# INVESTIGATIONS ON ROOT-KNOT NEMATODE (*Meloidogyne incognita*) ASSOCIATED WITH BLACK GRAM (*Vigna mungo* L.)

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***for the award of the Degree of***

*MASTER OF SCIENCE (Agriculture)*

in

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**BENGALURU** **JULY, 2016**

*Affectionately Dedicated to*

*My Beloved*

*Father- MOTHINAIK*

*Mother- DRAKSHAYANIBAI*

*SAVITRI*

**DEPARTMENT OF PLANT PATHOLOGY**
**UNIVERSITY OF AGRICULTURAL SCIENCES**
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## CERTIFICATE

This is to certify that, the thesis entitled **INVESTIGATIONS ON ROOT-KNOT NEMATODE (*Meloidogyne incognita*) ASSOCIATED WITH BLACK GRAM (*Vigna mungo* L.)** submitted by **Mr. HARILAL, D. M., ID. No. PALB 4254**, in partial fulfilment of the requirements for the award of the degree of **MASTERS OF SCIENCE (Agriculture) in PLANT PATHOLOGY** to the University of Agricultural Sciences, GKVK, Bengaluru, is a record of research work carried out by him during the period of his study in this university under my guidance and supervision and the thesis has not previously formed the basis of the award of any other degree, diploma, associate ship, fellowship or other similar titles.

BENGALURU
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*Bengaluru*

*July, 2016* ***HARILAL, D. M.***

**INVESTIGATIONS ON ROOT-KNOT NEMATODE (*Meloidogyne incognita*) ASSOCIATED WITH BLACK GRAM (*Vigna mungo* L.)**

**HARILAL, D. M.**

**ABSTRACT**

Root-knot nematode (*Meloidogyne incognita*) is a serious pest of pulse crops and is a major limiting factor in the production of black gram in many parts of the country, including Karnataka. Use of chemicals to manage nematodes affects soil bio-diversity and environment besides their residual toxicity. The present studies were undertaken to survey for the occurrence of root-knot nematode in major pulse growing areas in southern Karnataka districts, screening of available varieties/cultivars of black gram against root-knot nematode and effect of root knot nematode on nodulation, growth and yield parameter in black gram. In the field survey, predominant occurrence of total soil and root population of *M. incognita* (657/200cc soil and 71/5g of root) followed by *Helicotylenchus* sp.(70 and 15), *Rotylenchulus* sp. (30 and 28) and *Pratylenchus* sp. (22 and 17) were noticed in Chamarajanagar district followed by Tumkuru. Screening of fourteen black gram cultivars *viz.,* 2KU-60, ADT-05, BG-2, DU-1, G-333, IC-282007, IC-436545, K-5-572, KU-8-155, LKU-64, RASHMI, RU10-601, SU-509 and UH-04-04 under glass house condition, revealed that UH-04-04 was more susceptible and G-333 was resistant to *M. incognita. M. incognita* infection significantly reduced the number of rhizobial nodules, nitrogen content, root length, shoot length, pod number and plant weight. A greater reduction was observed when the *M. incognita* was established before the inoculation of the *Rhizobium* than the *M. incognita* and *Rhizobium* inoculated together or when the *Rhizobium* was established before the inoculation of *M. incognita*.

JULY, 2016

Department of Plant Pathology
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**(N. G. Ravichandra)**
Major Advisor

# ಉದ್ದಿನ ಬೆಳೆಯನ್ನು *(ವಿಗ್ನಾ ಮುಂಗೊ)* ಪೀಡಿಸುವ ಬೇರುಗಂಟು ಜಂತು ಹುಳುವಿನ (*ಮೆಲಾಯ್ಡೊಗೈನೆ ಇನ್ಕಾಗ್ನಿಟಾ*) ಅಧ್ಯಯನ


**ಹರಿಲಾಲ್, ಡಿ. ಎಮ್.**



## ಸಾರಾಂಶ

ಬೇರುಗಂಟು ಜಂತು ಹುಳುವು ದ್ವಿದಳ ಧಾನ್ಯಗಳ ಮಾರಕ ಪೀಡೆಯಾಗಿದೆ ಹಾಗು ಇದು ಕರ್ನಾಟಕ ಸಹಿತ ದೇಶದ ಎಲ್ಲ ಭಾಗಗಳಲ್ಲಿ ಉದ್ದಿನ ಬೆಳೆ ಇಳುವರಿಯನ್ನು ಕುಂಠಿತಗೊಳಿಸುವಲ್ಲಿ ಪ್ರಮುಖ ಪಾತ್ರವಹಿಸಿದೆ. ಜಂತು ಹುಳುವನ್ನು ನಿಯಂತ್ರಿಸಲು ಹೆಚ್ಚಾಗಿ ಔಷಧಿಗಳನ್ನು ಬಳಸುವುದರಿಂದ ಮಣ್ಣಿನಲ್ಲಿರುವ ಉಪಯುಕ್ತ ಸೂಕ್ಷ್ಮಾಣು ಜೀವಿಗಳು ಮತ್ತು ವಾತಾವರಣ ಮಲಿನಗೊಳ್ಳುತ್ತದೆ. ದ್ವಿದಳ ಧಾನ್ಯಗಳಲ್ಲಿ ಬರುವ ವಿವಿಧ ಪರಾವಲಂಭಿ ಜಂತು ಹುಳುಗಳ ಬಗ್ಗೆ ದಕ್ಷಿಣ ಕರ್ನಾಟಕ ಜಿಲ್ಲೆಗಳಲ್ಲಿ ಸಮೀಕ್ಷೆ ನಡೆಸಲಾಯಿತು. ಬೇರುಗಂಟು ಜಂತು ಹುಳುವಿನ ವಿರುದ್ಧ ಲಭ್ಯವಿರುವ ಉದ್ದಿನ ಬೆಳೆಯ ತಳಿಗಳನ್ನು ಜಂತುರೋಗ ನಿರೋಧಕ ಶಕ್ತಿಗೆ ಪರೀಕ್ಷೆ ಮಾಡಲಾಯಿತು ಹಾಗೂ *ರೈಜೊಬಿಯ್‌ಂ* ಗಂಟುಗಳ (ನೊಡುಲೇಶನ್), ಬೆಳವಣಿಗೆ ಮತ್ತು ಇಳುವರಿ ನಿಯತಾಂಕಗಳ ಮೇಲೆ ಜಂತು ಹುಳುವಿನ ಪರಿಣಾಮವನ್ನು ಅಧ್ಯಯನ ಮಾಡಲಾಯಿತು. ದ್ವಿದಳ ಧನ್ಯಗಳನ್ನು ಬೆಳೆಯುವ ಪ್ರದೇಶಗಳಲ್ಲಿ ನಡೆಸಲಾದ ಸಮೀಕ್ಷೆಯಲ್ಲಿ ಹೆಚ್ಚಿನ ಸಂಖ್ಯೆಯಲ್ಲಿ *ಮೆಲಾಯ್ಡೊಗೈನೆ ಇನ್ಕಾಗ್ನಿಟಾ* (೬೫೭/ ೨೦೦ ಸಿಸಿ ಮಣ್ಣು ಮತ್ತು ೭೧/೫ಗ್ರಾಂ ಬೇರು) ನಂತರ *ಹೇಲಿಕೊಟೈಲಿಂಕಸ್* ಪ್ರಭೇದ (೭೦ ಮತ್ತು ೧೫), *ರೋಟಿಲೆಂಕುಲಸ್* ಪ್ರಭೇದ (೩೦ ಮತ್ತು ೨೮) ಮತ್ತು *ಪ್ರಾಟಿಲೆಂಕಸ್* ಪ್ರಭೇದ (೨೨ ಮತ್ತು ೧೭) ಚಾಮರಾಜನಗರ ಜಿಲ್ಲೆಯಲ್ಲಿ ಕಂಡು ಬಂದವು. ಗಾಜಿನ ಮನೆಯಲ್ಲಿ ರೋಗ ನಿರೋಧಕ ಪರಿಕ್ಷೆ ಮಾಡಿದ ೧೪ ಉದ್ದಿನ ಬೆಳೆ ತಳಿಗಳಾದ೨ಕೆಯು –೬೦, ಎಡಿಟಿ–೦೫, ಬಿಜಿ–೨, ಡಿಯು –೧, ಜಿ–೩೩೩, ಐಸಿ೨೮೨೦೦೭, ಐಸಿ–೪೩೬೫೪೫, ಕೆ–೫–೫೭೨, ಕೆಯು –೮–೧೫೫, ಎಲ್‌ಕೆಯು –೬೪, ರಶ್ಮಿ, ಅರ್‌ಯು –೧೦–೬೦೧, ಎಸ್‌ಯು –೫೧೯ ಮತ್ತು ಯುಹೆಚ್ –೦೪–೦೪ ತಳಿಗಳಲ್ಲಿ ಯುಹೆಚ್–೦೪–೦೪ ತಳಿಯು ಅತೀ ಹೆಚ್ಚು ಬೇರುಗಂಟಿನ ಜಂತುಹುಳು ಸೊಂಕಿಗೆ ಒಳಗಾಗಿದೆ, ಜಿ–೩೩೩ ತಳಿಯು ರೋಗ ನಿರೋಧಕ ಶಕ್ತಿಯನ್ನು ಹೊಂದಿರುವುದು ತಿಳಿದು ಬಂದಿದೆ. *ರೈಜೊಬಿಯ್‌ಂ* ಗಂಟುಗಳ (ನೋಡ್ಯುಲ್) ಸಂಖ್ಯೆಗಳು ಮತ್ತು ಗಿಡದ ಉದ್ದವು ಜಂತುಹುಳು ಭಾದೆಯಿಂದಾಗಿ ಕಡಿಮೆಯಾಗಿರುವುದು ತಿಳಿದು ಬಂದಿರುತ್ತದೆ. *ಮೆಲಾಯ್ಡೊಗೈನೆ ಇನ್ಕಾಗ್ನಿಟಾ*ವನ್ನು *ರೈಜೊಬಿಯ್‌*ಂಗಿಂತು ಮುಂಚೆ ಗಿಡಕ್ಕೆ ಸೇರಿಸಿದಾಗ ಹೆಚ್ಚಿನ ಪ್ರಮಾಣದಲ್ಲಿ ಬೆಳವಣಿಗೆ ಮತ್ತು ಇಳುವರಿ ನಿಯತಾಂಕಗಳ ಇಳಿಕೆಯನ್ನು ಕಾಣಲಾಯಿತು.



ಸಸ್ಯರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗ,

ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾನಿಲಯ,

ಜಿ.ಕೆ.ವಿ.ಕೆ. ಬೆಂಗಳೂರು–೬೫

**ಮುಖ್ಯ ಸಲಹೆಗಾರರ ಸಹಿ**

(ಎನ್. ಜಿ. ರವಿಚಂದ್ರ)

# Investigations on root knot nematode (*Meloidogyne incognita)* associated with black gram (*Vigna mungo* L.)

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## Introduction

- Black gram (*Vigna mungo* L.) belongs to the family Fabaceae.
- In India Black gram is popular known as "Urad dal" and it is highly prized than other pulses.
- It is also cultivated in Pakistan, Afghanistan, Bangladesh and Myanmar.
- Black gram grows normally in 90-120 days and also enriches the soil with nitrogen.
- It is best source of protein, fat, carbohydrates, iron, folic acid, calcium, magnesium, potassium and vitamin B.
- It has medicinal properties which help to heal rheumatic pain, stiff shoulder and contracted knees (Indira and Kurup, 2012).
- The crop is prone to many diseases *viz.*, cercospora leaf spot, bacterial leaf blight, anthracnose, powdery mildew, root rot, yellow mosaic disease, leaf crinkle, and root-knot nematode (*Meloidogyne incognita*).

## Objectives

1. Survey for incidence of root knot nematode associated with pulse crops in Southern Karnataka.
2. Screening of available cultivars of black gram against root knot nematode.

## Material and Methods

**Survey and collection of samples**

- Collection of soil and root samples randomly from the rhizosphere of black gram crop grown in southern Karnataka.
- Analysis of soil and root samples for the presence of root-knot nematode and other plant parasitic nematodes was done by following combined Cobb's sieving and Baermann's technique.

**Screening of available cultivars**

- Healthy seeds of fourteen black gram cultivars were sown individually in pots containing 1kg autoclaved soil.
- After germination, freshly hatched 2nd stage juveniles (J2) of *M. incognita* , were inoculated around the sown seeds @ 2,000 J2/ Kg soil.
- The observations on the number of galls/root system were taken and the susceptible, tolerant and resistant levels of cultivars were recorded based on the Root-Knot Index on 0 to 5 scale.

**Root-knot index for *M. incognita***

| Number of galls | Scale (based on number of root-knot galls/root) | Reaction |
|---|---|---|
| 0 | 1 | Highly Resistant |
| 1-10 | 2 | Resistant |
| 11-30 | 3 | Moderately Resistant |
| 31-100 | 4 | Susceptible |
| 101 and above | 5 | Highly Susceptible |

- **Experimental details**
  Crop : Black gram
  Design : CRD
  Cultivars : 14
  Replication : 4
- The experiment was conducted in the glass house belonging to Department of Plant Pathology, UAS, GKVK, Bengaluru-65.

## Results

- Survey revealed that *Meloidogyne incognita* was predominant with a maximum population of 99.7/200 cc of soil and 46.46/ 5g of root respectively followed by *Helicotylenchus multicinctus, Rotylenchulus reniformis* and *Pratylenchus penetrans.* Highest population of nematodes were recorded in Chamarajanagar district followed by Tumkuru, Mandya, Hassana and Mysuru districts.
- G-333 showed least number of galls per plant (6 at 60 days and 7.5 at harvest) with lower gall index 2. ADT-05 and 2KU-60 were also found to be significantly superior with less gall index than other cultivars tested.

Plate 1: General view of the experiment in the glass house


Plate 2: Reaction of black gram cultivars against *Meloidogyne incognita*

## Discussion

- *M. incognita* was predominant in black gram, green gram and cowpea compared to other plant parasitic nematodes. Similar results were reported by (Ali and Sharma, 2003).
- The cultivars G-333, ADT-05 and 2KU-60 showed resistant reaction to *M. incognita*. This is in line with the work of Adegbite *et al*. (2005).

Plate 3: General view of infested black gram field

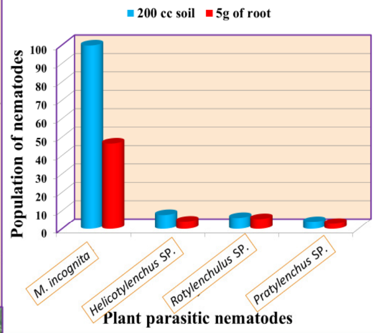


Fig.1 : Distribution of nematode spp. in soil and root samples of black gram

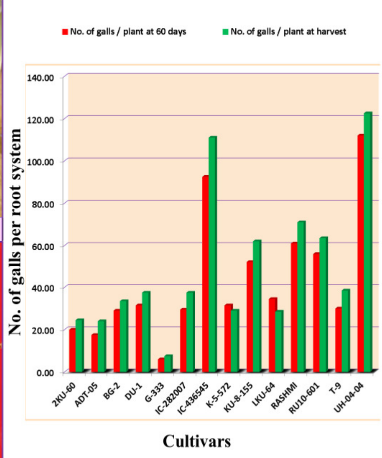


Fig.2: Reaction of black gram cultivars to *M. incognita* under pot culture

## Summary

- *M. incognita* was predominant in most areas surveyed compared to other plant parasitic nematodes with maximum population in Chamarajanagar district followed by Tumkuru, Mandya, Hassana and Mysuru districts.
- The cultivars G-333, ADT-05 and 2KU-60 were resistant to *M. incognita* than other cultivars tested.

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# I INTRODUCTION

Pulse occupies a unique position in the world's agriculture by virtue of its rich source of protein. The importance of pulses in the average Indian diet hardly needs to be emphasized as they constitute major source of protein for the predominantly vegetarian population of India. India is a major pulse producing country accounting for 28 per cent of total pulse production in the world. Pulses add average 30 kg nitrogen per hectare to the soil, besides providing nutritious fodder.

At present, in the world, pulses are grown on an area of 70.64 million hectares with a production and productivity of 59.28 million tonnes and 839 kg per hectare, respectively (Anon., 2014). India is the world's largest producer of pulses (18.5 million tonnes), largest importer (3.5 million tonnes) and largest consumer (22.0 million tonnes) (Anon., 2014).

Pulses are an affordable alternative to more expensive animal-based protein, ideal for improving diets in poorer parts of the country, where protein sources from milk is often five times more expensive than protein sourced from pulses. Beans, black gram, lentils, peas and chickpeas have been the cornerstone of global nutrition for centuries.

Black gram (*Vigna mungo* L.) belongs to the family Fabaceae, originated from India, where it has been in cultivation from ancient times and is one of the highly prized pulses of India and Pakistan. It has also been introduced to other tropical areas mainly by Indian immigrants. It is very nutritious as it contains high levels of protein (25 g/100 g), potassium (983 mg/100 g), calcium (138 mg/100 g), iron (7.57 mg/100 g), niacin (1.44 mg/100 g), thiamine (0.27 mg/100 g) and riboflavin (0.25 mg/100 g). Black gram complements the essential amino acids provided in most cereals and plays an important role in the diets of the people of India and Nepal. It has been shown to be useful in mitigating elevated cholesterol levels.

In India, it is mainly grown in the states of Madhya Pradesh, Maharashtra, Uttar Pradesh, Rajasthan, Karnataka and Bihar. In Karnataka, it is grown on an area of 1.02 lakh hectares with a production of 0.29 lakh tonnes (Anon., 2015). It is also used in making pastes for several south Indian dishes like idli, vada, dosa etc. Whether it be the very special "Dal makhni" of Punjab or the Vada Sambhar of South India, the taste rules the hearts of one and all alike. Indian immigrants have popularized the taste worldwide as well.

It is grown as rainfed crop in the warm plains as well as in the cool hills, up to an altitude of 2,000 metres. The cooking quality of black gram produced in the hills or in moist climate is claimed to be better. It prefers water retentive, stiff loamy or heavy soils and does well on both black cotton soils and brown alluviums. Being a proper leguminous crop, it has all the essential nutrients which makes it to turn into a fertilizer, with its ability to fix nitrogen it restores soil fertility as well. It proves to be a great rotation crop enhancing the yield of the main crop as well. For the best yields it should be grown in the seasons from mid-September to October and March to April and it grows normally in 90-120.

Nodulation is an ecologically and economically important plant phenomenon, in which the symbiosis between plants from the family Fabaceae and the bacterial genera rhizobia results in the development of nitrogen fixing nodules on host plant roots. The rhizobia-plant interaction is initiated by nod factors, which are produced by species-specific rhizobia. Nod factor alone is necessary and sufficient to produce many of the initial responses including ionic fluxes, root hair deformation, changes in gene expression and cortical cell differentiation (Weerasinghe, 2004).

Black gram suffers from many diseases namely, anthracnose (*Colletotrichum lindemuthianum*), bacterial leaf blight (*Xanthomonas phaseoli*), Cercospora leaf spot (*Cercospora canescens*), powdery mildew (*Erysiphe polygoni*), root rot (*Rhizoctonia solani*), rust (*Uromyces phaseoli*), stem canker (*Macrophomina phaseolina*), yellow mosaic (Mungbean Yellow Mosaic Virus) and leaf crinkle (Leaf Crinkle Virus) and plant parasitic nematodes. Among plant parasitic nematodes, root-knot nematodes are well known for their global distribution with wide host range and also involved in disease complexes.

The knowledge about biological nitrogen fixation at genetic, biochemical and physiological levels has expanded rapidly during past few decades. Even though numerous obstacles that limit maximum nitrogen fixation still remain unsolved, one of the biological factors affecting nodule formation and its functioning is the presence of phytonematodes in the rhizosphere (Taha, 1993), *Meloidogyne* spp. adversely affect nodulation and nitrogen fixation in pulses (Huang, 1987; Taha, 1993).

*Meloidogyne incognita has* been reported to be one of the major limiting factors in the production of pulses causing stunting of plants, reduction of nodules and yield losses to the tune of 30.1 to 67.5 per cent (Singh, 2015) and in black gram it causes about 12.40-46.19 per cent yield loss (Devi and Choudhary, 2014)

However, information with regard to the occurrence and distribution of root-knot nematode, resistant cultivar and effect on nodulation in black gram is lacking. Keeping the deficit of research in view, a study was undertaken with the following objectives

1. Survey for incidence of root knot nematode associated with pulse crops in southern Karnataka.

2. Screening of available cultivars of black gram for resistance against root knot nematode.

3. Effect of root knot nematode on nodulation, growth and yield parameter in black gram.

# II REVIEW OF LITERATURE

Black gram (*Vigna mungo* L.) is subjected to attack by many soil borne pathogens, among which root-knot nematode, *M. incognita* is a major constraint. Not much work has been done on this disease and literature available on these aspects is also meager. The detailed review on survey, screening and effect of *M. incognita* on nodulation, growth and yield parameter in black gram and other related crops is presented here under.

## 2.1 Survey for incidence of root knot nematode associated with pulse crops in southern Karnataka

Survey of soybean fields of Mannar, Sri Lanka was carried out by Lamberti and Ekanayake (1983), reported that *Meloidogyne incognita*, *M. areanaria*, *Rotylenchulus renifirmis*,. *Pratylenchus* sp., *Helicotylenchus* sp. and *Hoplolaimus* sp. were present in soybean field. Among these, *Meloidogyne incognita*, *M. incognita* caused severe growth reduction and the estimated yield loss of soybean due to this infection is around 45 per cent in infested fields.

A survey of 103 soybean fields was carried out to determine the incidence of *Meloidogyne* sp. in north-central Florida (Garcia and Rich, 1985). All fields sampled were infested with *Meloidogyne* sp. The most prevalent species was *M. incognita* found in 70 per cent of the soybean fields, followed by *M. javanica* in 24 per cent and *M. arenaria* in 6 per cent , the other plant-parasitic nematode genera commonly found in soil samples were *Pratylenchus, Criconemella, Trichodorus, Belonolaimus, Helicotylenchus, Xiphinema,* and *Hoplolaimus*.

Bernard and Jennings (1997) carried out a survey of *Meloidogyne incognita* in soybean, pasture and clover fields of southwestern Tennessee, *Meloidogyne incognita* was not found in a survey of pasture and soybean fields in southwestern Tennessee. The nematode's known range in the United States is restricted to the southwestern corner of Tennessee. However, its ability to infect many clovers could ensure its widespread, suitable hosts over much of the United States.

During a survey of black gram fields in the Aligarh district of Uttar Pradesh (Fazal *et al*., 1998) reported decline of the crop in some fields infested with both *Meloidogyne javanica* and *Rhizoctonia bataticola*.

Random surveys were taken in chickpea fields in Rajasthan and Infestations due to *Meloidogyne incognita* and *M. javanica* were observed in sandy soils of Jaipur, Jhunjhunu and Swai madhaopur both in rainfed and irrigated situations estimating 20.30 per cent yield loss. *Heterodera* was reported for the first time on chickpea and its incidence was recorded from Ajmer, Alwar, Bikaner, Jaipur, Nagaur, Sikar, Jhunjhunu, Swai Madhopur and Tonk districts. The lesion nematode (*Pratylenchus* sp.) was predominant in north eastern region (Ali and Sharma, 2003).

A survey was conducted by Olowe (2004) for *Meloidogyne incognita* in 248 major cowpea growing areas, spread over 31 States and the Federal Capital Territory in different ecological zones in Nigeria. The survey revealed the occurrence of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* singly or in combination in all the cowpea farms sampled. *M. incognita* (51.8 per cent ) was the most prevalent nematode followed by *M. javanica* (44.1 per cent ) and *M. arenaria* (4.1 per cent ). The greatest abundance of *M. javanica* (65.6 per cent ) was concentrated in the Sudan savannah of the core North (north-east and north-west) and that of *M. incognita* (82.1 per cent ) in the humid forest of the south Nigeria.

A nematode survey was conducted in the southernmost parts of Illinois by Allen *et al*. (2005). Among 294 fields surveyed eight nematode genera were detected, with four genera having considered being important soybean pathogens. Populations of *Meloidogyne incognita* were detected in 6 per cent of all fields surveyed in southern Illinois.

A survey of plant parasitic nematodes associated with pulse crops of Hamirpur district, Uttar Pradesh, India, was carried out by Ali *et al*. (2006). Totally 174 soil samples were collected around the roots of pulse crops, among them 63 soil samples of chickpea revealed the presence of *Hoplolaimus indicus* and *Tylenchorhynchus mashhoodi*, 21 soil samples collected from lentil, yielded five species of plant parasitic nematodes. Among them *T. mashhoodi* was most prominent and 72 soil samples of pigeon pea, yielded nine plant parasitic nematode species of which *T. mashhoodi* was the most abundant species.

A survey of the leguminous vegetables crops in seven districts of West Bengal, India, was done by Roy *et al*. (2007). Examination of root samples showed the presence of root-knot nematodes in all the seven districts on cowpea, dolichos bean, french bean and pea, but reniform nematode was found infecting cowpea and pea only. Analysis of soil samples showed the occurrence of 17 species of plant parasitic nematodes belonging to 8 genera. *Rotylenchulus* reniformis was found the most prominent followed by *Meloidogyne* sp.

*Meloidogyne incognita* is one of most important plant parasitic nematode of black gram with an extensive host range, wide area distribution with temperate area to the tropics of the world (Mahalik and Routray, 2009).

A survey was carried out in a cowpea (*Vigna unguiculata*) fields in the three primary agro-climatic zones of Burkina Faso in West Africa, 109 samples were collected, in those sample twelve plant-parasitic nematodes were identified, of which six appeared to have significant parasitic potential on cowpea based on their frequency and abundance, these included *Helicotylenchus* sp., *Meloidogyne* sp., *Pratylenchus* sp., *Scutellonema* sp., *Telotylenchus* sp. and *Tylenchorhynchus* sp. (Sawadogo *et al*., 2009).

The survey conducted by Kumar *et al*. (2010) in five districts of Rajasthan to know the different species of plant parasitic nematodes in cowpea, revealed that phytoparasitic nematode community structure of Udaipur district reflects *M. incognita* had maximum prominence value whereas in Rajsamand and Chittorgarh, the prominence value of *R. reniformis* was found maximum. In Jhunjhunu and Churu districts *Tylenchorhynchus* sp. had maximum prominence value.

A survey was carried out to know plant parasitic and other beneficial soil nematodes associated with various vegetables, cereal, oil-seed and pulses of 10 districts of Madhya Pradesh (Rathour *et al*., 2010). Analysis of nematode samples revealed 39 genera of plant parasitic and 9 genera of beneficial soil nematodes. Highest population of *M. incognita* was recorded, followed by *Helicotylenchus dihystera* and *Hoplolaimus indicus*.

Singh (2015) carried out a survey in pigeon pea fields of Bendelkhnad region of Uttar Pradesh and found thirteen nematode genera in the soil samples collected. Nematode genera observed were *Hoplolaimus*, *Tylenchorhynchus*, *Helicotylenchus*, *Hoplolaimus*, *Heterodera cajani*, *Pratylenchus*, *Meloidogyne* and *Rotylenchulus*. Out of eight districts surveyed, *Hoplolaimus* was most prominent in Jalaun district followed by Mahoba whereas *Tylenchorhynchus* was prominent in Kanpur district followed by Jalaun, *H. cajani* was most prominent in Mahoba district followed by Jalaun and Kanpur districts.

Anes and Gupta (2014) studied the distribution of plant parasitic nematodes in the soybean growing areas of India. Population analysis of 58 soil samples representing 23 districts of 14 states revealed the presence of *Helicotylenchus* sp. *Rotylenchulus* sp., *Pratylenchus* sp., *Hoplolaimus* sp., *Tylenchorhynchus* sp., *Heterodera* sp., *Rotylenchus reniformis*, *Hirschmanniella* sp., *Meloidogyne* sp. and *Trichodorus* sp., with varying population densities. Among the different plant parasitic nematodes recorded, spiral nematodes were found to be most widely distributed.

*Meloidogyne incognita* and *M. javanica* were the predominant nematode pests in local soybean production areas of South Africa (Fourie *et al*., 2015).

### 2.2 Screening of available cultivars of black gram for resistance against root knot nematode

Seven common bean cultivars were screened against *M. incognita*, among which cv. Contender was resistant to *M. incognita* races 2, 3 and 4, but it was only moderately resistant to race 1. Other common bean cultivars supported higher reproduction. A lower level of reproduction by race 4 occurred on common bean. cv. Manoa Wonder was resistant to all *Meloidogyne* sp. including *M. hapla*. The other cultivars ranged from moderately resistant to susceptible to *M. hapla* and they were less susceptible to *M. javanica* than to either race of *M. arenaria* or to *M. hapla*. Lima bean White Ventura N was very resistant to *M. arenaria* race 2 and to *M. javanica* (Hadisoeganda and Sasser, 1982).

Swanson and Gundy (1984) carried out screening of cowpea cultivars and the resistance was measured on the basis of nematode reproduction, cultivars like Magnolia blackeye, Mississippi purple, and Mississippi silver were more resistant to *M. incognita* than to *M. javanica*.

*M. chitwoodi* and *M. hapla* had no detrimental effect on any of the three cultivars of bean and six cultivars of peas tested (Santo and Ponti, 1985). Bean and pea cultivars were tolerant to *M. chitwoodi* and *M. hapla*. Only the growth of pea cultivar Dark Skin Perfection was adversely affected by *M. chitwoodi*

Choudhury *et al*. (1985) screened sixteen lines/varieties of four pulse crops. four each of mung bean (*Vigna radiata*), black gram (*Vigna mungo*), lentil (Lens culinaris) and kheshari (*Lathyrus sativus*) were screened for resistance to *Meloidogyne* sp. Mung bean cv. K-851, lentil L-79504 and L-79546 and kheshari K-3975 were resistant. Two lentil lines L-5 and L-79554 exhibited highly resistant reactions. The remaining mung bean, lentil and kheshari lines were found to be moderately susceptible and those of black gram were highly susceptible.

Moore *et al*. (1988) conducted a greenhouse study to evaluate twenty eight pigeon pea accessions resistance to *Meloidogyne incognita* race 3 and *M. javanica*. 24 of 28 accessions inoculated with *M. incognita* were free of galls and egg masses. When inoculated with *M. javanica* 10 of 28 pigeon pea accessions were not galled and egg masses were not observed.

Bouton *et al* . (1989) carried out a greenhouse screening of twelve alfalfa cultivars for resistance to southern root-knot nematode, all 12 alfalfa cultivars were differed in per cent age of resistance, but all had fewer galls than 'Lahontan' (susceptible check) and most were as resistant as 'Moapa 69' (resistant check).

Sharma *et al*. (1993) evaluated 66 accessions of *Cajanus*, *Rhynchosia*, and *Flemingia* (wild relatives of pigeon pea) for resistance to *M. javanica* under greenhouse. Thirty-five accessions had less than 10 galls, five accessions had very small or no galls. ICPW 92 was highly resistant to *M. javanica* and 38 other accessions were resistant. Accessions of *Flemingia* sp. and *Rhynchosia* sp. showed greater susceptibility than accessions of *Cajanus* sp.

Thirty-four pigeon pea cultivars and 227 germplasm accessions were evaluated for resistance to *M. javanica* based on number of galls, egg masses, size of galls and area of root covered with galls. Galls were not formed on 75 per cent of the cultivars and no egg masses were observed on the roots of four cultivars (UPAS 120, Pant A3, CO 1, and BDN 2); however, shoot mass of 64 per cent of the cultivars was reduced by *M.javanica*. Pant A3, ANM 504 and BDN 2 were identified as highly resistant to *M. javanica* (Sharma *et al*., 1994).

Sharma *et al*. (1994) screened 25 accessions, 173 advanced breeding lines and five chickpea cultivars for resistance to *M. javanica* in a green house. All the accessions and cultivars were found susceptible and all the tested breeding lines were highly susceptible to *M. javanica* and more than 25 per cent were killed.

Screening of different accessions of *Cajanus platycarpus*, a wild relative of pigeon pea, in the greenhouse for resistance to *R. reniformis* race A, *H. cajani* and *M. javanica* races 1 and 3 was done by Sharma (1995). Two accessions ICPWs 60 and 62 were resistant to *R. reniformis* race A. ICPWs 62, 69 and 70 were resistant to *H. cajani*. All the accessions were susceptible to *M. javanica* races (Race 1 produced bigger galls than those produced by race 3).

Bernard and Jennings (1997) evaluated the ability of *Meloidogyne trifoliophila* to infect 230 species and cultivars of plants in a green house. All clovers (*Trifolium* sp.) were severely galled regardless of species or cultivar. Most soybean cultivars were moderately to severely galled. Among other legumes, broad bean, garden pea, Korean lespedeza and sweet clover were good hosts, but alfalfa, bird's-foot trefoil, peanut and pole bean were poor or non-hosts. Among other plant families most Apiaceae (Umbelliferae) and Brassicaceae (Cruciferae) were galled.

Thirteen popular cultivars of chickpea were screened for their reaction to *F. oxysporum* f.sp. *ciceri*, *M incognita* alone and to their combinations (Rao and Krishnappa, 1999). All the tested cultivars were found highly susceptible to root-knot nematode and the reaction was not altered even in the presence of fungus.

Jain and Trivedi (2000) evaluated 47 chickpea cultivars for their reaction to *M. incognita,* all the varieties were found to be susceptible at varying degrees however, variety RSG 564 was found to be highly susceptible and RSG 617 least susceptible.

All chickpea cultivars screened for the resistance against *Meloidogyne incognita*, favored nematode development in terms of gall formation per root system and population densities in soil and root. Lower degree of gall formation per root system was observed in cv. Nes 95004 followed by Nes 950012, Nes 950193 compared to other cultivars. And Nes 90122, Nes 93127 and Nes 950174 limited nematode population density in soil and root (Hussain *et al*., 2001).

Choudhury *et al*. (2002) evaluated 149 varieties of cowpea against *M incognita*, 19 were resistant with gall index 1.1-2.0, 42 moderately resistant with gall index2.1-3.0, 61susceptible with gall index 3.1-4.0 and 27 highly susceptible with gall index 4.1-5.0.

Four hundred and twenty five varieties of tomato, pea, cowpea, coriander, fenugreek, cluster bean and bottle guard were screened against root-knot nematode *Meloidogyne incognita* in naturally infested field, cv. BCKV-2 of cowpea found to be resistant (Yadav and Chand, 2003).

Field studies were conducted to assess the reaction of 15 varieties of cowpea for resistance to a natural infestation of *Meloidogyne incognita* race 2 (Adegbite *et al*., 2005). The degree of reaction of the different genotypes to the nematode differed significantly, IT84S-2246-4 was found resistant.

Allen *et al*. (2005) evaluated 18 genotypes against *Meloidogyne incognita*, six out of 18 genotypes had less galling and nematode reproduction than other genotypes, five genotypes were resistant and four were moderately resistance based on gall index.

Thirty-three cultivars of lentil were screened for resistance to *M. incognita-F. oxysporum* disease complex (Haseeb *et al*., 2005). All the cultivars showed a wide range of response *i.e*. from resistant to susceptible to both pathogens. None of the cultivars was found highly resistant to any of the 2 pathogens. Nine cultivars were found resistant and 14 cultivars were found moderately resistant to *M. incognita*.

Green house studies were conducted for 23 selections of field pea (*Pisum sativum*) against *Meloidogyne incognita*. Out of 23 selections HFP-990713, Pant P-25 and HFP-0129 were resistant; Pant P-2005, NDP-2 and Pant P-42 were tolerant; LFP-305, HFP-8909, HFP-4, HUP-31, HFP-0128, Pant P-31, Pant P-40, LFP-363 and HFP-0118 were moderately resistant; HFP-0110, HUDP-28, HUDP-15, HUDP-27, HUP-30, HUP-2 and HUDP-26 were moderately susceptible; and only Ambika was susceptible to *M. incognita*. They observed that reproduction of nematode was favored on tolerant and susceptible cultivars but inhibited on resistant ones (Anita *et al*., 2006).

Kutywayo *et al*. (2006) assessed the response of cotton and soybean cultivars against *Meloidogyne javanica* and *Meloidogyne incognita* races 1 and 3 in Zimbabwe. Soybean cultivars were all susceptible to the three species except SNK60, which was resistant to *M. incognita* race 1.

Simon and Dass (2010) carried out a screening operation of different varieties/lines of chickpea (141), field-pea (55), lentil (41) and pigeon pea (70) against *Meloidogyne incognita*. Out of 141 varieties of chickpea only 8 were found moderately resistant (MR), rest of the chickpea lines were categorized as highly susceptible and susceptible, none of the screened variety of chickpea showed highly resistant (HR) and resistant (R) reaction. Out of 55 varieties of field-pea screened, only Pant P-74 was resistant and eight varieties were moderately resistant, while remaining showed susceptible or highly susceptible reaction. Out of Forty one varieties of lentil screened, nine varieties were shown resistant and twelve varieties shown moderately resistant, rest are susceptible and highly susceptible to *M. incognita*.

Field experiments were conducted for two consecutive years to access the reaction of 10 accessions of pigeon pea for resistance to natural infestation of *Meloidogyne incognita*. Out of 10 accessions none was resistant and none was immune to *M. incognita* under field conditions. Two accessions were tolerant under field conditions (Cc 10B and Cc 12), with grain yield ranging between 1356 and 1300 kg/ha in 2006 and 1300 and 1200 kg/ha in 2007, a gall index rating of <2 and a reproduction factor of >1, while the rest eight accessions were susceptible to *M. incognita* under field conditions with grain yield ranging between 1035 and 896 kg/ha in 2006 and 1025 and 865 kg/ha in 2007, with a gall index rating of >2 and a reproduction factor of >1. Based on reproduction factor, it was concluded that all the susceptible pigeon pea accessions supported greater nematode reproduction (Adegbite *et al*., 2011).

Mccord (2012) screened thirty genotypes against *M. chitwoodi* race 2 or *M. hapla* and the reproductive factor (RF) was considered. Among 30 genotypes tested, 16 had an RF of zero. For *M. hapla*, a large portion of genotypes (approximately 30 per cent ) also had low RF values, but a significant number also supported large numbers of nematodes.

Common bean was assessed for resistance against temperate root-knot nematodes (Wesemael and Moens, 2012). The tested cultivars were poor to good hosts for *Meloidogyne chitwoodi*, non-hosts or bad hosts for *Meloidogyne fallax* and excellent hosts for *Meloidogyne hapla*. Significantly penetration of *Meloidogyne hapla* took place over a longer period than that of *Meloidogyne chitwoodi* and *Meloidogyne fallax*.

Brahma and Borah (2013) screened thirty-six varieties of pea for their reaction to *M. incognita* under net house conditions. Pea variety Bonneville was taken as susceptible check. Out of 36 varieties, 26 varieties were found to be susceptible (Root-knot index=3.1- 4) and remaining 10 varieties were found highly susceptible (Root-knot index= 4.1- 5). None of the varieties was found resistant to *M. incognita.*

Fourteen varieties of soybean were evaluated against *Meloidogyne* sp., varieties TGX1485-1D, TGX1805-31F, TGX18436E and Cameroon were found resistant to the nematode infection (Ibiam *et al*., 2014).

Twenty-eight germplasm of black gram were tested against *Meloidogyne incognita* race-2, under greenhouse condition. Out of them 25 germplasm were susceptible with the gall index of 4 and 3 germplasm were highly susceptible with the gall index of 5. No resistant or moderately resistant genotypes were recorded (Devi and Choudhary, 2014).

Bruinsma and Antoniolli (2015) screened 12 soybean genotypes against *Meloidogyne incognita*. Among them CEPsBt 09036, CEPsBt 10129, CEPsRR 07224 genotypes were found susceptible, lowest gall index values were observed in genotypes CEPsBt 09036, CEPsBt 10129.

## 2.3 Effect of root knot nematode on nodulation, growth and yield parameter in black gram

Studies involving *Meloidogyne* and *Heterodera* sp. have shown that their invasion into the roots of leguminous hosts can completely prevent nodulation with subsequent reduction in plant growth (Robinson, 1961)

The effect of *Meloidogyne javanica* and *Heterodera trifolii* on number, size, structure and efficiency of nodules formed by *Rhizobium* on white clover roots was investigated by Taha and Raski (1969). The nitrogen fixation efficiency of nematode infected nodules was not impaired; however, earlier disintegration of nodules as a result of *M. javanica* infection ultimately deprived the plants of nitrogenous materials. The drastic reduction of the total nitrogen in nematode infected plants mirrored stunting of the entire plant due to nematode infection.

Balasubramanium (1971) observed that when the nematode population was high they interfered directly with the establishment of *Rhizobium japonicum* bacterium due to lowered production of root hairs in *M incognita* infected plants. It caused reduction in nodulation. Reduced number of nodules might also be due to overall reduction of the root system as a result of nematode infection.

Nematode induced reduction in symbiotic nitrogen fixation in leguminous plants was observed in white clover infected by *M. javanica* and *Heterodera trifolii* (Lehman *et al*., 1971).

Minchin and Pate (1973) observed that inoculation of nematodes ultimately led to the reduced production and supply of carbohydrates to the nodules to fix atmospheric nitrogen.

Bopaiah *et al*. (1976) observed significant reduction in the growth and dry weight of the shoot and root in mung bean in the treatments with nematode alone, and nematode followed by *Rhizobium* compared to the treatment with *Rhizobium* followed by the nematode.

Stimulated nodule formation in soybean infected with *M. hapla* and *Pratylenchus penetrans* was reported by (Hussey and Barker, 1976).

Trabulsi and Elsamca (1980) carried out a greenhouse experiment and histologic investigations to determine interactions between root-knot nematode and root-nodule. Cowpea plants inoculated with both *Rhizobia* and nematodes showed more severe nitrogen deficiency and retarded growth than plants inoculated with nematodes only or uninoculated plants. Nematode invasion reduced the numbers of nodules and inhibited nitrogen fixation by about 63 per cent in nodular tissue. Infected nodules contained different developmental stages of the nematode. Histological studies indicated that nematodes developed in nodular tissue, and they were found inside vascular bundles. Many giant cells with thickened walls were produced and exhibited the morphologic characteristics of transfer cells. The nematodes did not alter the structure of nodules, but bacteriodes did not develop adjacent to nematodes. Infected nodules deteriorated earlier than uninfected ones.

Trabulsi *et al*. (1980) carried out a study to know the effect of *Meloidogyne incognita* on nodulation and growth of soybean and found that root-knot nematode infestation significantly affected the growth of the plant, number of nodules and nitrogen fixation compared with the uninfected control and growth of soybean is markedly improved by the presence of *Rhizobium japonicum*.

A relative decrease of the constituent of leghemoglobin in soybean nodules infected by *H. glycines* was observed by Huang and Baker (1983).

Ko *et al*. (1984) studied the impact of *H. glycines* on the nodulation of soybean and suggested that nematodes would compete for photoassimilates coming from the shoot, and thus inhibiting nodulation.

*Meloidogyne incognita* caused reduction in the number of nodules, dry weight of nodules, dry weight of shoot, total nitrogen uptake and chlorophyll contents of leaves of mung bean (*Vigna radiaia* L.) cv. G-65. The functioning of nodules was adversely affected by nematode infection which could be attributed to reduction in the bacteroids, leghaemoglobin contents of nodules and reduced supply of photosynthate to the nodules (Chahal and Chahal, 1987).

*H. glycines* caused a reduction in the synthesis of leghemoglobin in *Rhizobium* nodules as well as alterations of its four components (Huang, 1987)

Root length, shoot length and the weight of plants were significantly reduced by root knot nematode infestation. Nematode inoculation in various combinations retarded the plant growth showing infestation by the nematode as the limiting factor irrespective of the sequence of inoculation in relation to the *Rhizobium*. However plants showed comparatively better growth when the nematode inoculation was delayed by fortnight. On the other hand, the delayed inoculation of *Rhizobium* was not sufficiently useful to the plants since the nematode had already established itself. Heavy damage to the tender roots of the young plants caused the growth retarding effect of the nematode. Nematode infestation significantly reduced the number of nodules, a greater reduction in number of nodules was observed when the nematode was established before the inoculation of the bacteria (51 per cent) than the nematode and *Rhizobium* inoculated together (30 per cent) or when the *Rhizobium* was established before the inoculation of nematode (12 per cent). (Sharma and Tiagi, 1990).

Dalal and Bhatti (1996) observed a significant reduction in plant growth characters at an inoculum level of 100 juveniles per plant and above in both the sets (*Rhizobium* treated and untreated), although significant reduction in weight and number of nodules was observed at 1000 juveniles/plant. With an increase in the inoculum level there was a corresponding increase in number of galls and egg masses.

Infection of nodulated roots by nematodes caused N deficiency symptom (Barker and McGawley, 1998).

Fazal *et al*. (1998) found that reduction in plant growth, nodulation and yield of black gram in the presence of nematode or fungus was directly and nematode multiplication was indirectly proportional to inoculum levels. Significant damage to plant growth, nodulation and yield due to pathogenic infection by *M. javanica* or *R. bataticola* even at a low inoculum level indicate that the crop is a good host for both pathogens.

Infection *M. incognita* could considerably affect the plant growth of soybean due to the formation of deformed galled roots, which inhibit the uptake and absorption of nutrients from soil (Ake and Ganepola, 2010). The nematode also affects the soybean nodulation due to the inhibition of the development of lateral roots and root hairs. The severity of this effect is directly proportional to the level of nematode infection.

Mahapatra and Swain (2001) found that *Meloidogyne incognita* alone could reduce 25 per cent of nodulation, whereas higher inoculum of nematode followed by *Fusarium oxysporum* significantly decreased bacterial nodulation in black gram.

Nematodes may change not only the amino acid composition of nodulated plants but also the concentration. In addition, the N fixation process is affected with consequent reduction of ureides, nematode (*M. javanica*) presence caused a more pronounced decrease in amino acid concentration, the small decrease in ureides represented a larger decrease of transported N (Carneiro *et al*., 2002).

The plants inoculated with *Rhizobium* + VAM and VAM alone significantly increased plant growth characters and number of nodules over the plants inoculated with nematode alone. All the treatments, except with *Rhizobium* + nematode were found to be effective in increasing plant growth parameters, number of nodules and number of pod. Rhizobium + VAM + nematode was found to be effective in reducing galls, egg masses on the roots of black gram and nematode population in soil over the plants inoculated with nematode alone (Mahanta and Phukan, 2007).

Ali (2009) carried out a study to estimate unavoidable yield losses in chickpea, field pea and lentil crops in the field trials. Root-knot nematode had incurred unavoidable yield losses up to 25.6 per cent in chickpea and 15 per cent each in pea and lentil.

In the presence of *Bradyrhizobium*, damage to black gram growth was significantly less, except in treatments where bacterial application followed nematode inoculations, compared to those where *Bradyrhizobium* was not applied (Bhat *et al*., 2009). Bacterial application appreciably increased the nitrogen content of root and shoot in all the treatments irrespective of the time of application. Leghaemoglobin, bacteriod and nitrogenase activities in nematode infected plants was higher in prior inclusion of *Bradyrhizobium* and least in treatments which were applied 10 days after nematode inoculation.

*Meloidogyne incognita* infestation resulted in significant decrease in the growth of black gram, root-nodule development, nitrogen contents of root and shoot and nitrogenase activity at all inoculum levels. *Bradyrhizobium* and *Paecilomyces lilacinus* resulted in significantly lesser damage to plant growth of black gram than the plants treated with bacteria at the time of inoculation with *Meloidogyne incognita*, followed by plants where bacteria and fungus was applied 10 days after nematode inoculation. *Bradyrhizobium* significantly increased the nitrogen content of root and shoot in all the treatments. Nitrogenase activities in nematode infected plants was higher in plants treated with *P. lilacinus* and *Bradyrhizobium* before and at the time of nematode inoculation in comparison to plants which were treated by *P. lilacinus* and *Bradyrhizobium* 10 days after nematode inoculation (Bhat *et al*., 2009)

Mahalik and Routray (2009) carried out an investigation to determine the effect of *Meloidogyne incognita* on growth traits of black gram. Experiment revealed that there was a reduction of flowering time (5.2 per cent), plant height (14.1 per cent), root length (14.02 per cent ), shoot dry weight (10.99 per cent ), root dry weight (4.24 per cent) and effective nodules (13.02 per cent ) due to *M. incognita* infection.

Infection by root-knot nematodes decreased the number of functional nodules in black gram either through inhibition in overall nodulation or premature conversion of functional nodules into nonfunctional ones due to nematode invasion (Akhtar *et al*., 2013).

Abbasi and Hisamuddin (2014) carried out an investigation to know the effect of *Meloidogyne incognita* on growth and biochemical parameters of a leguminous plant *viz*., *Vigna radiata* cv. PDM 139 under greenhouse condition. With the increase in inoculum level of *M. incognita*, there was a progressive decrease in growth and biochemical parameters of the crop, significant reduction in plant length, fresh and dry weight, leaf area, chlorophyll, seed protein, nitrogenase and leghaemoglobin contents in the root nodules at 400 $J_2$, while at higher inoculum levels *i.e*., 800 $J_2$ and 1,600 $J_2$, the reduction was more pronounced.

Reduced nitrogenase activity was observed in nematode infected nodules (Azhagumurugan and Rajan, 2015). Functioning of nitrogenase was directly related to leghaemoglobin content as it regulates the diffusion of oxygen.

Sahoo *et al*. (2016) studied the effect of *Rhizobium* and *Meloidogyne incognita* on black gram and assessed potential role of nematode parasitism on nodulation. Addition of *M. incognita* to *Rhizobium* treated plants adversely affected the nodulation process to the extent of 31.8 per cent which clearly indicated that *M. incognita* acted as a limiting factor in nodule formation process.

# III MATERIAL AND METHODS

The experiments were conducted to study the occurrence of root-knot nematode on pulse crops, screening of available genotypes and effect of *M. incognita* on nodulation, growth and yield parameter in black gram at the Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bengaluru. The details of material used and the procedures adopted are described in this chapter.

## 3.1 Survey for incidence of root knot nematode associated with pulse crops in southern Karnataka

A survey was conducted in major pulse growing areas of southern Karnataka to study the occurrence of different plant parasitic nematodes. Fields showing uneven patches with yellowing, stunted growth, delayed flowering and root galling were selected for collection of soil and root samples.

### 3.1.1 Collection of soil and root samples

Soil and root samples from 5 to 10 spots were collected randomly with the aid of shovel in the root zone of standing black gram, cowpea and green gram. Later a composite sample of 200 cc soil and 5 g roots were collected in a polythene bag with proper labeling. Information pertaining to the crop, locality, soil condition etc., was also collected along with the samples as mentioned below in the data sheet.

**Survey data Sheet**

**Survey for major plant parasitic nematodes associated with pulse crops grown under field condition**

1. Name of reporter :

2. Sample Number :

3. Date of collection :

4. Name of the cultivar :

5. Locality: a. Village :

   b. Taluk :

   c. District :

6. Name of the crop :

7. Total cropped area :

8. Stage of the crop :

9. Condition of the crop :

a) Symptoms if any: I. Aboveground :

II. Root system :

b) Percentage of plants infected :

c) Total area infected :

10. Previous crop :

11. Soil type :

12. Meteorological data :

13. Other diseases :

14. Any other information :

(Plant protection chemicals,

Fertilizer etc., applied)

The collected samples were preserved under refrigerated condition and later analyzed for the presence of plant parasitic nematodes.

**3.1.2. Estimation of nematode population in soil samples**

Soil sample of 200 cc were washed thoroughly and processed using combined "Cobb's sieving and Baermann's funnel method" (Ayoub, 1977) as given below.

1. Two hundred cc of soil was taken in 1000 ml beaker and sufficient quantity of water was added to make soil solution.

2. This was stirred thoroughly and allowed to stand for heavier particles to settle down.

3. Then the soil solution was passed through a set of sieves of 100, 250, 325 and 400 mesh sizes, respectively.

4. Residue from 325 and 400 mesh sieves were collected and poured over a tissue paper spread on a wire gauge and placed on Baermann's funnel.

5. Level of water in the Baermann's funnel was maintained to keep the tissue paper wet and left undisturbed for 48 hr.

6. After incubation of 48 hr. the volume of suspension was made to 200 ml, out of which 10 ml was pipetted out and used for counting of various plant parasitic nematodes present. Nematode population from this was finally estimated for 200 cc soil.

### 3.1.3. Estimation of nematode population in root samples

Nematode population in 5 g of roots was estimated by root incubation method (Ayoub, 1977) as explained below:

**Procedure**

1. Roots were gently washed to remove adhering soil particles.

2. Washed roots were cut into small bits of 2.5 cm and split longitudinally.

3. Then placed over tissue paper spread on a wire gauge and kept in a Petri plate filled with water.

4. Level of water was maintained in Petri plate and left undisturbed for 48 hours.

5. Later, the suspension in the Petri plate was collected and observed for nematodes using stereo binocular microscope.

### 3.1.4. Maintenance and multiplication of pure culture

### 3.1.5. Soil sterilization

1. Red sandy loam soil free from lumps and stones was collected, sieved and mixed thoroughly with compost in 6:1 proportion.

2. Cleaned soil mixture was transferred to a cement tank and four percent formalin was added (for every 9” soil bed) for sterilization.

3. Soil bed was made airtight by covering with polythene sheet.

4. Polythene sheet was removed after 48 hr. of fumigation and then the soil was spread to facilitate escape of toxic fumes, if any.

5. Sterilized soil was tested to confirm that no living nematodes exist in it. Soil was stored in clean polythene bags for further use in the investigation.

### 3.1.6. Maintenance of cultures of root-knot nematode (*Meloidogyne incognita*)

Culture of *M. incognita* from black gram field was collected and maintained at greenhouse belonging to AICRP (Nematodes), Department of Plant pathology, University of Agriculture Sciences, GKVK, Bengaluru. Well matured single egg mass was separated from the gall and kept in a Syracuse dish containing distilled water for hatching. Later, hatched larvae were used for inoculating to black gram seedlings grown in sterilized soil for buildup of the single egg mass culture of *M. incognita*.

### A. Identification of root-knot nematode species

1. The roots infested with root-knot nematode were gently washed free of soil

2. The females were dissected out from well-developed galls of the root under stereo-binocular microscope and transferred to Petri plate containing water. The posterior portion of the female was cut with a perennial pattern-cutting knife (Taylor and Netscher, 1974) and the body contents were cleaned.

3. Cleaned posterior portion of the female was further trimmed and transferred on to a drop of glycerin taken on a clean microscopic slide.

4. A cover slip was placed on it, sealed with nail polish and observed under stereo-binocular microscope. The species confirmation was done based on the perennial pattern as described by Chitwood (1949).

### B. Taxonomic position of nematode

| | |
|---|---|
| Phylum | : Nematoda |
| Class | : Phasmidia (Secernentia) |

| | |
|---|---|
| Order | : Tylenchida |
| Sub order | : Tylenchina |
| Sub family | : Heteroderoidea |
| Family | : Heteroderidae |
| Genus | : *Meloidogyne* |
| Species | : *incognita* |

### 3.2. Screening of available cultivars of black gram for resistance against root knot nematode

#### 3.2.1. Experimental site

A pot culture experiment was carried out in the greenhouse belonging to the Department of Plant Pathology, University of Agriculture Sciences, GKVK, Bengaluru. The field is situated at $13^0$N latitude and 77' $38^0$ E longitude and an altitude of 899 meters above the mean sea level.

#### 3.2.2. Soil characteristics

Soil characteristics of the experimental location are red sandy loam in texture. Soils have low to medium water holding capacity in the profile.

#### 3.2.3. Screening

The black gram cultivars including the local cultivars and other lines were screened for their reaction to *M. incognita* in glass house belonging to Department of Plant Pathology, University of Agricultural Sciences, GKVK campus, Bengaluru-65. A pot study was conducted to assess the level of resistance or susceptibility of the prevailing cultivars to the root-knot nematode. A total of fourteen cultivars *viz*., 2KU-60, ADT-05, BG-2, DU-1, G-333, IC-282007, IC-436545, K-5-572, KU-8-155, LKU-64, RASHMI, RU10-601, T-9 and UH-04-04 were screened against the root-knot nematode. The cultivars were collected from Department of Genetics and Plant breeding, UAS, GKVK, Bengaluru.

The severity of the disease was recorded at monthly intervals. Five plants were selected in each accession of black gram and assessed for the tolerance to the diseases. The observations on the number of galls/root system were taken and the susceptible, tolerant and resistant levels of cultivars or lines were recorded based on the Root-Knot Index on 0 to 5 scale.

### 3.2.4. Root-knot index for *M. incognita* (Anon, 1993)

| Number of galls | Scale (based on number of root-knot galls/root) | Reaction |
|---|---|---|
| 0 | 1 | HR (Highly Resistant) |
| 1-10 | 2 | R(Resistant) |
| 11-30 | 3 | MR(Moderately Resistant) |
| 31-100 | 4 | S(Susceptible) |
| 101 and above | 5 | HS(Highly Susceptible) |

### 3.2.5. Effect of root knot nematode on nodulation, growth and yield parameter in black gram

A pot experiment was conducted in the glasshouse belonging to Department of Plant Pathology, University of Agricultural Sciences, GKVK campus, Bengaluru to evaluate effect of *M. incognita* on nodulation, growth and yield parameter.

The *Rhizobium* culture for black gram was procured from Dept. of Agricultural Microbiology**,** University of Agricultural Sciences, GKVK campus, Bengaluru and a culture of *M. incognita* maintained on tomato plants under AICRP (Nematodes) was used for inoculation.

**Experimental details**

Crop : Black gram

Variety : T-9

Duration : 60 days

Design : CRD

Soil type : Sandy loam soil

Treatments : 6

Replication : 4

**Treatment details:**

$T_1$ = *M. incognita* alone

$T_2$ = *Rhizobium* alone

$T_3$ = *M. incognita* + *Rhizobium*

$T_4$ = *M. incognita* followed by *Rhizobium* after two weeks

$T_5$ = *Rhizobium* followed by *M. incognita* after two weeks

$T_6$ = Untreated check

### 3.2.6 Sowing

The uniformly sized seeds of black gram were selected for sowing. Nematode free sterilized mixture of soil, vermicompost and sand was filled in 15cm diameter earthen pots with a capacity of 2 kg soil. After germination the seedlings were thinned off to maintain one seedling per pot. The pots were watered regularly whenever required. Four replications were maintained for each treatment with three plants in each replication. The pots were maintained at 25-30°C for 60 days under glasshouse conditions.

### 3.2.7. Imposition of treatments

Treatments as listed above were replicated four times and applied to one week old seedlings.

### 3.2.8. Effect of *M. incognita* on nodulation

Observations were recorded sixty days from the first inoculation on height and fresh and dry weight of roots and shoots. The effect on bacterial nodulation was estimated by counting the number of nodules on the root. The rating of root-knot index of each plant was recorded on the basis of 0-5 scale.

### 3.2.9. Observations recorded

**Growth parameters of the host**

Plant height (cm) : Recorded from the base to the tip of the leaf at 30 days interval up to harvest (centimeter).

2) Number of leaves : Counted at different interval.

4) Root length (cm) : Recorded from base of the stem to the tip of the roots, at harvest in centimeter.

5) Root weight (g) : Fresh and dry root weights were recorded after termination of the experiment. After uprooting the plants, the root and portion of the plants were cut and dried in an oven at 60ºC till the constant weight is reached and the dry weight was noted down.

6) Shoot weight (g) : Fresh and dry shoot weights were recorded after termination of the experiment. After uprooting the plants, shoot portion of the plants were cut and dried in an oven at 60ºC till the constant weight is reached and the dry weight was noted down.

6) Nodule number : Counted at different interval.

7) Nitrogen content (Per cent): Recorded from dry shoot and root samples.

8) Number of pods : Counted at different interval.

**On the nematode**

1) Nematode Population in soil : The nematode population in soil was estimated at 30 days interval up to harvest.

Procedure:

I. Two hundred cc soil was taken in a plastic pan and sufficient quantity of water was added to make the soil solution.

II. This was stirred thoroughly and allowed to stand for a minute for the heavier particles to settle down.

III. Then the soil solution was passed through a set of sieves of 100, 250, 325 and 400 mesh sizes respectively.

IV. Residues from 325 and 400 mesh sieves were collected and poured over tissue paper on a wire gauge placed on a Baerman's funnel.

V. Level of water in the funnel was maintained to keep the tissue paper wet and left undisturbed for 48 hours.

The suspension was then collected in a beaker and volume was made to 200 ml. The suspension was stirred and ten ml of aliquot was drawn and transferred to a counting dish and examined under stereo binuclear microscope and nematodes were counted. The nematode counts from this were converted to 200 cc soil by multiplying with a common factor 20.

2) Nematode Population in root: The nematode population in root was estimated at 30 days interval up to harvest.

3) Number of galls per root system: Recorded at the time of harvest by counting number of galls per root system.

4) Number of egg masses per root system: Recorded at the time of harvest. The number of egg masses of *M .incognita* per root system was counted after exposing the infected roots to 0.25 per cent tryphan blue for 3 minutes.

### 3.2.10. Effect of *M. incognita* on nitrogen content.

#### Sowing

Surface sterilized black gram cv. T-9 seeds were sown singly in l5cm polythene lined earthen pots. 2000 freshly hatched J2 of *M. incognita* per Kg of soil were inoculated into the root zone of each plant. The plants were fed biweekly, throughout the experimental period with N-free Hoagland nutrient solution and watered as required.

To study the effect of *M. incognita* on nitrogen content in black gram. Plants were uprooted at 30 and 60 days from the first inoculation. Shoots and roots were oven dried, powdered and nitrogen content was estimated by Kjeldhal method.

#### Kjeldhal method

The Kjeldahl method consists of three steps digestion, distillation and titration. The sample is first digested in strong sulfuric acid in the presence of a catalyst, which helps in the conversion of the amine nitrogen to ammonium ions.

1. The ammonium ions are then converted into ammonia gas, heated and distilled.

2. The ammonia gas is led into a trapping solution where it dissolves and becomes an ammonium ion once again

3. Finally the amount of the ammonia that has been trapped is determined by titration with a standard solution and a calculation made.

**Step one: Digestion**

1. Weighed out approximately 1g of the sample containing protein and placed the sample into a digestion flask, along with 12-15 ml of concentrated sulfuric acid ($H_2SO_4$).

2. Seven grams of potassium sulfate and a catalyst, usually copper were added.

3. Digestion tube/flask and mixture were brought to a "rolling boil" (about 370°C to 400°C) using a heating block.

4. Heating was carried until mixture in the tube/flask produced white fumes and then continued the heating for about 60-90 min.

**Step two: Distillation**

The purpose of the distillation is to separate the ammonia (that is, the nitrogen) from the digestion mixture.

1. Raised the pH of the mixture using sodium hydroxide (45 per cent NaOH solution). This had the effect of changing the ammonium ($NH_4^+$) ions (which are dissolved in the liquid) to ammonia ($NH_3$), which is a gas.

2. Separated the nitrogen away from the digestion mixture by distilling the ammonia (converting it to a volatile gas, by raising the temperature to boiling point) and then trapped the distilled vapors in a special trapping solution of about 15 ml HCl (hydrochloric acid).

3. Removed the trapping flask and rinsed the condenser with water so as to make sure that all the ammonia has been dissolved.

**Step Three: Titration**

As the ammonia dissolved in the acid trapping solution, it neutralized some of the HCL it finds there. What acid is left is "back titrated", that is titrated with a standard, known solution of base (usually NaOH). In this way the amount of ammonia distilled off from the digestive solution is calculated and hence the amount of nitrogen in the protein determined.

**Determination of Ammonia**

1. Indicator dye is added to the acid/ammonia trapping solution. This dye turned a strong color, indicating that a significant amount of the original trapping acid is still present.

2. Standard solution of NaOH (sodium hydroxide) is added into the buret and slowly, slowly added small amounts of the sodium hydroxide solution to the acid solution with the dye.

3. Observed for the point at which the dye turns orange, indicating that the "endpoint" has been reached and that now all the acid has been neutralized by the base.

4. Recorded the volume of the neutralizing base (sodium hydroxide solution) that was necessary to reach the endpoint.

5. Performed a calculation to find the amount of ammonia and thus nitrogen that came from the original sample.

# IV EXPERIMENTAL RESULTS

A preliminary survey was undertaken on the occurrence of root-knot nematode in the rhizosphere of pulse crops in southern Karnataka districts namely; Chamarajanagara, Hassana, Mysuru, Mandya and Tumakuru. The available cultivars of black gram were evaluated for their reaction against *M. incognita* and effect of nematode on the nodulation, growth and yield parameter in black gram were studied under greenhouse conditions. The results obtained are presented in this chapter.

**Symptomatology**

The *M. incognita* infested pulse crops exhibited characteristic symptoms like stunted growth coupled with slight chlorosis of the foliage. When these plants were uprooted moderate to severe galling was noticed along with severely reduced root growth. Mortality of the infested plants was also noticed during the survey and is depicted in Plate 1.

**Identification**

Root-knot nematode populations obtained from infested plants from various locations in southern Karnataka districts were identified based on the Perennial Pattern technique as described by Chitwood (1949).

**4.1 Survey for incidence of root knot nematode associated with pulse crops in southern Karnataka**

A preliminary survey was carried out on the occurrence of root-knot nematode associated with the rhizosphere of major pulse crops like black gram, green gram and cow pea in southern Karnataka districts. The major pulse growing areas are Chamarajanagara, Hassana, Mysuru, Mandya and Tumakuru. The soil and root samples collected from these places were brought to the laboratory and analyzed for the presence of root-knot nematode and the results are presented in Table 1, 2, 3, 4, 5 and 6 and Map.1.

The survey revealed that in 26 samples of each crop *M. incognita* was predominant in black gram with a maximum total soil population of 2593 followed by *Helicotylenchus* sp. (198), *Rotylenchulus* sp. (156) and *Pratylenchus* sp. (98).

**4.1.1 Black gram**

In Chamarajanagara district, maximum soil population (114/200 cc soil) of *M. incognita* was recorded in Shivapura followed by Honnur (113 /200cc soil), Katanavadi (110 /200cc soil), Alur and Mallligehalli (109 /200cc soil) and the least population was recorded at Nagavalli (102 /200cc soil). In Hassana district, maximum soil population (114/200 cc soil) of *M. incognita* was recorded at Alisandra followed by Thimmalapura (102/200cc soil), Kanihalli (97/200cc soil), Bisalihalli (89/200cc soil) and the least was at Hiriyur (78/200cc soil).

In Tumkuru district, maximum soil population (119/200 cc soil) of *M. incognita* was recorded at Madhihalli followed by Aralikere (112/200cc soil), Puttamadhihalli (108/200cc soil), Ganganaghatta (103/200cc soil), keragodu (102/200cc soil) and the least was recorded at Nonavinakere (98/200cc soil). In Mandya district, maximum soil population (105/200 cc soil) of *M. incognita* was recorded at Uthukere followed by Huskur and Ankanathapur (97/200cc soil), Kulgere (89/200cc soil), Mallanathapura (83/200cc soil) and the least was recorded at Hucche gowdana doddi (78/200cc soil).

In Mysuru district, maximum soil population (96/200 cc soil) of *M. incognita* was recorded at Chamalapura followed by Kottegala (87/200cc soil) and the least was recorded at Krishnapura (83/200cc soil). Among the districts, maximum total soil population (657) of *M. incognita* was recorded in Chamarajanagara district followed by Tumkuru district (642), Mandya district (549), Hassana district (480) and the least was recorded at Mysuru district(266) (Table 1).

The root samples collected from the rhizosphere of black gram in southern Karnataka districts were analyzed for nematode population. The samples revealed *M. incognita* as a predominant nematode with a maximum total root population (323) followed by *Rotylenchulus* sp. (90), *Helicotylenchus* sp. (77) and *Pratylenchus* sp. (72). In Chamarajanagara district, maximum root population (17/5 g root) of *M. incognita* was recorded in Shivapura followed by Honnur (16/5 g root), Mallligehalli (14/5 g root), Katanavadi (9/5 g root), Nagavalli (8/5 g root) and the least was recorded at Alur (7/5 g root).

In Hassana district, maximum root population (15/5 g root) of *M. incognita* was recorded at Alisandra followed by Thimmalapura (13/5 g root), Bisalihalli (11/5 g root), Hiriyur (9/5 g root) and the least was recorded at Kanihalli (8/5 g root). In Tumkuruu district, maximum root population (17/5 g root) of *M. incognita* was recorded at Madhihalli followed by Aralikere (16/5 g root), Ganganaghatta and keragodu (13/5 g root), Nonavinakere (11/5 g root) and the least was recorded at Puttamadhihalli (8/5 g root). In Mandya district, maximum root population (18/5 g root) of *M. incognita* was recorded at Ankanathapura followed by Hucche gowdana doddi (16), Mallanathapura (15 g root), Uthukere (13/5 g root), Kulgere (13/5 g root) and the least was recorded at Huskur (10/5 g root).

In Mysuru district, maximum root population (14/5 g root) of *M. incognita* was recorded at Kottegala followed by Krishnapura (10/5 g root) and the least was recorded at Chamalapura (8/5 g root). The maximum root population (85) of *M. incognita* was recorded in Mandya district, followed by Tumkuru district (79), Chamarajanagara district (71), Hassana district (56) and the least population was recorded at Mysuru

**Table 1. Soil population of major plant parasitic nematodes associated with the rhizosphere of black gram in southern Karnataka**

| Locality | | Nematode population per 200 cc soil | | | | |
|---|---|---|---|---|---|---|
| | | *Meloidogyne incognita* | *Helicotylenchus* spp. | *Rotylenchulus* spp. | *Pratylenchus* spp. | **Total** |
| **CHAMARAJANAGAR DISTRICT** | | | | | | |
| 1 | Nagavalli | 102 | 12 | 5 | 3 | 125 |
| 2 | Shivapura | 114 | 13 | 5 | 2 | 117 |
| 3 | Alur | 109 | 25 | 6 | - | 120 |
| 4 | Katanavadi | 110 | 9 | 4 | 9 | 119 |
| 5 | Mallligehalli | 109 | 7 | 5 | 8 | 103 |
| 6 | Honnur | 113 | 4 | 5 | - | 87 |
| | **Total** | 657 | 70 | 30 | 22 | |
| **HASSANA DISTRICT** | | | | | | |
| 1 | Kanihalli | 97 | 11 | 4 | 7 | 119 |
| 2 | Bisalihalli | 89 | 5 | 8 | - | 102 |
| 3 | Hiriyur | 78 | 7 | 7 | 8 | 100 |
| 4 | Thimmalapura | 102 | 4 | 7 | 13 | 126 |
| 5 | Alisandra | 114 | 10 | 9 | - | 133 |
| | **Total** | 480 | 37 | 35 | 28 | |
| **TUMKUR DISTRICT** | | | | | | |
| 1 | Madhihalli | 119 | 4 | 4 | - | 110 |
| 2 | Aralikere | 112 | 9 | 6 | 28 | 157 |
| 3 | Puttamadhihalli | 108 | 11 | 6 | 3 | 129 |
| 4 | Ganganaghatta | 103 | 12 | - | 1 | 123 |
| 5 | Nonavinakere | 98 | - | 6 | - | 115 |
| 6 | keragodu | 102 | 3 | 7 | 3 | 126 |
| | **Total** | 642 | 39 | 29 | 35 | |
| **MANDYA DISTRICT** | | | | | | |
| 1 | Uthukere | 105 | 4 | 4 | 1 | 128 |
| 2 | Ankanathapura | 97 | - | 5 | 4 | 121 |
| 3 | kulgere | 89 | 4 | 5 | - | 117 |
| 4 | Huskur | 97 | 12 | 0 | - | 115 |
| 5 | Mallanathapura | 83 | - | 20 | - | 118 |
| 6 | Hucche gowdana doddi | 78 | 11 | 9 | 7 | 129 |
| | **Total** | 549 | 31 | 43 | 12 | |
| **MYSURU DISTRICT** | | | | | | |
| 1 | Krishnapura | 83 | 6 | 9 | - | 98 |
| 2 | Chamalapura | 96 | 7 | 5 | 1 | 109 |
| 3 | Kottegala | 87 | 8 | 5 | - | 100 |
| | **Total** | 266 | 21 | 19 | 1 | |
| | Grand total | 2593 | 198 | 156 | 98 | 3045 |
| | Mean | 99.7 | 7.61 | 6 | 3.76 | 117 |

**Table 2. Root population of major plant parasitic nematodes associated with the rhizosphere of black gram in southern Karnataka**

| Locality | | Nematode population per 5g of root | | | | |
|---|---|---|---|---|---|---|
| | | *Meloidogyne incognita* | *Helicotylenchus* spp. | *Rotylenchulus* spp. | *Pratylenchus* spp. | Total |
| **CHAMARAJANAGAR DISTRICT** | | | | | | |
| 1 | Nagavalli | 8 | 4 | 5 | - | 12 |
| 2 | Shivapura | 17 | 1 | 5 | 4 | 27 |
| 3 | Alur | 7 | - | 4 | 4 | 15 |
| 4 | Katanavadi | 9 | - | 9 | - | 18 |
| 5 | Mallligehalli | 14 | 5 | 3 | 8 | 30 |
| 6 | Honnur | 16 | 5 | 2 | 1 | 24 |
| | **Total** | 71 | 15 | 28 | 17 | |
| **HASSANA DISTRICT** | | | | | | |
| 1 | Kanihalli | 8 | - | 2 | - | 10 |
| 2 | Bisalihalli | 11 | - | 3 | 3 | 17 |
| 3 | Hiriyur | 9 | 9 | 2 | 1 | 21 |
| 4 | Thimmalapura | 13 | 3 | 2 | 4 | 22 |
| 5 | Alisandra | 15 | 7 | 4 | - | 26 |
| | **Total** | 56 | 19 | 13 | 8 | |
| **TUMKUR DISTRICT** | | | | | | |
| 1 | Madhihalli | 17 | 4 | 5 | 9 | 35 |
| 2 | Aralikere | 16 | 7 | - | - | 23 |
| 3 | Puttamadhihalli | 8 | - | 3 | 1 | 12 |
| 4 | Ganganaghatta | 14 | - | - | - | 14 |
| 5 | Nonavinakere | 11 | 7 | 2 | - | 20 |
| 6 | keragodu | 13 | - | 3 | 2 | 18 |
| | **Total** | 79 | 18 | 13 | 12 | |
| **MANDYA DISTRICT** | | | | | | |
| 1 | Uthukere | 13 | - | 9 | 2 | 24 |
| 2 | Ankanathapura | 18 | 8 | - | - | 26 |
| 3 | kulgere | 13 | - | 9 | 9 | 31 |
| 4 | Huskur | 10 | - | - | 7 | 17 |
| 5 | Mallanathapura | 15 | 3 | 6 | 6 | 30 |
| 6 | Hucche gowdana doddi | 16 | 4 | 5 | - | 25 |
| | **Total** | 85 | 15 | 29 | 24 | |
| **MYSURU DISTRICT** | | | | | | |
| 1 | Krishnapura | 10 | 4 | 4 | 5 | 23 |
| 2 | Chamalapura | 8 | 6 | 3 | 2 | 19 |
| 3 | Kottegala | 14 | - | - | 4 | 18 |
| | **Total** | 32 | 10 | 7 | 11 | |
| | Grand total | 323 | 77 | 90 | 72 | 562 |
| | Mean | 12.43 | 2.96 | 3.46 | 2.76 | 21.61 |

### 4.1.2 Green gram

The samples collected from the rhizosphere of green gram in southern Karnataka districts were analyzed for nematode population. The samples revealed *M. incognita* as a predominant nematode with a maximum total soil population (2550) followed by *Helicotylenchus* sp. (158), *Rotylenchulus* sp. (118) and *Pratylenchus* sp. (53).

In Chamarajanagara district, maximum soil population (119/200 cc soil) of *M. incognita* was recorded in Honnur followed by Katanavadi (103/200cc soil), Mallligehalli (102/200cc soil), Nagavalli (97/200cc soil), Shivapura (89/200cc soil) and the least population was recorded at Alur (78/200cc soil). In Hassana district, maximum soil population (114/200 cc soil) of *M. incognita* was recorded at Bisalihalli followed by Thimmalapura (110/200cc soil), Hiriyur and Alisandra (109/200cc soil) and the least was at Kanihalli (102/200cc soil). In Tumkuru district, maximum soil population (125/200 cc soil) of *M. incognita* was recorded at Nonavinakere followed by Aralikere (119/200cc soil), Puttamadhihalli (112/200cc soil), Ganganaghatta (108/200cc soil) and the least was recorded at keragodu (98/200cc soil).

In Mandya district, maximum soil population (107/200 cc soil) of *M. incognita* was recorded at Ankanathapura followed by Mallanathapura (105/200cc soil), Uthukere and kulgere (102/200cc soil), Hucche gowdana doddi (101/200cc soil) and the least was recorded at Huskur(83/200cc soil). In Mysuru district, maximum soil population (87/200 cc soil) of *M. incognita* was recorded at Chamalapura followed by Krishnapura (86/200cc soil) and the least was recorded at Kottegala (83/200cc soil). Among the districts, maximum soil population (600) of *M. incognita* was recorded in Mandya district followed by Chamarajanagara district (588), Tumkuru district (562), Hassana district (544) and the least was recorded at Mysuru district (256) (Table 3).

The root samples collected from the rhizosphere of green gram in southern Karnataka districts were analyzed for nematode population. The samples revealed *M. incognita* as a predominant nematode with a maximum total root population (294) followed by *Helicotylenchus* sp. (83), *Pratylenchus* sp. (83) and *Rotylenchulus* sp. (59). In Chamarajanagara district, maximum root population (18/5 g root) of *M. incognita* was recorded in Katanavadi followed by Nagavalli (16/5 g root), Alur (14/5 g root), Shivapura (13/5 g root), Honnur (11/5 g root) and the least was recorded at Mallligehalli (7/5 g root). In Hassana district, maximum root population (16/5 g root) of *M. incognita* was recorded at Kanihalli followed by Bisalihalli (13/5 g root), Hiriyur (12/5 g root), Alisandra (9/5 g root) and the least was recorded at Thimmalapura (5/5 g root).

In Tumkuruu district, maximum root population (15/5 g root) of *M. incognita* was recorded at Ganganaghatta followed by Aralikere (13/5 g root), Puttamadhihalli (12/5 g root), Nonavinakere (8/5 g root), Madhihalli (5/5 g root) and the least was recorded at keragodu (3/5 g root). In Mandya district, maximum root population (19/5 g root) of *M. incognita* was recorded at Mallanathapura followed by Ankanathapura (14/5 g root), Huskur (12/5 g root), kulgere (7/5 g root), Hucche gowdana doddi (5/5 g root) and the least was recorded at Uthukere (4/5 g root). In Mysuru district, maximum root population (19/5 g root) of *M. incognita* was recorded at Krishnapura followed by Chamalapura (16/5 g root) and the least was recorded at Kottegala (8/5 g root).

The maximum root population (79) of *M. incognita* was recorded in Chamarajanagara district followed by Mandya district (61), Tumkuru district (56), Hassana district (55) and the least population was recorded at Mysuru district (43) (Table 4).

**4.1.3 Cowpea**

The soil samples collected from the rhizosphere of cowpea in southern Karnataka districts were analyzed for nematode population. The samples revealed *M. incognita* as a predominant nematode with a maximum total soil population (2373) followed by *Helicotylenchus* sp. (106), *Rotylenchulus* sp. (50) and *Pratylenchus* sp. (30). In Chamarajanagara district, maximum soil population (106/200 cc soil) of *M. incognita* was recorded in Alur, followed by Shivapura (102/200cc soil), Katanavadi (98/200cc soil), Honnur (93/200cc soil), Mallligehalli (87/200cc soil) and the least population was recorded at Nagavalli (83/200cc soil).

In Hassana district, maximum soil population (103/200 cc soil) of *M. incognita* was recorded at Alisandra followed by Thimmalapura (95/200cc soil), Kanihalli (91/200cc soil), Bisalihalli (87/200cc soil) and the least was at Hiriyur (88/200cc soil). In Tumkuru district, maximum soil population (108/200 cc soil) of *M. incognita* was recorded at Aralikere followed by Madhihalli (104/200cc soil), Puttamadhihalli (92/200cc soil), Nonavinakere (86/200cc soil), keragodu (84/200cc soil) and the least was recorded at Ganganaghatta (83/200cc soil). In Mandya district, maximum soil population (96/200 cc soil) of *M. incognita* was recorded at Huskur followed by Mallanathapura (91/200cc soil), Ankanathapura (88/200cc soil), Uthukere and Hucche gowdana doddi (83/200cc soil) and the least was recorded at kulgere (74/200cc soil).

In Mysuru district, maximum soil population (94/200 cc soil) of *M. incognita* was recorded at Krishnapura followed by Chamalapura (91/200cc soil) and the least was recorded at Kottegala (83/200cc soil). Among the districts, maximum soil population (569) of *M. incognita* was recorded in Chamarajanagara district followed by Tumkuru district (557), Mandya district (515), Hassana district (464) and the least was recorded at Mysuru district (268) (Table 5).

The root samples collected from the rhizosphere of cowpea in southern Karnataka districts were analyzed for nematode population. The samples revealed *M. incognita* as a predominant nematode with a maximum total root population (272) followed by *Helicotylenchus* sp.(73), *Rotylenchulus* sp. (69) and *Pratylenchus* sp. (62). In Chamarajanagara district, maximum root population (16/5 g root) of *M. incognita* was recorded in Nagavalli followed by Alur (14/5 g root), Shivapura (11/5 g root),

**Table 3. Soil population of major plant parasitic nematodes associated with the rhizosphere of green gram in southern Karnataka**

| Locality | | Nematode population per 200 cc soil | | | | |
|---|---|---|---|---|---|---|
| | | *Meloidogyne incognita* | *Helicotylenchus* spp. | *Rotylenchulus* spp. | *Pratylenchus* spp. | **Total** |
| **CHAMARAJANAGAR DISTRICT** | | | | | | |
| 1 | Nagavalli | 97 | 9 | 5 | - | 111 |
| 2 | Shivapura | 89 | 7 | - | 7 | 103 |
| 3 | Alur | 78 | 4 | - | - | 82 |
| 4 | Katanavadi | 103 | 11 | 9 | 8 | 131 |
| 5 | Mallligehalli | 102 | 5 | 12 | 13 | 132 |
| 6 | Honnur | 119 | 7 | 15 | - | 141 |
| | **Total** | 588 | 43 | 41 | 28 | |
| **HASSANA DISTRICT** | | | | | | |
| 1 | Kanihalli | 102 | 4 | 7 | 3 | 116 |
| 2 | Bisalihalli | 114 | 4 | - | - | 118 |
| 3 | Hiriyur | 109 | 10 | - | - | 119 |
| 4 | Thimmalapura | 110 | 3 | 3 | 1 | 117 |
| 5 | Alisandra | 109 | 9 | - | 4 | 122 |
| | **Total** | 544 | 30 | 10 | 8 | |
| **TUMKUR DISTRICT** | | | | | | |
| 1 | Madhihalli | - | 12 | 9 | - | 21 |
| 2 | Aralikere | 119 | 15 | 8 | - | 142 |
| 3 | Puttamadhihalli | 112 | - | 9 | - | 121 |
| 4 | Ganganaghatta | 108 | 3 | - | 7 | 118 |
| 5 | Nonavinakere | 125 | - | 8 | 2 | 135 |
| 6 | Keragodu | 98 | 4 | 12 | - | 114 |
| | **Total** | 562 | 34 | 46 | 9 | |
| **MANDYA DISTRICT** | | | | | | |
| 1 | Uthukere | 102 | 4 | - | - | 106 |
| 2 | Ankanathapura | 107 | 4 | 9 | - | 120 |
| 3 | Kulgere | 102 | 9 | - | 3 | 114 |
| 4 | Huskur | 83 | 12 | 5 | - | 100 |
| 5 | Mallanathapura | 105 | 7 | - | 2 | 114 |
| 6 | Hucche gowdana doddi | 101 | 5 | - | - | 106 |
| | **Total** | 600 | 41 | 14 | 5 | |
| **MYSURU DISTRICT** | | | | | | |
| 1 | Krishnapura | 86 | 4 | 3 | - | 93 |
| 2 | Chamalapura | 87 | - | - | 1 | 88 |
| 3 | Kottegala | 83 | 6 | 4 | 2 | 95 |
| | **Total** | 256 | 10 | 7 | 3 | |
| | Grand total | 2550 | 158 | 118 | 53 | 2879 |
| | Mean | 98.07 | 6.07 | 4.53 | 2.03 | 110 |

**Table 4. Root population of major plant parasitic nematodes associated with the rhizosphere of green gram in southern Karnataka**

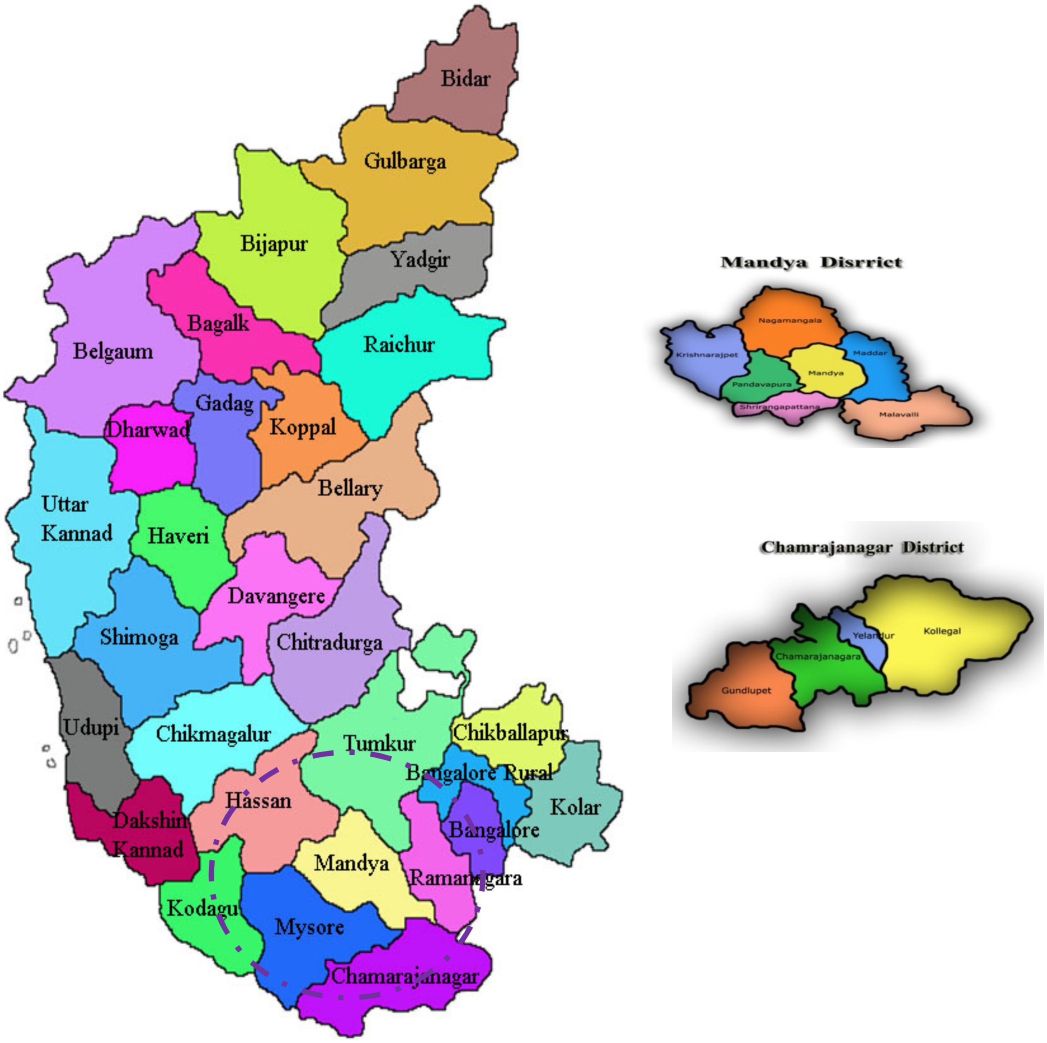
| Locality | | Nematode population per 5g root | | | | |
|---|---|---|---|---|---|---|
| | | *Meloidogyne incognita* | *Helicotylenchus* spp. | *Rotylenchulus* spp. | *Pratylenchus* spp. | **Total** |
| **CHAMARAJANAGAR DISTRICT** | | | | | | |
| 1 | Nagavalli | 16 | 3 | 9 | 5 | 33 |
| 2 | Shivapura | 13 | 5 | 1 | 5 | 24 |
| 3 | Alur | 14 | 9 | 5 | 1 | 29 |
| 4 | Katanavadi | 18 | - | - | - | 18 |
| 5 | Mallligehalli | 7 | 5 | - | 2 | 14 |
| 6 | Honnur | 11 | 6 | 4 | 9 | 30 |
| | **Total** | 79 | 28 | 19 | 22 | |
| **HASSANA DISTRICT** | | | | | | |
| 1 | Kanihalli | 16 | - | 5 | 8 | 29 |
| 2 | Bisalihalli | 13 | - | 4 | - | 17 |
| 3 | Hiriyur | 12 | - | - | 4 | 16 |
| 4 | Thimmalapura | 5 | 8 | 5 | 6 | 24 |
| 5 | Alisandra | 9 | 6 | - | 7 | 22 |
| | **Total** | 55 | 14 | 14 | 25 | |
| **TUMKUR DISTRICT** | | | | | | |
| 1 | Madhihalli | 5 | 7 | 2 | 6 | 20 |
| 2 | Aralikere | 13 | 6 | 3 | 2 | 24 |
| 3 | Puttamadhihalli | 12 | - | - | - | 12 |
| 4 | Ganganaghatta | 15 | 3 | 2 | 4 | 24 |
| 5 | Nonavinakere | 8 | 2 | 1 | 3 | 14 |
| 6 | Keragodu | 3 | - | 3 | - | 6 |
| | **Total** | 56 | 18 | 11 | 15 | |
| **MANDYA DISTRICT** | | | | | | |
| 1 | Uthukere | 4 | 4 | 2 | 2 | 12 |
| 2 | Ankanathapura | 14 | 3 | - | - | 17 |
| 3 | Kulgere | 7 | - | 3 | 7 | 17 |
| 4 | Huskur | 12 | 5 | 3 | 3 | 23 |
| 5 | Mallanathapura | 19 | 4 | 2 | - | 25 |
| 6 | Hucche gowdana doddi | 5 | - | 2 | 4 | 11 |
| | **Total** | 61 | 16 | 12 | 16 | |
| **MYSURU DISTRICT** | | | | | | |
| 1 | Krishnapura | 19 | 4 | 1 | - | 24 |
| 2 | Chamalapura | 16 | - | 2 | 3 | 21 |
| 3 | Kottegala | 8 | 3 | - | 2 | 13 |
| | **Total** | 43 | 7 | 3 | 5 | |
| | Grand total | 294 | 83 | 59 | 83 | 519 |
| | Mean | 11.3 | 3.19 | 2.26 | 3.19 | 19.96 |

**Table 5. Soil population of major plant parasitic nematodes associated with the rhizosphere of cowpea in southern Karnataka**

| Locality | | Nematode population per 200 cc soil | | | | |
|---|---|---|---|---|---|---|
| | | *Meloidogyne incognita* | *Helicotylenchus* spp. | *Rotylenchulus* spp. | *Pratylenchus* spp. | **Total** |
| **CHAMARAJANAGAR DISTRICT** | | | | | | |
| 1 | Nagavalli | 83 | 12 | 5 | - | 100 |
| 2 | Shivapura | 102 | 9 | - | 3 | 114 |
| 3 | Alur | 106 | - | - | 2 | 108 |
| 4 | Katanavadi | 98 | 4 | 10 | - | 112 |
| 5 | Mallligehalli | 87 | - | 6 | 2 | 95 |
| 6 | Honnur | 93 | 3 | - | 1 | 97 |
| | **Total** | 569 | 28 | 21 | 8 | |
| **HASSANA DISTRICT** | | | | | | |
| 1 | Kanihalli | 91 | 9 | 3 | - | 103 |
| 2 | Bisalihalli | 87 | 12 | 2 | - | 101 |
| 3 | Hiriyur | 88 | - | - | - | 88 |
| 4 | Thimmalapura | 95 | 4 | - | 2 | 101 |
| 5 | Alisandra | 103 | - | 5 | 1 | 109 |
| | **Total** | 464 | 25 | 10 | 3 | |
| **TUMKUR DISTRICT** | | | | | | |
| 1 | Madhihalli | 104 | 4 | 1 | - | 109 |
| 2 | Aralikere | 108 | 4 | - | - | 115 |
| 3 | Puttamadhihalli | 92 | - | 2 | 2 | 96 |
| 4 | Ganganaghatta | 83 | 12 | - | - | 95 |
| 5 | Nonavinakere | 86 | 6 | 2 | 3 | 97 |
| 6 | keragodu | 84 | - | - | 2 | 86 |
| | **Total** | 557 | 26 | 5 | 7 | |
| **MANDYA DISTRICT** | | | | | | |
| 1 | Uthukere | 83 | 3 | 5 | 4 | 95 |
| 2 | Ankanathapura | 88 | 9 | - | 2 | 99 |
| 3 | kulgere | 74 | - | 4 | - | 78 |
| 4 | Huskur | 96 | 2 | - | - | 98 |
| 5 | Mallanathapura | 91 | 3 | - | 1 | 95 |
| 6 | Hucche gowdana doddi | 83 | - | - | - | 83 |
| | **Total** | 515 | 17 | 9 | 7 | |
| **MYSURU DISTRICT** | | | | | | |
| 1 | Krishnapura | 94 | 4 | 3 | 3 | 104 |
| 2 | Chamalapura | 91 | - | - | - | 91 |
| 3 | Kottegala | 83 | 6 | 2 | 2 | 93 |
| | **Total** | 268 | 10 | 5 | 5 | |
| | Grand total | 2373 | 106 | 50 | 30 | 2559 |
| | Mean | 91.26 | 4.07 | 1.92 | 1.15 | 98.42 |

**Table 6. Root population of major plant parasitic nematodes associated with the rhizosphere of cowpea in southern Karnataka**

| Locality | | Nematode population per 5g root | | | | |
|---|---|---|---|---|---|---|
| | | *Meloidogyne incognita* | *Helicotylenchus* spp. | *Rotylenchulus* spp. | *Pratylenchus* spp. | **Total** |
| **CHAMARAJANAGAR DISTRICT** | | | | | | |
| 1 | Nagavalli | 16 | 2 | 2 | 5 | 25 |
| 2 | Shivapura | 11 | 3 | - | 4 | 18 |
| 3 | Alur | 14 | - | 7 | - | 21 |
| 4 | Katanavadi | 9 | 2 | 3 | 5 | 19 |
| 5 | Mallligehalli | 8 | 1 | - | - | 9 |
| 6 | Honnur | 6 | 3 | 4 | 2 | 15 |
| | **Total** | 64 | 11 | 16 | 16 | |
| **HASSANA DISTRICT** | | | | | | |
| 1 | Kanihalli | 7 | 4 | - | - | 11 |
| 2 | Bisalihalli | 13 | - | 2 | 3 | 18 |
| 3 | Hiriyur | 12 | 3 | 9 | 2 | 26 |
| 4 | Thimmalapura | 17 | 7 | 4 | 5 | 33 |
| 5 | Alisandra | 9 | 5 | 3 | 3 | 20 |
| | **Total** | 58 | 19 | 18 | 13 | |
| **TUMKUR DISTRICT** | | | | | | |
| 1 | Madhihalli | 18 | 5 | 2 | 2 | 27 |
| 2 | Aralikere | 13 | 4 | - | - | 17 |
| 3 | Puttamadhihalli | 10 | - | 7 | 3 | 20 |
| 4 | Ganganaghatta | 4 | - | 3 | 3 | 10 |
| 5 | Nonavinakere | 3 | 5 | - | 2 | 10 |
| 6 | keragodu | 6 | 4 | 4 | 2 | 16 |
| | **Total** | 54 | 18 | 16 | 12 | |
| **MANDYA DISTRICT** | | | | | | |
| 1 | Uthukere | 3 | 6 | 4 | - | 13 |
| 2 | Ankanathapura | 9 | - | - | 4 | 13 |
| 3 | kulgere | 16 | 3 | 3 | 3 | 25 |
| 4 | Huskur | 13 | 2 | 7 | - | 22 |
| 5 | Mallanathapura | 14 | 3 | 3 | 4 | 24 |
| 6 | Hucche gowdana doddi | 11 | 2 | - | 3 | 16 |
| | **Total** | 66 | 16 | 17 | 14 | |
| **MYSURU DISTRICT** | | | | | | |
| 1 | Krishnapura | 10 | 6 | - | 4 | 20 |
| 2 | Chamalapura | 13 | - | 2 | - | 15 |
| 3 | Kottegala | 7 | 3 | - | 3 | 13 |
| | **Total** | 30 | 9 | 2 | 7 | |
| | Grand total | 272 | 73 | 69 | 62 | 476 |
| | Mean | 10.46 | 2.8 | 2.65 | 2.38 | 18.30 |




**Map 1: Occurrence of *M. incognita* associated with major pulse crops in southern Karnataka**

**Yellowing**

**Stunting**

**Galling on the roots**

**Reduced root growth**

**Plate 1: Symptoms of root-knot nematode (*M. incognita*) infestation on black gram**

**Infested roots of green gram**

**Plate 2: Symptoms of root-knot nematode (*M. incognita*) infestation on green gram and cowpea**

**Table 7. Identification of *Meloidogyne incognita***

| Characters | Original description Eisenback (1985) | Observed |
|---|---|---|
| Body | Male : Elongated larvae.<br>Female: Typical saccate, spheroid with a distinct neck. | Male: Elongated larvae.<br>Female: Spheroid with a distinct neck. |
| Stylet | Male: Strong with rounded knob.<br>Female : More slender than in males or larvae but with strong basal knob. | Male: Strong rounded knob<br>Female: Slender stylet. |
| Oesophagous | With very large median bulb followed by short isthmus. | Large median bulb followed by short isthmus. |
| Excretory pore | Often seen with part of excretory tube in the area between posterior stylet knobs and oppose to median bulb. | Often seen with part of excretory tube in the area between posterior stylet knobs and oppose to median bulb. |
| Vulva and anus | Female: Typically opposite to neck. Perennial patterns of females typically somewhat oval with a high, irregular, squarish dorsal arch composed of closely spaced wavy lines. | Perennial pattern: oval with a high, squarish, dorsal irregular arch with closely spaced wavy lines. |
| Spicules | Very near the terminus of males. | Very near the terminus of males. |

(9/5 g root), Mallligehalli (8/5 g root) and the least was recorded at Honnur (6/5 g root).

In Hassana district, maximum root population (17/5 g root) of *M. incognita* was recorded at Thimmalapura followed by Bisalihalli (13/5 g root), Hiriyur (12/5 g root), Alisandra (9/5 g root) and the least was recorded at Kanihalli (7/5 g root). In Tumkuru district, maximum root population (18/5 g root) of *M. incognita* was recorded at Madhihalli followed by Aralikere (13/5 g root), Puttamadhihalli (10/5 g root), keragodu (6/5 g root) Ganganaghatta (4/5 g root) and the least was recorded at Nonavinakere (3/5 g root). In Mandya district, maximum root population (16/5 g root) of *M. incognita* was recorded at Kulgere followed by Mallanathapura (14/5 g root), Huskur (13/5 g root), Hucche gowdana doddi (11/5 g root), Ankanathapura (9/5 g root) and the least was recorded at Uthukere (3/5 g root).

**Table 8. Identification of *Helicotylenchus* sp.**

| Characters | Original description | Observed |
|---|---|---|
| Body | Spirally coiled, lateral field with 4 incisures. Cephalic region low or elevated, continuous or slightly offset, rounded or anteriorly flattened, generally annulated but never longitudinally striated. | Spirally coiled, cephalic region anteriorly flattened and annulated. |
| Stylet | Robust, about 3-4 times maximum width of cephalic region. | Robust. |
| Oesophagous | Hoplolaimoid, median bulb rounded with average sized valve. | Hoplolaimoid and median bulb rounded. |
| Excretory pore | Behind hemizonid, near base of isthmus. | Behind hemizonid. |
| Tail | Male: Tail short, conical with a distinct terminal hyaline portion. Bursa enveloping entire tail tip, rarely subterminal.<br>Female: Short hemispherical, dorsally convex-conoid, with or without a ventral or terminal projection. | Male: Tail short, conical.<br>Female: Short hemispherical. |

In Mysuru district, maximum root population (13/5g root) of *M. incognita* was recorded at Chamalapura followed by Krishnapura (10/5 g root) and the least was recorded at Kottegala (7/5g root). The maximum total root population (66) of *M. incognita* was recorded in Mandya district followed by Chamarajanagara district (64), Hassana district (58), Tumkuru district (54) and the least population was recorded at Mysuru district (30) (Table 6).

Since black gram was more commonly infested with *M. incognita* in most areas surveyed, further studies were carried out on black gram.

## 4.2 Screening of available cultivars of black gram for resistance against root knot nematode

A pot experiment was conducted under glasshouse belonging to Department of

**Table 9. Identification of *Rotylenchulus* sp.**

| Characters | Original description | Observed |
|---|---|---|
| Body | Male : Vermiform, arcuate to spiral upon fixation.<br>Female: Kidney shaped, with an irregular, less swollen neck. | Male: Vermiform, spiral upon fixation.<br>Female: Kidney shaped. |
| Stylet | Male: Regressed.<br>Female : 2-3 times lip region widths long. Orifice of dorsal gland usually one stylet length behind stylet base. | Regressed. |
| Oesophagous | Male: Regressed. | Regressed. |
| Tail | Elongate-conoid, with prominent hyaline terminal portion. | Conoid |
| Spicules | Slender, lacking distal flanges | Slender |

**Table 10. Identification of *Pratylenchus* sp.**

| Characters | Original description | Observed |
|---|---|---|
| Body | Female: Sclerotization massive. | Sclerotized body. |
| Stylet | Less long, with round, anteriorly flat or indented basal knobs. | Round, anteriorly flat. |
| Oesophagous | Usually less than two body widths long, extending over intestine mostly ventrally. | Short |
| Tail | Male: Have a bent tail.<br>Female: Straight tail. | Straight tail. |

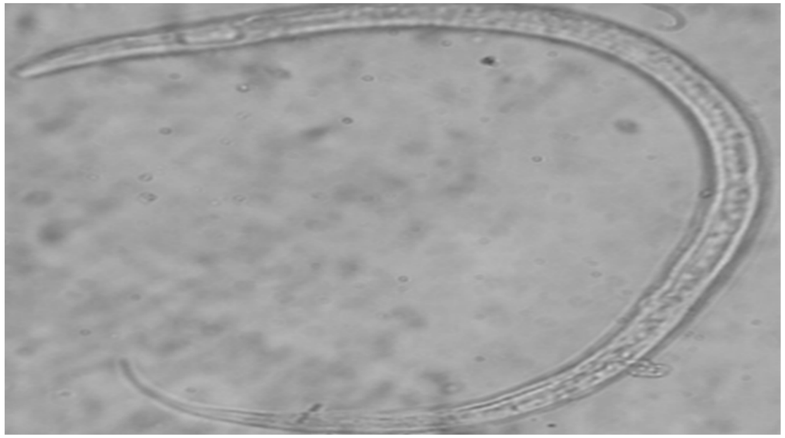
Plant Pathology, University of Agriculture Sciences, GKVK Bengaluru, to screen fourteen black gram cultivars to assess their reaction to the *M. incognita*.

Fourteen black gram cultivars evaluated were 2KU-60, ADT-05, BG-2, DU-1, G-333, IC-282007, IC-436545, K-5-572, KU-8-155, LKU-64, RASHMI, RU10-601, SU-509 and UH-04-04. The data is presented in (Table 11; Plate 4 and 5; Fig. 2a and 2b).

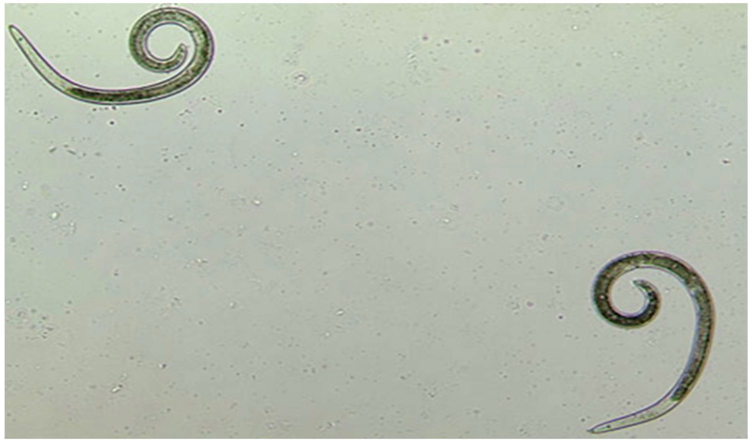
Among fourteen cultivars evaluated UH-04-04 recorded maximum number of galls per plant (112 and 122.5 galls at 60 days and at harvest respectively) with gall indices of 5.0 indicating its high susceptibility to *M. incognita* followed by IC-436545 (92.5 – 111galls at 60 days and at harvest respectively) with gall indices of 4 and 5 at 60 days and at harvest respectively, RASHMI (61 and 71 galls at 60 days and at harvest respectively) with gall indices of 4, RU10-601 (56 and 63.5 galls at 60 days and at harvest respectively) with gall indices of 4, KU-8-155 (52 – 62 galls at 60 days and at harvest respectively) with gall indices of 4, LKU-64 (34.5 – 28.5 at 60 days and at harvest respectively) with gall indices of 3 and 4 at 60 days and at harvest respectively.

Cultivar K-5-572 (31.5 and 29 galls at 60 days and at harvest respectively) with gall indices of 3 and 4 at 60 days and at harvest respectively, DU-1 (31.5 – 37.5 galls at 60 days and at harvest respectively) with gall indices of 3.6 and 4 at 60 days and at harvest respectively, T-9 (30 – 38.5 galls at 60 days and at harvest respectively) with gall indices of 3.3 and 4 at 60 days and at harvest respectively, IC-282007 (29.5 – 37.5 galls at 60 days and at harvest respectively) with gall indices of 3.3 and 4 at 60 days and at harvest respectively, BG-2 (29 – 33.5 galls at 60 days and at harvest respectively) with gall indices of 3.3 and 4 at 60 days and at harvest respectively, 2KU-60 (20–24.5 galls at 60 days and at harvest respectively) with gall indices of 3, ADT-05 (17.5–24 at 60 days and at harvest respectively) with gall indices of 3 and G-333 showed least number of galls per plant (6 at 60 days and 7.5 at harvest) with gall index of 2.

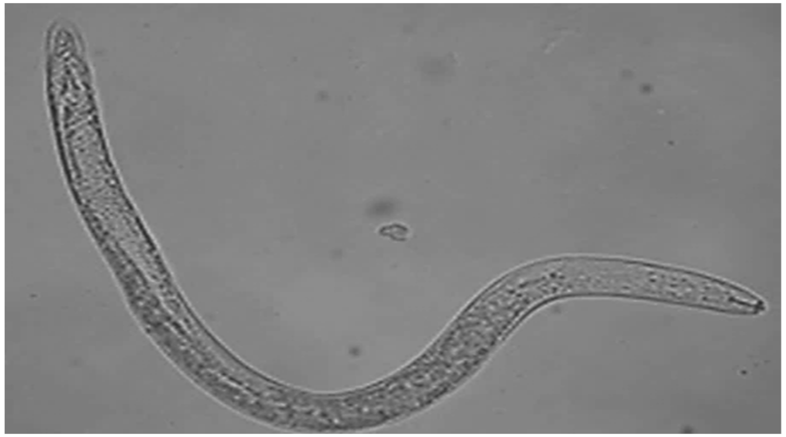
To sum up, G-333 (Resistant) was found significantly superior over the rest, with least gall index while, ADT-05 and 2KU 60 were moderately resistant, BG-2, DU-1, IC-282007, K-5-572, KU-8-155, LKU-64, RASHMI, RU10-601 and SU-509 were susceptible and UH-04-04 and IC-436545 were highly susceptible to *M. incognita*.



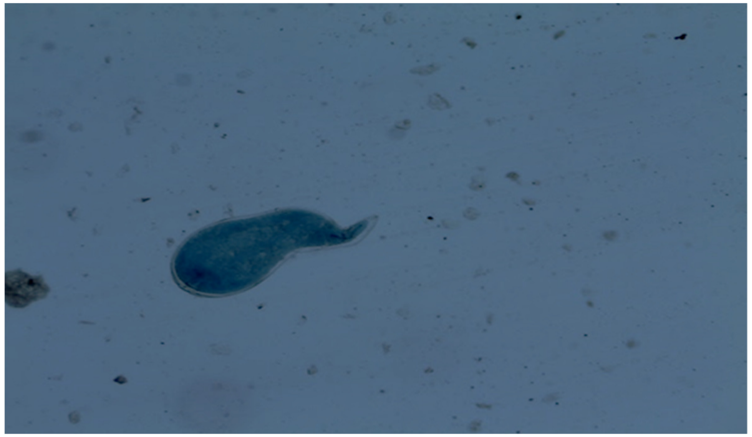
*Meloidogyne incognita*



*Helicotylenchus spp.*



***Pratylenchus* spp.**



***Rotylenchulus* spp.**

**Plate 3: Major plant parasitic nematode associated with pulse crops**

**Table 11. Screening of black gram cultivars against *M. incognita***

| Cultivars | At 60 DAS | | At harvest | | Disease reaction |
|---|---|---|---|---|---|
| | Number of galls/plant | Root-Knot Index | Number of galls/plant | Root-Knot Index | |
| 2KU-60 | 20.00 | 3 | 24.50 | 3 | Moderately resistant |
| ADT-05 | 17.50 | 3 | 24.00 | 3 | Moderately resistant |
| BG-2 | 29.00 | 3.3 | 33.50 | 4 | Susceptible |
| DU-1 | 31.50 | 3.6 | 37.50 | 4 | Susceptible |
| G-333 | 6.00 | 2 | 7.50 | 2 | Resistant |
| IC-282007 | 29.50 | 3.3 | 37.50 | 4 | Susceptible |
| IC-436545 | 92.50 | 4 | 111.00 | 5 | Highly Susceptible |
| K-5-572 | 31.50 | 4 | 29.00 | 3 | Moderately resistant |
| KU-8-155 | 52.00 | 4 | 62.00 | 4 | Susceptible |
| LKU-64 | 34.50 | 4 | 28.50 | 3 | Moderately resistant |
| RASHMI | 61.00 | 4 | 71.00 | 4 | Susceptible |
| RU10-601 | 56.00 | 4 | 63.50 | 4 | Susceptible |
| T-9 | 30.00 | 3.3 | 38.50 | 4 | Susceptible |
| UH-04-04 | 112.00 | 5 | 122.50 | 5 | Highly Susceptible |
| S. Em ± | 1.66 | - | 1.50 | - | - |
| C.D. at 5% | 4.81 | - | 4.36 | - | - |

## 4.3 Effect of root knot nematode on nodulation, growth and yield parameter in black gram

### 4.3.1 Nodulation

Effect of various treatments on the number of nodules per root system was recorded after termination of the experiment and data is presented in Table 12. The minimum number of root nodules were recorded when inoculation of *M. incognita* before two weeks of *rhizobium* (28) followed by *M. incognita* + *Rhizobium* applied simultaneously (40), inoculation of rhizobium before two weeks of *M. incognita* (51) and rhizobium alone (57). In general, *M. incognita* treated plants recorded significantly lower number of nodules compared to other treatments. *M. incognita* infestation significantly reduced the number of nodules per plant (Table 12). A greater reduction in number of nodules was observed when the *M. incognita* was established before the inoculation of the *Rhizobium* than the *M. incognita* and *Rhizobium* inoculated together or when the *Rhizobium* was established before the inoculation of *M. incognita*.

### Estimation of nitrogen

Observations on the effect of different treatments on nitrogen fixation in black gram were recorded at 30 and 60 days after sowing and data is presented in table 13 and Fig.4. In all *M. incognita* treatments nitrogen content was significantly reduced compared to plants inoculated with *Rhizobium* only. Maximum reduction in the nitrogen content of shoot (31.6 per cent) and root (25.1 per cent) occurred in plants inoculated with *M. incognita* alone.

### In shoot

At 30 days after sowing the maximum reduction of nitrogen content in plant shoot (24.70 per cent) over *Rhizobium* was recorded in plants treated with *M. incognita* alone. Among different treatments, maximum reduction of nitrogen (24.70 per cent) was recorded in plants treated with *M. incognita* alone with a per cent nitrogen content of 1.20 in shoot, followed by inoculation of *M. incognita* before two weeks of *rhizobium* (16.0 per cent) with a per cent nitrogen content of 1.37 in shoot, *M. incognita* + *Rhizobium* (12.90 per cent) with a per cent nitrogen content of 1.43 in shoot and inoculation of *Rhizobium* before two weeks of *M. incognita* (11.40 per cent) with a per cent nitrogen content of 1.46 in shoot.

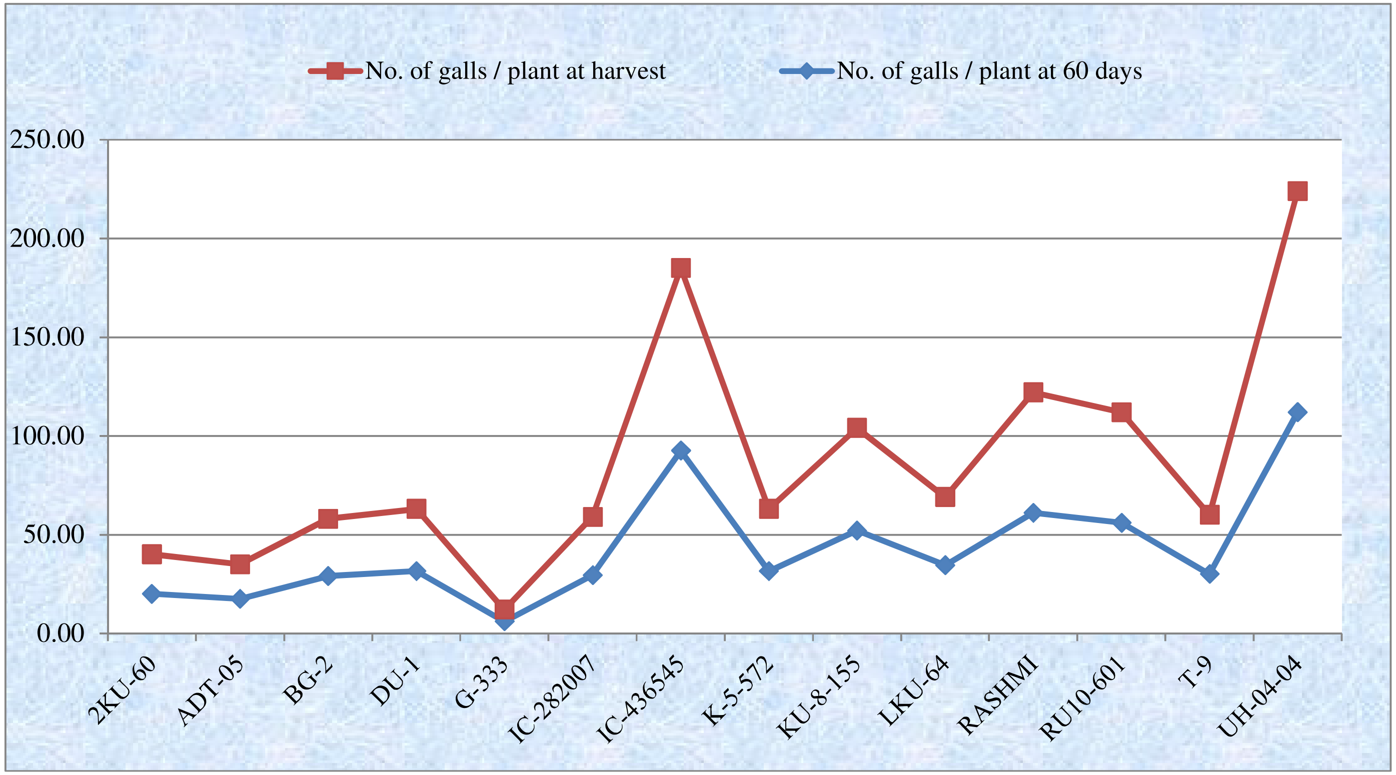



**Fig. 2a: Reaction of black gram cultivars to *M. incognita***

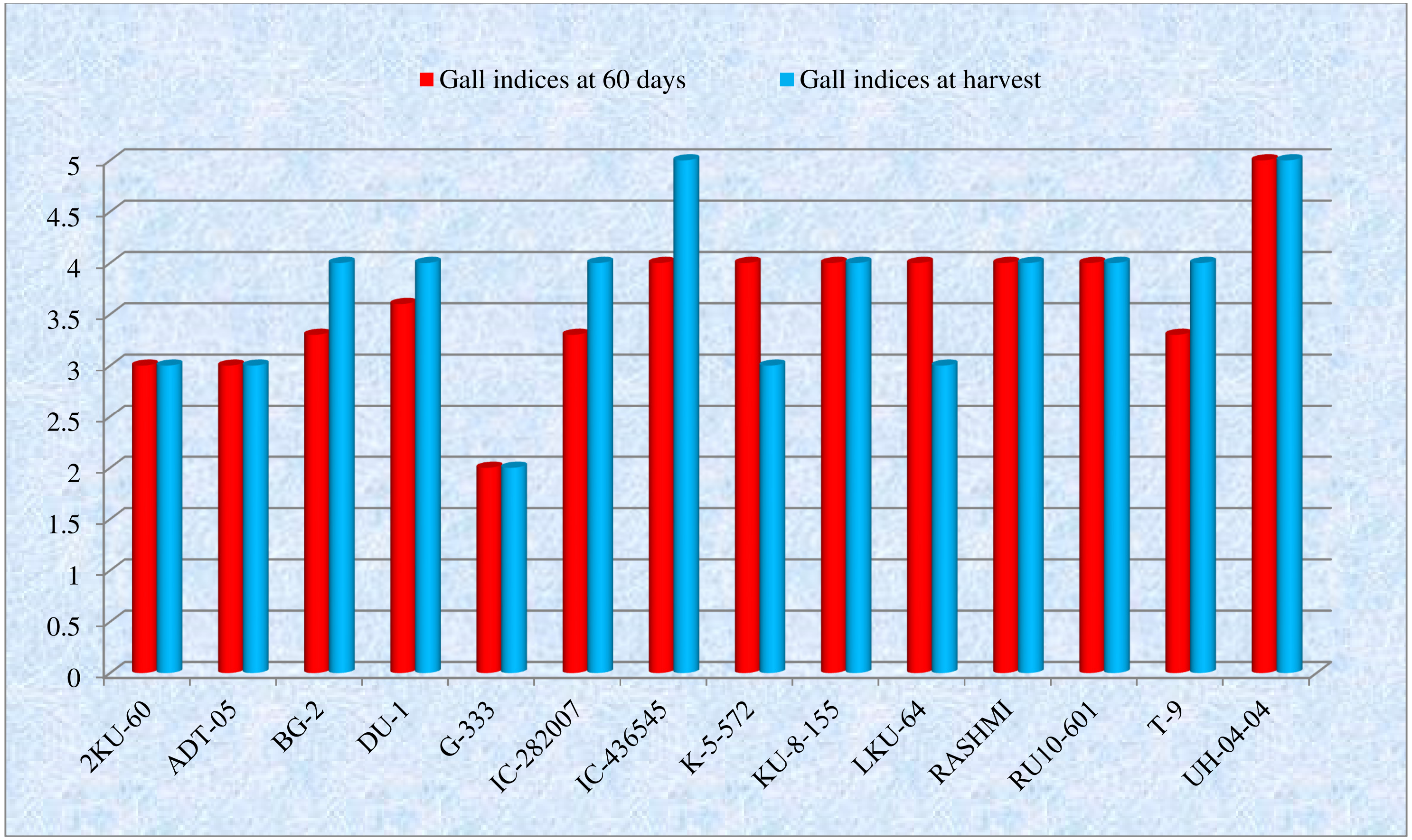



**Fig. 2b: Reaction of black gram cultivars to *M. incognita***


**Plate 4. Infestation of *M. incognita* on black gram cultivars**

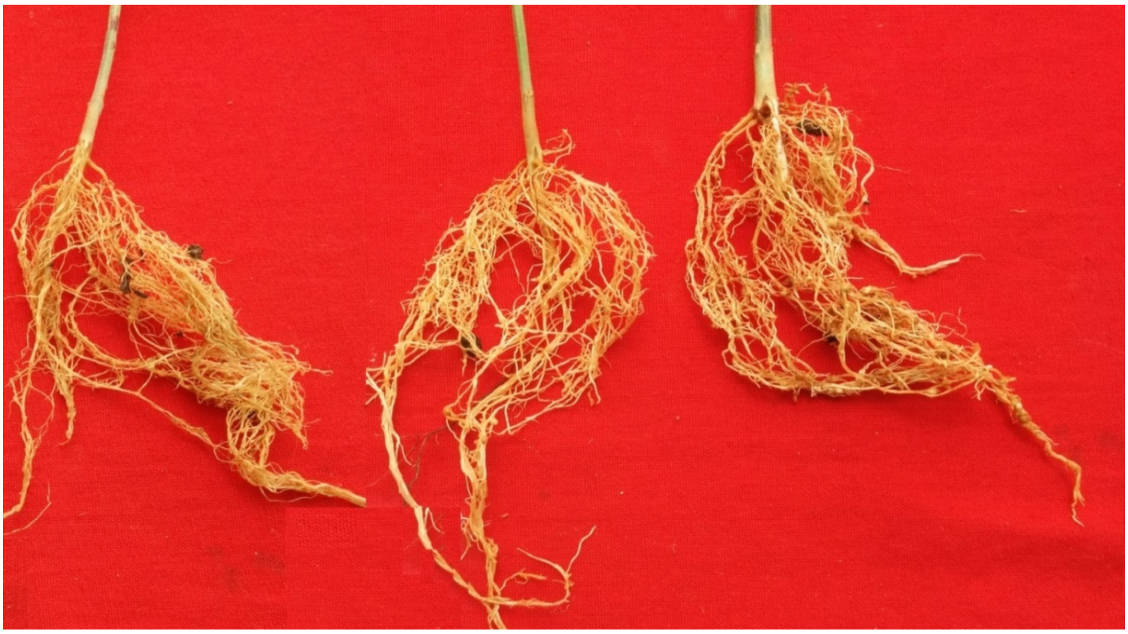


**Plate 5a: Resistant cultivar (G-333)**

**Plate 5b: Highly susceptible cultivar (UH-04-04)**

**Table 12: Effect of *M. incognita* and *Rhizobium* on galling and nodulation in black gram**

| Treatment | No. of nodules per plants | No. of galls per plant | Final soil population per pot | Root- Knot Index |
|---|---|---|---|---|
| ***M. incognita* alone** | 0.00 (0.71) | 114.00 (10.70) | 545.00 (44.84) | 5 |
| ***Rhizobium* alone** | 57.00 (7.57) | 0.00 (0.71) | 0.00 (0.71) | 1 |
| ***M. incognita* + *Rhizobium*** | 40.00 (6.34) | 62.00 (7.91) | 410.00 (37.16) | 4 |
| ***M. incognita* followed by *Rhizobium* after two weeks** | 28.00 (5.35) | 88.00 (8.28) | 450.00 (8.48) | 4 |
| ***Rhizobium* followed by *M. incognita* after two weeks** | 51.00 (7.18) | 68.00 (8.26) | 337.00 (40.09) | 4 |
| **Untreated check** | - | - | - | - |
| **S. Em±** | 0.009 | 0.02 | 0.05 | - |
| **CD at 5%** | 0.03 | 0.06 | 0.15 | - |

*Figures in parenthesis indicate $\sqrt{x + 0.5}$ values

At 60 days after sowing maximum reduction of nitrogen content in plant shoot (31.63 per cent) over *Rhizobium* was recorded in plants treated with *M. incognita* alone. Among different treatments, maximum reduction of nitrogen (31.63 per cent) was recorded in plants treated with *M. incognita* alone with a per cent nitrogen content of 1.34 in shoot, followed by inoculation of *M. incognita* before two weeks of *rhizobium* (25.68 per cent) with a per cent nitrogen content of 1.46 in shoot, inoculation of *rhizobium* before two weeks of *M. incognita* (24.83 per cent) with a per cent nitrogen content of 1.48 in shoot and *M. incognita* + *Rhizobium* (24.66 per cent) with a per cent nitrogen content of 1.43 in shoot.

**In root**

At 30 days after sowing maximum reduction of nitrogen content in plant shoot (24.70 per cent) over *Rhizobium* was recorded in plants treated with *M. incognita* alone. Among different treatments, maximum reduction of nitrogen (28.80 per cent) was recorded in plants treated with *M. incognita* alone with a per cent nitrogen content of 1.12 per cent in root, followed by inoculation of *M. incognita* before two weeks of *rhizobium* (21.20 per cent) with a per cent nitrogen content of 1.24 in root, *M. incognita* + *Rhizobium* (14.40 per cent) with a per cent nitrogen content of 1.35 in root and inoculation of *rhizobium* before two weeks of *M. incognita* (8.7 per cent) with a per cent nitrogen content of 1.44 in root.

At 60 days after sowing the maximum reduction of nitrogen content in plant shoot (31.63 per cent) over *Rhizobium* was recorded in plants treated with *M. incognita* alone. Among different treatments, maximum reduction of nitrogen (25.10 per cent) was recorded in plants treated with *M. incognita* alone with a per cent nitrogen content of 1.23 in root, followed by inoculation of *M. incognita* before two weeks of *rhizobium* (22.60 per cent) with a per cent nitrogen content of 1.27 in root, *M. incognita* + *Rhizobium* (15.90 per cent) with a per cent nitrogen content of 1.38 in root and inoculation of *rhizobium* before two weeks of *M. incognita* (13 per cent) with a per cent nitrogen content of 1.42 in root.

**4.3.2 Growth and yield parameters**

The effect of different treatments on the shoot length (cm) was recorded at 60 days. The data on shoot length is presented in Table 14; Fig. 5, Plate 6a and 6b. There was a significant difference among the treatments with respect to shoot length. It ranged from 21.63 to 36.60 cm. Maximum reduction in shoot length was recorded in *M. incognita* alone (20.48 cm) followed by inoculation of *M. incognita* before two weeks of *Rhizobium* (21.63 cm), *M. incognita* + *Rhizobium* simultaneously (25.20 cm), untreated check (28.25cm), inoculation of *rhizobium* before two weeks of *M. incognita* (31.23cm) and *Rhizobium* inoculated alone (36.60cm).

The effect of different treatments on the fresh shoot weight was recorded at 60 days and data presented in Table 11. There was a significant difference among the

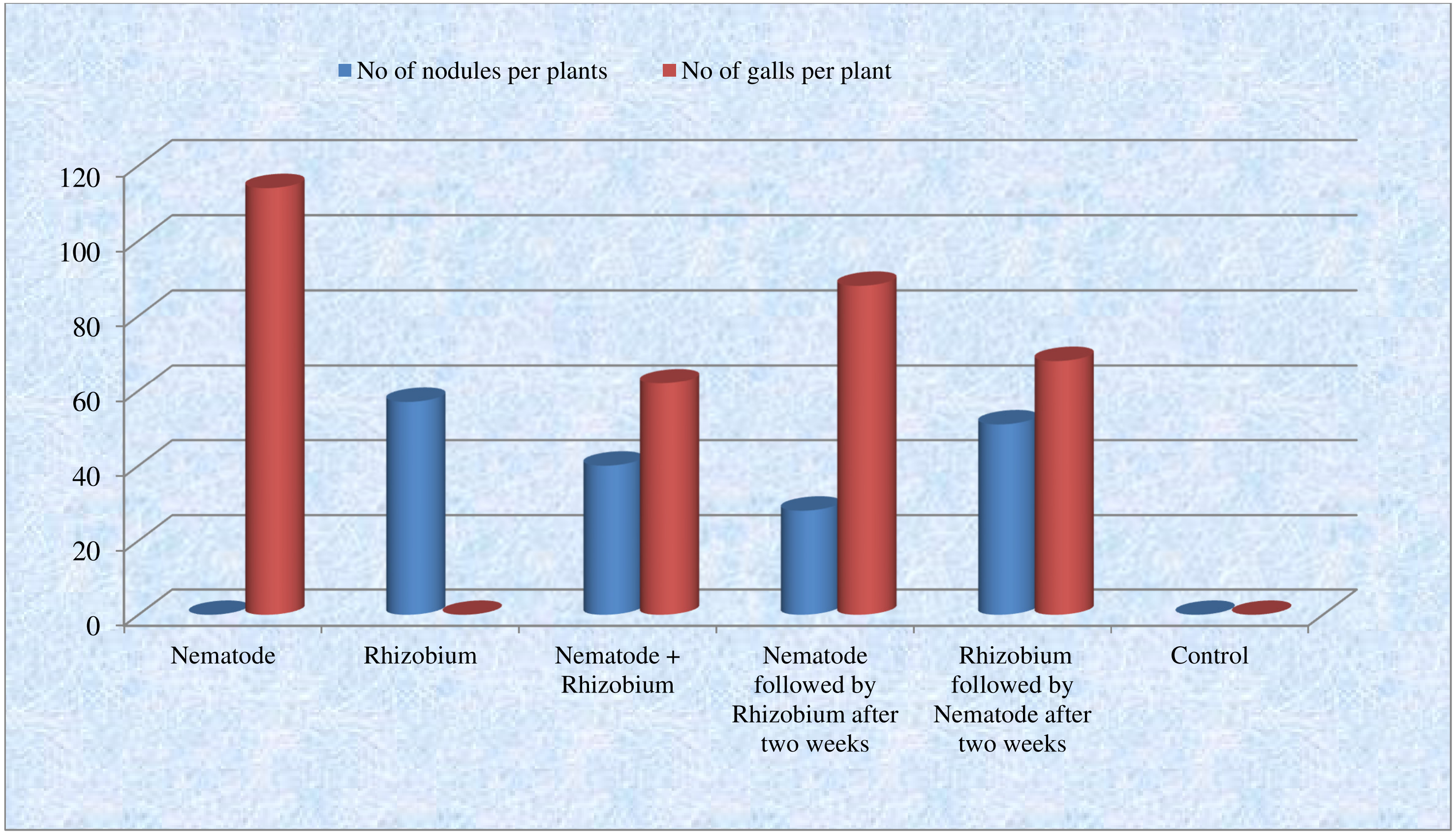



**Fig 3: Interaction of *Meloidogyne incognita* and *Rhizobium* on galling and nodulation in black gram**

treatments with respect to fresh shoot weight. Fresh shoot weight of plants ranged from 4.21g to 6.28g it was less when inoculated *M. incognita* before two weeks of *Rhizobium* (4.21 g) followed by *M. incognita* alone (4.30 g), *M. incognita* + *Rhizobium* simultaneously (4.65 g), untreated check (5.38 g), inoculation of *Rhizobium* before two weeks of *M. incognita* (5.40 g) and *Rhizobium* alone (6.28 g).

Effect of different treatments on the dry shoot weight was recorded at 60 days after sowing and data is presented in Table 14. There was a significant difference among the treatments with respect to dry shoot weight. It ranged from 2.94 g to 5.63 g. The maximum reduction in dry shoot weight was recorded in *M. incognita* alone (2.94 g) alone followed by inoculation of *M. incognita* before two weeks of *Rhizobium* (3.46 g), *M. incognita* + *Rhizobium* (3.74 g), untreated check (4.16 g), inoculation of *Rhizobium* before two weeks of *M. incognita* (4.73 g) and *Rhizobium* alone (5.63 g).

Effect of different treatments on the root length of black gram plants under glass house condition was recorded at 60 days after sowing and data is presented in (Table 14**;** Plate 6; Fig.5). There was a significant difference among the treatments with respect to the root length. Root length of plants ranged from 11.58 cm to 21.40 cm. The maximum reduction of root length was observed in *M. incognita* alone (11.58 cm) followed by inoculation of *M. incognita* before two weeks of *rhizobium* (12.68 cm), *M. incognita* + *Rhizobium* (14.48 cm), untreated check (15.68 cm), inoculation of *rhizobium* before two weeks of *M. incognita* (16.35 cm) and *Rhizobium* alone (21.40 cm).

**Table 13: Effect of *M. incognita* on symbiotic nitrogen fixation in black gram**

| Treatments | Nitrogen (%) | | | | | |
|---|---|---|---|---|---|---|
| | Shoot days after inoculation | | | Root days after inoculation | | |
| | 30 | 60 | Mean | 30 | 60 | Mean |
| ***M. incognita* alone** | 1.20 (6.29) | 1.34 (6.66) | 1.27 | 1.12 (6.07) | 1.23 (6.36) | 1.17 |
| ***Rhizobium* alone** | 1.68 (7.45) | 1.96 (8.05) | 1.82 | 1.57 (7.21) | 1.64 (7.35) | 1.61 |
| ***M. incognita* + *Rhizobium*** | 1.43 (6.95) | 1.43 (6.95) | 1.46 | 1.35 (6.66) | 1.38 (6.74) | 1.36 |
| ***M. incognita* followed by *Rhizobium* after two weeks** | 1.37 (3.54) | 1.46 (6.98) | 1.40 | 1.24 (2.78) | 1.27 (6.46) | 1.25 |
| ***Rhizobium* followed by *M. incognita* after two weeks** | 1.46 (6.87) | 1.48 (6.98) | 1.46 | 1.44 (6.88) | 1.42 (6.85) | 1.43 |
| **Untreated check** | 1.30 (6.73) | 1.38 (6.35) | 1.34 | 1.20 (5.26) | 1.25 (6.41) | 1.04 |
| **S. Em±** | 0.46 | 0.11 | - | 0.32 | 0.17 | - |
| **CD at 5%** | 1.37 | 0.33 | - | 0.95 | 0.51 | - |

* Figures in parenthesis indicate arc sine values

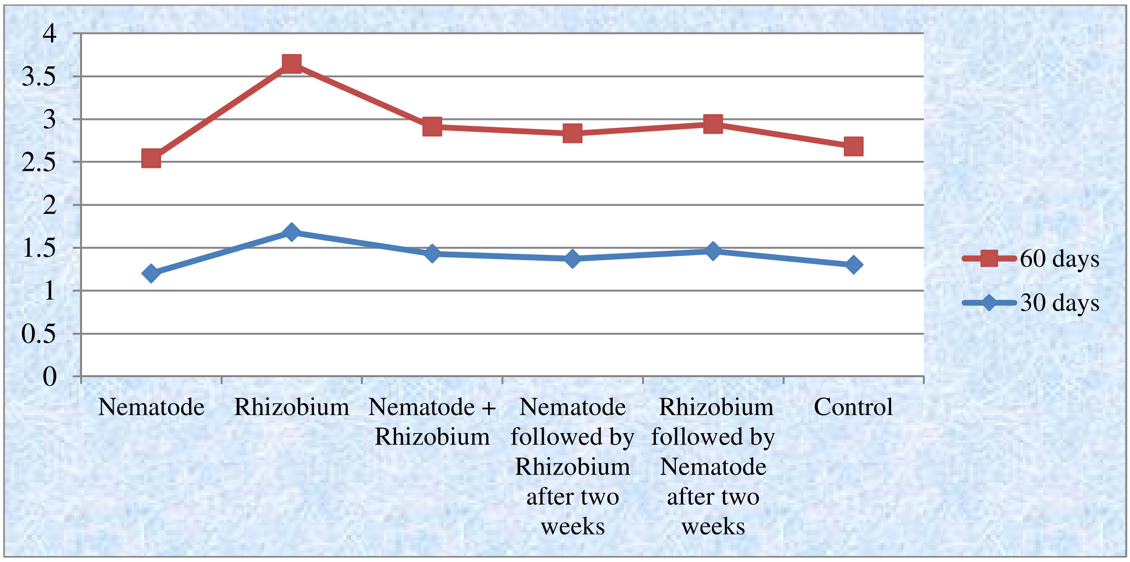



**Fig 4a: Nitrogen content in black gram shoots of different treatments**

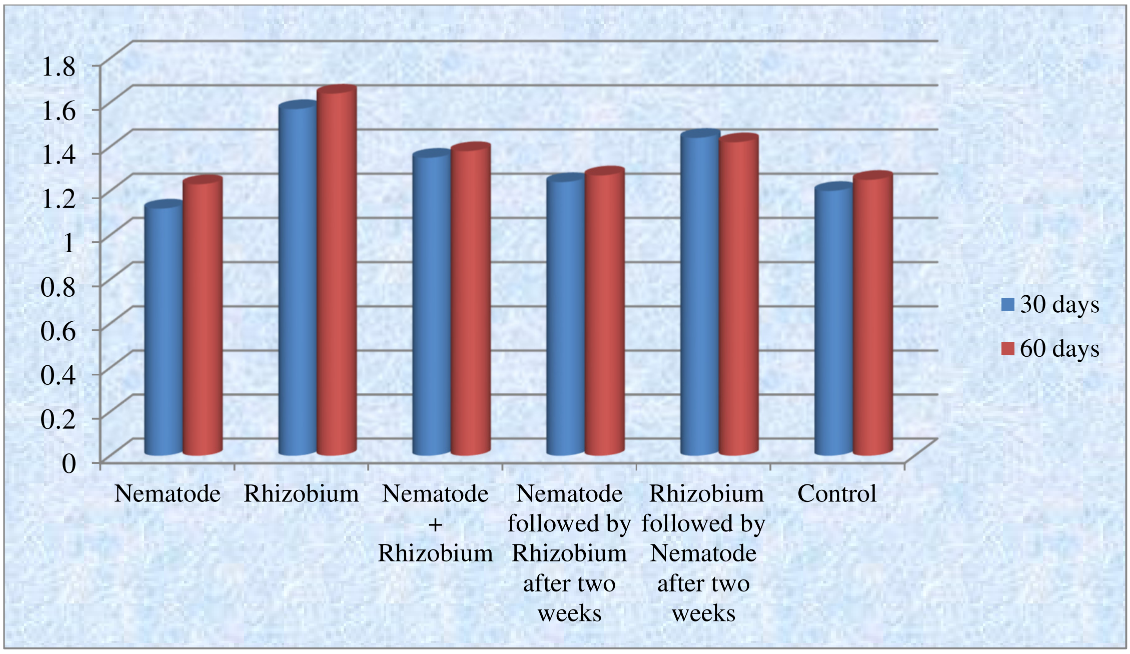



**Fig 4b: Nitrogen content in black gram roots of different treatments**

The effect of different treatments on the fresh root weight was recorded at 60 days after sowing and data is presented in Table 14**.** There was a significant difference among the treatments with respect to the fresh root weight. Fresh root weight of plants ranged from 2.33 g to 5.45 g. The maximum reduction of fresh root weight was recorded in *M. incognita* alone (2.33 g) followed by *M. incognita* + *Rhizobium* simultaneously (2.58 g), inoculation of *M. incognita* before two weeks of *rhizobium* (3.16 g), untreated check (3.28 g), inoculation of *rhizobium* before two weeks of *M. incognita* (3.73 g) and *Rhizobium* alone (5.45 g).

Effect of different treatments on the dry root weight was recorded at 60 days after sowing and data is presented in Table 14. There was a significant difference among the treatments with respect to the dry root weight. Dry root weight of plants ranged from 2.16g to 4.19g. The maximum reduction in dry root weight was recorded in *M. incognita* alone (2.16 g) followed by *M. incognita* + *Rhizobium* simultaneously (2.23 g), inoculation of *M. incognita* before two weeks of *rhizobium* (2.64 g), Untreated check (3.19 g), inoculation of *rhizobium* before two weeks of *M. incognita* (3.38 g) and *Rhizobium* alone (4.19g).

Effect of different treatments on number of pods of black gram plants was recorded at harvest and data is presented in Table 14. There was a significant difference among the treatments with respect to pod number. Number of pods in black gram plants ranged from 13.67 to 22.62/plant. The maximum reduction in number of pods was recorded in *M. incognita* alone (13.67) followed by inoculation of *M. incognita* before two weeks of *rhizobium* (14.60), *M. incognita* + *Rhizobium* simultaneously (15.33), inoculation of *rhizobium* before two weeks of *M. incognita* (17.00), untreated check (20.10) and *Rhizobium* alone (22.62).

In general, *M. incognita* treated plants recorded significantly less dry and fresh root weight, root and shoot length, fresh and dry shoot weight and lesser number of pods compared to other treatments.

### 4.3.3 Nematode parameters

Effect of various treatments on the number of galls per root system was recorded after termination of the experiment and data presented in Table 12 and Fig. 3.

The maximum number of galls was recorded when *M. incognita* inoculated alone (114 galls/root system). Among different treatment imposed plants, *M. incognita* alone recorded maximum number of galls (114.00 galls/root system) followed by inoculation of *M. incognita* before two weeks of *rhizobium* (88.00 galls/root system), inoculation of *rhizobium* before two weeks of *M. incognita* (68.00 galls/root system) and *M. incognita* + *Rhizobium* (62.00 galls/root system).

Infestation by *M. incognita* reduced the number of nodules on primary and

**Table 14: Effect of *M. incognita* and *Rhizobium* on plant growth parameters and yield of black gram**

| Treatments | Shoot length (cm) | Shoot weight (g) | | Root length (cm) | Root weight (g) | | No. of pods/plant |
|---|---|---|---|---|---|---|---|
| | | Fresh | Dry | | Fresh | Dry | |
| ***M. incognita*** **alone** | 20.48 | 4.3 | 2.94 | 11.58 | 2.33 | 2.16 | 13.67 |
| ***Rhizobium*** **alone** | 36.6 | 6.28 | 5.63 | 21.4 | 5.45 | 4.19 | 22.62 |
| ***M. incognita*** **+ *Rhizobium*** | 25.2 | 4.65 | 3.74 | 14.48 | 2.58 | 2.23 | 15.33 |
| ***M. incognita*** **followed by *Rhizobium* after two weeks** | 21.63 | 4.21 | 3.46 | 12.68 | 3.16 | 2.64 | 14.6 |
| ***Rhizobium*** **followed by *M. incognita* after two weeks** | 31.23 | 5.4 | 4.73 | 16.35 | 3.73 | 3.38 | 17 |
| **Untreated check** | 28.25 | 5.38 | 4.16 | 15.68 | 3.28 | 3.19 | 20.1 |
| **S. Em±** | 0.13 | 0.08 | 0.06 | 0.13 | 0.02 | 0.28 | 0.49 |
| **CD @ 5%** | 0.39 | 0.24 | 0.18 | 0.39 | 0.06 | 0.83 | 1.46 |

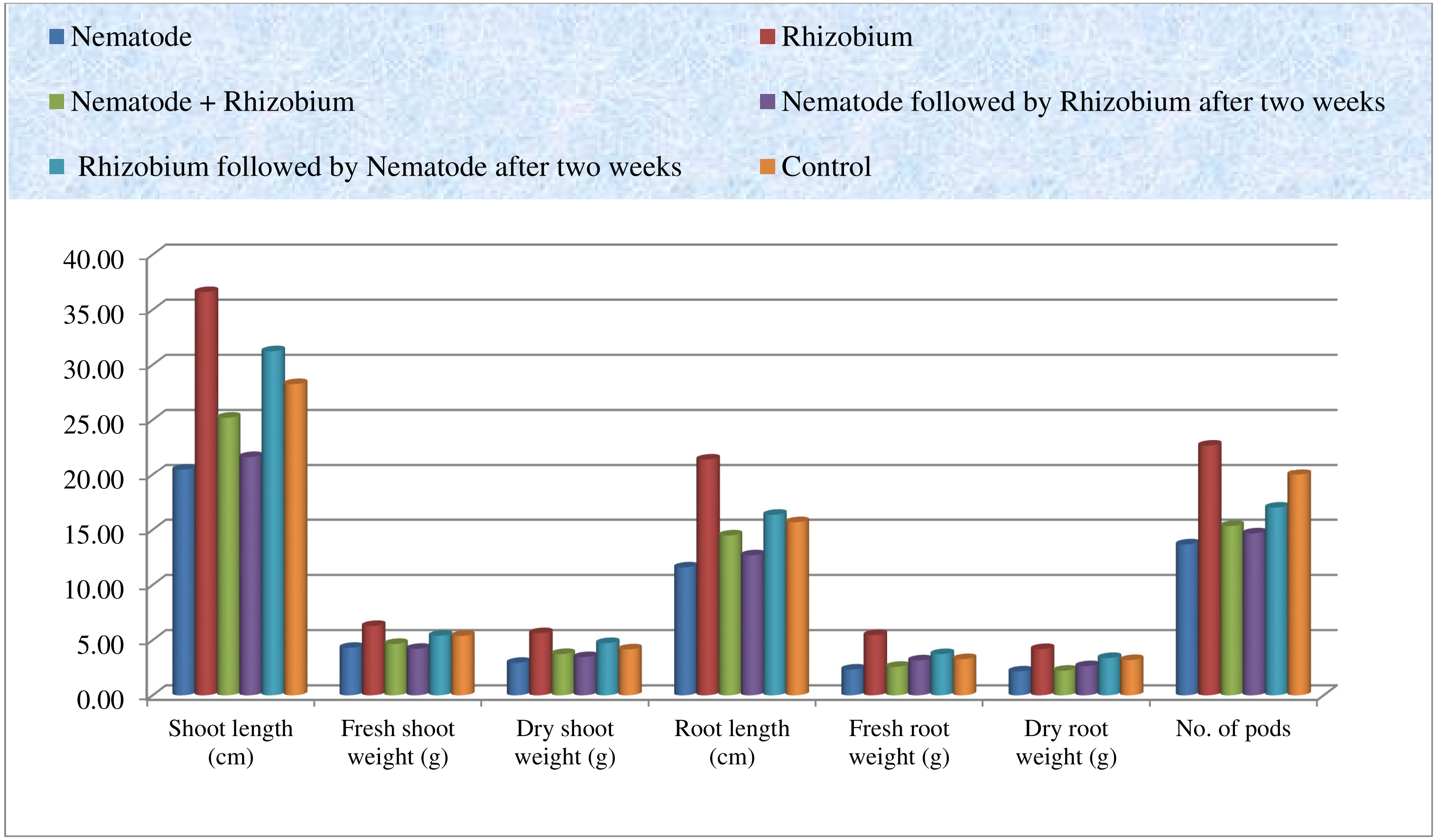



**Fig 5: Effect of *M. incognita* and *Rhizobium* on plant growth and yield parameters of black gram**

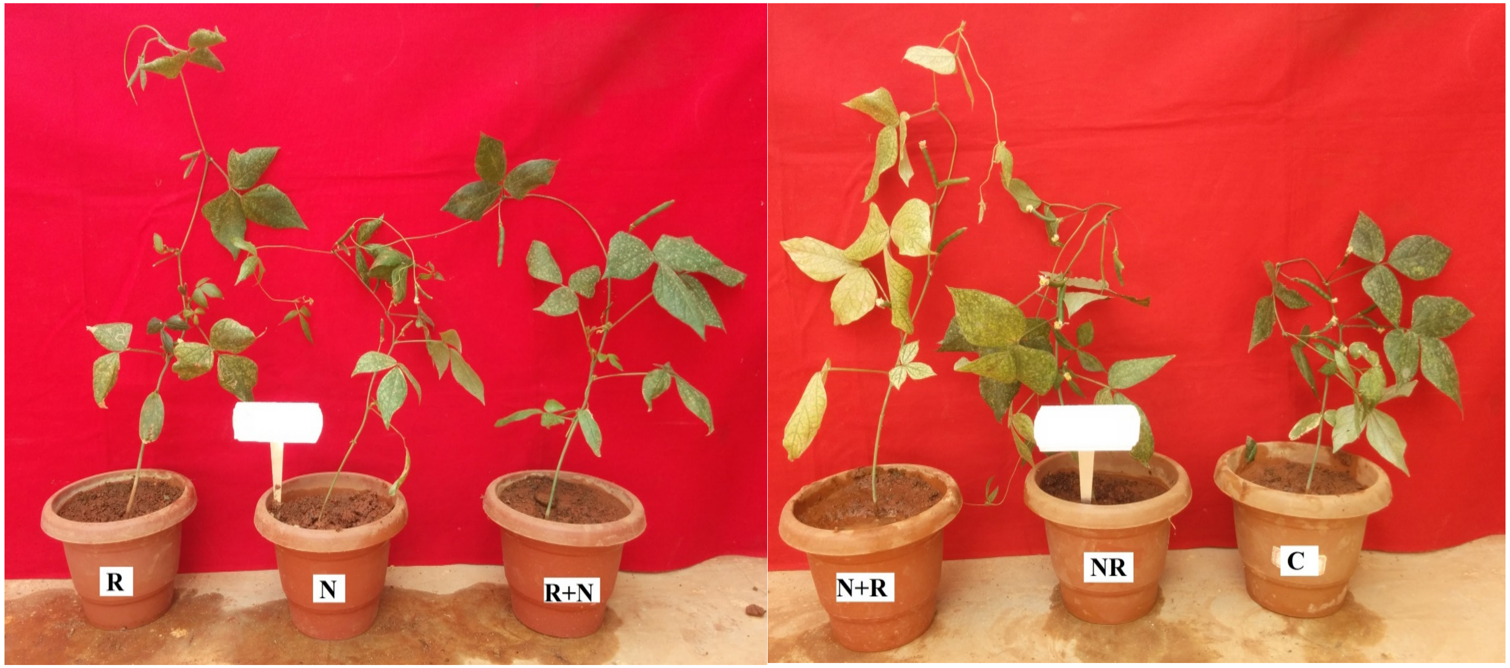



**Plate 6a: Effect of different treatments on shoot development of black gram**

R=Rhizobium, N=Nematode, R+N=Rhizobium followed by Nematode after two weeks, N+R=Nematode followed by Rhizobium after two weeks, NR=Nematode + Rhizobium simultaneously and C=Untreated check.

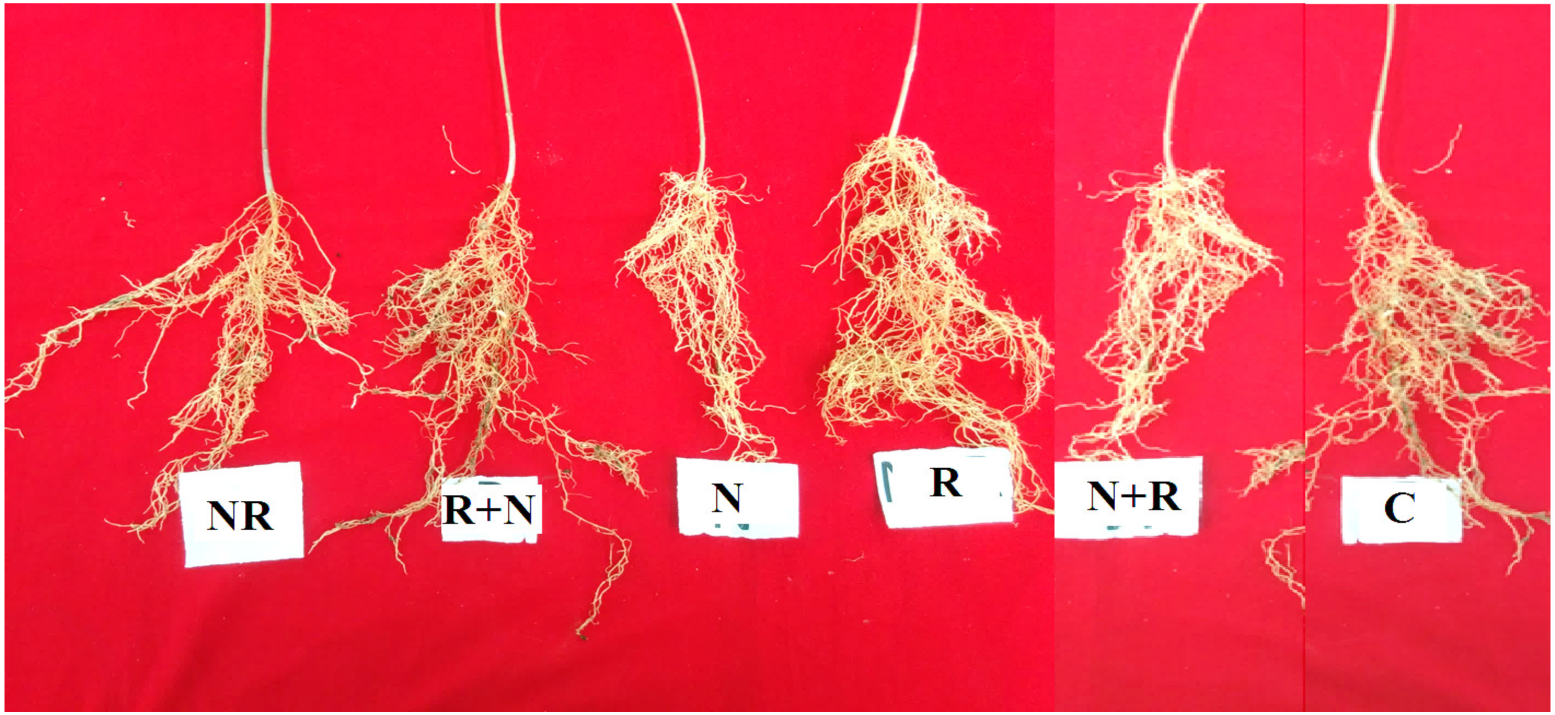



**Plate 6b: Effect of different treatments on root development in black gram**

NR=Nematode and Rhizobium simultaneously, R+N=Rhizobium followed by Nematode after two weeks, N=Nematode alone, R=Rhizobium alone, N+R=Nematode followed by Rhizobium after two weeks, and C=Untreated check.

secondary root system significantly in all the treatments. Gall number was significantly higher in unbacterized plants than on bacterized ones. Application of *Rhizobium* prior to or simultaneously with *M. incognita* invariably resulted in comparatively decreased rate of gall development on the roots than in treatments where *Rhizobium* followed *M. incognita* inoculation.

*M. incognita* population in 200 cc soils of different treatments were recorded at 60 days after sowing and data is presented in table 12 and Fig. 3. The maximum number of *M. incognita* was recorded in treatment inoculated with nematode alone (545.00/200cc soil) followed by inoculation of *M. incognita* before two weeks of *rhizobium* (450.00/200cc soil), *M. incognita* + *Rhizobium* applied simultaneously (410.00/200cc soil) and inoculation of *rhizobium* before two weeks of *M. incognita* (337.00/200cc soil).

Final soil population of juveniles was significantly lower in plants inoculated with *M. incognita* and *Rhizobium* either together or *Rhizobium* first and *M. incognita* afterwards or vice-versa, compared to the *M. incognita* inoculation alone.

Root-knot index was significantly lower in plants inoculated with *M. incognita* and *Rhizobium* either combined or *Rhizobium* first and *M. incognita* afterwards or vice-versa, compared to the *M. incognita* inoculation alone.

# V DISCUSSION

The root-knot nematode, *M. incognita* is one of the most serious plant parasitic nematode causing considerable loses in the yields of pulse crops especially chickpea, black gram, cowpea, green gram and pigeon pea. In black gram alone it causes about 12.40-46.19 per cent yield loss. *M. incognita* adversely affects nodulation and nitrogen fixation in pulses. Use of resistant cultivars for the management of nematode population is expected to be a vital management component in the future. The survey also shows that this nematode is widespread in most of the black gram growing areas.

Keeping in view, the hazardous consequences of the use of chemicals for the contro1 of nematodes, different popularly grown cultivars were evaluated and effect of *M. incognita* on nodulation, nitrogen fixation and yield parameters in black gram was evaluated.

## 5.1 Survey for incidence of root knot nematode associated with pulse crops in southern Karnataka

A survey was carried out to identify the occurrence of *M. incognita* in the root zone of pulse crops in southern Karnataka districts. The major pulse growing areas like Chamarajanagara, Hassana, Mysuru, Mandya and Tumakuru were surveyed. The soil and root samples collected from all the places were brought to the laboratory and analyzed for the presence of root-knot nematode.

The analysis of soil samples from the black gram growing areas of southern Karnataka districts revealed that maximum total soil population of *M. incognita* (657) was recorded at Chamarajanagara district followed by Tumkuru district (642), Mandya district (549), Hassana district (480) and the least was recorded at Mysuru district (266). However, the population of *Helicotylenchus* sp., *Rotylenchulus* sp. and *Pratylenchus* sp. was comparatively very less.

The root samples collected from the root zone of black gram growing areas of southern Karnataka districts revealed that maximum root population of *M. incognita* was recorded in Mandya district (85), followed by Tumkuru district (79), Chamarajanagara district (71), Hassana district (56) and the least population was recorded at Mysuru district (32). However, the population of *Helicotylenchus* sp., *Rotylenchulus* sp. and *Pratylenchus* sp. was comparatively very less.

The analysis of soil samples from the green gram growing areas of southern Karnataka districts revealed that maximum total soil population of *M. incognita* was recorded in Mandya district (600) followed by Chamarajanagara district (588), Tumkuru district (562), Hassana district (544) and the least was recorded at Mysuru district (256). However, the population of *Helicotylenchus* sp., *Rotylenchulus* sp. and *Pratylenchus* sp.was comparatively very less.

The root samples collected from the root zone of green gram growing areas of southern Karnataka districts revealed that maximum root population of *M. incognita* was recorded in Chamarajanagara district (79), Mandya district (61), followed by

Tumkuru district (56), Hassana district (55) and the least population was recorded at Mysuru district (43). However, the population of *Helicotylenchus* sp., *Rotylenchulus* sp. and *Pratylenchus* sp. was comparatively very less.

The analysis of soil samples from the cowpea growing areas of southern Karnataka districts revealed that maximum total soil population of *M. incognita* was recorded in Chamarajanagara district (569) followed by Tumkuru district (557), Mandya district (515), Hassana district (464) and the least was recorded at Mysuru district (268). However, the population of *Helicotylenchus* sp., *Rotylenchulus* sp. and *Pratylenchus* sp. was comparatively very less.

The root samples collected from the root zone of cowpea growing areas of southern Karnataka districts revealed that maximum root population of *M. incognita* was recorded in Mandya district (66), followed by Chamarajanagara district (64), Hassana district (58), Tumkuru district (54) and the least population was recorded at Mysuru district (30). However, the population of *Helicotylenchus* sp., *Rotylenchulus* sp. and *Pratylenchus* sp. was comparatively very less.

The present results are in conformity with the findings of survey made by Fazal *et al*. (1998), Mahalik and Routray (2009), Roy *et al*. (2007), and Sawadogo *et al*. (2009). who have reported occurrence of plant parasitic nematodes like *Meloidogyne incognita*, *Rotylenchulus reniformis* and *Pratylenchus* nematodes being predominant affecting black gram and other pulse crops in India (Rajasthan, Uttar Pradesh and West Bengal) and several parts of world causing significant yield loss in pulse production.

The above results with respect to the occurrence of *M. incognita* in cowpea are in conformity with the findings of Olowe (2004) who reported that occurrence of *M. incognita*, *M. javanica* and *M. arenaria* singly or in combination in all the cowpea farms sampled. In overall distribution, *M. incognita* (51.8 per cent) was the most prevalent nematode species compare to all. The increase in nematode population may be due to continuous mono culturing of pulse and other susceptible crops and environmental conditions.

## 5.2. Screening of available cultivars of black gram for resistance against root knot nematode

Plant response to *M. incognita* was measured by amount of galling and Root Knot Index. There was considerable variation in response against *M. incognita* among the cultivars of black gram screened.

Fourteen black gram cultivars used were 2KU-60, ADT-05, BG-2, DU-1, G-333, IC-282007, IC-436545, K-5-572, KU-8-155, LKU-64, RASHMI, RU10-601, SU-509 and UH-04-04 were screened against *M. incognita*, which showed significant difference.

Cultivar G-333 recorded least number of galls (6 galls/plant at 60 DAS and 7.5 galls/plant at harvest respectively) with mean root-knot index of 2 at 60 days after sowing and 2 at the time of harvest, followed by ADT-05 (17.5 galls/plant at 60 DAS and 24 galls/plant at harvest), with mean root-knot index of 3 at 60 days after sowing and 3.0 at the time of harvest, 2KU-60 (20 galls/plant at 60 DAS and 24.5 galls/plant at harvest), with mean root-knot index of 3 at 60 days after sowing and 3.0 at the time of harvest as compared to the other cultivar.

Cultivar UH-04-04 which recorded highest number (112 galls/plant at 60 days and 122.5 galls/plant at harvest) of galls per plant and more root-knot index (5.0 at 60 DAS and 5.0 at harvest, respectively) indicating it's highly susceptibility to *M. incognita*.

Present results are in line with the results obtained by Choudhury *et al*. (1985) who reported that black gram cultivars K-851, L-79504 and L-79546 and K-3975 were resistant to *M. incognita*. Devi and Choudhary (2014) did not found any resistant or moderately resistant genotypes of black gram cultivar tested against *Meloidogyne incognita*, race-2. Rahman *et al*. (2004) evaluated some black gram and pigeon pea varieties for resistance against *M. incognita* and no resistant varieties were recorded. Devi and Choudhary (2014) evaluated twenty eight genotypes of black gram for resistance against *M. incognita*, out of which 25 were susceptible (Gall Index=4) and 3 were highly susceptible (Gall Index=5) and no resistant varieties were recorded. The resistance may be due to physiological character of varieties.

Thus, breeding programs would be best served to select resistant genotypes based on root-knot index in preliminary evaluations, followed by selection based on nematode reproduction in advanced evaluations (Hussey and Janssen, 2004).

## 5.3 Effect of root knot nematode on nodulation, growth and yield parameter in black gram

### 5.3.1 Effect of root knot nematode on nodulation

*M. incognita* infection significantly reduced the number of rhizobial nodules. A greater reduction in number of nodules was observed when the *M. incognita* was established before the inoculation of the bacteria than the *M. incognita* and *Rhizobium* inoculated together or when the *Rhizobium* was established before the inoculation of *M. incognita*, which are in line with the Kumar and Vadivelu (1993). The reduction in bacterial nodulation in *M. incognita* infected plants were explained by Mahanta *et al.* (2007) that it may be due to antagonistic competition phenomenon between *M. incognita* larvae and bacteria.

Suppressed nodulation was related to the size of the root system and thus confirms the findings of Taha and Raski (1969) and Verdego *et al*. (1988). They also reported fewer nodules on plants infested with *M. incognita* were due to reduced root system.

*M. incognita* infestation diminish the number of nodules on primary and secondary root system significantly in all the treatments and followed the trend as that of plant growth similar results were obtained by Bhat *et al*. (2009) who reported that infected nodules deteriorated more quick1y than the healthy ones. The life of an individual nodule, unlike the root, was of short duration (Nutman, 1958).

Thirty days old shoots of black gram were estimated for nitrogen content. Among different treatments, maximum reduction of nitrogen (24.70 per cent) was recorded in plants treated with *M. incognita* alone with a per cent nitrogen content of 1.20 per cent in shoot, followed by inoculation of *M. incognita* before two weeks of rhizobium (16.0 per cent) with a per cent nitrogen content of 1.37 per cent in shoot, *M. incognita* + *Rhizobium* (12.90 per cent) with a per cent nitrogen content of 1.43 per cent in shoot and inoculation of rhizobium before two weeks of *M. incognita* (11.40 per cent) with a per cent nitrogen content of 1.46 per cent in shoot.

Sixty days old shoots of black gram were estimated for nitrogen content. Among different treatments, maximum reduction of nitrogen (31.63 per cent) was recorded in plants treated with *M. incognita* alone with a per cent nitrogen content of 1.34 per cent in shoot, followed by inoculation of *M. incognita* before two weeks of rhizobium (25.68 per cent) with a per cent nitrogen content of (1.46 per cent) in shoot, inoculation of rhizobium before two weeks of *M. incognita* (24.83 per cent) with a per cent nitrogen content of 1.48 per cent in shoot and *M. incognita* + *Rhizobium* (24.66 per cent) with a per cent nitrogen content of 1.43 per cent in shoot.

Thirty days old roots of black gram were estimated for nitrogen content. Among different treatments, maximum reduction of nitrogen (28.80 per cent) was recorded in plants treated with *M. incognita* alone with a per cent nitrogen content of 1.12 per cent in root, followed by inoculation of *M. incognita* before two weeks of rhizobium (21.20 per cent) with a per cent nitrogen content of 1.24 per cent in root, *M. incognita* + *Rhizobium* (14.40 per cent) with a per cent nitrogen content of 1.35 per cent in root and inoculation of rhizobium before two weeks of *M. incognita* (8.7 per cent) with a per cent nitrogen content of 1.44 per cent in root.

Sixty days old roots of black gram were estimated for nitrogen content. Among different treatments, maximum reduction of nitrogen (25.10 per cent) was recorded in plants treated with *M. incognita* alone with a per cent nitrogen content of 1.23 per cent in root, followed by inoculation of *M. incognita* before two weeks of rhizobium (22.60 per cent) with a per cent nitrogen content of 1.27 per cent in root, *M. incognita* + *Rhizobium* (15.90 per cent) with a per cent nitrogen content of 1.38 per cent in root and inoculation of rhizobium before two weeks of *M. incognita* (13 per cent) with a per cent nitrogen content of 1.42 per cent in root.

In all *M. incognita* treatments the nitrogen content was significantly reduced compared with plants inoculated with *Rhizobium* only. Maximum reduction in the nitrogen content of shoot and root occurred in plants inoculated with *M. incognita* alone (Table 10). Similar results were obtained by Taha and Raski (1969) who reported *M. incognita* induced reduction in symbiotic nitrogen fixation in leguminous plants. Lehman *et al*. (1971) reported that *M. incognita* induced the reduction in nitrogen fixation in white clover infected by *M. javanica* and *Heterodera trifoli* in soybean infected by *H. glycines* and cowpea infected by *M. incognita* and *H. cajani* (Sharma and Sethi, 1976).

Similar results were observed by Bergesen (1961) who reported that *M. incognita* altered the function of nodule development. It adversely affected the nitrogenase activity in nodules as well as nitrogen uptake of root and shoot. Foremost function of nitrogenase present in bacteriod is to reduce dinitrogen to ammonia, this may be one of the reasons for reduced fixations of dinitrogen due to *M. incognita* infestation (Chahal and Rewari, 1977).

**5.3.2 Effect of root knot nematode on growth and yield parameter in black gram**

Shoot length of black gram was recorded at 60 days, which varied in all the treatments compared to untreated check (28.25 cm). There was a significant difference among the treatments with respect to shoot length. It ranged from 21.63 to 36.60 cm. Maximum reduction in shoot length was recorded in *M. incognita* alone (20.48 cm) followed by inoculation of *M. incognita* before two weeks of *rhizobium* (21.63 cm), *M. incognita* + *Rhizobium* simultaneously (25.20 cm), untreated check (28.25cm), inoculation of *rhizobium* before two weeks of *M. incognita* (31.23cm) and *Rhizobium* inoculated alone (36.60cm).

Fresh shoot weight was varied in all the treatments compared to untreated check (5.38 g). There was a significant difference among the treatments with respect to fresh shoot weight. Fresh shoot weight of plants ranged from 4.21g to 6.28g. it was less when inoculated *M. incognita* before two weeks of *Rhizobium* (4.21 g) followed by *M. incognita* alone (4.30 g), *M. incognita* + *Rhizobium* simultaneously (4.65 g), untreated check (5.38 g), inoculation of *Rhizobium* before two weeks of *M. incognita* (5.40 g) and *Rhizobium* alone (6.28 g).

Dry shoot weight was recorded at 60 days after sowing and it significantly differed in all the treatments compared to untreated check (4.16 g). There was a significant difference among the treatments with respect to dry shoot weight. It ranged from 2.94 g to 5.63 g. The maximum reduction in dry shoot weight was recorded in *M. incognita* alone (2.94 g) alone followed by inoculation of *M. incognita* before two weeks of *Rhizobium* (3.46 g), *M. incognita* + *Rhizobium* (3.74 g), untreated check (4.16 g), inoculation of *Rhizobium* before two weeks of *M. incognita* (4.73 g) and *Rhizobium* alone (5.63 g).

At the time of observation, all the treatments differed significantly over untreated check (15.68 cm). There was a significant difference among the treatments with respect to the root length. Root length of plants ranged from 11.58 cm to 21.40 cm. The maximum reduction of root length was observed in *M. incognita* alone (11.58 cm) followed by inoculation of *M. incognita* before two weeks of rhizobium (12.68 cm), *M. incognita* + *Rhizobium* (14.48 cm), untreated check (15.68 cm), inoculation of rhizobium before two weeks of *M. incognita* (16.35 cm) and *Rhizobium* alone (21.40 cm).

Fresh root weight of black gram was recorded at 60 days after sowing and it was significantly differed in all the treatments compared to untreated check (3.28 g). There was a significant difference among the treatments with respect to the fresh root weight. Fresh root weight of plants ranged from 2.33 g to 5.45 g. The maximum reduction of fresh root weight was recorded in *M. incognita* alone (2.33 g) followed by *M. incognita* + *Rhizobium* simultaneously (2.58 g), inoculation of *M. incognita* before two weeks of rhizobium (3.16 g), untreated check (3.28 g), inoculation of rhizobium before two weeks of *M. incognita* (3.73 g) and *Rhizobium* alone (5.45 g).

Dry weight of root was recorded at 60 days after sowing and was significantly differed in all the treatments compared to untreated check (3.19 g). There was a significant difference among the treatments with respect to the dry root weight. Dry root weight of plants ranged from 2.16g to 4.19g. The maximum reduction in dry root weight was recorded in *M. incognita* alone (2.16 g) followed by *M. incognita* + *Rhizobium* simultaneously (2.23 g), inoculation of *M. incognita* before two weeks of rhizobium (2.64 g), untreated check (3.19 g), inoculation of rhizobium before two weeks of *M. incognita* (3.38 g) and *Rhizobium* alone (4.19g).

Number of pods was recorded at harvest and was significantly differed in all the treatments compared to untreated check (20.10). There was a significant difference among the treatments with respect to pod number. Number of pods in black gram plants ranged from 13.67 to 22.62. The maximum reduction in number of pods was recorded in *M. incognita* alone (13.67) followed by inoculation of *M. incognita* before two weeks of rhizobium (14.60), *M. incognita* + *Rhizobium* simultaneously (2.23), inoculation of rhizobium before two weeks of *M. incognita* (17.00), untreated check (20.10) and *Rhizobium* alone (22.62).

Root and shoot length and the weight of plants were significantly reduced by *M. incognita* infestation. *M. incognita* inoculation in various combinations retarded the plant growth showing infestation by the *M. incognita* as the limiting factor irrespective of the sequence of inoculation in relation to the *Rhizobium*. However, plants showed comparatively better growth when the *M. incognita* inoculation was delayed by a fortnight. On the other hand, the delayed inoculation of *Rhizobium* was not sufficiently useful to the plants since the *M. incognita* had already established itself (Table 11). Heavy damage to the tender roots of the young plants appeared to be the possible explanation for the growth retarding effect of the *M. incognita*. Similar results were obtained by Bopaiah *et al*. (1976) who reported significant reduction in the growth and dry weight of the shoot and root in mung bean in the treatments with *M. incognita* alone and *M. incognita* followed by *Rhizobium* compared to the treatment with *Rhizobium* followed by the *M. incognita*. Wallace (1971) observed that when the *M. incognita* population was high there was a severe effect on root growth, root hair development and that these changes reduce water and mineral absorption and translocation in the plant. These effects result in lower photosynthesis rate and reduced plant growth.

The growth of black gram is markedly reduced by the presence of *Meloidogyne incognita* which adversely affected growth and yield parameters, nodule development and nitrogen fixation. Similar results were obtained by Trabulsi *et al*. (1980) when plants were inoculated with *Rhizobia* but in absence of *M. incognita*, cv. lAC-2 developed the largest number of nodules and fixed the most nitrogen. Root-knot *M. incognita* infestation resulted in a considerable decrease in the number of nodules and nitrogen fixation, the least effect with cv. Jupiter. Nodules on *M.*

*incognita*-infested roots were swollen and brown in colour, compared with the normal whitish to light pink colour on uninfested plants (Bhat, 2009).

**Future line of investigation:**

- Understanding resistance mechanisms and genetic basis in black gram cultivars to *M. incognita*.

- Elucidation of the molecular and physiological pathways induced by nematode and *Rhizobium* in black gram for understanding their economically important relationships.

# VI SUMMARY

The results obtained on various studies conducted during the present investigation with root-knot nematode infesting black gram are summarized in this chapter.

Survey in southern Karnataka districts of major pulse (Black gram, Green gram and Cowpea) growing areas like Chamarajanagara, Hassana, Mandya, Mysuru and Tumakuru revealed the occurrence of root-knot nematode (*Meloidogyne incognita*) in rhizosphere of the pulse crops. Analysis of soil from the rhizosphere of black gram recorded maximum total soil population (657) of *M. incognita* was recorded in Chamarajanagara district followed by Tumkuru district (642), Mandya district (549), Hassana district (480) and the least was recorded at Mysuru district(266) and The maximum root population (85) of *M. incognita* was recorded in Mandya district, followed by Tumkuru district (79), Chamarajanagara district (71), Hassana district (56) and the least population was recorded at Mysuru district (32).

Analysis of soil from the rhizosphere of green gram recorded maximum soil population (600) of *M. incognita* was recorded in Mandya district followed by Chamarajanagara district (588), Tumkuru district (562), Hassana district (544) and the least was recorded at Mysuru district (256) and maximum total root population (79) of *M. incognita* was recorded in Chamarajanagara district followed by Mandya district (61), Tumkuru district (56), Hassana district (55) and the least population was recorded at Mysuru district (43)

Analysis of soil from the rhizosphere of cowpea recorded maximum soil population (569) of *M. incognita* was recorded in Chamarajanagara district followed by Tumkuru district (557), Mandya district (515), Hassana district (464) and the least was recorded at Mysuru district (268) and maximum total root population (66) of *M. incognita* was recorded in Mandya district followed by Chamarajanagara district (64), Hassana district (58), Tumkuru district (54) and the least population was recorded at Mysuru district (30).

The lowest number of galls (6/plant at 60 DAS and 7.5/plant at harvest respectively) were recorded from the roots of G-333(Resistant) cultivar with root-knot indices of 2.0 at 60 DAS and 2.0 at harvest respectively followed by ADT-05 which recorded galls of 17.5/plant at 60 DAS and 24/plant at harvest, with root-knot indices of 3.0 at 60 DAS and 3.0 at harvest, respectively. The variety UH-04-04 was highly susceptible to root knot nematode, recorded higher number of galls (112/plant at harvest) with higher root-knot index (5.0).

*M. incognita* infection significantly reduced the number of rhizobial nodules. A greater reduction in number of nodules was observed when the *M. incognita* was established before the inoculation of the bacteria than the *M. incognita* and *Rhizobium* inoculated together or when the *Rhizobium* was established before the inoculation of *M. incognita*.

In all *M. incognita* inoculated treatments the nitrogen content was significantly reduced compared with plants inoculated with *Rhizobium* only. Maximum reduction in the nitrogen content of shoot (31.6 per cent) and root (25.1 per cent) occurred in plants inoculated with *M. incognita* alone.

Root length, shoot length, pod number and plant weight were significantly reduced by *M. incognita* infestation. *M. incognita* inoculation in various combinations retarded the plant growth showing infestation by the *M. incognita* as the limiting factor irrespective of the sequence of inoculation in relation to the *Rhizobium*. However, plants showed comparatively better growth when the *M. incognita* inoculation was delayed by a fortnight.

Among different treatment imposed plants, *M. incognita* alone inoculated recorded maximum number of galls (114.00 galls/root system) followed by inoculation of *M. incognita* before two weeks of rhizobium.

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