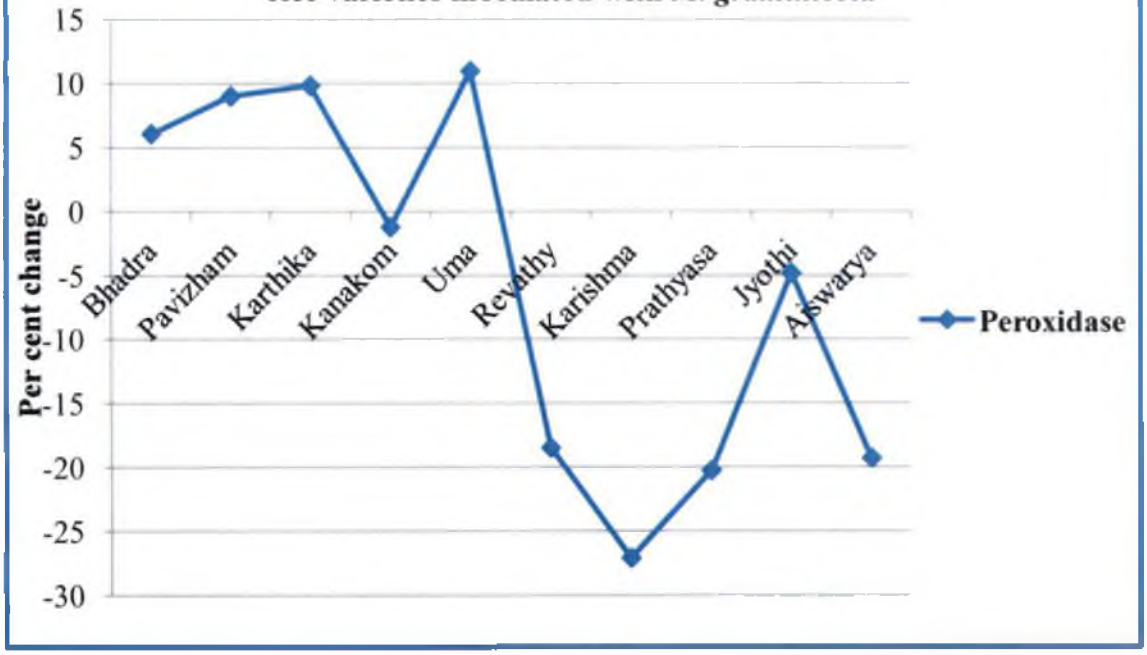


Fig 16. Per cent change in polyphenol oxidase content in roots of various rice varieties inoculated with *M. graminicola*

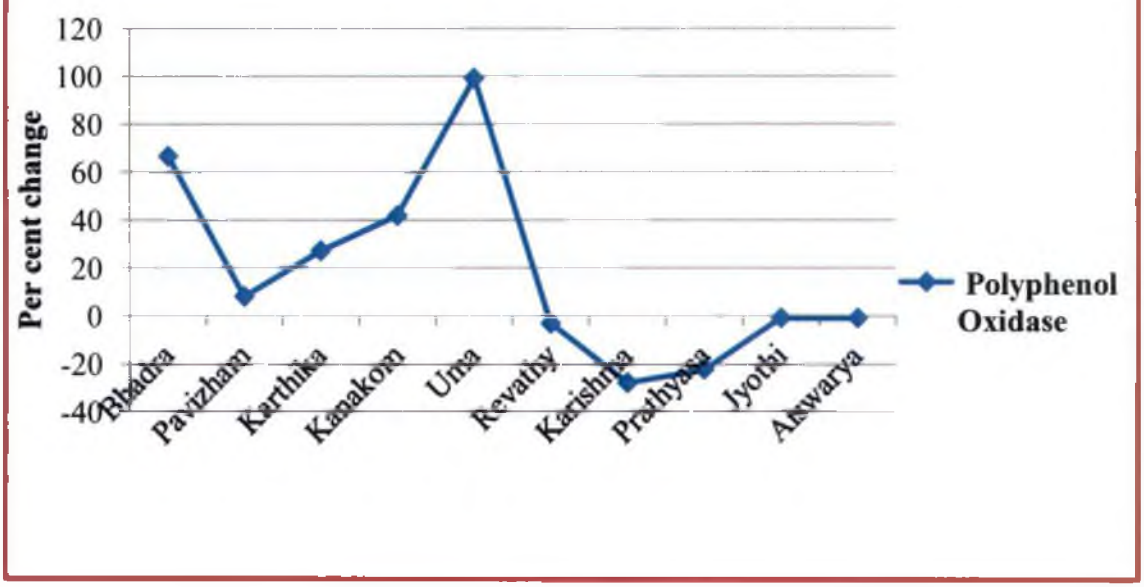
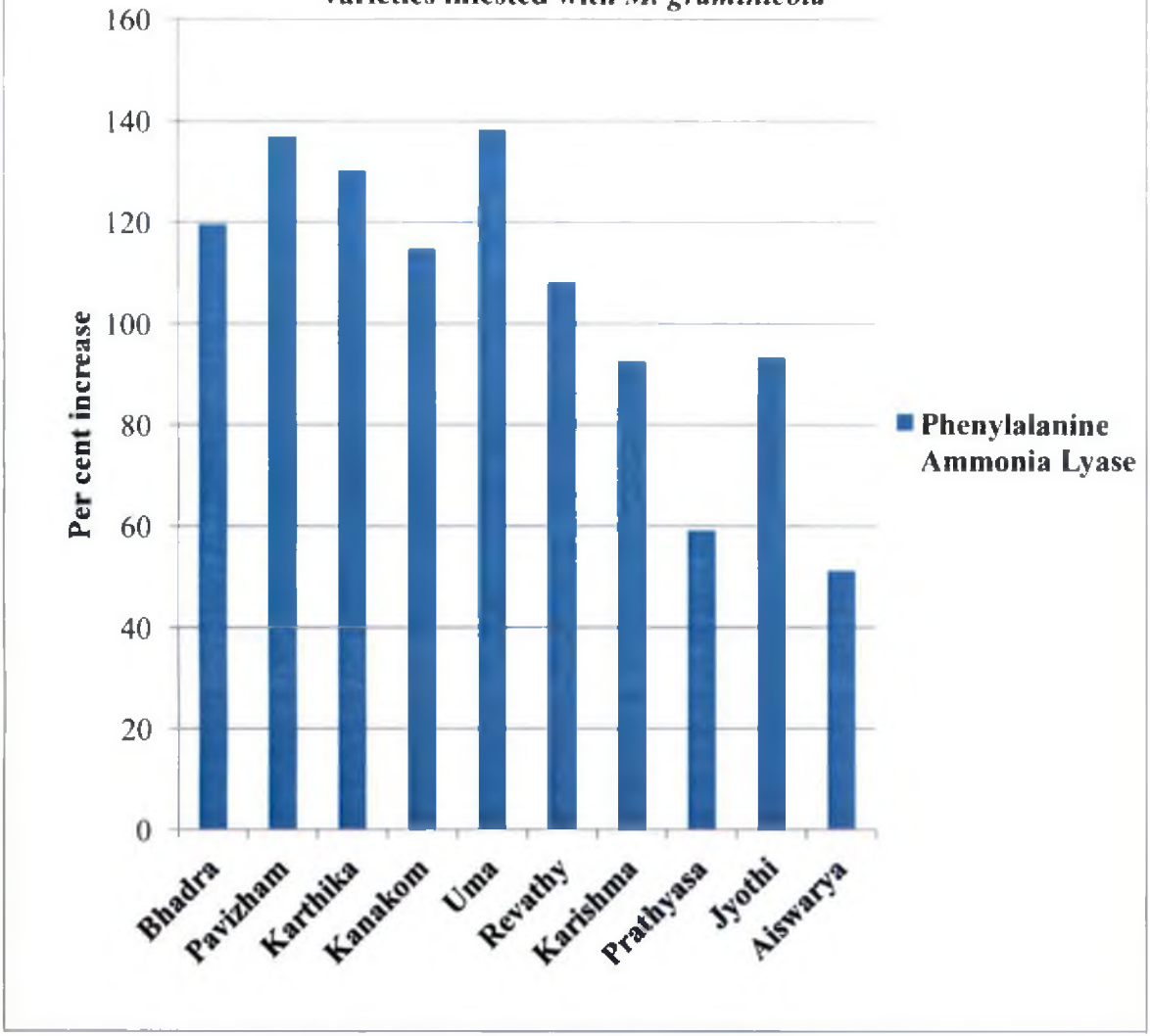


Fig 17. Per cent change in PAL content in roots of various rice varieties infested with *M. graminicola*



5.7 GROWTH PARAMETERS OF VARIETIES

Changes in fresh weight of plant, plant height and dry weight of shoot and root were observed in different rice varieties inoculated with *M. graminicola.* Highest fresh weight of plant was observed in variety Uma (5.69 g) and lowest was in Aiswarya (3.65 g). The dry weight of soot and root was highest in variety Uma (2.22 g and 1.22 g) and minimum was seen in Karishma (1.69 g and 0.39 g). No significant variation was observed in plant height due to the infestation of *M. graminicola.*

Similarly, Khan *et al.* (2012) found that considerable degree of reduction in plant growth was seen in different rice cultivars due to the infestation of root knot nematode. The rice cultivars Pusa-44 and Sugandh-5 showed significantly lower plant height, shoot and root dry weight with increasing nematode population. Win *et al.* (2013) reported that at 8 WAI the plant height and root length were significantly reduced in the inoculated plant of the rice varieties Yatanartoe, MR 9 and Saytanar 1 compared with the uninoculated plants.

5.8 NEMATODE POPULATION

Observable variation was seen in the nematode population obtained from the roots and rhizosphere of the different rice varieties. Among the varieties lowest population of *M. graminicola* was recorded from the rhizosphere of Uma (1885.20) and highest population was observed in Karishma (2362.60). Prathyasa (2243.60) and Aiswarya (2213.20) supported large population of the nematodes in the rhizhosphere. Lowest population of *M. graminicola* in root was observed in Uma (35.00) and was on par with that of Karthika and Bhadra. A significantly higher population was recorded from the root samples of variety Karishma (53.20) which was on par with that of variety Aiswarya (52.80) and TN1 (51.40).

Reproduction potential of nematode was lowest in Uma and was on par with Karthika. In Bhadra the nematode had a reproduction rate of 20.74 and was on par with that of Pavizham (20.63) and Karthika (19.76). Highest reproduction rate of

nematode was seen in Karishma (24.15) and was significantly different from all other varieties. Khan *et al.* (2012) reported that highest soil population of *M. graminicola* was recorded in the root zone of rice cvs. R-Dhan and Sugandh and lowest population was recorded from the rice cv. Abhishek. Khan *et al.*, (2014) found that one month after planting, the *M. graminicola* population in soil was almost double the initial population and further increased to approximately four times the initial population at harvest. Out of 10 rice varieties of Manipur screened, the variety Lamyanba showed maximum soil population (7500), root population (3500) and total population (11000) at harvest (Devi, 2014).

SUMMARY

6. SUMMARY

Rice root knot nematode, *M. graminicola* happens to be the most important pest and is prevalent in major rice producing countries of the world. The recently encountered *M. graminicola* in nursery and main field of rice is considered as a newly emerging problem in Kerala. Basic information on the biochemical and histopathological alterations, yield and growth characters of rice at different inoculum levels of *M. graminicola* is essential for effective management of the nematode problem. In view of the wide spread occurrence of the nematode in rice in the state, a pot culture study was conducted to determine the biochemical and histopathological alterations occur in rice due to the infestation of *M. graminicola*. Ten important rice varieties in Kerala were screened to identify sources of resistance to the nematode. Enzymatic activity, growth characters and nematode population in rhizhosphere and root samples of those varieties were also determined through a pot culture experiment. The salient results of the study are summarized below.

A progressive increase in phenol content, defense enzymes *viz.*, Peroxidase (PO), Polyphenol Oxidase (PPO) and Phenylalanine Ammonia Lyase (PAL) from both leaf and root samples after 45 days of nematode inoculation were observed with increase in inoculum levels. The maximum increase was observed in plants inoculated with 10,000 J_2/ pot. Higher phenol production was seen in root than in leaves. The phenol content increased 87.05 per cent in root and 22.37 per cent in leaf compared to uninoculated plants. PO, PPO and PAL activity increased 70.79, 60.75 and 94.69 per cent in root and 62.16, 32.57 and 61.36 per cent in leaf respectively.

The various levels of nematode population showed reduction in pH, chlorophyll a and b, NPK content and micronutrients *viz.*, Fe, Cu, Zn and Mn of leaf samples compared to that of the uninoculated plants. Plants inoculated with 10,000 J_2

showed low pH (5.69) compared to that of the uninoculated plants and was significantly different from all other treatments.

Minimum chlorophyll a, b and total chlorophyll content was observed in 10,000 J_2 inoculated plants (0.19, 0.38 and 0.686 mg per g tissue respectively). The chlorophyll a and b content in leaf samples of plants inoculated with 10,000 J_2 reduced 40.67 and 27.55 per cent over uninoculated plants.

The content of N, P and K was much reduced in the leaf samples of rice plants inoculated with *M. graminicola*. The percentage reduction of N, P and K content in plants inoculated with 10,000 J_2 were 12.80, 48.57 and 40.82 per cent over uninoculated plants. Similarly, the concentration of micronutrients *viz.*, Fe, Cu, Zn and Mn in rice leaves were significantly reduced with increasing nematode population levels. The plants inoculated with 10,000 J_2 recorded the lowest Fe (82.79 ppm), Zn (27.34 ppm), Cu (30.92 ppm) and Mn (29.34 ppm) compared to all other treatments.

The grain quality was also affected by the infection of root knot nematode. The starch, protein and total sugar content of grain decreased with increase in nematode inoculum levels. The maximum reduction was observed in plants inoculated with 10,000 J_2/ pot. The starch, protein and total sugar content decreased 41.44, 41.89 and 44.95 per cent respectively in plants inoculated with 10,000 J_2 per pot compared to that of the uninoculated plants.

A progressive decrease in plant growth and yield parameters were observed with increase in population levels of nematode. The maximum percentage reduction in plant height (20.81 per cent), fresh weight of plant (35.56 per cent), dry weight of root (55.69 per cent) and shoot (34.67 per cent) and the yield parameters like thousand seed weight (16.77 per cent) and number of seeds per panicle (28.77 per cent) were observed in plants inoculated with 10,000 J_2 over uninoculated plants.

The accumulation of polysaccharides, proteins and nucleic acids (RNA and DNA) increased with increase in nematode population levels. The maximum accumulation was observed in plants inoculated with 10,000 J_2/pot. Among the epidermal, cortical and stelar cells higher accumulation of polysaccharides was seen in cortical cells and more accumulation of nucleic acid was seen in stelar cells of the roor sections. In *M. graminicola* infected root sections recorded increase in size of epidermal, cortical and stelar cells and the cells in stelar region were irregularly arrainged. Giant cells were formed near the xylem and phloem cells and the xylem and phloem cells were disorganized due to nematode infection.

In studying the varietal reaction of ten popular rice varieties grown in Kerala *viz.*, Bhadra, Pavizham, Karthika, Kanakom, Uma, Revathy, Karishma, Prathyasa, Jyothi and Aiswarya against *M. graminicola*, all the varieties were susceptible to the nematode. Minimum number of galls (51.8) and egg mass (64.60) per plant was observed in the variety Uma and was on par with Pavizham. According to gall index, all the varieties were rated as susceptible to root knot nematodes. The egg mass index was highest in Karishma (4.29) and lowest in variety Uma (2.94). Based on egg mass index all the varieties were highly susceptible except Uma.

Variation was observed in PO, PPO and PAL activity of different rice varieties. Maximum PO (6.82 OD per g /min), PPO (1.77 OD per g/min) and PAL (3.55 OD per g/min) contents were observed in variety Uma and minimum observed in variety Karishma. Changes in fresh weight of plant, plant height and dry weight of shoot and root were observed in different rice varieties inoculated with *M. graminicola.* Highest fresh weight of plant was observed in variety Uma (5.69 g) and lowest was in Aiswarya (3.65 g). The dry weight of soot and root was highest in variety Uma (2.22 g and 1.22 g) and minimum was seen in Karishma (1.69 g and 0.39 g). No significant variation was observed in plant height due to the infestation of *M. graminicola.*

After 45 days of inoculation, the nematode population in root and soil were worked out. Highest nematode population in root and rhizhosphere was seen in variety Karishma. In variety Uma, the nematode population in soil (1885.20) and root (35.00) was lowest and was on par with Karthika. The reproduction rate also lowest (19.38) in variety Uma. Based on the results of the study, the variety Uma showed better performance against root knot nematode, *M. graminicola* than all other varieties.

REFERENCES

7. References

Abbasi and Hisamuddin. 2014. Effect of different inoculum levels of *Meloidogyne incognita* on growth and biochemical parameters of *Vigna radiate. Asian J. Nematol.*, 3(1): 15-20.

Alam, L., Sattar, A. and Janadhana, K. K. 1991. Changes in phenol and peroxidase in the leaves of *Java citrenella* infected with *Creruvularia andeapogenis. Boil Plant.*, 33: 211-215.

Arayarungsarit, L., Chongkid, B., Suwanbutr, S. and Weerapat, P. 1985. Reaction of some upland rices to root-knot nematodes in rubber plantation fields. *Int. Rice Res. Newsl.*, 10: 23-24.

Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris. Pl. Physiol.*, 24:1.

Arrigoni, O., Zacheo, G., Blave-Zaches, T., Arrigoni-Liso R. and Lamberti, C. 1981. Changes of superoxide dismutase and peroxidase activities in pea roots infested by *Heterodera goettingiana.* Institute di Botanica dell University dagli Studi and Institute to dimematologia Agraria, C.N.R. Bari, Italy, pp. 189-195.

Azam, T. 2009. Studies on the efficacy of *Paecilomyces lilacinus* and *Cassia tora* against the root knot nematode (*Meloidogyne incognita*) on tomato (*Lycopersicon esculentum*) in fly ash amended soil. Ph.D. Thesis, Aligarh Muslim University, Aligarh, India.

Azam, T. and Hisamuddin, 2008. Histopathological study of the roots of tomato infected with *Meloidogyne incognita*. *Proceedings of the 31st All India Botanical Conference and International Symposium on Plant biology and Environment: Changing Scenario*, December 17-19, 2008, Department of Botany, University of Allahabad, Allahabad, India, pp.67.

Azam, T., Hisamuddin., Singh, S. and Robab, M. I. 2011. Effect of different inoculum levels of *Meloidogyne incognita* on growth and yield of *Lycopersicon esculentum* and internal structure of infected root. *Arch. Phytopathol. Plant Prot.*, 44: 1829-1839.

Bajaj, K. L. and Mahajan. R. 1977. Phenolic compounds in tomato susceptible and resistant to *M. incognita* (Kofoid et whit) chitwood. *Nematol. Medit.*, 5: 329-333.

Bajaj, K. L., Singh, P. and Mahajan, R. 1985. Changes induced by *Meloidogyne incognita* in superoxide dismutase, peroxidase and polyphenol oxidase activity in tomato roots. *Biochem. Physiol. Pflanz.*, 180: 543-546.

Bajaj, H. K. Dabur, K. P. 2000. *Cyperus deformis*, a new recorded of rice root knot nematode, *Meloidogyne graminicola*. *Indian J. Nematol.*, 30(2): 256.

Bergeson, G. B. 1966. Mobilsation of minerals to the infection site of root-knot nematode. *Phytopathol.*, 56: 1287-1289.

Berliner, J., Pokhare, S. S., Mishra, C., Jena, M. and Singh, O. N. 2014. Screening of rice germplasm lines against rice root knot nematone *M. graminicola*. *Oryza.*, 51(2): 177-178.

Bose, L. K., Sahu, S. C., Mishra, C. D. and Ratho, S. N. 1998. Molecular polymorphism between the rice root knot nematode resistant and susceptible cultivars. *Oryza.*, 35: 190-192.

Bray, G. G. and Thorpe, W. V. 1954. Analysis of phenolic compounds of interest in metabolism. *Methods Biochem. Ann.*, 1: 27-52.

Bridge, J., Michel, L. and Plowright, R. A. 1990. Nematode parasites of rice. In: Luc, M., Sicora, R. A., Bridge, J. (eds.), *Plant parasitic nematodes in subtropical and tropical agriculture.* CABI, UK, pp. 69-108.

Bridge, J., Plowright, R. A. and Peng, D. 2005. Nematode parasites of rice. In: *Plant parasitic nematodes in subtropical and tropical agriculture.* 2nd ed. (Luc, M., Sikora, R. A. and Bridge, J. (eds). CABI Publishing, CAB International, Wallingford, UK. pp. 87-130.

Buangsuwon, D. T., Rujirachoon, G., Brawn, A. J. and Taylor, A. L. 1971. Rice diseases and pests of Thailand. Thailand: Rice Protection Research Centre, Ministry of Agriculture, pp. 61-67.

Chakrabarti, U. and Mishra, S. D. 2002. Evaluation of biochemical parameters for screening resistance of chickpea cultivars against *Meloidogyne incognita. Indian J. Nematol.*, 32: 26-29.

Chandel, S. T., Gaur, H. S. and Alam, M. M. 2001. Histopatholoy of wheat roots infected with root knot nematode, *Meloidogyne triticoryzae. Pak. J. Nematol.*, 19: 67-70.

Charles, J. S. K. and Venkitesan, T. S. 1993. Pathogenicity of *Heterodera oryzicola* (Nemata: Tylenchina) towards banana (Musa AAB cv. Nendran). *Fundam. Appl. Nematol.*, 16(4): 359-365.

Choudhary, K., Chawla, N., Kaur, S. and Jindal, S. 2013. Analysis of biochemical parameters in tomato fruits before and after inoculation with root knot nematode (*Melodogyne incognita*). *Veg. Sci.*, 40(2): 178-181.

Cobb, N. A. 1918. Estimating nematode population of the soil.*U.S. Department of Agriculture.* Technical Circulation1: 1-48.

Coyne, D. L. and Plowright, R. A. 2000. Nematode treats intensifying smallholder upland production in the Guinea Savannah of Cote d' Ivoire. *Trop. Sci.*, 40(2): 67-74.

Dangal, N. K., Sharma-Poudyal, D., Shrestha, S. M., Adhikari,C., Duxbury, J. M. and Lauren, J. G. 2008. Evaluation of organic amendments against rice root knot nematode at seedling stage of rice. *Nepal J. Sci. Technol.*, 9: 21-27.

Das, K., Zhao, D., Waele D. D., Tiwari, R. K. S., Shrivastava, D. K., and Arvind Kumar. 2011. Reactions of traditional upland and aerobic rice genotypes to rice root knot nematode (*Meloidogyne graminicola*). *J. Plant Breed. Crop Sci.*, 3: 131-137.

Devrajan, K. and Seenivasan, N. 2002. Biochemical changes in banana roots due to *Meloidogyne incognita* infected with *Paecilomyces lilacinus. Curr. Nematol.*, 13: 1-5.

Devrajan, K., Rajendran, G. and Seenivasan, N. 2003. Nutrient status and photosynthetic efficiency of banana (*Musa* sp.) by *Meloidogyne incognita* infected with *Pasteuria penetrans*. *Nematologia Mediterranea.*, 31: 197-200.

Devi, A. N., Ponnuswami, V., Sundararaju, P., Soorianathasundaram, K., Sathiamoorthy, S., Uma, S. and Van den bergh. 2007. Phenylalanine Ammonia Lyase and Total Phenol Content in Resistant Banana to *Pratylenchus coffeae*. *Indian J. Nematol.*, 37(2): 149-155.

Devi, G and Thakur, N. S. A. 2007. Screening of Rice Germplasm/Varieties for resistance against root-knot nematode (*Meloidogyne graminicola). Indian J. Nematol.*, 37:1.

Devi, L. J. 2014. Evaluation of some common rice varieties of Manipur for resistance against rice root knot nematode, *Meloidogyne graminicola. J. Glob. Biosci.*, 3(1): 374-378.

Dickerson, D. P., Pascholati, S. F., Hagerman, A. K., Butler, L. G. and Nicolson, R. L. 1984. Phenyl alanine ammonia lyase hydroxyl cinnamate, CoA Ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. *Physiol. Plant Path.*, 25: 111-123.

Dropkin, V. H. and King, R. C. 1956. Studies on plant parasitic nematodes homogeneously labeled with radio phosphorus. *Exp. Parasitol.*, 5: 469-480.

Dutta, T. K., Ganguly, A. K. and Gaur, H. S., 2012. Global status of rice root-knot nematode, *Meloidogyne graminicola. Afr. J. Microbiol. Res.*, 6(31): 6016-6021.

Ekanayake, H. M. R. K., Di Vito, M. and Vovlus, N. 1985. Histological changes caused by *M. incognita* in tomato plant roots. *Trop. Agric.*, 144: 89-97.

FAOSTAT, 2013. FAO Statistical Year Book 2013 World Food and Agricultural. Food and Agriculture Organization of the United Nations, Rome, 307p.

Farahat, A. A., Alsayed, A. A., El-Beltagi, H. S. and Mahfoud, N. M. 2012. Impact of organic and inorganic fertilizers on nematode reproduction and biochemical alterations on tomato. *Not. Sci. Biol.*, 4(1): 58-66.

Farkas, G. L. and Kiraly, Z. 1962. Role of phenolic compounds in the physiology of plant diseases and disease resistance. *Phytopathol.*, 44: 8-15.

Fogain, R. 1996. Screen house evaluation of *Musa* for susceptibility to *Radopholus similis:* Evaluation of plantains MB and diplod M, AB and BB. *Proceedings of the workshop held in Kuala Lumpur*, 2-5 Oct 1995, Malaysia, NASI, pp. 1-16.

Ganguly, S. and Dasgupta, D. R. 1982. Cellular responses and changes in phenols in resistant and susceptible tomato varieties inoculated with the root-knot nematode, *Meloidogyne incognita. Indian J. Nematol.*, 44: 166-171.

Ganguly, A. K. and Dasgupta, D. R. 1983. Chemical changes in brinjal plant induced by root knot nematodes. *Indian J. Nematol.*, 45: 45-47.

Ganguly, S. and Dasgupta, D. R. 1984. Sequential development of PPO (E.C.1.14.18) in resistant and susceptible tomatoes inoculated with the root knot nematode, *Meloidogyne incognita. Nematologia Mediterranea.*, 12: 15-22.

Ganguly, S., Misra, R. L. and Mishra, S. D. 1991. New disease complex of tuberose (*Polianthes tuberosa*) involving root-knot nematode, *Meloidogyne incognita* and a mite species. *Curr. Nematol.* 4:113-114.

Gauo, Y. and Wang, X. 2004. Effect of root-knot nematode on the absorption of micro-elemnts in ginger. *China J. Agric. Sci.*, 17: 185-188.

Gaur, H. S. and Pankaj. 2010. Root-knot nematode infestation in rice. In: Khan, M. R. and Jairajpuri, M. S (eds), *Nematode Infestations, Part I: Food Crop*, NASI, pp. 72-90.

Giebel, J. 1973. Enzyme phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) activities in potato roots & their significance in potato resistant to *Heterodera rostochiensis. Nematologica.*, 19: 1-6.

Golden, A. M. and Birchfield, W. 1965. *Meloidogyne graminicola* (Heteroderidae) a new species of koot-knot nematode from grass. *Proc. Helminthol. Soc. Wash.* 32: 228-231.

Goodman, R. N., Kiraly, E. and Ziatlin, M. 1967. The biochemistry and physiology of infectious plant diseases. D. Van Nosti and Co., Princeton, New Jersey, 354p.

Gopinath, K. V. 2001. Studies on root knot nematode *Meloidogyne incognita* on tomato and its management. M.Sc. (Ag) Thesis, University of Agricultural sciences, Bangalore, 108p.

Haseeb, A. and Srivatava, N. K. 1992. Studies of the K- increased susceptibility of the host plant. *Afr. Asian J. Nematol.*, 3: 165-169.

Hassan, M. G., Pant, V. R., and Devi, L. S. 2004. Infestation of rice root-knot nematode (*Meloidogyne graminicola*) associated with different varieties of rice in Allahabad District of Uttar Pradesh, India. *Indian J. Nematol.*, 34: 227.

Hatchkis, R. D. 1948. Microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparation. *Arch. Biochem.*, 16: 131-141.

Hofmann, J., Wieczorek, K., Blo¨Chl, A. and Grundler, F. M. W. 2007. Sucrose supply to nematode-induced syncytia depends on the apoplasmic and the symplasmic pathway. *J. Exp. Bot.*, 58: 1591-1601.

Hunter, A. H. 1958. Nutrient absorption and translocation of phosphorus as influenced by the root knot nematode, *Melodogyne incognita acrita. Soil Sci.*, 85: 245-250.

Hussey, R. S. and Williamson, V. M. 1997. Physiological and molecular aspects of nematode parasitism. In: Barker, K. R., Pederson, G. A. and Windhan, G. L. (eds), *Plant and Nematode Interactions.* Proceedings of an international workshop, American Society of Agronomy, Madison, WI, USA., pp: 87-108.

Jackson, M. L. 1973. Nitrogen determination for soils and plant tissue. Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi.

Jain, R. K., Mathur, K. N. and Singh, R. V. 2007. Estimation of losses due to plant parasitic nematodes on different crops in India. *Indian J. Nematol.* 37:217-219.

Jain, R. K., Khan, M. R. and Kumar, V. 2011. Rice root knot nematode (*Meloidogyne graminicola*) infestation in rice. *Arch. Phytopathol. Plant Prot.*, 45(6): 635-645.

Jairajpuri, M. S. and Baqri, Q. H., 1991. Nematode Pest of Rice. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India, 66p.

Jaiswal, R. K. and Singh, K. P. 2010. A technique for studying the life cycle of *Meloidogyne graminicola* in rice roots. *Int. Rice Res. Notes.*, 35: 117- 185.

Jena, R. N. and Rao, Y. S. 1976. Nature of root-knot nematode (*Meloidogyne graminicola*) resistance in rice (*Oryza sativa* L.). Isolation of resistant varieties. Proc. *Indian Acad.*, 83: 177-184.

Jena, R. N. and Rao, Y. S. 1977. Nature of resistance in rice *(Oryza sativa)* to root-knot nematode *(Meloidogyne graminicola)*- Histopathology of nematode infestation. *Proc. Indian Acad. Sci.,* 86 : 87-91.

Jonathan, E. I and Rajendran, G. 2002. Assessment of avoidable yield loss in banana due to root knot nematode *Meloidogyne incognita. Indian J. Nematol.*, 30: 162-164.

Karpinski, S., Gabrys, H., Mateo, A., Karpinska, B. and Muttineaux, P. M. 2003. Light perception in plant disease defence signaling.*Cuur. Opin. Plant Biol.*, 6: 390-396.

Khan, M. R., Ghosh, S. and Bhattacharya, S. P. 2004. Weed hosts of rice root-knot nematode, *Meloidogyne graminicola* from West Bengal. *Environ. Ecol.*, 22:583-584.

Khan, A., Bilqees, F. S., Khatoon, N. and Riyazuddin, 2007. Histopathology of Jujube (*Zizyphus maurieiana* Lam.) roots infected with *Meloidogyne javanica*. *Pak. J. Nematol.*, 22: 143-149.

Khan, M. R. and Jairajpuri, M. S. 2010. Nematode infestation in food crops- national scenario. In: Khan, M. R. and Jairajpuri, M.S. (eds), *Nematode Infestations Part I- Food Crops*. The National Academy of Sciences, India. pp. 1-16.

Khan, M. R. and Anwer, A. 2011. Occurrence of rice root-knot nematode and yield loss assessment in Aligarh and Hathras districts of Uttar Pradesh, India. *Indian J. Nematol.*, 41 (1): 34-40.

Khan, M. R., Ashraf, T. and Shahid, S. 2012. Evaluation for relative susceptibility of rice against field population of *Meloidogyne graminicola*. *Indian J. Nematol.*, 42: 46-52.

Khan, M. R., Haque, Z. and Kausar, N. 2014. Management of the root knot nematode, *Meloidogyne graminicola* infesting rice in the nursery and crop field by integrating seed priming and soil application treatments of pesticides. Crop Prot., 63: 15-25.

Korayem, A. M., Mohamed, M. M. M. and Abou-Hussein, S. D. 2013. Damage threshold or root knot nematode, *Meloidogyne arenaria* on peanut in relation to date of planting and irrigation system. *Can. J. Plant Pathol.*, 1: 117-124.

Kumar, P. S., Ramakrishnan, S. and Jonathan, E. I. 2007. Life cycle, varietal reaction, biochemical alteration and histopathology of rice root-knot nematode, *Meloidogyne graminicola. Indian J. Nematol.*, 37: 165-171.

Kumar, B. R., Pankaja, N. S. and Mahesh, Y. S. 2008. Histopathological and histochemical variations in rice roots infected with root knot nematode. *Curr. Biot.*, 2(3): 323- 336.

Langrimini., L. M. and Rothstain, S. 1987. Tissue specificity of tobacco peroxidase isozymes and their induction by wounding and tobacco mosaic virus infection. *Plant Physiol.*, 84: 438-442.

Longley, J. B. 1952. Effectiveness of schiffs variance in the periodic Schiff and Feulgem nucleus techniques. *Stain Tech.*, 27: 161-169.

Loveys, R. R. and Bird, A. F. 1973. The influence of nematodes on photosynthesis in tomato plants. *Physiol. Plant Pathol.*, 3: 525-529.

MacGowan, J. B. and Langdon, K. R. 1989. Hosts of the rice root-knot nematode, *Meloidogyne graminicola. Nematol. Cir.* 172p.

Main, I. H. and Khan, M. M. A. 1995. Effects of inoculum level on the post penetration development of *Meloidogyne graminicola* in rice root. *Bangladesh J. Sci. Ind. Res.*, 30: 55-64.

Mayer, A. M., Harel, E. and Shaul, R. B. 1965. Assay of catechol oxidase, a critical comparison of methods. *Phytochem.*, 5: 783-789.

Mayer, A. M. and Harel, E. 1979. Polyphenol oxidases in plants. *Phytochem.*, 18: 193-215.

Mazia, D., Brower, P. A. and Alfert, M. 1953. The cytochemical staining and measurement of protein with mercuric bromophenol blue. *Biol. Bulletin.*, 104: 57-67.

Melakeberhan, H., Brooke, R. C. and Webster, J. M. 1986. Relationship between physiological response of French beans of different age to *Meloidogyne incognita* and subsequent yield loss. *Plant Pathol.*, 35: 203-213.

Melo, G. A., Shimizu, M. M. and Mazzafera, P. 2006. Polyphenoloxidase activity in coffee leaves and its role in resistance against the coffee leaf miner and coffee leaf rust. *Phytochem.*, 67: 277-285.

Mhatre, P. H., Malik, P. S. K., Kaur, S., Singh, A.K., Mohan, S. and Sirohi, A. 2015. Histopathological changes and evaluation of resistance in Asian rice (*Oryza sativa* L.) against rice root knot nematode, *Meloidogyne graminicola* Golden and Birch. *Indian J. Genet.*, 75(1): 41-48.

Miller, A. J. and Cramer, M. 2004. Root nitrogen acquisition and assimilation. *Plant soil.*, 274: 1-36.

Mishra, C. D. and Mohanty K. C. 2007. Role of phenolics and enzymes in imparting resistance to rice plants against root-knot nematode, *Meloidogyne graminicola. Indian J. Nematol.*, 37: 131-134.

Mishra, C. D. and Mohanty, K. C. 2008. Biochemical changes in susceptible and resistant rice varieties due to infection by *Meloidogyne graminicola* Golden and Birchfield. *Crop Prot.*, 45(3): 226-229.

Mohanty, K. C. and Pradhan, A. K. 1989. Quantitative estimation of free aminoacids and amides in resistant and susceptible greengram varieties inoculated with root knot nematode, *Meloidogyne incognita. Indian J. Nematol.,* 19: 74-76.

Mohanty, K. C., Mahapatra, N. and Patnaik, N. C. 1992. Reaction of rice cultivars to root-knot nematode. *Meloidogyne graminicola. Indian J. Nematol.,* 22: 67-69.

Mohanty, K. C., Swain, S. C. and Pradhan, T. 1995. Biochemical variations in resistant and susceptible brinjal varieties influenced by root knot nematode, *Meloidogyne incognita. Indian J. Nematol.*, 25: 142-146.

Mohanty, K. C., Pradhan, G. C. and Panigrahi, D. 1997. Post infection biochemical changes in susceptible and resistant rice cultivars due to root-knot nematode *Meloidogyne graminicola. Oryza.,* 34: 67-69.

Molinari, S. 1995. Difference in isoperoxidase activities of tomato roots susceptible and resistant to root-knot nematodes. *Nematologia Mediterranea.*, 23(2): 271-281.

Mote, U. N., Dasgupta, D. R. and Ganguly, A. K. 1990. Sequential development of deaminases in resistant and susceptible tomato varieties infested with root-knot nematode, *Meloidogyne incognita. Indian J. Nematol.,* 20: 127-137.

Mukhopadhyay, A. K. and Khan, M. R. 2000. Nematode pests in rice-wheat-legume cropping systems. *Proceedings of Review and Planning Meeting and Training Workshop*; April 5-10, 1999; New Delhi: Division of Nematology, Indian Agricultural Research Institute. Rice-wheat-consortium Paper Series 7, New Delhi, India: Rice-Wheat Consortium for Indo-Gangetic Plains and Patancheru, Andrapradesh, India: ICRISAT. Distribution of *Meloidogyne graminicola* and other nematodes in rice-based cropping system in West Bengal. pp. 20-21.

Mulk, M. M. 1976. *Meloidogyne graminicola*, C.I.H. Descriptions of plant-parasitic Nematodes Set 6. *Nematologia Mediterranea.*, 87:1-7.

Muzzafera, P., Goncalves, W. and Feinandes, J. A. R. 1989. Phenols, peroxidase and (polyphenol oxidase in resistance of coffee to *Meloidogyne incognita. Bragantia. Indian J. Nematol.*, 48: 131-142.

Myint, Y. Y. 1981. Country report on root knot nematode in Burma. In: Sasser, J. N. (ed.) *Proceedings of the third research planning conference on root knot nematode, Meloidogyne sp.* 20-24 July 1981, Jakarta, Indonesia. Institute of Agriculture, Burma, pp. 163-170.

Narayan, Y. D. and Reddy, D. D. R. 1980. The role of nitrogen, amino acids and phenols in resistance of tomato to root knot nematodes. *Nematologia Mediterranea.*, 8: 51-57.

Nayak, D. K. and Mohanty, K. C. 2010. Biochemical Changes in Brinjal Induced by Root-Knot Nematode, *Meloidogyne incognita. Indian J. Nematol., 40*(1): 43-47.

Niyaz, T. and Hisamuddin, 2009. Histology of interation of *Paecilomyces lilacinus* with *Meloidogyne incognita* on *Eclipta alba* (L.). *Arch. Phytopathol. Plant prot.*, 42: 829-834.

Niyaz, T., Azam, T. and Hissamuddin, 2011. Histopathological responses and damage potential in roots of *Eclipta alba* L. caused by *Meloidogyne incognita*. *Libyan Agric. Res. Center J. Int.*, 2: 118-122.

Oteifa, B. A. and Elgindi, D. M. 1962. Influence of parasitic duration of *Meloidogyne javanica* (Treub) on host nutrient uptake. *Nematologica.*, 8: 216-220.

Padgham, J. L., Mazid, M. A., Duxbury, J. M., Abawi, G. S. and Hossain, M. 2004. Yield loss caused by *Meloidogyne graminicola* on lowland rainfed rice in Bangladesh. *J. Nematol.*, 36: 42-48.

Patel, B. A., Patel, D. J., Patel, R. G. and Talati J. G. 2001. Biochemical changes induced by infection of *Meloidogyne* spp. in chickpea. *Intern. Chickpea Pigeonpea Newslett.*, 8: 13-14.

Patil, J., Miller, A. J. and Gaur, H. S. 2013. Effect of nitrogen supply form on the invasion of rice roots by the root-knot nematode, *Meloidogyne graminicola*. *Nematol.*, 15: 483-492.

Patil, J. 2014. Relationship between population density of root-knot nematode, *Meloidogyne graminicola* and the growth and nutrient uptake of rice plant. *Int. J. Plant Sci.*, 27(1): 130-138.

Patil, J. P. and Gaur, H. S. 2014. Relationship between population density of root-knot nematode, *Meloidogyne graminicola* and the growth and nutrient uptake of rice plant. *Vegetos*.. 27(1): 130-138.

Plowright, R. A. and Bridge, J. 1990. Effect of *Meloidogyne graminicola* (Nematoda) establishment growth and yield of rice cv. IR36. *Nematologica*., 36: 81-89.

Poudyal, D. S. 2001. The rice root knot nematode: Its distribution and impact on rice-wheat system. M.Sc. (Ag) thesis, IAAS,Rampur, Chitwan, Nepal, 96p.

Poudyal, D. S., Pokharel, R. R., Shrestha, S. M. and Khatri-Chhetri, G. B. 2002. Population and effect of rice root knot nematode in diseased and healthy looking rice plants and their distribution in rice field. *J. Inst. Agric. Anim. Sci.* 23: 9-14.

Poudyal, D. S., Pokharel, R. R., Shrestha, S. M. and Khatri-Chhetri, G. B. 2004. Evaluation of common Nepalese rice cultivars against rice root-knot nematode. *Nepal Agric. Res. J.*, 5: 33-37.

Poudyal, D. S., Pokharel, R. R., Shrestha, S. M. and Khatri- Chetri, G. B. 2005. Effect of inoculum density of rice root knot nematode on growth of rice cv. Musali and nematode development. *Aust. Plant Pathol.*, 34: 181-185.

Pokharel, R. R. 2007. Characterization of root-knot populations from rice-wheat fields in Nepal and reactions of selected rice and wheat germplasm to *Meloidogyne graminicola* (Ph.D. Dissertation). Ithaca, New York, USA: Cornell University.

Poornima, K. and Vadivelu, S. 1998. Pathogenisity of *Meloidogyne incognita* to turmeric (*Curcuma longa* L.). Proceedings of the 3rd international symposium of Afro-Asian society of Nematologists, April16-19. 1998, Coimbatore, pp: 29-31.

Prasad, J. S., Panwar, M. S. and Rao, Y. S. 1986. Screening of some rice cultivars against root knot nematode, *Meloidogyne graminicola. Indian J. Nematol.*, 16(1): 112-213.

Prasad, J. S., Panwar, M. S. and Rao, Y. S. 1990. Influence of root-knot nematode infection in rice under simulated rainfed lowland situation. *Nematol. Medit.*, 18: 195-197.

Prasad, J. S., Varaprasad, K. S., Panwar, M. S., Pandhi, N. N. and Pathak, K. N. 2000. An effective method for screening rice varieties against root-knot nematode, *Meloidogyne graminicola. Indian J. Nematol.*, 30: 210-215.

Prasad, J. S., Vishakanta, and Gubbaiah. 2001. Outbreak of root knot nematode, *Meloidogyne graminicola* in Mandya district, Karnataka state and farmers' perceptions. p. 73–74. Paper presented at National Congress on Centenary of Nematology in India - appraisal and future plans; 2001 Dec 5–7; New Delhi, India: Nematological Society of India. p.176.

Prasad, J. S., Vijayakumar, C. H. M., Sankar, M. Varaprasad, K. S, Prasad, M. and Kondala, R. Y. 2006. Root-knot nematode resistance in advanced back cross populations of rice developed for water stress conditions. *Nematol. Mediterr.*, 34: 3-8.

Prasad, J. S., Somasekhar, N. and Varaprasad, K. S., 2010. Nematode infestation in Paddy. In: Khan, M. R., Jairajpuri, M. S. (eds.), *Nematode infestations, Part I: Food Crop*. NASI, pp. 17-71.

Praveen, H. M. 2002. Studies on the root knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood on Gherkin and its management. M. Sc. (Ag) thesis, University of Agricultural sciences, Bangalore, 113p.

Praveen, K., Haseeb, A. and Shukla, P. K. I. 2006. Pathogenic potential of *Meloidogyne incognita* on *Mentha arvensis* cv. Gomti. *Indian J. Nematol.*, 36: 177-180.

Praveen, R. 2006. Studies on *Ocimum sanctum* (L) infected with root knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood. Ph.D. Thesis, Aligarh Muslim University, Aligarh, India.

Prot, J. C. 1994. Effects of economic and policy changes on the status of nematode rice pests in Vietnam and the Philippines. *Fundamental Appl. Nematol.*, 17(3):195-198.

Prot, J. C., Villanueva, L. M. and Gergon, E. B. 1994. The potential of increased nitrogen supply to mitigate growth and yield reduction of upland rice cultivar UPL Ri5 caused by *Meloidogyne graminicola*. *Fund. Appl. Nematol.*, 7: 445-454.

Prot, J. C. and Matias, D. 1995. Effects of water regime on the distribution of *Meloidogyne graminicola* and other root-parasitic nematodes in a rice field toposequence and pathogenicity of *M. graminicola* on rice cultivar UPL R15. *Nematologica.*, 41: 219-228.

Qudassi, S., Khan, F., Mazid, M., Khan, T. A. Ansari, S. and Arshad, M. 2014. Quality status of nutrients, vitamins and hormones under pathological threats in tomato: Basic scenario. *Int. J. Med. Chem. Anal.*, 4(1): 12-21.

Rajasekar, S. P., Ganguly, A. K. and Swain, S. C. 1997. Quantitative changes in superoxide dismutase, catalase and peroxidase with reference to resistance in tomato to *Meloidogyne incognita. Indian J. Nematol.*, 27: 79-85.

Ramakrishnan, S., Varadharajan, G. and Sutharsan, P. D. 1984. TKM9 is resistant to rice-root nematode. *Int. Rice Res. Newsl.*, 9: 20.

Ramakrishnan, S. and Rajendran, G. 1998. Effect of individual and concomitant initial inoculum of *Meloidogyne incognita* and *Rotylenchulus reniformis* on growth, physiology and nutrient content of papaya (*Carica papaya* L.). *Proceedings of the 3rd international symposium of Afro-Asian society of Nematologists*, April16-19. 1998, Coimbatore, pp: 17-28.

Rani, I. C., Veeraragavathatham, and Sanjutha, S. 2008. Analysis on biochemical basis of root knot nematode (*Meloidogyne incognita*) resistance in tomato (*Lycopersicon esculentum* Mill.). *Res. J. Agric. Biol. Sci.*, 4(6): 866-870.

Rao, Y. S. and Israel, P. 1973. Life history and bionomics of isolates of *Meloidogyne graminicola* in rice. *Indian J. Nematol.* 7: 98-99.

Rao, Y. S., Prasad, J. S. and Panwar, M. S. 1986. Nematode problems in rice: Crop losses, symptomatology and management. In : Swarup, G and Dasgupta, D. R. (eds), *Plant Parasitic Nematodes of India Problems and Progress,* IARI, New Delhi. pp. 279-299.

Rao, Y. S., Jayaprakash, A. and Mohanty J. 1988. Nutritional disorders in rice due to infestation by *Heterodera oryzicola* and *Meloidogyne graminicola*. *Rev. Nématol.*, 11:375-380.

Robab, M. I., Hisamuddin, S. and Azam, T. 2010. Histopathology of roots of *Glycine max* (L.) Merrill induced by root-knot nematode (*Meloidogyne incognita*). *Arch. Phytopathol. Plant Prot.*, 43: 1758-1767.

Sadasivam, S. and Manickam, A. 2008. Biochemical methods, (second edition). New Age International Publishers, New Delhi, 256 p.

Sasser, J. N. 1989. Plant parasitic nematodes: the farmer's hidden enemy. Raleigh:North Carolina State University Graphics, 115p.

Sayed, M., Khan, A., Khaatoon, N., Bilqees, F. M. and Samad, M. A. 2010. Histopathology of mango roots infected by root knot nematode. *Pak. J. Nematol.*, 28: 335-340.

Schindler, A. F. 1961. A sample substitute for a Baermann funnel. Plant Dis. Rep., 45: 747-748.

Sempio, C., Della, T. C., Ferranti, F., Barberine, B. and Draoli, F. 1975. Defense mechanisms in beans resistant to rust. *Phytopathol.*, 83 : 244-66.

Shah, J. J. and Raju, C. 1977. Histopathology of ginger (*Zingiber officinale*) infected by soil nematode, *Meloidogyne* sp. *Phyton.*, 16: 79-84.

Sharma, O. N. 1993. Enhancement of biological defense mechanisms in resistance and its activation in susceptible tomato cultivars infested with root-knot nematode, *Meloidogyne incognita*. *Plant Dis. Res.*, 8: 47-53.

Sheela, M. S., Jiji, T. and Nisha, M. S. 2005. A new record of *Meloidogyne graminicola* on Rice, *Oryza sativa* in Kerala. *Indian J. Nematol.*, 35(2): 218.

Shukla, Y. M. and Chakraborthy, M. K. 1988. Biochemical studies on response of tobacco and tomato plants to root-knot nematode infection. *Tob. Res.*, 14: 43-50.

Siddiqui, I. A. and Taylor, D. P., 1970. Histopathogenesis of galls induced by *Meloidogyne naasi* in wheat roots. *J. Nematol.*, 2: 239-247.

Siddiqui, Y., Ali, A. and Naidu, Y. 2014. Histopathological changes induced by *Meloidogyne incognita* in some ornamental plants. *Crop Prot,*, 65: 216-220.

Silva, R. V., Oliveira, R. D. L., Nascimento, K. J. T. and Rodrigues, F. A. 2010. Biochemical responses of coffee resistance against *Meloidogyne exigua* mediated by silicon. *Plant Pathol.*, 59: 586-593.

Singh, S., Dhawan, S. C. and Goswami, B. K. 2007. Histopathology of cowpea roots co-infected with root knot nematode, *Meloidogyne incognita* and wilt fungus, *Fusarium oxysporum*. *Indian J. Nematol.*, 37: 156-160.

Singh, S., Abbasi and Hisamuddin. 2013. Histopathological response of *Lens culinaris* roots towards root knot nematode, *Meloidogyne incognita*. *Pak. J. Biol. Sci.*, 16(7): 317-324.

Sirohi, A. and Dasgupta, D. R. 1993 a. Mechanism of resistance in cowpea to the root-knot nematode, *Meloidogyne incognita* Race 1: I: Early induction of Phenylalanine ammonia lyase (EC.4.3.1.5.) and chlorogenic acid. *Indian J. Nematol.*, 23: 31-41.

Sirohi, A. and Dasgupta, D. R. 1993 b. Mechanism of resistance in cowpea to the root-knot nematode, *Meloidogyne incognita* Race 1: II: *de Novo* synthesis of Phenyl alanine ammonia lyase (EC.4.3.1.5.). *Indian J. Nematol.*, 23: 42-52.

Soriano, I. R., Espiritu, M. J., Schmit, V., Brar, D. and Reversat, G. 1998. Resistance to rice root knot nematode *Meloidogyne graminicola* in *Oryza longistaminata* and *Oryza glaberrima. Philipp. J. Crop Sci.* 23:89.

Soriano, I. R., Schmit, V., Brar, D. S., Prot, J. and Reverstat, G. 1999. Resistance to rice root-knot nematode *Meloidogyne graminicola* identified in *O. longistaminata* and *O. glaberrima. Nematol.*, 1: 395-398.

Srivasthava, S. K. 1987. Peroxidase and polyphenol oxidase in *Brassica juncea* plants infected with *Macrophomina phaseolina* (Tassi) Goid and their implication in disease resistance. *Phytopath.*, 120: 249-254.

Srivastava, A., Rana, V., Rana, S., Singh, D. and Singh, V. 2011. Screening of rice and wheat cultivars for Resistance aginst Root knot Nematode, *Meloidogyne graminicola* (Golden and Birchfield) in Rice-Wheat cropping system. *J. Rice Res.*, 4: 1-2.

Sundararaju, P. and Suba, K. P. 2006. Biochemical changes in banana plants induced by *Pratylenchus coffeae* and *Meloidogyne incognita. Indian J. Nematol.*, 36(2): 256-259.

Swain, B. and Prasad, J. S. 1988. Chlorophyll content in rice as influenced by the root knot nematode, *Meloidogyne graminicola* infection. *Curr. Sci.*, 57: 895-896.

Swain, S. C., Ganguly, A. K. and Umarao. 2004. Race specific biochemical responses in differential hosts against rootknot nematode, *Meloidogyne incognita. Indian J. Nematol.*, 34: 26-32.

Taiz, L. and Zeiger, E. 2002. *Plant Physiology*. 3rd ed., Sinaur Associates Inc, Sunderland, MA, USA, 290 pp.

Takamiya, K. I., Tsuchiya, T and Ohta, A. 2000. Degradation pathway(s) of chlorophyll: what has gene cloning revealed? *Trends in Plant Science.*, 5: 426-431.

Tandon, R. S. and Kumar, P. 1979. Histological changes of *Lycopersicon esculentum* roots parasitized with *M. lucknowika. Indian J. Nematol.*, 9: 169-180.

Taylor, D. P. 1976. Histopathology of *Meloidogyne incognita* galls in the stems of rosette *Hibiscus sabdariffa. J. Nematol.*, 9: 169-180.

Valette, C., Nicole, M., Sarah, J. L., Boisseau, M., Boher, B., Fargette, M. and Geiger, J. P. 1997. Ultrastructure and cytochemistry of interactions between banana and the nematode, *Radopholus similis. Fundamental Appl. Sci.*, 20: 65-77.

Varanasi, A. V. and Talati, J. G. 2014. Effect of exogenous salicylic acid on plant defense mechanism in chickpea (Cicer arientinumL.) against infection of Meloidogyne incognita. *Indian J. Agric. Biochem.*, 27(1): 52-59.

Vast, P. H., Caswell, E. P., and Zasoskic, R. J. 1998. Effects of two endoparasitic nematodes (*Pratylenchus coffeae* and *Meloidogyne konaensis*) on ammonium and nitrate uptake by Arabica coffee (*Coffea arabica* L.). *Appl. Soil Ecol.*, 10: 171-178.

Venkatesan, M., Gaur, H. S. and Datta, S. P. 2013. Effect of Root-Knot Nematode, *Meloidogyne graminicola* on the Uptake of Macronutrients and Arsenic and Plant Growth of Rice. *Vegetos.*, 26(2): 112-120.

Verma, K. K. and Jain, R. K. 2006. Nutrient uptake as affected by inorganic fertilizers under *Meloidogyne incognita* infested conditions in cotton. *Indian J. Nematol.*, 36: 60-64.

Vlot, A. C., Dempsey, D. M. A. and Klessig, D. F. 2009. Salicylic acid: a multifaceted hormone to combat disease. *Annu Rev Phytopathol.*, 47: 177-206.

Win, P. P., Kyi, P. P., Maung, Z. T. Z. and De Waele, D. 2013. Evaluation of the host response of lowland and upland rice varieties from Myanmar to the rice root knot nematode *Meloidogyne graminicola*. *Arch. Phytopathol. Plant Prot.*, 47 (7): 869-891.

Wuyts, N., Lognay, G., Sagi, L., De Waele, D. and Swennen, R. 2005. Secondary metabolites in roots and implications for nematode resistance in banana (*Musa* spp.). In: Banana root system: Towards a better understanding for its productive management -Turner, D.W. (ed.); Rosales, F.E. (ed.), p. 238-246, International Symposium, San Jose (CRI), 2003/II/03-05, INIBAP, Montpellier, France.

Xu, X. Y., McGrath, S. P., Meharg, A. and Zhao, F. J. 2008. Growing rice aerobically markedly decreases arsenic accumulation. *Environ. Sci. Tech.*, 42: 5574-5579.

Yasmeen, N. 2002. Histopathological studies on *Leucantha* infected with *Meloidogyne incognita* and *Pythium aphanidermatum* in fly ash amended soil. Ph.D. Thesis, Aligarh Muslim University, Aligarh, India.

Yik, C. P. and Birchfield, W. 1979. Host studies and reaction of cultivars to *Meloidogyne graminicola*. *Phytopathol.*, 69: 497-499.

APPENDICES

APPENDIX I

Buffer for peroxidase and polyphenol oxidase analysis

0.1 M Sodium Phosphate buffer (pH 6.5)

Stock Solutions

A: 0.2 M solution of monobasic sodium phosphate (27.8 g in 1000 ml)

B: 0.2 M solution of di basic sodium phosphate (53.65 g $Na_2HPO_4.7\ H_2O$ in 1000 ml)

68.5 ml of A mixed with 31.5 ml of B diluted to a total of 200 ml.

APPENDIX II

Buffer for Phenylalanine Ammonia Lyase (PAL) analysis

0.1 M Borate buffer (pH 8.8)

Stock Solutions

A: 0.2 M solution boric acid (12.4 g in 1000 ml)

B: 0.05 M solution of borax (19.05 g in 1000 ml)

50 ml of A is mixed with 30 ml of B, diluted to a total of 200 ml.

APPEDIX III

Anthrone reagent for starch analysis

Anthrone reagent made by dissolving 200 mg of anthrone in 100 ml ice cold 95 % concentrated sulphuric acid.

APPENDIX IV

Schiff's reagent for localization of total insoluble polysaccharides

Basic fuchsine powder	: 1 gm
Distilled water	: 90 ml
1N HCL	: 15 ml
Potassium metabisulphate	: 1.8 g
Activated charcoal	: 1 g

APPENDIX V

Mercuric bromophenol blue stain for localization of total proteins

Mercuric chloride	: 10 g
Bromophenol blue	: 100 g
Absolute alcohol	: 100 ml

APPENDIX VI

Toluedine blue stain for localization of nucleic acid (RNA and DNA)

Toluedine blue powder : 0.5 g

Benzoate buffer (pH- 4.0) : 100 ml

APPENDIX VII

Benzoate buffer for preparation of Toluedine blue stain

Benzoate buffer (pH- 4)

Stock solutions

A: 100 ml of 0.1 M benzoic acid

B: 28.63 ml of 0.22 M sodium benzoate

77.74 ml of A is mixed with 22.26 ml of B

BIOCHEMICAL AND HISTOPATHOLOGICAL ALTERATIONS DUE TO ROOT- KNOT NEMATODE, *Meloidogyne graminicola* IN RICE (*Oryza sativa* L.) AND VARIETAL REACTIONS.

DARSANA. V. S. LAL

(2013-11-160)

Abstract of the thesis

Submitted in the partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

2015

Department of Agricultural Entomology
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM - 695 522
KERALA, INDIA

ABSTRACT

An experiment entitled "Biochemical and histopathological alterations due to root- knot nematode, *Meloidogyne graminicola* in rice (*Oryza sativa* L.) and varietal reactions" was undertaken at Department of Agricultural Entomology, College of Agriculture, Vellayani, during 2013-14. A progressive increase in phenol content, defense enzymes *viz.* Peroxidase (PO), Polyphenol Oxidase (PPO), Phenylalanine Ammonia Lyase (PAL) from both leaf and root after 45 days of nematode inoculation were observed with increase in inoculum levels.

The chlorophyll a and b, NPK content and micronutrients *viz.* Fe, Cu, Zn and Mn were decreased with increase in nematode population. The plants inoculated with 10,000 J_2 showed low pH (5.69) compared to the uninoculated plant and was significantly different from all other treatments. The starch, protein and total sugar content decreased 41.44, 41.89 and 44.95 per cent respectively in plants inoculated with 10,000 J_2 compared to uninoculated plants. A progressive decrease in plant growth and yield parameters were observed with increase in population levels of nematode. The maximum reduction in plant height, fresh weight of plant, dry weight of root and dry weight of shoot and the yield parameters like thousand seed weight and number of seeds per panicle were observed in plants inoculated with 10,000 J_2.

Histochemical studies revealed that an accumulation of polysaccharides, proteins and nucleic acid in epidermal, cortical and stelar region of root progressively increased with increase in population density of nematode. Giant cells were formed near the xylem and phloem cells. The xylem and phloem vessels were disorganized.

In studying the varietal reaction of ten popular rice varieties of Kerala against *M. graminicola*, all the varieties were suceptible to the nematode. Minimum number of galls (51.8) and egg mass (64.60) per plant was observed in the variety Uma and was on par with Pavizham. On the basis of egg mass index, all other varieties were highly susceptible except Uma. The PO and PPO activity of Uma, Karthika, Pavizham and Bhadra were higher than that of TN1 (Suceptible check). In Uma, the nematode population in soil and root was minimum and on par with Karthika and the reproduction rate also minimum in Uma.

Based on the results of the study, *M. graminicola* can be considered as a potential threat to the cultivation of rice. Uma showed better performance against root knot nematode than all other varieties.