# BIOCHEMICAL CHANGES DUE TO ROOT- KNOT NEMATODE, *Meloidogyne incognita* (KOFOID AND WHITE) CHITWOOD IN GINGER (*Zingiber officinale* Roscoe).

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**(2014-11-225)**

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**DEPARTMENT OF AGRICULTURAL ENTOMOLOGY**

**COLLEGE OF AGRICULTURE**

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*by*

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**(2014-11-225)**

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

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# DECLARATION

I, hereby declare that this thesis entitled **"Biochemical changes due to root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood in ginger *(Zingiber officinale* Roscoe)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Date : **(2014-11-225)**

# CERTIFICATE

Certified that this thesis entitled **"Biochemical changes due to root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood in ginger *(Zingiber officinale* Roscoe)"** is a record of bonafide research work done independently by Mr. Sunilkumar. B. C. (2014-11-225) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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| | |
|---|---|
| & | And |
| *et al*. | And other co workers |
| @ | At the rate of |
| Cm | Centimeter |
| CD | Critical difference |
| cc | Cubic centimetre |
| cv. | Cultivar |
| °C | Degree Celsius |
| d S | desi simen |
| dia. | Diameter |
| $ | Dollars |
| EMI | Egg Mass Index |
| EC | Electrical conductivity |
| Fig. | Figure |
| GI | Gall Index |
| G | Gram |
| ha | Hectare |
| HR | Highly resistant |
| h. | Hours |
| Kg | Kilo gram |
| l. | Litre |
| M | Meter |
| MT | Metric tones |
| µg | Micro gram |

| | |
|---|---|
| µl | Micro litre |
| mg | Milli gram |
| ml | Milli litre |
| min | Minutes |
| MR | Moderately resistant |
| M | Molar |
| MAI | Months After Inoculation |
| *viz.* | Namely |
| nm | Nano meter |
| pH | Negative logarithm of hydrogen ions |
| No. | Number |
| OD | Optical density |
| % | Per cent |
| PO | Peroxidase |
| PAL | Phenylalanine ammonia lyase |
| PPO | Polyphenol oxidase |
| psi | Pounds force per square inch |
| rpm | Revolution per minute |
| RKN | Root-knot nematode |
| $J_2$ | Second stage juvenile |
| Sec | Seconds |
| Sl. No. | Serial number |
| sp. or spp. | Species (Singular and plural) |
| S | Susceptible |
| *i.e.* | that is |
| wt. | Weight |

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# INTRODUCTION

# 1. INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is an annual herb belonging to the family Zingiberaceae. India is the largest producer and exporter of ginger to more than 50 countries, accounting more than 45 % of world production. Ginger occupies fourth position among the spices in India with a production of 6,55,000 MT of ginger from 1,32,000 ha area and fifth position in terms of quality. (National Horticulture Board, 2014). It is grown in many countries of the tropics and subtropics. It is one of the mainstay in Indian spice account and is used widely in food preparations, beverages, confectionery and medicines. Mascolo *et al*. (1989) reported that the rhizome contains biologically active compounds such as gingerol, 6-shogoals, zingiberene, bisabolene and several other types of lipids that are responsible for the characteristics medicinal properties.

Though grown all over India, the finest quality ginger comes from Kerala due to its congenial climate and a rich earthy soil. Ginger cultivation in Kerala is confined to an area of 9,000 ha and contribute to 10.5 % of India's production (National Horticulture Board, 2014). Cochin Ginger and Calicut Ginger are famous Indian dry ginger varieties in the world market.

Nematodes constitute an important group of microscopic organisms, the existence of which is intricately woven with man and his activities. Chitwood, (2003) reported that plant parasitic nematodes are highly destructive plant pathogens causing worldwide losses exceeding $ 125 billion annually. Plant parasitic nematodes belonging to 17 genera were reported on ginger (Sundararaju *et al.,* 1979; Rama and Dasgupta, 1985; Kaur, 1987) and the most important are *Meloidogyne* spp., *Radopholus similis* Cobb and *Pratylenchus coffeae* Zimmermann. Ginger is attacked by a wide range of plant parasitic nematodes that cause damage to the root system directly and or through transmission of fungal pathogens, leading to

substantial yield loss. *Meloidogyne* spp. is one of the most devastating and widespread nematode pests of agricultural crops **(**Sasser, 1989). Sheela *et al.* (1995) reported that *Meloidogyne* spp., *R. similis* and *Pratylenchus* spp. were the major nematodes species found in the rhizosphere of ginger in Kerala. Several species of root knot nematodes *viz. Meloidogyne arenaria* Chitwood, *Meloidogyne hapla* Chitwood, *M. incognita* and *Meloidogyne javanica* Chitwood have been reported as parasites of ginger in various countries. Bridge *et al.* (2005) reported that the root-knot nematode has exceedingly wide host range and attack almost all cereal, vegetable, pulse, fiber, fruit and beverage crops.

*M. incognita* is associated with almost all the cultivated spice crops in India as well as in Kerala resulting in severe crop losses particularly in ginger. *M. incognita* is the most widely distributed and serious pest of ginger (Charles, 1978). The primary symptom of root-knot nematode infection is a formation of typical root galls on the roots of susceptible host plants. Nutrient and water uptake are substantially reduced because of the damaged root system, resulting in weak and low-yielding plants (Abad *et al.*, 2003). *M. incognita* alters the metabolic processes of the host which are manifested in the form of cellular, physiological and biochemical changes occurring in the infected host. Williamson and Gleason (2003) reported that root-knot nematode caused measurable changes in the morphology and physiology of the host.

This endoparasitic nematode, spend a major part of their life cycle embedded in the roots of host plants and is therefore exposed to a variety of host defense responses (Jones *et al.*, 2007). Second stage juveniles ($J_2$) penetrate the roots closely behind the root tip region and then become sedentary. Later they inject secretions into undifferentiated procambial cells near the head of $J_2$ to become multi-nucleate and to form specialized feeding cells called giant cells (Hussey and Grundler, 1998). *M. incognita* infected ginger plants show stunting, chlorosis, poor tillering and

necrosis of leaves. Characteristic root galls and lesions that lead to rotting are generally seen in roots. The infected rhizomes have brown, water soaked areas in the outer tissues. Nematode infection aggravates rhizome rot disease. The losses due to infection by nematodes in ginger varieties differ on the extent of infection and the ability of the cultivars to resist/tolerate infection.

The changes in the physiological, biochemical, growth and yield parameters of infected host decides whether the host becomes susceptible or resistant to nematode attack. In this context, an intimate knowledge of nematode physiology and biochemistry along with its host is absolutely essential for developing plant resistance against the nematodes. In recent past, some progress has also been made in this direction to understand, the basic biochemical mechanism of plant nematode interactions by several workers (Ganguly and Dasgupta, 1983; Mohanty *et al.,* 1995; Darsana *et al.,* 2015).

The defense enzymes *viz*., peroxidase, phenylalanine ammonia lyase, polyphenol oxidase activities and phenol content were found to be higher in *M. incognita* infected resistant varieties. The peroxidase activity decreased in nematode infected susceptible roots compared to nematode resistant roots. Sequential development of polyphenol oxidase increased in nematode infected resistant rice varieties (Kumar *et al*., 2007). The growth and yield parameters decreased in nematode infected susceptible plants compared to nematode resistant plants. Since work on biochemical changes in response to nematode infection in ginger is limited in kerala, the present study entitled "Biochemical changes due to root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood in ginger *(Zingiber officinale* Roscoe)" was taken up with the following objectives.

1. To determine the biochemical changes in ginger due to the infection of *M. incognita.*
2. To assess the changes in growth and yield parameters of ginger due to *M. incognita* infection.
3. To screen five important ginger varieties against *M. incognita.*

# REVIEW OF LITERATURE

# 2. REVIEW OF LITERATURE

*Meloidogyne incognita* (Kofoid and White) Chitwood is one of the most devastating and widespread nematode pests of agricultural crops and hence recent research is focused on this nematode. Literature pertaining to the changes in biochemical, growth parameters and screening of ginger varieties against *M. incognita* is reviewed below.

## 2.1 ROOT KNOT NEMATODE (*M. incognita*) INFECTING GINGER

### 2.1.1 Economic Importance and Yield Loss

Charles (1978) reported that *M. incognita* was the most important plant parasitic nematode infecting ginger causing an yield loss of 46.4 per cent in Kerala. Sudha and Sundararaju (1986) observed a reduction of 75 per cent rhizome weight in ginger with an initial inoculum level of 10,000 $J_2$ of *M. incognita* per plant over a period of six months under potted conditions. In another study on ginger, Sheela *et al.* (1995) found that an avoidable yield loss of 43 per cent with an initial population level of 166 *M. incognita* juveniles per 250 g soil. *M. incognita* significantly reduced the height, root biomass and yield of ginger with an initial inoculum level of 200 $J_2$ /100 cc soil (Ramana and Eapen, 2001).

### 2.1.2 Life Cycle

*M. incognita* are sedentary endoparasites, which spend a major part of their life cycle embedded in the roots of host plants. The female deposits single-celled eggs in a gelatinous mass at or near the root surface. Embryonation of the egg begins immediately and continues until worm-shaped juveniles hatches. This juvenile is about 1/60 of an inch long and is in the second stage, having passed through one

moult within the egg. It then migrates either into the soil or to a different location in the root.

Nematodes pass through an embryonic, four juvenile ($J_1$-$J_2$) and an adult stage. Juveniles of *Meloidogyne* sp. that hatch from eggs as vermiform are $J_2$. The first molt occurs within the eggs. Newly-hatched juveniles have a short free-living stage in the soil and do not feed during the free-living stage, but use lipids stored in the gut. They may reinvade the host plants of their parent or migrate to find a new host root (Eisenback and Triantaphyllou, 1991).

Many reports are available on the biology of various species of these nematodes on tomato; *M. incognita* and *M. arenaria* (Dement'eva, 1980), *M. javanica* and *M. acrita* (Pogrebnova, 1983), *M. hapla* (Stephan, 1983) and others. Dement'eva and Sadykin (1982) conducted a study on a highly susceptible tomato cultivar (Teplichnyl-200). *M. incognita* completed its life cycle in 45 days during 1977 when soil temperature was 21-30 $^0$C. A moderately susceptible cultivar took 57 days in 1977 and 55 days in 1978. Similar results were found with *M. arenaria* also.

The biology and life cycle of *M. incognita* on carnation showed that most of $J_2$ invaded the roots of carnation within two days after the plants inoculated with nematodes (Kavitha, 2012). In general *M. incognita* took 25.66 days to produce galls, egg masses (28 days) and contained 398 eggs/egg mass. Totally, the nematode took 24-34 days to complete its life cycle on carnation.

## 2.2 BIOCHEMICAL CHANGES IN GINGER DUE TO *M. incognita*

### 2.2.1 Plant Growth and Yield Parameters

In a study conducted by Ahmed *et al.,* 2009 on Mungbean, plant growth was severely affected by *M. javanica* infection in terms of shoot length and shoot weight of plants.

Ray *et al*. (1995) reported that yield loss in turmeric due to the *M. incognita* infection was 35.97 per cent in 1991 and 31.25 per cent in 1992 (average 33.61 per cent) and yield loss in ginger due to this nematode was 23.97 per cent in 1991 and 28.67 per cent in 1992 (average 26.32 per cent).

The plant response to *M. incognita* parasitism induces morphological and physiological changes that affect photosynthetic processes in french bean (Melakeberhan *et al.,* 1986). Jonathan and Rajendran (2000) carried out pathogenicity test of *M. incognita* on banana and found that reduction in plant growth parameters *viz.,* plant height, pseudostem girth and number of leaves occurred at 1,000 and 10,000 juveniles per kg soil. In a study conducted by Bhatt *et al.* (2001) on sunflower infected with *M. incognita*, there was a reduction in plant growth parameters with a population of 2500 $J_2$ per 200 cc soil. Increase in the population density of *M. javanica* was negatively correlated with growth of tomato and pepper (Mekete *et al.,* 2003).

Kheir *et al.* (2004) found significant reduction in growth of banana cultivars inoculated with more than 1,000 $J_2$ of *M. incognita* per plant. Azam *et al.* (2010) reported that there was a significant reduction of plant height, fresh weight and dry weight of shoot at highest inoculum level of *M. incognita* (20,000 $J_2$ $pot^{-1}$) on *Lagenaria siceraria*. In a study conducted by Anamika (2015), there was a decrease

in growth parameters *viz.,* shoot length and shoot weight with increase in inoculums levels of *M. incognita* on tomato, brinjal, spinach and beet root.

Patil and Gaur (2014) reported that the rice cultivars Pusa- 44 and sugandh- 5 showed significantly lower plant height, shoot and root dry weight with increasing inoculums levels of *M. graminicola*. Abbasi and Hisamuddin (2014) found that plant length, fresh weight of the whole plants and dry weight of shoot and root decreased significantly with an increase in inoculum levels of *M. incognita* in *Vigna radiata*.

Mhatre *et al*. (2015) reported that the *M. graminicola* infection in rice leads to poor growth and 50-75 % reduction in root system of rice.

Darsana *et al.,* (2015) found that significant reduction in plant height, fresh weight and dry weight of shoot in rice due to infection of *M. graminicola* at 500 $J_2$ onwards.

### 2.2.2 Biochemical Changes

#### *2.2.2.1 Phenol Content*

Phenolic compounds played a major role in the defense mechanism of the plants against various infectious agents. The increase in phenolic compounds during the infection period leads to the rapid break down of bound phenols or switching over of phenols to different pathways leading to the formation of various compounds like lignin which plays significant role in resistant reaction.

Phenolic compounds associated with *M. incognita* injury, leads to the browning of plant tissues (Sharma *et al*., 1990). In a study on apple, Pitcher *et al.* (1960) observed that there was a distinct correlation between degree of plant resistance to *Pratylenchus penetrans* and amount of phenolic compounds present in

plant tissues. Giebel (1974) found that the phenolic substances were the best known factors in susceptible-resistant response of potato towards the infection of *Globodera rostochiensis*.

Sempio *et al.,* 1975 observed that phenolic compounds are converted by increased peroxidase activity to quinines in resistant bean cultivars and quinines are reported to be more toxic to pathogen causing rust.

Bajaj and Mahajan (1977) reported that the oxidized forms of phenolic compounds occurring in high concentration in roots of resistant tomato plants and it contributes to the *M. incognita* resistance by creating a toxic environment for nematode penetration and multiplication.

Ganguly and Dasgupta (1982) reported that in resistant tomato varieties, the non-toxic phenolic glycosides was hydrolysed by β-glycosidase enzymes secreted by *M. incognita* and the resultant product prevented localized parasitization or even caused the death of the nematode.

The *M. incognita* infected tomato plant showed more accumulation of phenolic compounds and activity of oxidative enzymes like peroxidise and polyphenol oxidase (Bajaj *et al.,* 1983).

The phenolic compounds increased in *M. incognita* infected resistant varieties compared to susceptible varieties of tomato (Narayan and Reddy 1980; Mote *et al*., 1990; Choudhary *et al*., 2013) and chickpea (Chakrabarti and Mishra, 2002).

Vlot *et al*. (2009) found that salicylic acid activate host resistance to pathogens by inducing the synthesis of PR proteins and defense related phenolic substances and enzymes.

Varanasi and Thalati (2014) reported that the induction of total phenols was more in chickpea roots than in leaves, as roots are the primary sites of infection for *M. incognita*. The increase in total phenol was higher at 10,000 $J_2$/plant.

### *2.2.2.2 Peroxidase, Polyphenol Oxidase and Phenylalanine Ammonia Lyase Activity*

Inducible defense against nematodes included accumulation of peroxidase **(**Ibrahim, 1991), polyphenol oxidase and superoxide dismutase (Zacheo and Zacheo, 1995). The *M. incognita* infection alters the plant enzymes *viz.,* peroxidase, polyphenol oxidase, catalase and phenylalanine ammonia lyase. The concentration of these enzymes were less in susceptible varieties compared to resistant varieties (Sharma, 1993; Molinari, 1995). Patel *et al.* (2001) observed that the *Meloidogyne* spp. have the ability to induce synthesis of peroxidase, polyphenol oxidase and total phenol in roots of chickpea (*Cicer arietinum* L.).

Devrajan and Srenivasan (2002) found that the synthesis of peroxidase and polyphenol oxidase in the roots of banana (cv. Robusta) due to infection of *M. incognita*.

In a study conducted by Sundararaju and Suba (2006), the *M. incognita* infection increases phenol content, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activity in the roots of banana.

Peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activities significantly increased in roots of *M. exigua* Goeldi inoculated coffee plants compared to the roots of uninoculated plants (Silva *et al*., 2010).

Korayem *et al.* (2012) found that the infection of *M. incognita* in sugarbeet genotypes induced the activities of polyphenol oxidase, peroxidase, superoxide dismutase and catalase enzymes.

Darsana (2015) reported that there was a increase in activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase with increase in inoculum levels of *M. graminicola.* Higher enzymatic activity was recorded at inoculum level of 10,000 $J_2$/pot.

#### *2.2.2.2.1 Peroxidase*

Peroxidase played a vital role in alleviating free radical toxicity in plant tissues. Following the entry of nematode, an increased peroxidase level in the infected plant suggested a great de novo synthesis of peroxidases since nematode do not exudate peroxidase (Glasiov *et al.,* 1967). Increased peroxidase activity in roots of pea (*Pisum sativum*) enhanced the resistant response against *Heterodera goettingiana* (Arrigoni *et al.,* 1981).

Shukla and Chakraborty (1988) reported that peroxidise activity were increased in resistant cultivars of tomato and tobacco up to five times compared to susceptible plants at 10 days after inoculation with *M. incognita*. The enzymatic activities were reported to decrease thereafter to normal levels within few days.

Kumar *et al*. (2007) reported that peroxidase activity decreased in roots of rice cultivars susceptible to *M. graminicola* compared to roots of resistant cultivars.

Varanasi and Talati (2014) found that the peroxidase activity increased with increase in infection levels of *M. incognita* in chickpea. Significantly higher peroxidase acitivity was recorded at 10,000 $J_2$/plant.

#### *2.2.2.2.2 Phenylalanine Ammonia Lyase*

Phenylalanine ammonia lyase was considered as the most important enzyme in disease resistance. The activity levels of phenylalanine ammonia lyase and anionic peroxidase induced early resistance response to *M. incognita* infecting tomato (Brueske, 1980; Zacheo *et al.*, 1993).

The enzyme activity of phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) was higher in the resistant potato variety than the susceptible one and the enzyme activity was greater in the roots of *Globodera rostochiensis* infected plant than in the healthy one (Giebel, 1973). Infection of *M. incognita* increased the polyphenol oxidase activity in banana (Devrajan and Srenivasan, 2002). Devi *et al*. (2007) reported that there was an increase in polyphenol oxidase activity in resistant varieties of banana in response to *Pratylenchus coffeae* infection compared to susceptible varieties.

The rice root-knot nematode, *M. graminicola* induced the synthesis of PAL and TAL enzymes in the affected tissue of rice. The higher activity of both enzymes were recorded in the roots of resistant variety Ramakrishna than in the susceptible variety Annapurna (Mishra and Mohanty, 2007).

#### *2.2.2.2.3 Polyphenol Oxidase*

Ganguly and Dasgupta (1984) reported an increased polyphenol oxidase activity in resistant varieties of tomato in response to *M. incognita* infection. Hasan and Saxena (1997) showed that polyphenol oxidase activity increased by 16-24 per cent in resistant cultivars of tomato. However, in susceptible cultivars it was 12-18 per cent.

Nagesh *et al.* (1998) observed higher polyphenol oxidase activity and indole-acetic acid oxidase activity in *M. incognita* infected tomato roots of both the susceptible (Pusa Ruby) and the resistant (Mangala) cultivars compared to healthy cultivars.

### *2.2.2.3 Starch and Protein*

Mohanty and Pradhan (1989) reported that the *M. incognita* infected plants showed decreased protein content and increased free amino acids and amides. They also observed that the susceptible green gram plant showed low protein content and high level of amino acids after inoculation of nematodes.

Sawant and Gawai (2011 reported that there was a reduction of protein content in fruits of papaya due to the infection of pathogens *viz.*, *Rhizopus, Aspergillus, Penicillium*, *Fusarium* etc. In a study conducted by Saxena and Karan (1991), there was a gradual loss of protein and carbohydrate content in sesame and sunflower seeds as a result of infection by pathogens like *A. flavus* and *A. niger* during storage.

Korayem *et al*. (2013) observed that crude protein and fat content decreased in peanut seeds infected with *M. arenaria*. *Meloidogyne incognita* infected green gram seeds showed low crude protein and fat content compared to healthy plants (Abbasi and Hissamuddin, 2014).

Patil and Gaur (2014) reported that the rice grains produced on plants infected with *M. graminicola* had less weight and had poorer nutrient qualities, such as amylose and protein content.

The protein and starch content of rice decreased with increase in inoculum levels of *M. graminicola* when compared to uninoculated plants (Darsana *et al.,* 2015).

### *2.2.2.4 Crude Fiber , Oleoresin and Total Ash*

Assessment of ginger varieties based on fibre content is important since low fibre content is considered as one of the desirable processing qualities of ginger. Presence of ample amounts of dietary fiber in ginger might be advantageous as they are known to reduce serum cholesterol level, reduce risk of coronary heart disease and lower the risks from hypertension and constipation (Ishida *et al*., 2000). Total ash content is also important to ascertain deposition of mineral matter in a biological system. Oleoresin represents the sum total of aromatic and pungent principles of ginger. This attribute also greatly varies with varieties, rhizome maturity and method of extraction. One of the protein constituent amino acids, *viz*., phenylalanine is considered as the precursor for biosynthesis of gingerol, the pungent principle of ginger (Denniff *et al*., 1980). Winterton and Richardson (1965) reported that the fibre content of rhizome increased with advancement of maturity.

Farag (1990) observed that the *A. flavus* affect the chemical compositions including crude fibre content of sesame and soybean. Choudhari and Kareppa (2013) reported that the crude fibre, gingerols, shagaols, oleoresin, volatile oil, non volatile ether extract, alcohol soluble extract, cold water soluble extract, total ash, water soluble ash and acid insoluble ash content of infected ginger plant reduced due to infection of *Fusarium* spp.

## 2.3 VARIETAL SCREENING

### 2.3.1 Reaction of Varieties

Cucumber varieties were tested for resistance to *M. arenaria* and *M. javanica*, using soil infested with 10,000-12,000 nematode eggs and juveniles /kg soil. Cucumber var. Ralda and pepper var. Clovis proved highly resistant, cucumber var. Capris were more resistant than the respective, susceptible standards BelyiNaliv, Leila and Biblos (Khelu *et al*., 1989).

Cho *et al.* (1996) screened 33 carnation cultivars for resistance to the *M. incognita.* Eight cultivars (Kappa, Echo, Rara, Izu Pink, Target, Castelaro, Antalia, and Desio) had 0 to 1.5 galls/ root system and were highly resistant to *M. incognita.* Eleven cultivars had 3 to 25.4 galls/root system and were categorized as moderately resistant. The remaining 14 cultivars, with more than 42 galls, were categorized as susceptible.

Praveen and Nanjegowda (2004) screened gherkin cultivars, against *M. incognita*. Among them Stemora and PS 64487 recorded least number of galls 34 and 57 respectively and egg masses of 13 and 28 respectively. Out of six cucumber varieties evaluated against *M. incognita*, Poinsette was moderately resistant to *M. incognita.* Cv. N.C-42 and NC-43 were resistant to *M. arenaria* and *M. javanica* respectively.

Mohamed and Hasabo (2005) evaluated five different cotton (*Gossypium barbadense* L.) cultivars against *M. incognita* under greenhouse conditions and found that only Giza 86 cultivar showed resistance because of induced biochemical changes after nematode infection and sequential development of chitinase, peroxidase and acid phosphatase in both cultivars (Giza 86 and Giza 89 race 2000) but the level of these compounds was much higher in roots of Giza 86 as

compared to susceptible Giza 89 race 2000 which supported lower population of *M. incognita*. These results indicate the role of these enzymes in the resistance mechanism of cotton against nematode infection.

Sharma *et al*. (2006) studied the reactions of 23 selections of field pea (*Pisum sativum* L.) against *M. incognita* by inoculating 2000 $J_2$/pot. 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, HFP-0118 were moderately resistant; HFP-0110, HUDP-28, HUDP-15, HUDP-27, HUP-30, HUP-2 and HUDP-26 were moderately susceptible and the variety Ambika was susceptible to *M. incognita*.

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

Malhotra *et al.* (2012) screened five cultivars of chilli, *Capsicum annuum* L. for resistance against *M. incognita* in pot experiments. Three-week-old seedlings were inoculated with 1000 freshly hatched juveniles of *M. incognita*. The cultivar Pusa Jwala was assessed as moderately resistant with the minimum number of galls and variety PC-1 was highly susceptible with maximum number of galls. All the other varieties exhibited variable degree of susceptibility between Pusa Jwala and PC-1.

Ramya *et al*. (2013) screened 20 varieties and hybrids of sunflower seed by inoculating two juveniles per CC soil. Average number of galls per root system ranged from 6.7 (CO-1) to 63.7 (CO-3). Only one cultivar CO-1 was found resistant

to *M. incognita* with an average gall number of 6.7. Five lines (DRSF-113, CO-4, CO-5, PKV-SF-9 and KBSH-41) were found moderately resistant with an average gall number of 15.3 to 30.3. Fourteen lines were found susceptible with an average gall number of 30.7 to 63.

Okorocha *et al.* (2014) screened seven ginger varieties for its resistance to root-knot nematode, *Meloidogyne* spp. Among the seven varieties tested Maran, U.G I and Rio de Jenairo were resistant while U.G II, Himachal Pradesh and St. Vincent were susceptible and Wynad Local was tolerant.

Mohanta *et al.* (2015) screened a total of seventy turmeric cultivars to identify the resistance to *M. incognita*. Among the cultivars tested Dugirala, PTS-31, Ansitapani, PTS-42, PTS-47 were found to be resistant. 361 Gorakhpur, 328 Sugandham and PTS-21 were rated as moderately resistant and remaining cultivars were susceptible.

Nayak and Pandey (2015) screened one hundred fifty brinjal varieties/cultivars against *M. incognita.* Twenty varieties had shown resistant reaction with least gall index of 1.1 to 2.0, fifty eight varieties/cultivars moderately resistant with gall index of 2.1 to 3.0. Forty seven varieties were susceptible with gall index of 3.1 to 4.0 and remaining twenty five varieties were highly susceptible with highest gall index of 4.1 to 5.0 against the *M. incognita*.

### 2.3.2 Gall Index and Egg Mass Index

Level of root galling was directly related to the number of females of *M. hapla* within the roots of alfalfa seedlings (Elgin *et al.,* 1973).

Cho *et al.* (1996) screened 33 carnation cultivars against *M. incognita.* Cultivars with 0 to 1.5 galls/ root system was designated as highly resistant, those

with 3 to 25.4 galls/root system as moderately resistant. However, cultivars with more than 42 galls was designated as susceptible. Khan and Anwer (2011) reported that symptoms of *M. graminicola* infection in roots of rice exhibited spiral, terminal or hooked galls which is characteristics of that nematode. Chakrabarti and Mishra (2002) reported that there was a negative correlation between number of galls and amount of phenolics in chickpea roots infected with *M. incognia*.

Ramya *et al*. (2013) observed that out of 20 released varieties and hybrids of sunflower, only one cultivar CO-1 was found resistant to root-knot nematode with an average gall number of 6.7. Five lines (DRSF-113, CO-4, CO-5, PKV-SF-9 and KBSH-41) were found moderately resistant with an average gall number of 15.3 to 30.3. Fourteen lines were found susceptible with an average gall number of 30.7 to 63.

Berliner *et al*. (2014) categorized the rice cultivars as resistant or susceptible to *M. graminicola* on the basis of gall index. Out of 414 rice genotypes screened, only two cultivars 127-28-1-1-1 and 183-6- 1-1-3 were found resistant with score two.

Khan *et al*. (2014) reported that the rice variety PS-5 grown in the *M. graminicola* infested field showed extensive terminal galls which varied in number from 73 to 81 galls per root system.

A large variation in number of galls caused by *M. graminicola* per root system in different rice cultivars was observed by Mhatre *et al*. (2015). They also reported that among the tested cultivars, Abhishek exhibited the best resistance response with 2 galls/plant and Bangla Patni with more than 100 galls/plant.

Nayak and Pandey (2015) found that resistant brinjal varieties showed least gall index (1.1 to 2.0), moderately resistant varieties showed gall index of 2.1 to 3.0,

susceptible varieties had gall index of 3.1 to 4.0 and highly susceptible varieties with highest gall index of 4.1 to 5.0 against the *M. incognita*.

### 2.3.3 Nematode Population

Poudyal (2001) reported that final $J_2$ population in soil and root, egg density and root knot index differed significantly with initial levels of *M. graminicola* at 150 days after inoculation.

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

Devi (2014) observed that the *M. graminicola* infected rice variety Lamyanba showed maximum soil population (7500), root population (3500) and total population (11000) compared to other variety Dharam.

Khan *et al*. (2014) found that the *M. graminicola* population in the soil was almost double the initial population after one month of planting, and further increased to approximately four times the initial population at harvest (4 months).

# MATERIALS AND METHODS

# 3. MATERIALS AND METHODS

Pot culture experiments were conducted in College of Agriculture, Vellayani during 2014-2016 to determine the biochemical changes in ginger due to root knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood and to determine the varietal reaction of five important varieties of ginger grown in Kerala against *M. incognita*.

## **3.1** MAINTENANCE AND MULTIPLICATION OF PURE CULTURES

### 3.1.1. Soil Sterilization

Field soil was collected, sieved and mixed thoroughly with compost in 2:1:1 proportion. Cleaned soil mixture was sterilized by using an autoclave at 121 °C at 15 psi for 15 minutes.

### 3.1.2 Collection and Maintenance of Cultures of *M. incognita*

Tomato roots infected with *M. incognita* were collected from root knot infested fields of College of Agriculture, Vellayani. Viable single egg masses were handpicked from the infected roots and kept on two layers of tissue paper supported by aluminium wire gauge in 10 cm sized Petri dishes filled with fresh water for obtaining required number of $J_2$ (second stage juveniles). The population of $J_2$ of *M. incognita* in the suspension was determined by counting $J_2$ in 5 ml aliquots. Three such counts were made and the average of 3 counts was taken as population in 5 ml suspension. The $J_2$ concentration was adjusted to required number of juveniles per ml of suspension by adding required quantity of sterile water.

Earthern pots of 30 cm dia. was filled with sterilized potting mixture and maintained in net house. Tomato plants were raised in these pots and the rhizosphere was inoculated with 5 ml of $J_2$/pot. Pure culture of *M. incognita* was raised from

single egg mass collected from infested roots of tomato. It was further multiplied in sterilized soil on tomato. The nematode was identified by observing perennial pattern of the female nematodes (Chitwood, 1949). Subculturing was done periodically to ensure availability of sufficient population of infective juveniles for inoculation by following the standard procedures.

### 3.1.3 Identification of Root-Knot Nematode Species

The root systems with well developed galls were selected from pure culture plants maintained under net house of Dept. of Nematology, College of Agriculture Vellayani.

The galled roots were immersed in a beaker containing 0.1 per cent boiling cotton blue/lactophenol solution for two minutes and then it was cooled by washing in running tap water. Roots were dipped in plain lactophenol and left over night for clearing. The root galls were dissected under a stereo binocular microscope with the help of a sharp needle to release a female nematode which was then transferred to drop of lacto phenol taken on a glass slide, the posterior portion of the nematode was carefully cut with a sharp blade and the body contents were cleaned. Cleaned posterior portion of the nematode was further trimmed and transferred to drop of glycerin on a clean microscopic slide. A cover slip was placed on it, sealed with nail polish and observed under a stereo binocular microscope (Taylor and Netscher, 1974). The species was confirmed as *M. incognita* based on the perennial pattern as described by Chitwood (1949).

### 3.1.4 Estimation of Soil Nematode Population.

Soil sample of 200 cc was washed thoroughly and processed using combined "Cobb's sieving and Modified Baermann's funnel method" (Schindler, 1961) as given below.

Soil sample of 200 cc capacity was taken in 1000 ml beaker and sufficient quantity of water was added to make soil solution. This was stirred thoroughly and allowed to stand for heavier particles to settle down. Then the soil solution was passed through a set of sieves of 100, 250, 325 and 400 mesh sizes, respectively. Residue from 325 and 400 mesh sieves were collected and poured over a tissue paper spread on a wire gauge and placed on Petri dish. Level of water in the Petri dish was maintained to keep the tissue paper wet and left undisturbed for 48 hours. After 48 hours of incubation, the volume of suspension in the Petri dish was collected. The population of juveniles in the suspension was determined by counting $J_2$ in 5 ml aliquot. Juvenile population from this was finally estimated for 200 cc soil.

### 3.1.4 Estimation of Nematode Population in Root Samples

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

Roots were washed and cut into small bits of 2.5 cm and split longitudinally. These were then placed over tissue paper spread on a wire gauge and kept in a Petri dish. Level of water in the Petri dish was maintained to keep the tissue paper wet and left undisturbed for 48 hours. Later, the suspension in the Petri dish was collected and observed for root knot nematodes using stereo binocular microscope.

## **3.2.** BIOCHEMICAL CHANGES IN GINGER

Biochemical analysis of ginger plants with different nematode inoculum were carried out in 30 cm diameter earthen pots. Rhizomes of ginger variety IISR Varada were planted in pots filled with steam sterilized soil. Ten days after planting pots were inoculated with freshly hatched juveniles at the rate of 0, 100, 500, 1000 and 10,000 $J_2$ per pot through the holes around the plant within the radius of two centimeters and plugged with the sterilized soil soon after inoculation. To maintain

soil moisture in the pot, regular watering was done. Each treatment was replicated five times and the pots were arranged in Complete Randomized Block Design (Plate 1.). Uninoculated set of plants served as control. There were five sets of pots as given below:

$T_1$: 100 $J_2$/ pot

$T_2$: 500 $J_2$/ pot

$T_3$: 1000 $J_2$/ pot

$T_4$: 10,000 $J_2$/ pot

$T_5$: Uninoculated plant

After six months of inoculation, the following parameters were taken into account for describing the results. Biochemical analysis was carried out to estimate the changes in the total phenols, protein, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, pH of the rhizome extract, starch, crude fibre, oleoresin and total ash content in rhizome and leaves.

### 3.2.1 Growth and Yield Parameters

#### 3.2.1.1 *Fresh Weight of Shoot*

Shoot weight of plants at six months after inoculation (MAI) was assessed after removing the root portion of the plant. Then it was weighed and the mean of fresh shoot weight per plant was ascertained. It was expressed in g plant$^{-1}$.

Plate 1. General view of pot culture experiment

#### 3.2.1.2 *Dry Weight of Shoot*

Shoot weight of the plants were taken at six MAI by cutting off the shoots. Shoots were dried in oven till constant weight obtained and weighing the oven dried shoot separately for each plant. Mean shoot dry weight per plant was determined and it was expressed in g plant$^{-1}$**.**

#### 3.2.1.3 *Height of Plant*

The height of plant was assessed at six MAI. Height was measured from the base of the stem to the tip of the largest leaf and the average height was calculated. It was expressed in centimeter (cm).

#### *3.2.1.4 Number of Tillers*

The number of tillers was assessed at six MAI. Number of tillers was counted from each plant.

#### *3.2.1.5 Rhizome Yield*

Yield of rhizome was assessed at six MAI.

### 3.2.2 Biochemical Observations

#### *3.2.2.1 pH of the Rhizome Extract*

Ten gram of rhizome sample was taken and crushed by using pestle and mortar. The ground samples were added to beaker containing 80 ml distilled water. The samples were shaked in a laboratory rotary shaker for 1 h, and centrifuged Pruthi, (1999). The pH of supernatant was measured using a wide range of laboratory pH meter (using buffer solutions of pH 4 and pH 7).

### *3.2.2.2 Estimation of Peroxidase (PO) in Leaf and Rhizome*

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

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

### *3.2.2.3 Estimation of Polyphenol Oxidase (PPO) in Leaf and Rhizome*

Polyphenol oxidase activity was determined as per the procedure given by Mayer *et al.* (1965). Leaf/Rhizome sample of one g was homogenized in 5 ml of 0.1 M sodium phosphate buffer (pH 6.5) to which a pinch of polyvinyl pyrrolidone (PVP) was added. The homogenization was done at 4 $^0$C using a pre-chilled mortar and pestle. The homogenate was filtered through a muslin cloth and centrifuged at 5000 rpm for 15 min at 4 $^0$C. The supernatant was used as the enzyme extract for the assay of PPO activity.

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

of 0.01 M catechol. The observations were recorded in a spectrophotometer (Systronics UV-VIS spectrophotometer 118). The change in absorbance was recorded at 495 nm at 30 seconds interval up to 180 sec. PPO activity was expressed as change in the absorbance of the reaction mixture $min^{-1}$ $g^{-1}$ of tissue on fresh weight basis.

### *3.2.2.4 Estimation of Phenylalanine Ammonia Lyase (PAL) in Leaf and Rhizome*

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

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

### *3. 2.2.5 Estimation of Phenol in Leaf and Rhizome*

The phenol content was estimated following the procedure described by Bray and Thorpe (1954).

Leaf/Rhizome sample (one g) was homogenized in 10 ml of 80 per cent

ethanol. The homogenate was then centrifuged at 10,000 rpm for 20 min, supernatant was saved and the residue was extracted with 5 times the volume of 80 per cent ethanol and centrifuged as above. The supernatant was saved and evaporated to dryness in a boiling water bath. The residue was dissolved in 5 ml of distilled water. An aliquot of 0.2 ml was pipetted out and made up to 3 ml with distilled water. Folin- Ciocalteau reagent (0.5 ml) was added and 2 ml of 20 per cent sodium carbonate solution was added to each tube after 3 min. This was mixed thoroughly and kept in boiling water for 1 min. The reaction mixture was cooled and absorbance was measured at 650 nm against a reagent blank. Standard curve was prepared using different concentrations of catechol. Phenol content was expressed as mg catechol equivalent of enzyme activity per g leaf tissue on fresh weight basis.

#### *3.2.2.6 Estimation of Protein*

Total soluble protein content was estimated as per the procedure described by Bradford (1976).

Rhizome sample weighing one g was homogenized in 10 ml of 0.1 *M* sodium acetate buffer (pH 4.7) (Appendix III) and centrifuged at 5000 rpm for 15 min at 4 $^{o}$C. The supernatant was stored for the estimation of soluble protein. The reaction mixture consisted of 0.5 ml enzyme extract, 0.5 ml distilled water and 5 ml of diluted (5 times) dye solution (Appendix III). The absorbance was read at 595 nm in a spectrophotometer against reagent blank. Bovine serum albumin was used as the protein standard. The protein content was expressed as mg albumin equivalent of soluble protein per g on fresh weight basis.

#### *3.2.2.7 Estimation of Starch*

The starch content in dried rhizome was estimated by anthrone method (Sadasivam and Manickam, 2008) and expressed in percentage.

Dried rhizome sample weighing 0.5 g was homogenized in hot 80 per cent ethanol to remove sugars and centrifuged and retained the residue. The residue was repeatedly washed with hot 80 per cent ethanol till the washing does not give colour with anthrone reagent (Appendix IV). The residue was dried over a water bath. To that residue 5 ml of water and 6.5 ml of 52 per cent perchloric acid were added and extracted at 0 $^0$C for 20 min. It is then centrifuged and supernatant was collected. The procedure was repeated using fresh perchloric acid and the supernatant was pooled and made up to 100 ml. Pipette out 0.1 or 0.2 ml of the supernatant and the volume was made up to one ml with water. The standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and made up the volume to 1 ml in each tube with water. 4 ml of anthrone reagent was added to each tube and heated for eight minutes in a boiling water bath. Then it was cooled and the intensity of green to dark green colour was measured at 630 nm.

### *3.2.2.8 Estimation of Crude Fibre*

Crude fibre content in dried rhizome was estimated as per the procedure described by Maynard (1970) and expressed in percentage.

In order to remove any fat content in the rhizome sample of ginger, the dried rhizome samples were powdered using a blender and two g of the powdered material was extracted with ether. It was boiled at an initial temperature of 35-38 $^0$C and finally at 52 $^0$C. After extraction with ether, 2 g of dried material was boiled with 200 ml of sulphuric acid for 30 min with bumping chips. Then it was filtered through the muslin cloth and thereafter washed with boiling water until it was no longer acidic. Then it was boiled with 200 ml of sodium hydroxide solution for 30 min. and filtered through muslin cloth, further it was washed with 25 ml of boiling 1.25 per cent $H_2SO_4$, followed by 50 ml portion of water and 25 ml alcohol. Residues were removed for 2 h at 130±15 ºC and cooled in desiccators and reweighed.

### *3.2.2.9 Estimation of Oleoresin*

Oleoresin content in dried rhizome was estimated as per the procedure described by Pruthi, (1999).

Ginger oleoresin was obtained by extraction of 30 g of dried ginger powder with acetone by soxhlet extraction. The solvent was carefully removed by distillation and the concentrated extracts (oleoresin) were collected. The difference between the empty flask and flask with oleoresin was used in obtaining the oleoresin content yield and it was expressed in percentage.

### *3.2.2.10 Estimation of Total Ash*

Total ash content in dried rhizome was estimated as per the procedure described in official analytical chemist (AOAC), 1990 and expressed in percentage.

Two gram of the sample was weighed into porcelain crucible, this was transferred into the muffled furnace at 550 $^{o}$C and left for about four hours. About this time it had turned to white ash. The crucible and its content were cooled to about 100 $^{o}$C in air then to room temperature in a dessicator and weighed.

$$\text{Per cent Ash content} = \frac{\text{weight of ash}}{\text{Original wt. of sample}} \times 100$$

### *3.2.2.11 Estimation of Gingerol and Shagaol*

Gingerol and shagaol of healthy and infected rhizomes were estimated from Spices Board of India, Cochin. Samples were analysed chemically according to official analytical chemist (AOAC) method (1990).

## **3.3** VARIETAL REACTION

Ginger varieties *viz*., Athira, Karthika, IISR Mahima, IISR Rejatha and IISR Varada were collected from the College of Horticulture, Vellanikara, Kerala. Rhizomes were planted in pots filled with steam sterilized soil. Ten days after planting, pots were inoculated with freshly hatched juveniles at the rate of 10,000 $J_2$ per pot by making holes around the plant within the radius of two centimeters and plugged with the sterilized soil soon after inoculation. The plants were maintained with five replications for each variety in completely randomized design under net house. To maintain soil moisture in the pot, regular watering was done.

A similar set of pots were maintained simultaneously for subjecting the plant sample to study the biochemical alterations induced by *M. incognita* in resistant/susceptible cultivars of ginger. The experiment was terminated at 6 MAI and the plants were observed for egg mass index and root knot index.

Five popular ginger varieties of Kerala *viz*., IISR Mahima, IISR Rejatha, IISR Varada, Athira and Karthika were screened for comparing the relative susceptibility/tolerance to root-knot nematode, studying the biochemical changes and growth parameters. The trial was laid out in Completely Randomized Design with five replications.

### **3.3.1 Nematode Population in Varieties**

#### **3.3.1.1** ***No. of Egg Mass per Plant***

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

### 3.3.1.2 *Egg Mass Index*

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

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

The Egg mass indexing in varietal screening was done as follows:

| Egg mass index | Host reaction |
|---|---|
| 0 | Highly resistant |
| 0.1-1.0 | Resistant |
| 1.1-2.0 | Moderately resistance |
| 2.1-3.0 | Susceptible |
| 3.1 and above | Highly Susceptible |

### 3.3.1.3 *No. of Galls per Plant*

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

#### 3.3.1.4 *Root Knot Index*

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

| Observation | Root knot index | Host reaction |
|---|---|---|
| 0 galls/ plant | 1 | Highly resistant |
| 1-10 galls/ plant | 2 | Resistant |
| 11-30 galls/ plant | 3 | Moderately resistance |
| 31-100 galls/ plant | 4 | Susceptible |
| Above 100 galls/ plant | 5 | Highly susceptible |

### 3.3.2 Population of Nematode

#### 3.3.2.1 *Nematode Population in Soil*

Soil samples were collected from each pot after six months of nematode inoculation and nematode was extracted from the respective soil samples following the method of combined Cobb's sieving and Modified Baermann's funnel method (Schindler, 1961). The population of the nematode thus extracted was counted under a stereoscopic microscope.

#### 3.3.2.2 *Nematode Population in Roots*

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

#### 3.3.2.3 *Nematode Population in Rhizome*

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

## 3.4 STATISTICAL ANALYSIS

Data generated from the experiment were subjected to statistical analysis applying ANOVA technique and significance was tested by 'F' test. In the cases where the effects were found to be significant, critical difference values were calculated for each observation using table't' values at 5 per cent level of significance. Then, the significance of treatments was compared with the critical difference values.

# RESULTS

# 4. RESULTS

An experiment to assess the biochemical changes due to root- knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood in ginger was undertaken at Department of Agricultural Entomology, College of Agriculture, Vellayani, during 2014-2016. The biochemical changes in ginger due to infection of *M. incognita* and the varietal reaction of five important ginger varieties in Kerala were evaluated. The results of the experiments are presented in this chapter.

## 4.1 BIOCHEMICAL CHANGES IN GINGER DUE TO *M. incognita*

### 4.1.1 Growth Parameters of Ginger

The effect of varying population densities of *M. incognita* on growth parameters of ginger is shown in Table 1, Fig 1 and Plate 2.

#### *4.1.1.1 Plant Height*

Population of *M. incognita* at different densities tested resulted in progressive reduction in the height of plants at six months after inoculation (MAI) compared to the uninoculated plants (91.20 cm). Maximum reduction was seen in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (56.20 cm) and it was significantly superior to all other treatments. Plants inoculated with 100 $J_2$ $pot^{-1}$ (87.40 cm) was on par with that of uninoculated plants. The height of the plants inoculated with 100, 500, 1000 and 10,000$J_2$/ pot decreased 4.17 per cent, 11.62 per cent, 22.37 per cent and 38.38 per cent respectively, when compared to the uninoculated plants. The height of plants inoculated with 500 $J_2$ $pot^{-1}$ (80.6 cm) was on par with that of plants inoculated with 100 $J_2$ $pot^{-1}$ (87.40 cm).

Table 1. Effect of different inoculum levels of *M. incognita* on plant growth parameters of ginger at six months after inoculation

| Treatments ( No. of $J_2$/pot) | Plant growth parameters | | | |
|---|---|---|---|---|
| | Plant height (cm)* | Fresh weight of shoot (g plant$^{-1}$)* | Dry weight of shoot (g plant$^{-1}$)* | No. of tillers per plant* |
| T1 (Uninoculated plant) | 91.20 | 340.20 | 68.11 | 13.60 |
| T2 (100) | 87.40 | 313.80 | 60.24 | 13.00 |
| T3 (500) | 80.60 | 285.60 | 53.13 | 11.60 |
| T4 (1000) | 70.80 | 228.40 | 44.15 | 10.20 |
| T5 (10,000) | 56.20 | 193.80 | 37.61 | 08.00 |
| CD (0.05) | 9.336 | 46.446 | 6.282 | 2.062 |

Mean of five replications*


**Plate 2. Effect of different inoculums levels of *M. incognita* in ginger**

#### *4.1.1.2 Fresh Weight of Shoot*

The fresh weight of shoot decreased with increase in inoculum levels of *M. incognita* at six MAI compared to the uninoculated plants. The fresh weight of plants inoculated with 100 $J_2$ $pot^{-1}$ (313.80 g) was on par with that of uninoculated plants (340.20 g). The plants inoculated with 10,000 $J_2$ $pot^{-1}$ (193.80 g) was significantly superior to other treatments. However, it was on par with that of plants inoculated with 1000 $J_2$ $pot^{-1}$ (228.40 g). The plants inoculated with 500 $J_2$ $pot^{-1}$ (285.60 g) was on par with that of plants inoculated with 100 $J_2$ $pot^{-1}$.

#### *4.1.1.3 Dry Weight of Shoot*

Dry weight of shoot decreased with increase in inoculum levels of *M. incognita* at six MAI. The dry weight of plants inoculated with 100 $J_2$ $pot^{-1}$ (60.24 g) was found to be on par with uninoculated plants. The minimum dry weight of shoot was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (37.61g) and it was significantly superior to all other treatments. The shoot weight of plants inoculated with 10,000 $J_2$/ pot decreased by 44.78 per cent when compared to that of uninoculated plants. The plants inoculated with 500 (53.13 g) and 1000 $J_2$ $pot^{-1}$ (44.15 g) were significantly different from other treatments.

#### *4.1.1.4 Number of Tillers per Plant*

Number of tillers decreased with increase in nematode inoculum levels at 6 MAI. Maximum reduction was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (8.00) and also it was significantly superior to all other treatments. The number of tillers in plants inoculated with 100 $J_2$ $pot^{-1}$ (13.00) was on par with that of plants inoculated with 500 $J_2$ $pot^{-1}$ and uninoculated plants. The percentage decrease in number of tillers in plants inoculated with 10,000, 1000, 500, and 100 $J_2$ $pot^{-1}$ were 41.10 per cent, 25.00 per cent, 14.70 per cent and 4.42 per cent respectively compared to uninoculated plants. The number of tillers in plants inoculated with 1000 $J_2$ $pot^{-1}$ (10.20) was on par with that of plants inoculated with 500 $J_2$ $pot^{-1}$ (11.60).

### 4.1.2 Yield Parameters of Ginger

Changes in yield parameters of ginger at different inoculum levels of *M.incognita* is shown in Table 2 and Fig 2.

#### *4.1.2.1 Fresh Weight of Rhizome*

A progressive reduction was seen in fresh weight of rhizome with increasing inoculum levels of *M. incognita* at six MAI. The weight of rhizome in plants inoculated with 100 $J_2$ $pot^{-1}$ (390 g) was on par with uninoculated plants (408.40 g). The percentage reduction of fresh rhizome weight in plants inoculated with 10,000 (242.20 g), 1000 (286.00 g), 500 (333.00 g), and 100 $J_2$ $pot^{-1}$ were 40.60 per cent, 29.90 per cent, 18.40 per cent and 4.51 per cent respectively compared to uninoculated plants. The plants inoculated with 10,000 $J_2$ $pot^{-1}$ was significantly superior to other treatments. However, it was on par with that of plants inoculated with 1000 $J_2$ $pot^{-1}$.

#### *4.1.2.2 Dry Weight of Rhizome*

A progressive reduction was seen in the dry weight of rhizome with increasing inoculum levels of *M. incognita* at six MAI. The dry weight of rhizome in plants inoculated with 100 $J_2$ $pot^{-1}$ (78.20 g) was on par with that of uninoculated plants. The plants inoculated with 10,000 (43.30 g), 1000 (52.50 g) and 500 $J_2$ $pot^{-1}$ (64.20 g) was significantly different from other treatments. Maximum reduction in dry weight of rhizome was observed in plants treated with 10,000 $J_2$ $pot^{-1}$ and it was significantly superior to other treatments. The percentage decrease in dry weight of rhizome in plants inoculated with 10,000, 1000, 500, and 100 $J_2$/pot were 47.18, 36.18 per cent, 21.50 per cent and 4.40 per cent respectively over uninoculated plants.

Table 2. Effect of different inoculum levels of *M. incognita* on yield parameters of ginger at six months after inoculation

| Treatments ( No. of $J_2$/pot) | Fresh weight of rhizome (g plant$^{-1}$)* | Dry weight of rhizome ( g plant$^{-1}$)* |
|---|---|---|
| T1 (Uninoculated plant) | 408.40 | 81.80 |
| T2 (100) | 390.00 | 78.20 |
| T3 (500) | 333.00 | 64.20 |
| T4 (1000) | 286.00 | 52.20 |
| T5 (10,000) | 242.20 | 43.20 |
| CD (0.05) | 44.587 | 4.454 |

Mean of five replications*

### 4.1.3 Biochemical Changes

#### *4.1.3.1 pH and Electrical Conductivity in Rhizome and Leaf*

Changes in pH and electrical conductivity in rhizome and leaf samples at different inoculum levels of *M. incognita* is shown in Table 3, Fig 7 and 8.

##### *4.1.3.1.1 pH of Rhizome*

A progressive decrease in pH of rhizome samples was observed with increase in nematode inoculum levels at six MAI. The lowest pH was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (6.37). The pH of the rhizome sample of plants inoculated with 1000 $J_2$ $pot^{-1}$ (6.56) was on par with that of the plants inoculated with 500 $J_2$ $pot^{-1}$ (6.73). The pH of leaf in plants inoculated with 10,000 $J_2$ $pot^{-1}$ was significantly superior to other treatments and it was on par with that of plants inoculated with 1000 $J_2$ $pot^{-1}$. The percentage reduction of pH in plants inoculated with 10,000 $J_2$ $pot^{-1}$ was 9.4 per cent when compared to uninoculated plants. The plants inoculated with 100 $J_2$ $pot^{-1}$ (6.86) was found to be non significant and it was on par with that of the plants inoculated with 500 $J_2$ $pot^{-1}$ and uninoculated plants (6.97).

##### *4.1.3.1.2 pH of Leaf*

The pH of leaf samples decreased with increase in nematode inoculum levels at six MAI. The lowest pH value was observed in plants inoculated with 10,000 $J_2$/ pot (5.70). The plants inoculated with 100 $J_2$ $pot^{-1}$ was on par with that of plants inoculated with 500 $J_2$ and uninoculated plants. The pH of the leaf sample of plants inoculated with 1000 $J_2$ $pot^{-1}$ (5.95) was on par with that of the plants inoculated with 500 $J_2$ (6.18). The plants inoculated with 10,000 $J_2$ $pot^{-1}$ was significantly superior to other treatments and it was on par with that of plants inoculated with 1000 $J_2$ $pot^{-1}$. The percentage decrease in the pH of leaf sample in

plants inoculated with 1000 and 10,000 $J_2$ $pot^{-1}$ was 7.98 per cent and 11.81 per cent respectively compared to that of uninoculated plants.

| Treatments ( No. of $J_2$/pot) | pH | | Electrical conductivity (d S $m^{-1}$) | |
|---|---|---|---|---|
| | Rhizome* | Leaf * | Rhizome* | Leaf * |
| T1 (Uninoculated plant) | 6.97 | 6.47 | 0.284 | 0.280 |
| T2 (100) | 6.86 | 6.34 | 0.282 | 0.280 |
| T3 (500) | 6.73 | 6.18 | 0.281 | 0.278 |
| T4 (1000) | 6.56 | 5.95 | 0.276 | 0.274 |
| T5 (10,000) | 6.37 | 5.70 | 0.272 | 0.272 |
| CD (0.05) | 0.270 | 0.288 | 0.0040 | 0.0070 |

Table 3. Effect of different inoculum levels of *M. incognita* on the pH and electrical conductivity in rhizome and leaf samples of ginger at six months after inoculation

Mean of five replications*

#### *4.1.3.1.3 Electrical Conductivity of Rhizomes*

A progressive decrease in EC of rhizome samples was observed with increase in nematode inoculum levels at six MAI. The lowest EC was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (0.272 d S $m^{-1}$) followed by plants inoculated with 1000 $J_2$ $pot^{-1}$. The EC of the rhizome sample of plants inoculated with 1000 $J_2$ $pot^{-1}$ (0.276 d S $m^{-1}$) was on par with that of the plants inoculated with 500 $J_2$ $pot^{-1}$ (0.281 d S $m^{-1}$). The EC of rhizome samples inoculated with 10,000 $J_2$ $pot^{-1}$ was significantly superior to other treatments. However, it was on par with that of plants inoculated with 1000 $J_2$ $pot^{-1}$. The percentage reduction in EC of rhizome sample in plants inoculated with 10,000 $J_2$ $pot^{-1}$ was 4.23 per cent with that of uninoculated plants.

#### *4.1.3.1.4 Electrical Conductivity of Leaf*

The EC of leaf samples decreased with increase in nematode inoculum levels at six MAI. The lowest EC was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (0.272 d S $m^{-1}$). The EC of the leaf sample of plants inoculated with 1000 $J_2$ $pot^{-1}$ (0.274 d S $m^{-1}$) was on par with that of the plants inoculated with 500 $J_2$ (0.278 d S $m^{-1}$). The EC of leaf in plants inoculated with 10,000 $J_2$ $pot^{-1}$ was significantly superior to other treatments. However, it was on par with that of plants inoculated with 1000 and 500 $J_2$ $pot^{-1}$. The percentage decrease in the EC of leaf sample in plants inoculated with 1000 and 10,000 $J_2$ $pot^{-1}$ was 2.14 per cent and 2.85 per cent respectively compared to that of uninoculated plants.

### *4.1.3.2 Peroxidase, Polyphenol Oxidase and Phenylalanine Ammonia Lyase Activity in Rhizome*

Changes in peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activity in rhizome at different inoculum levels of *M. incognita* is shown in Table 4 and Fig 3.

Table 4. Effect of different inoculum levels of *M. incognita* on the peroxidase, polyphenol oxidase, phenylalanine ammonia lyase activities in rhizome

| Treatments ( No. of $J_2$/pot) | Enzymatic activity in rhizome samples (Changes in absorbance) | | |
|---|---|---|---|
| | PO* ( $min^{-1}$ $g^{-1}$ tissue) | PPO* ( $min^{-1}$ $g^{-1}$ tissue) | PAL* (cinnamic acid $min^{-1}$ $g^{-1}$ tissue) |

samples of ginger at six months after inoculation

| | | | |
|---|---|---|---|
| T1 (Uninoculated plant) | 5.51 | 0.47 | 10.53 |
| T2 (100) | 5.96 | 0.52 | 10.75 |
| T3 (500) | 6.63 | 0.56 | 13.46 |
| T4 (1000) | 7.63 | 0.64 | 14.67 |
| T5 (10,000) | 8.82 | 0.74 | 16.70 |
| CD (0.05) | 0.546 | 0.088 | 0.611 |

Mean of five replications*

#### *4.1.3.2.1 Peroxidase (PO) Activity in Rhizome*

Plants inoculated with various levels of nematode population showed progressive increase in the peroxidase activity compared to uninoculated plants. However the treatment having 100 $J_2$ $pot^{-1}$ (5.96 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) showed no significant difference in activity of PO when compared to control (5.51 changes in absorbance $min^{-1}$ $g^{-1}$ tissue). The peroxidase activity of rhizome in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (8.82 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) was significantly superior to all other treatments. The percentage increase of PO content in rhizome of plants inoculated with 10,000, 1000 $J_2$ and 500 $J_2$ $pot^{-1}$ was 59.88 per cent, 38.37 per cent and 20.31 per cent respectively.

#### *4.1.3.2.2 Polyphenol Oxidase (PPO) Activity in Rhizome*

The polyphenol oxidase content of rhizome increased with increasing nematode population levels at six MAI. The highest activity of enzyme was observed at 10,000 $J_2$ $pot^{-1}$ (0.74 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and recorded an increase of 58.29 percent over the uninoculated plants (0.47 changes in absorbance $min^{-1}$ $g^{-1}$ tissue). The PPO content of rhizome at different inoculum level was significantly different from that of uninoculated plants. The plants inoculated with 10,000 and 1000 $J_2$ $pot^{-1}$ were found to be significantly different compared to other treatments. However, the PPO content in rhizome of plants inoculated with 100 $J_2$ $pot^{-1}$ (0.52 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) was on par with that of plants inoculated with 500 $J_2$ $pot^{-1}$ (0.56 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and uninoculated plants. The percentage increase of PPO content in rhizome of plants inoculated with 1000 $J_2$ and 500 $J_2$ $pot^{-1}$ was 37.02 per cent and 19.57 per cent respectively.

#### *4.1.3.2.3 Phenylalanine Ammonia Lyase (PAL) Activity in Rhizome*

Phenylalanine ammonia lyase activity of rhizome increased with the increase in nematode inoculum levels at six MAI. The PAL activity of rhizome in plants inoculated with different inoculum levels of nematodes were significantly different from uninoculated plants (10.53 changes in cinnamic acid $min^{-1}$ $g^{-1}$ tissue) except the plants inoculated with 100 $J_2$ $pot^{-1}$ (10.75 changes in cinnamic acid $min^{-1}$ $g^{-1}$ tissue). The maximum PAL activity was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (16.70 changes in cinnamic acid $min^{-1}$ $g^{-1}$ tissue). The percentage increase of PAL activity of rhizome in plants inoculated with 500, 1000 and 10,000 $J_2$ $pot^{-1}$ were 27.85 per cent, 39.38 per cent and 58.60 per cent respectively. The plants inoculated with 10,000 $J_2$ $pot^{-1}$ was significantly superior compared to other treatments.

#### *4.1.3.3 Peroxidase, Polyphenol Oxidase and Phenylalanine Ammonia Lyase Activity in Leaf*

Changes in peroxidase, phenylalanine ammonia lyase and polyphenol oxidase activity in leaf samples at different inoculum levels of *M. incognita* is shown in Table 5 and Fig 4.

##### *4.1.3.3.1 Peroxidase (PO) Activity in Leaf*

The PO activity in leaf samples increased with increase in nematode inoculum levels at six MAI. The highest PO activity was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (3.15 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and followed by plants inoculated with 1000 (2.71 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and 500 $J_2$ $pot^{-1}$ (2.57 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and were at par with each other. The PO activity of plants inoculated with 100 (2.07 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and 500 $J_2$ $pot^{-1}$ was on par with that of the uninoculated plants. The percentage increase in PO activity of plants inoculated with 10,000 $J_2$ was 57.93 per cent over uninoculated plants.

##### *4.1.3.3.2 Polyphenol Oxidase Activity in Leaf*

The PPO activity in leaf samples also increased with increase in nematode inoculum levels at six MAI. The plants inoculated with 10,000 $J_2$/pot showed maximum PPO activity (0.60 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and which

| Treatments ( No. of $J_2$/pot) | Enzymatic activity in leaf samples (Changes in absorbance) | | |
|---|---|---|---|
| | PO* ( $min^{-1}$ $g^{-1}$ tissue) | PPO* ( $min^{-1}$ $g^{-1}$ tissue) | PAL* (cinnamic acid $min^{-1}$ $g^{-1}$ tissue) |
| T1 (Uninoculated plant) | 1.99 | 0.36 | 1.58 |
| T2 (100) | 2.07 | 0.40 | 1.63 |
| T3 (500) | 2.57 | 0.44 | 1.82 |
| T4 (1000) | 2.71 | 0.50 | 2.20 |
| T5 (10,000) | 3.15 | 0.60 | 2.71 |
| CD (0.05) | 0.625 | 0.103 | 0.336 |

Table 5. Effect of different inoculum levels of *M. incognita* on the peroxidise (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) activities in leaf samples of ginger at six months after inoculation

Mean of five replications*

was on par with that of plants inoculated with 1000 $J_2$ $pot^{-1}$ (0.50 changes in absorbance $min^{-1}$ $g^{-1}$ tissue). The PPO activity of plants inoculated with 100 and 500 $J_2$ $pot^{-1}$ were on par with each other**.** The percentage increase in PPO activity in plants inoculated with 500, 1000 and 10,000 $J_2$ $pot^{-1}$ was 20.44 per cent, 37.57 per cent and 64.64 per cent respectively over uninoculated plants.

#### *4.1.3.3.3 Phenylalanine Ammonia Lyase Activity in Leaf*

The PAL activity of leaf also increased with increase in nematode population at six MAI. The plants inoculated with 10,000 $J_2$ $pot^{-1}$ (2.71 changes in cinnamic acid $min^{-1}$ $g^{-1}$ tissue) was significantly superior to other treatments followed by plants inoculated with 1000 (2.20 changes in cinnamic acid $min^{-1}$ $g^{-1}$ tissue) and 500 $J_2$ $pot^{-1}$ (1.82 changes in cinnamic acid $min^{-1}$ $g^{-1}$ tissue). The plants inoculated with 100 $J_2$ $pot^{-1}$ showed lowest PAL activity (1.63 changes in cinnamic acid $min^{-1}$ $g^{-1}$ tissue) and it was found to be on par with that of uninoculated plants. The plants inoculated with 500, 1000 and 10,000 $J_2$ $pot^{-1}$ was significantly different from other treatments. The percentage increase in the PAL activity in plants inoculated with 10,000 $J_2$ $pot^{-1}$ was 71.64 per cent compared with that of uninoculated plants.

### *4.1.3.4 Phenol Content in Rhizome and Leaf*

Changes in phenol content in rhizome and leaf samples of ginger at different inoculum levels of *M. incognita* is shown in Table 6, Fig 5 and 6.

#### *4.1.3.4.1 Phenol Content in Rhizome*

Population of *M. incognita* at different densities tested, resulted in progressive increase in phenol content of rhizome in plants at six MAI compared to that of uninoculated plants (2.57 mg $g^{-1}$ tissue). The maximum phenol content was seen in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (3.93 mg $g^{-1}$ tissue). The phenol

content of rhizome in plants inoculated with 100, 500 and 1000 $J_2$ $pot^{-1}$ were 2.70, 3.14 and 3.52 mg per g tissue respectively. The phenol contents of plants

| Treatments ( No. of $J_2$/pot) | Phenol content (mg $g^{-1}$ tissue) | |
|---|---|---|
| | Rhizome samples* | Leaf samples* |

| | | |
|---|---|---|
| T1 (Uninoculated plant) | 2.57 | 0.56 |
| T2 (100) | 2.70 | 0.60 |
| T3 (500) | 3.14 | 0.69 |
| T4 (1000) | 3.52 | 0.87 |
| T5 (10,000) | 3.93 | 1.04 |
| CD (0.05) | 0.600 | 0.218 |

Table 6. Effect of different inoculum levels of *M. incognita* on the phenol content in rhizome and leaf samples of ginger at six months after inoculation

Mean of five replications*

inoculated with 100 and 500 $J_2$ $pot^{-1}$ was on par with that of the uninoculated plants. However, all other treatments were found to be significant when compared to uninoculated plants. Plants inoculated with 1000 $J_2$ $pot^{-1}$ was on par with that of plants inoculated with 10,000 $J_2$ $pot^{-1}$.

#### *4.1.3.4.2 Phenol Content in Leaf*

A progressive increase in phenol content of leaf samples were observed with increase in nematode inoculum levels at six MAI. The phenol content of plants inoculated with 100 $J_2$ $pot^{-1}$ (0.60 mg $g^{-1}$ tissue) was on par with that of uninoculated plants (0.56 mg $g^{-1}$ tissue) and 500 $J_2$ $pot^{-1}$ (0.69 mg $g^{-1}$ tissue). The percentage increase in the phenol content of leaf was higher in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (86.33 per cent) followed by the plants inoculated with 1000 $J_2$ $pot^{-1}$ (56.11 per cent), when compared to uninoculated plants. The phenol content of leaf was highest in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (1.04 mg $g^{-1}$ tissue) and was significantly superior to all other treatments.

### *4.1.3.5 Starch and Protein Content in Rhizome*

Effect of different levels of *M. incognita* on starch and protein content in rhizome sample is shown in Table 7 and Fig 9.

#### *4.1.3.5.1 Starch Content of Rhizome*

The starch content of rhizome exhibited reduction at different nematode inoculum levels when compared to uninoculated plants at six MAI. The starch content of rhizome in the plants inoculated with 100 $J_2$ $pot^{-1}$ (17.95 per cent) was found to be on par with uninoculated plants (18.56 per cent). The starch content of rhizome in all the treatments were significantly different from other treatments except plants inoculated with 100 $J_2$ $pot^{-1}$. The percentage decrease in starch content of rhizome in plants inoculated with 500, 1000 and 10,000 $J_2$ $pot^{-1}$ was 10.22, 19.65 and 31.15 respectively compared with that of uninoculated plants.

Table 7. Effect of different inoculum levels of *M. incognita* on the starch and protein content in rhizome of ginger at six months after inoculation

| Treatments (No. of $J_2$/pot) | Starch * (%) | Protein * (%) |
|---|---|---|
| T1 (Uninoculated plant) | 18.56 | 4.18 |
| T2 (100) | 17.95 | 3.92 |
| T3 (500) | 16.6 | 3.62 |
| T4 (1000) | 14.91 | 2.78 |
| T5 (10,000) | 12.78 | 2.52 |
| CD (0.05) | 1.224 | 0.323 |

Mean of five replications*

The starch content of rhizome in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (12.78 per cent) was significantly superior to all other treatments.

#### *4.1.3.5.2 Protein Content of Rhizome*

Plants inoculated with *M. incognita* in varying inoculum levels resulted in corresponding decrease in protein content of rhizome at six MAI. The protein content of plants inoculated with 500 $J_2$ $pot^{-1}$ (3.62 per cent) was on par with that of the plants inoculated with 100 $J_2$ $pot^{-1}$ (3.92 per cent). The percentage decrease in the protein content of rhizome was higher in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (39.78 per cen)t followed by the plants inoculated with 1000 $J_2$/pot (33.56 per cent), when compared to uninoculated plants. The protein content of rhizome was lowest in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (2.52 per cent) and it was significantly superior to other treatments.

### *4.1.3.6 Crude Fibre, Oleoresin, Total Ash, Gingerol and Shagaol Content in Rhizome*

Changes in crude fibre, oleoresin, total ash, gingerol and shagaol content in grain sample due to infection of *M. incognita* is given in Table 8 and Fig 10.

#### *4.1.3.6.1 Crude Fibre Content of Rhizome*

Plants treated with different inoculum levels of *M. incognita* resulted in a corresponding decrease in the percentage of crude fibre in the rhizome compared to uninoculated plants at six MAI. However, crude fibre present in plants inoculated with 100 $J_2$ $pot^{-1}$ was on par with that of plants inoculated with 500 $J_2$ $pot^{-1}$. The percentage reduction of crude fibre content in rhizome of plants inoculated with 100 and 500 $J_2$ $pot^{-1}$ was 5.70 per cent and 5.49 per cent respectively. The plants inoculated with 10.000 $J_2$ $pot^{-1}$ (4.69 per cent) was

significantly superior to other treatments. Percentage decrease in plants inoculated with 10.000 $J_2$ $pot^{-1}$ was 19.01 per cent over uninoculated plants.

Table 8. Effect of different inoculum levels of *M. incognita* on crude fibre, total ash, oleoresin, gingerol and shagaol content in rhizome samples of ginger at six months after inoculation

| Treatments ( No. of $J_2$/pot) | Crude fibre* (%) | Total ash* (%) | Oleoresin* (%) | Gingerol* (%) | Shagaol* (%) |
|---|---|---|---|---|---|
| T1 (Uninoculated plant) | 5.79 | 1.76 | 4.98 | 0.73 | 0.15 |
| T2 (100) | 5.70 | 1.70 | 4.86 | 0.71 | 0.14 |
| T3 (500) | 5.49 | 1.59 | 4.46 | 0.68 | 0.12 |
| T4 (1000) | 5.17 | 1.41 | 3.95 | 0.59 | 0.11 |
| T5 (10,000) | 4.69 | 1.24 | 3.52 | 0.51 | 0.10 |
| CD (0.05) | 0.410 | 0.104 | 0.717 | 0.079 | 0.016 |

Mean of five replications*

Plants inoculated with 1000 $J_2$ $pot^{-1}$ was on par with that of plants inoculated with 500 $J_2$ $pot^{-1}$.

#### *4.1.3.6.2 Oleoresin Content of Rhizome*

Plants treated with different levels of nematode population showed reduction in oleoresin content of rhizome samples compared to uninoculated plants at six MAI. Lowest content of oleoresin in rhizome sample was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (3.52 per cent) and was significantly superior to other treatments. However, it was on par with that of plants inoculated with 1000 $J_2$ $pot^{-1}$. The percentage decrease in oleoresin content of rhizome sample in plants inoculated with 1000 and 10,000 was 20.79 and 29.41 respectively over the uninoculated plants. The oleoresin content of rhizome in plants inoculated with 1000 $J_2$ $pot^{-1}$ (3.95 per cent) was on par with that of plants inoculated with 500 $J_2$ (4.46 per cent). The oleoresin content of rhizome in plants inoculated with 100 $J_2$ $pot^{-1}$ (4.86 per cent) was found to be on par with 500 $J_2$ and uninoculated plants.

#### *4.1.3.6.3 Total Ash Content ofRhizome Sample*

Total ash content of rhizome samples decreased with increase in nematode inoculum levels at six MAI. Maximum reduction was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (1.24 per cent) and it was significantly superior to all other treatments. The total ash content of rhizome sample in plants inoculated with 100 $J_2$ $pot^{-1}$ (1.70 per cent) was found to be on par with uninoculated plants. The percentage decrease in total ash content of rhizome samples in plants inoculated with 10,000 and 1000 $J_2$/ pot were 29.93 and 20.12 respectively. The plants inoculated with 500, 1000 and 10,000 $J_2$ $pot^{-1}$ were significantly different when compared to other treatments.

#### *4.1.3.6.4 Gingerol Content of Rhizome*

The gingerol content of rhizome exhibited reduction at different nematode inoculum levels when compared to uninoculated plants at 6 MAI. The plants inoculated with 100 $J_2$ $pot^{-1}$ (0.71 per cent), 500 $J_2$ $pot^{-1}$ (0.68 per cent) and uninoculated plants (0.73 per cent) were on par with each other. The gingerol content of rhizome in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (0.59 per cent) was significantly superior to all other treatments. Percentage decrease in plants inoculated with 10.000 $J_2$ $pot^{-1}$ was 31.01 per cent over uninoculated plants.

#### *4.1.3.6.5 Shagaol Content of Rhizome*

The different inoculum levels of *M. incognita* resulted in a corresponding decrease in the percentage of shagaol content of rhizome compared to uninoculated plants at 6 MAI. The plants inoculated with 10.000 $J_2$ $pot^{-1}$ (0.10 per cent) was significantly superior to other treatments. Percentage decrease in plants inoculated with 10.000 $J_2$ $pot^{-1}$ was 34.24 per cent compared to uninoculated plants. The plants inoculated with 100 $J_2$ $pot^{-1}$ (0.14 per cent) was on par with that of uninoculated plants (0.15 per cent). The plants inoculated with 1000 $J_2$ $pot^{-1}$ (0.11 per cent) was on par with that of plants inoculated with 500 $J_2$ $pot^{-1}$ (0.12 per cent).

### 4.1.4 Population of Nematode

Changes in nematode population in ginger at different inoculum levels of *M. incognita* is shown in Table 9.

#### *4.1.4.1 Number of Galls*

A progressive increase was seen in the number of galls with increasing inoculum levels of *M. incognita* at 6 MAI (Plate 3, 4, and 5). Highest number of galls per plant was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (85.00 $plant^{-1}$) and it was significantly superior to all other treatments. Plants inoculated

Table 9. Effect of different inoculum levels of *M. incognita* on population of nematode in ginger at six months after inoculation

| Treatments ( No. of $J_2$/pot) | Nematode population | | | Adult females/ plant* | No. of galls/ plant* | Egg mass/ plant* |
|---|---|---|---|---|---|---|
| | Soil (200cc)* | Root (5g)* | Rhizome (200cc)* | | | |
| T1 (Uninoculated plant) | 0 | 0 | 0 | 0 | 0 | 0 |
| T2 (100) | 584.20 | 32.40 | 22.80 | 17.60 | 19.60 | 11.20 |
| T3 (500) | 1154.40 | 65.60 | 34.80 | 30.80 | 35.20 | 23.00 |
| T4 (1000) | 2124.60 | 104.58 | 59.40 | 64.20 | 61.80 | 40.40 |
| T5 (10,000) | 3673.60 | 185.50 | 81.20 | 93.60 | 85.00 | 56.80 |
| CD (0.05) | 179.146 | 12.863 | 5.297 | 6.630 | 5.380 | 4.090 |

Mean of five replications*

Plate 3. Healthy ginger roots

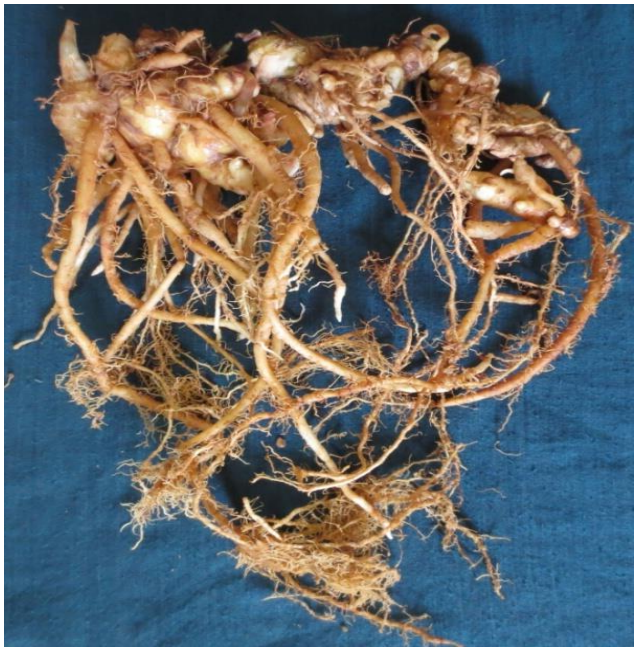


Plate 4. Infected ginger roots

T2 (100 $J_2$/pot)

T3 (500 $J_2$/pot)

T4 (1000 $J_2$/pot)

T5 (10,000 $J_2$/pot)

T1 (Control)

**Plate 5. Symptoms on roots of ginger at different inoculum levels of *M. incognita***

with 500 and 1000 $J_2$ $pot^{-1}$ showed 35.20 and 61.80 galls per plant respectively and both treatments were significantly different compared to other treatments. Lowest number of galls was recorded in plants inoculated with 100 $J_2$ $pot^{-1}$ (19.60 $plant^{-1}$).

#### *4.1.4.2 Number of Egg Mass*

The number of egg masses increased significantly with increase in inoculum levels of *M. incognita* at 6 MAI. Plants inoculated with 500 and 1000 $J_2$ $pot^{-1}$ showed 23.00 and 40.40 egg mass per plant respectively and both treatments were significantly different compared to other treatments. The plants inoculated with 100 $J_2$ $pot^{-1}$ (11.20 $plant^{-1}$) showed lowest number of egg mass. Highest number of egg mass was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (56.80 $plant^{-1}$) and it was significantly superior to all other treatments (Plate 6.)

#### *4.1.4.3 Nematode Population in Soil (200cc soil)*

A progressive increase was recorded in the nematode population with increasing inoculum levels of *M. incognita* at six MAI. Highest nematode population in soil was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (3673.60) and it was significantly superior to all other treatments. Plants inoculated with 500 and 1000 $J_2$ $pot^{-1}$ showed population of 1155.40 and 2124.60 respectively in soil and both treatments were significantly different compared to other treatments. Lowest nematode population was seen in plants inoculated with 100 $J_2$ $pot^{-1}$ (584.20).

#### *4.1.4.4 Nematode Population in Rhizome (10g)*

The nematode population in rhizome increased significantly with increase in inoculum levels of *M. incognita* at six MAI. The plants inoculated with 100 $J_2$ $pot^{-1}$ (22.80) showed lowest nematode population in rhizome. Highest nematode population was recorded in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (81.20) in rhizome and it was significantly superior to all other treatments. The plants

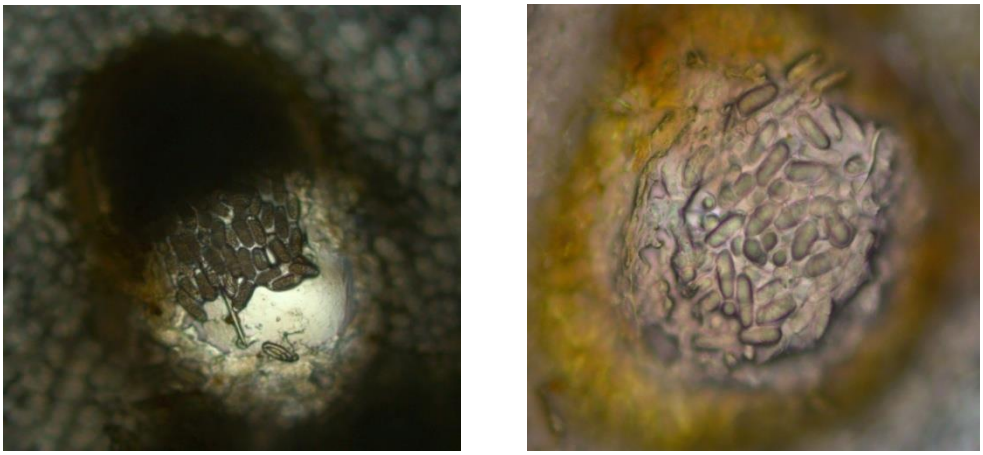


Plate 6. Egg mass inside the root system

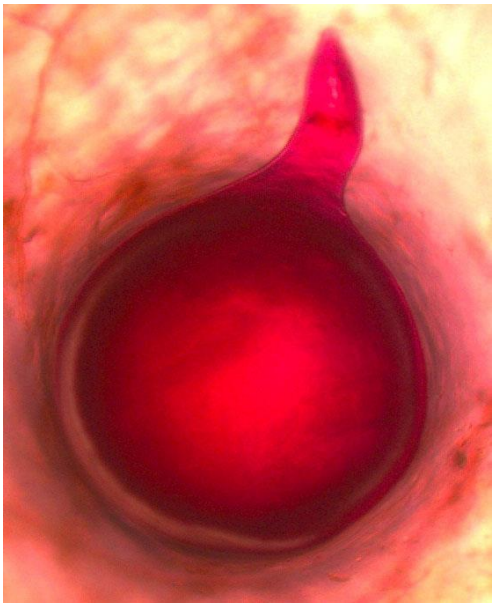
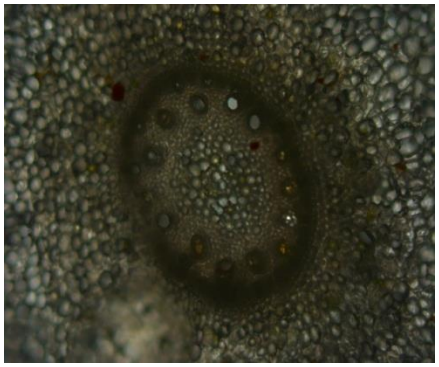
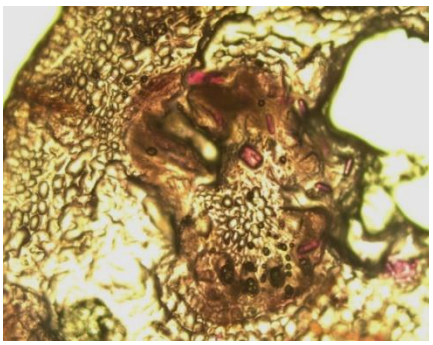


Plate 7. Adult female inside root

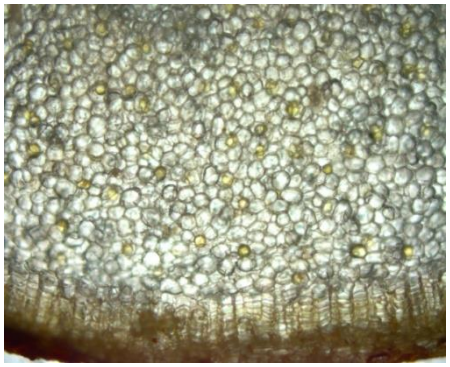


a. Healthy root section



b. Infected root section

Plate 8. Cross section of ginger rhizome



a. Healthy rhizome

b. Infected rhizome

Plate 9. Cross section of ginger rhizome

inoculated with 500 and 1000 $J_2$ $pot^{-1}$ showed a population of 34.80 and 59.40 respectively.

#### *4.1.4.5 Nematode Population in Root (10g)*

The nematode population in root increased significantly with increase in inoculum levels of *M. incognita* at six MAI. Highest nematode population was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$(185.50) in root and it was significantly superior to all other treatments. The plants inoculated with 100 $J_2$ $pot^{-1}$ (32.40) showed lowest nematode population in root. Plants inoculated with 500 and 1000 $J_2$ $pot^{-1}$ showed population of 65.60 and 104.80 respectively in root samples and both treatments were significantly different compared to other treatments.

#### *4.1.4.6 Adult Females (per plant)*

The population of adult females in root increased significantly with increase in inoculum levels of *M. incognita* at six MAI. Highest number of adult females was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (93.60) and it was significantly superior to other treatments. The plants inoculated with 500 and 1000 $J_2$ $pot^{-1}$ showed population of 30.80 and 64.20 respectively and both treatments were significantly different compared to other treatments. The plants inoculated with 100 $J_2$ $pot^{-1}$ (17.6) showed lowest number of adult females (Plate 7.)

## **4.2** VARIETAL SCREENING

### 4.2.1 Reaction of Varieties

#### *4.2.1.1 Galls and Gall Index*

Number of galls and gall index in roots of different ginger varieties due to infection of *M. incognita* is shown in Tables 10 and Plate 10.

Table 10. Effect of *M. incognita* on number of galls, gall index and host reaction of different ginger varieties at six months after inoculation

| Varieties | Number of galls per plant* | Gall index | Host reaction |
|---|---|---|---|
| Athira | 50.20 | 4 | S |
| Karthika | 106.80 | 5 | HS |
| IISR Mahima | 28.60 | 3 | MH |
| IISR Rejatha | 85.80 | 4 | S |
| IISR Varada | 93.80 | 4 | S |
| CD (0.05) | 18.238 | - | - |

Mean of five replications*


Karthika


IISR


IISR Mahima


IISR Rejatha


Athira

**Plate 10. Symptoms on roots of ginger varieties due to *M. incognita***

Among the varieties, highest number of galls was recorded in the variety Karthika (106.8 plant$^{-1}$) followed by varieties IISR Rejatha (85.8 plant$^{-1}$) and IISR Varada (93.8 plant$^{-1}$) and also were found to be on par with each other. The lowest number of galls was recorded in the variety IISR Mahima (28.6 plant$^{-1}$) and was significantly different from other varieties. The variety Karthika showed a gall index of 5 and was rated as highly susceptible variety to *M. incognita*. However, the varieties Athira, IISR Rejatha and IISR Varada were rated as susceptible with a gall index 4. The variety IISR Mahima showed a gall index of 3 and was moderately resistant to *M. incognita*.

#### *4.2.1.2 Egg Mass and Egg Mass Index*

Number of egg mass and egg mass index in roots of different ginger varieties due to infection of *M. incognita* is shown in Tables 11.

Highest number of egg mass was seen in the variety Karthika (80.10 plant$^{-1}$) and was statistically significant to other varieties. The lowest number of egg mass per plant was seen in variety IISR Mahima (21.45 plant$^{-1}$). The number of galls per plant in the variety IISR Rejatha (63.75 plant$^{-1}$) was on par with that of variety IISR Varada (70.35 plant$^{-1}$). The varieties Karthika, IISR Rejatha and IISR Varada showed a egg mass index of 4, 3.18 & 3.51 respectively and was rated as highly susceptible to *M. incognita*. However, the varieties Athira and IISR Mahima were rated as moderately resistant with a egg mass index 1.88 and 1.07 respectively.

### 4.2.2 Enzymatic Activity in Rhizome and Leaf of Different Ginger Varieties

#### *4.2.2.1 Peroxidase (PO), Polyphenol Oxidase (PPO) and Phenylalanine Ammonia Lyase (PAL) Activity in Rhizome*

Changes in PO, PPO and PAL activity in rhizome of different ginger varieties due to infection of *M. incognita* is shown in Table 12.

Table 11. Effect of *M. incognita* on number of egg mass, egg mass index and host reaction of different ginger varieties at six months after inoculation

| Varieties | Number of egg mass per plant* | Egg mass index | Host reaction |
|---|---|---|---|
| Athira | 37.65 | 1.88 | MR |
| Karthika | 80.10 | 4.00 | HS |
| IISR Mahima | 21.45 | 1.07 | MR |
| IISR Rejatha | 63.75 | 3.18 | HS |
| IISR Varada | 70.35 | 3.51 | HS |
| CD (0.05) | 7.122 | - | - |

Mean of five replications*

Table 12. Effect of *M. incognita* on peroxidase, polyphenol oxidase, phenylalanine ammonia lyase activities in rhizome samples of different ginger varieties at six months after inoculation

| Varieties | Enzymatic activity in rhizome samples (Changes in absorbance) | | |
|---|---|---|---|
| | PO* ( $min^{-1}$ $g^{-1}$ tissue) | PPO* ( $min^{-1}$ $g^{-1}$ tissue) | PAL* (cinnamic acid $min^{-1}$ $g^{-1}$ tissue) |
| Athira | 8.33 | 0.86 | 16.81 |
| Karthika | 6.92 | 0.70 | 13.59 |
| IISR Mahima | 9.02 | 0.94 | 17.78 |
| IISR Rejatha | 7.34 | 0.80 | 16.16 |
| IISR Varada | 8.04 | 0.77 | 15.62 |
| CD (0.05) | 0.665 | 0.131 | 0.673 |

Mean of five replications*

Highest PO activity was recorded by variety IISR Mahima (9.02 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and it was on par with that of varieties Athira (8.33 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and IISR Varada (8.04 changes in absorbance $min^{-1}$ $g^{-1}$ tissue). Lowest PO activity was observed in variety Karthika (6.92 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and it was on par with that of variety IISR Rejatha (7.34 changes in absorbance $min^{-1}$ $g^{-1}$ tissue).

Lowest PPO activity was observed in variety Karthika (0.70 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and was on par with that of Rajatha (0.80 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and IISR Varada (0.77 changes in absorbance $min^{-1}$ $g^{-1}$ tissue). The PPO activity was highest in variety IISR Mahima (0.94 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and it was significantly superior to other varieties. However, it was on par with that of the variety Athira (0.86 changes in absorbance $min^{-1}$ $g^{-1}$ tissue).

Highest PAL activity was observed in variety IISR Mahima (17.78 changes in cinnamic acid $min^{-1}$ $g^{-1}$ tissue). Lowest PAL activity was observed in variety Karthika (13.59 changes in cinnamic acid $min^{-1}$ $g^{-1}$ tissue) and it was on par with that of variety IISR Rejatha (16.16 changes in cinnamic acid $min^{-1}$ $g^{-1}$ tissue) and IISR Varada (15.62 changes in cinnamic acid $min^{-1}$ $g^{-1}$ tissue). The variety IISR Mahima was significantly different to other treatments and it was on par with that of variety Athira and IISR Rejatha.

#### *4.2.2.2 Peroxidase (PO), Polyphenol Oxidase (PPO) and Phenylalanine Ammonia Lyase (PAL) Activity in Leaf*

Changes in PO, PPO and PAL activity in leaf of different ginger varieties due to infection of *M. incognita* is shown in Table 13.

Highest PO activity was observed in variety IISR Mahima (2.63 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) followed by Athira (2.47 changes in absorbance

$min^{-1}$ $g^{-1}$ tissue), IISR Rejatha (2.78 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and IISR Varada (2.36 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and all are on par with

Table 13. Effect of *M. incognita* on peroxidase, polyphenol oxidase, phenylalanine ammonia lyase activities in leaf samples of different ginger varieties at six

| Varieties | Enzymatic activity in leaf samples (Changes in absorbance) | | |
|---|---|---|---|
| | PO* ( $min^{-1}$ $g^{-1}$ tissue) | PPO* ( $min^{-1}$ $g^{-1}$ tissue) | PAL* (cinnamic acid $min^{-1}$ $g^{-1}$ tissue) |
| Athira | 2.47 | 0.58 | 3.47 |

months after inoculation

| | | | |
|---|---|---|---|
| Karthika | 1.95 | 0.48 | 3.17 |
| IISR Mahima | 2.63 | 0.60 | 4.02 |
| IISR Rejatha | 2.78 | 0.50 | 3.83 |
| IISR Varada | 2.36 | 0.56 | 3.55 |
| CD (0.05) | 0.434 | 0.071 | 0.472 |

Mean of five replications*

each other. Lowest PO activity was observed in variety Karthika (1.95 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and it was on par with that of variety IISR Varada.

The PPO activity was highest in variety IISR Mahima (0.60 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and it was on par with that of the variety Athira (0.58 changes in absorbance $min^{-1}$ $g^{-1}$) and IISR Varada (0.56 changes in absorbance $min^{-1}$ $g^{-1}$). Lowest PPO activity was observed in variety Karthika (0.48 changes in absorbance $min^{-1}$ $g^{-1}$) and it was significantly different from other varieties. However, it was on par with that of IISR Rejatha (0.50 changes in absorbance $min^{-1}$ $g^{-1}$).

Lowest PAL activity was observed in variety Karthika (3.17 changes in cinnamic acid $min^{-1}$ $g^{-1}$) and was on par with that of varieties Athira (3.47 changes in cinnamic acid $min^{-1}$ $g^{-1}$) and IISR Varada. Highest PAL activity was observed in variety IISR Mahima (4.02 changes in cinnamic acid $min^{-1}$ $g^{-1}$) and was on par with that of variety IISR Rejatha (3.83 changes in cinnamic acid $min^{-1}$ $g^{-1}$) and IISR Varada (3.55 changes in cinnamic acid $min^{-1}$ $g^{-1}$).

### 4.2.3 Growth Parameters of Varieties

The change in plant growth parameters of different ginger varieties due to infection of *M. incognita* is shown in Table 14 and Plate 11.

The highest plant height was seen in variety Athira (84.8 cm) and it was significantly superior to other varieties. The lowest plant height was seen in variety Karthika (52 cm) and it was significantly different from other varieties. The variety IISR Mahima (63.6 cm) was on par with that of variety IISR Rejatha (72.4 cm) and IISR Varada (62.6 cm).

Lowest fresh weight of shoot was observed in variety Karthika (122.2 g) and it was significantly different from other varieties. The highest fresh weight of

shoot was observed in variety Athira (238 g) and it was on par with that of IISR Mahima (216.4 g) and IISR Rejatha (207.2 g).

| Varieties | Plant growth parameters | | | |
|---|---|---|---|---|
| | Plant height (cm)* | Fresh weight of shoot (g plant$^{-1}$)* | Dry weight of shoot ( g plant$^{-1}$)* | No. of tillers per plant* |
| Athira | 84.80 | 238.00 | 40.20 | 14.80 |

| | | | | |
|---|---|---|---|---|
| Karthika | 52.00 | 122.20 | 21.40 | 8.80 |
| IISR Mahima | 63.60 | 216.40 | 34.40 | 11.60 |
| IISR Rejatha | 72.40 | 207.20 | 32.80 | 10.40 |
| IISR Varada | 62.60 | 196.20 | 30.80 | 10.80 |
| CD (0.05) | 9.310 | 39.795 | 6.548 | 2.188 |

Table 14. Effect of *M. incognita* on plant growth parameters of ginger varieties at six months after inoculation

Mean of five replications*


**Plate 11. Effect of *M. incognita* on different varieties of ginger**

Highest dry weight of shoot was observed in variety Athira (40.2 g) and it was on par with that of variety IISR Mahima (34.4 g). Dry weight of shoot was lowest in variety Karthika (21.4 g) and it was significantly different from other varieties. The variety IISR Mahima was on par with varieties IISR Rejatha (32.80 g) and IISR Varada (30.80 g).

The number of tillers was lowest in variety Karthika (8.8) and it was on par with that of varieties IISR Rejatha (10.40) and IISR Mahima (11.6). Highest number of tillers was observed in variety Athira (14.8) and it was significantly superior to other varieties. The variety IISR Varada (10.80) was on par with varieties IISR Rejatha and IISR Mahima.

### 4.2.4 Yield Parameters of Varieties

The change in yield parameters of different ginger varieties due to infection of *M. incognita* is shown in Table 15.

Lowest fresh weight of rhizome yield was observed in the case of variety Karthika (234.8 g) and it was on par with that of varieties IISR Mahima (306.00 g) and IISR Rejatha (291.00 g). The highest fresh weight of rhizome was observed in variety Athira (348.2 g) and it was on par with variety IISR Mahima (306.0 g).

Highest dry weight of rhizome was observed in the case of variety Athira (67.40 g) and it was statistically superior to other varieties. Dry weight of rhizome was lowest in variety Karthika (51.20 g) and it was on par with that of varieties IISR Varada (50.50 g) and IISR Rejatha (51.60 g).

### 4.2.5 Nematode Population

Results on the preference of *M. incognita* for different varieties of ginger assessed in terms of nematode population characteristics is shown in Table 16.

Table 15. Effect of *M. incognita* on yield parameters of ginger varieties at six months after inoculation

| Varieties | Fresh weight of rhizome (g/plant)* | Dry weight of rhizome (g/plant)* |
|---|---|---|
| Athira | 348.20 | 67.40 |
| Karthika | 234.80 | 51.20 |
| IISR Mahima | 306.00 | 60.60 |
| IISR Rejatha | 291.00 | 51.60 |
| IISR Varada | 278.00 | 50.80 |
| CD (0.05) | 53.698 | 6.011 |

Mean of five replications*

Table 16. Effect of *M. incognita* on nematode population in different ginger varieties at

| Varieties | Nematode population (6 MAP) | | | Adult Females per plant* |
|---|---|---|---|---|
| | Soil (200 cc)* | Root (10 g)* | Rhizome (10g)* | |
| Athira | 3274.80 | 82.60 | 50.20 | 53.40 |

six months after inoculation

| | | | | |
|---|---|---|---|---|
| Karthika | 4005.20 | 164.60 | 83.60 | 114.60 |
| IISR Mahima | 2994.20 | 57.40 | 30.60 | 36.20 |
| IISR Rejatha | 3587.80 | 106.20 | 68.60 | 98.60 |
| IISR Varada | 3702.40 | 114.60 | 74.20 | 106.00 |
| CD (0.05) | 250.130 | 15.460 | 12.520 | 12.520 |

Mean of five replications*

#### *4.2.5.1 Nematode Population in Soil* **(200cc soil)**

Significant difference was observed in population of the $J_2$ recorded from the rhizosphere of the varieties tested at 6 MAI. Among the varieties screened lowest population of *M. incognita* was recorded from the rhizosphere of IISR Mahima (2994.2) and it was significantly superior to other varieties. The variety IISR Varada supported a soil population of 3702.4 and was on par with that of the variety IISR Rejatha (3587.7). Higher population of nematode was observed in variety Karthika (4005.2) and it was significantly different from all other varieties.

#### *4.2.5.2 Nematode Population in Root (10g)*

Significant variation was also seen in the nematode population obtained from the root of the different ginger varieties. The variety IISR Mahima had the lowest population (57.4**)** and it was significantly superior to all other varieties. A significantly higher population was recorded from the root samples of variety Karthika (164.6). The rhizome population obtained from the varieties IISR Rejatha (106.2) and IISR Varada (114.6) were on par with each other.

#### *4.2.5.3 Nematode Population in Rhizome (10g)*

Significant variation was recorded in the nematode population obtained from the rhizome of the different ginger varieties. The lowest population was seen in variety IISR Mahima (30.6**)** and it was on par with that of Athira (50.2). Higher population was recorded from the rhizome samples of variety Karthika (83.60) and it was on par with that of variety IISR Varada (74.20).

#### *4.2.5.4 Adult females ( Per plant)*

A significantly lower number of adult females were recorded from the root samples of variety IISR Mahima (36.2). Highest number of adult females were observed in variety Karthika (114.6) followed by varieties IISR Rejatha (98.6) and IISR Varada (106.0).

# DISCUSSION

# 5. DISCUSSION

The root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood is the most serious and widely distributed pest of ginger. Information on the biochemical alterations, yield and growth characters of ginger at different population density of nematode and the varietal reaction studies are essential for the cost effective management of the pest. The varietal reaction studies also help to identify the best varieties against *M. incognita*.

The results on the evaluation of biochemical alterations in growth and yield characters due to different inoculum levels of *M incognita* and the varietal screening are discussed in this chapter under different headings.

## 5.1 BIOCHEMICAL CHANGES IN GINGER DUE TO *M. incognita*

### 5.1.1 Growth Parameters

The plant growth parameters *viz*., plant height, number of tillers, fresh weight of shoot and dry weight of shoot decreased significantly with increasing inoculum levels of *M incognita* at 6 MAI. Highest reduction of growth parameters was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$. This reduction in plant height, number of tillers and fresh weight of plant is because of poor development of lateral roots infected with higher nematode numbers. The shoot weight of 10,000 $J_2$ inoculated plants was reduced by 43.03 per cent when compared to uninoculated plants. This was in accordance with the findings of Abbasi and Hisamuddin (2014), who found that plant height, fresh weight of plant and dry weight of shoot decreased significantly with increasing levels of *M incognita* in green gram. The plant height of green gram inoculated with 800 $J_2$ $pot^{-1}$and 1600 $J_2$ $pot^{-1}$decreased significantly by 32.02 and 38.05 per cent respectively over the uninoculated plants (Figure 1.). The rice cultivars non-basmati Pusa-44 and basmati Sugandh-5 showed significantly lower plant height and shoot dry weight with increasing inoculum levels of *M. graminicola* (Patil and Gaur, 2014). Darsana *et al.* (2015) reported that the plant growth parameters

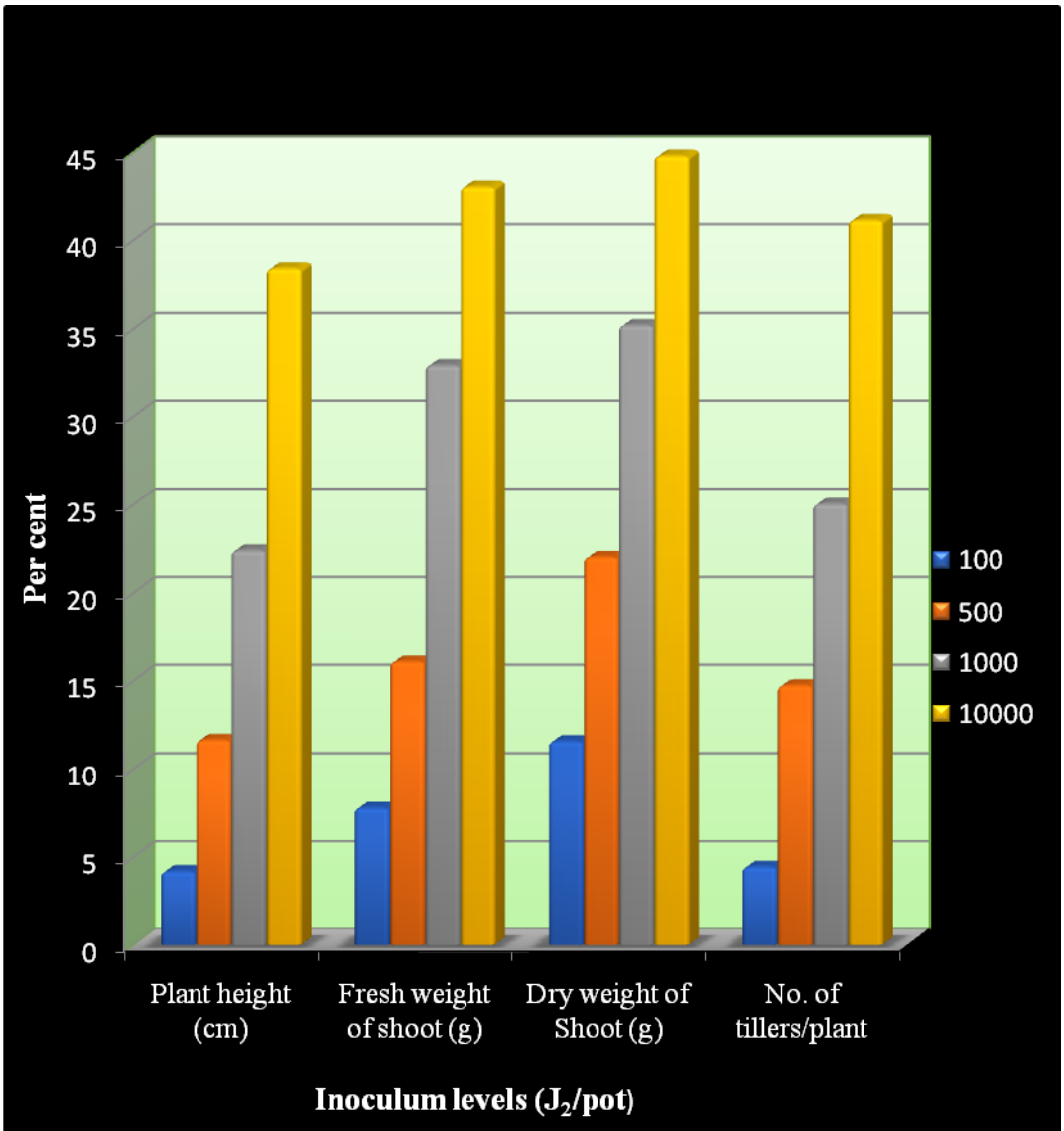



Figure 1. Per cent decrease in plant growth parameters at different inoculum levels of *M. incognita*

of rice *viz*., plant height, fresh weight of plant, dry weight of root and shoot decreased with increasing inoculum levels of *M. graminicola*.

### 5.1.2 Yield Parameters

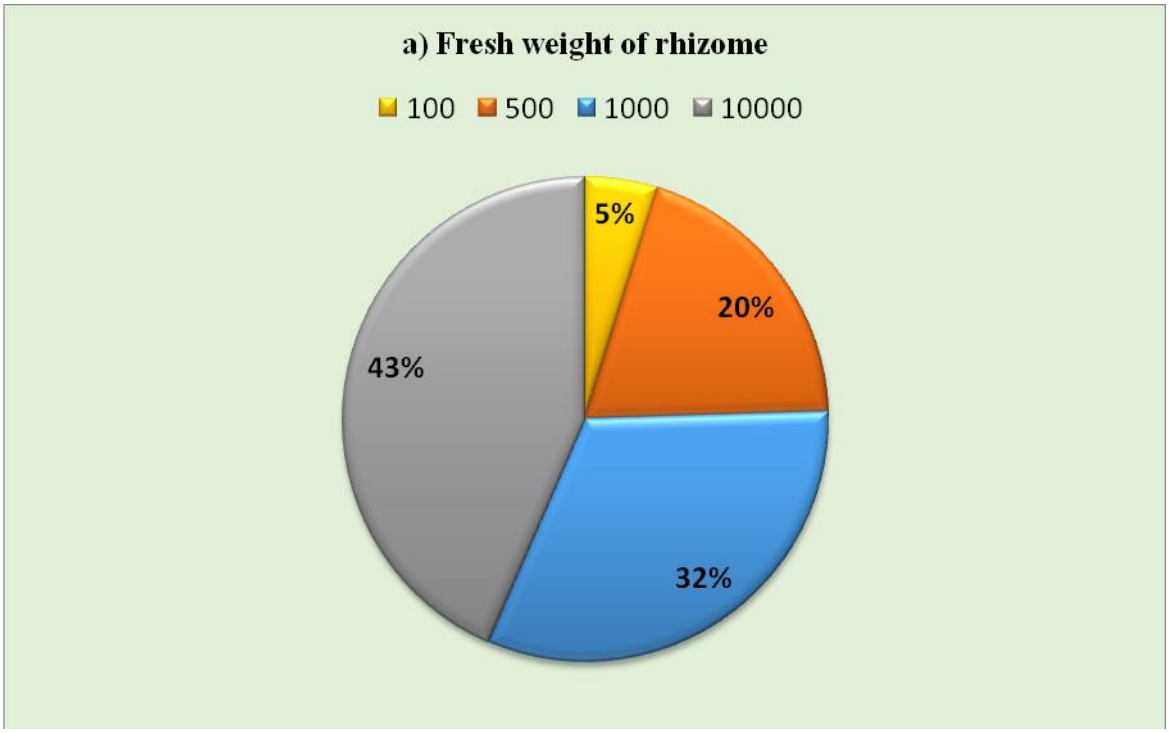
The yield parameters *viz*., fresh weight and dry weight of rhizome decreased with increasing inoculum levels of *M incognita*. Highest reduction of yield parameters was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ and it was significantly different from all other treatments.

The percentage reduction in fresh weight of rhizome in plants inoculated with 10,000, 1000, 500, and 100 $J_2$ per pot were 40.60 per cent, 29.90 per cent, 18.40 per cent and 4.51 per cent respectively compared to uninoculated plants. The percentage decrease in dry weight of rhizome in plants inoculated with 10,000 $J_2$ $pot^{-1}$ was 47.18 per cent when compared to uninoculated plants (Figure 2.). This was in accordance with the findings of Ray *et al*. (1995), they reported that yield loss due to *M. incognita* infection in turmeric and ginger was 35.97 and 23.97 per cent respectively. Sudha and Sundararaju (1986) observed a reduction of 75 % rhizome weight with an initial inoculum level of 10,000 $J_2$ of *M. incognita* per plant over a period of six months under potted conditions in ginger. In another study on ginger, Sheela *et al.* (1995) found that an avoidable yield loss of 43 % with an initial population level of 166 *M. incognita* juveniles per 250 g soil.

### 5.1.3 Biochemical Changes

The present study showed that *M. incognita* infection brought about biochemical changes in rhizomes. This was more pronounced due to infection of *M. incognita* during their growth and metabolism that causes deterioration of the content of the rhizome. Increased enzyme activity in infected plants offering high

degrees of protection from nematode invasion and development. The activity of the enzymes were higher in rhizomes than in leaves of ginger. The results



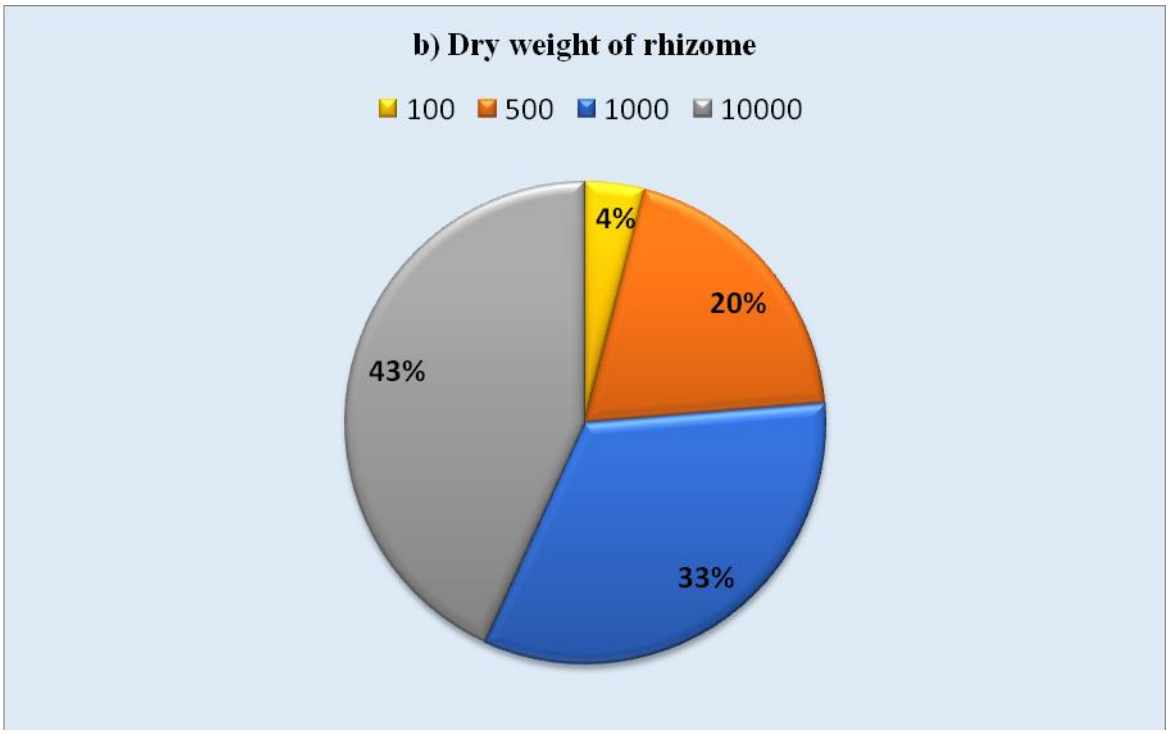



Figure 2. Per cent decrease in yield parameters of ginger at different inoculum levels of *M. incognita*

suggested that plants responded to *M. incognita* by adopting biochemical strategies to withstand the adverse effects of infection.

### *5.1.3.1 Peroxidase (PO), Polyphenol Oxidase (PPO) and Phenylalanine Ammonia Lyase (PAL) Activity*

PO, PPO and PAL are the defense enzymes in plants and they synthesis when the plant is infected by any pathogen. The PO, PPO and PAL activity in leaf and rhizome samples increased with increasing inoculum levels of *M incognita* at six MAI. The PO activity of rhizome and leaf samples in plants inoculated with 10,000 $J_2$ increased 59.88 and 57.93 per cent respectively compared to that of the uninoculated plants. The PAL activity of rhizome and leaf samples in plants inoculated with 10,000 $J_2$ increased 58.61 and 71.64 per cent respectively over the uninoculated plants (Figure 3 and 4.). The PPO activity of rhizome increased 58.29 per cent in plants inoculated with 10,000 $J_2$ over uninoculated plants. These results are in good harmony with those obtained by Devrajan and Srenivasan (2002), who observed that the inoculation of *M. incognita* increased the PPO activity in banana. Shukla and Chakraborthy (1988) found that PO activity increased in *M. incognita* infected resistant tomato cultivars up to 5 times than that of healthy plants. Peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activities significantly increased in roots of *M. exigua* Goeldi inoculated coffee plants compared to the roots of uninoculated plants (Silva *et al*., 2010).

### *5.1.3.2 Phenol Content*

Population of *M. incognita* at different densities tested, resulted in progressive increase in phenol content of rhizome and leaf at six MAI when compared to that of uninoculated plants. The highest phenol content was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ and it was significantly superior

to all other treatments. The per cent increase in the phenol content of leaf and rhizome in plants inoculated with 10,000 $J_2$/pot was 86.33 and 52.88 per cent

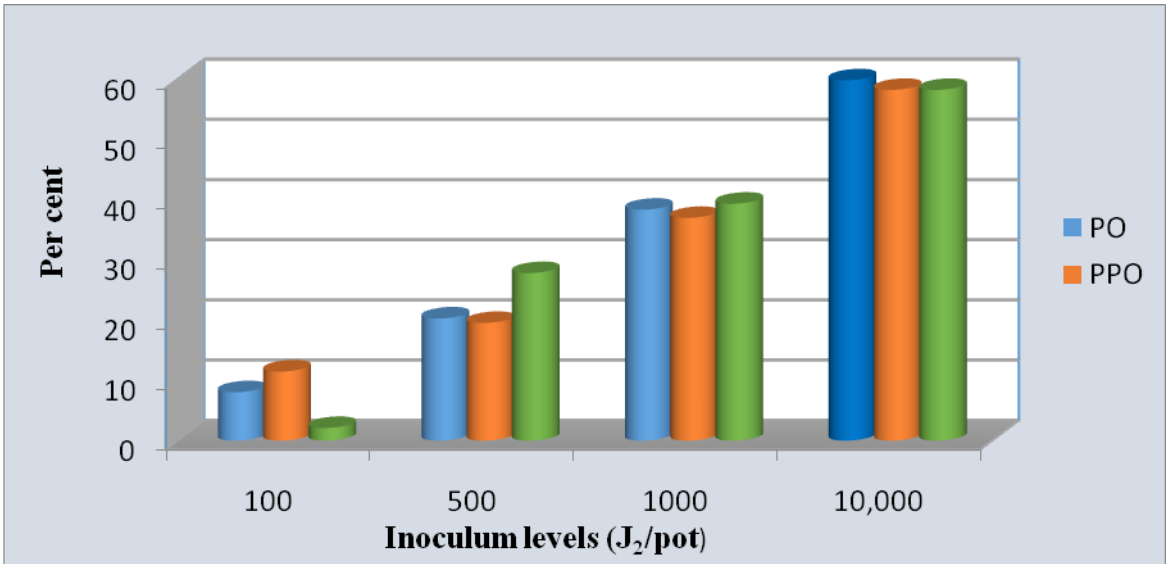



Figure 3. Per cent increase in PO, PPO and PAL in rhizome at different inoculum levels of *M. incognita*

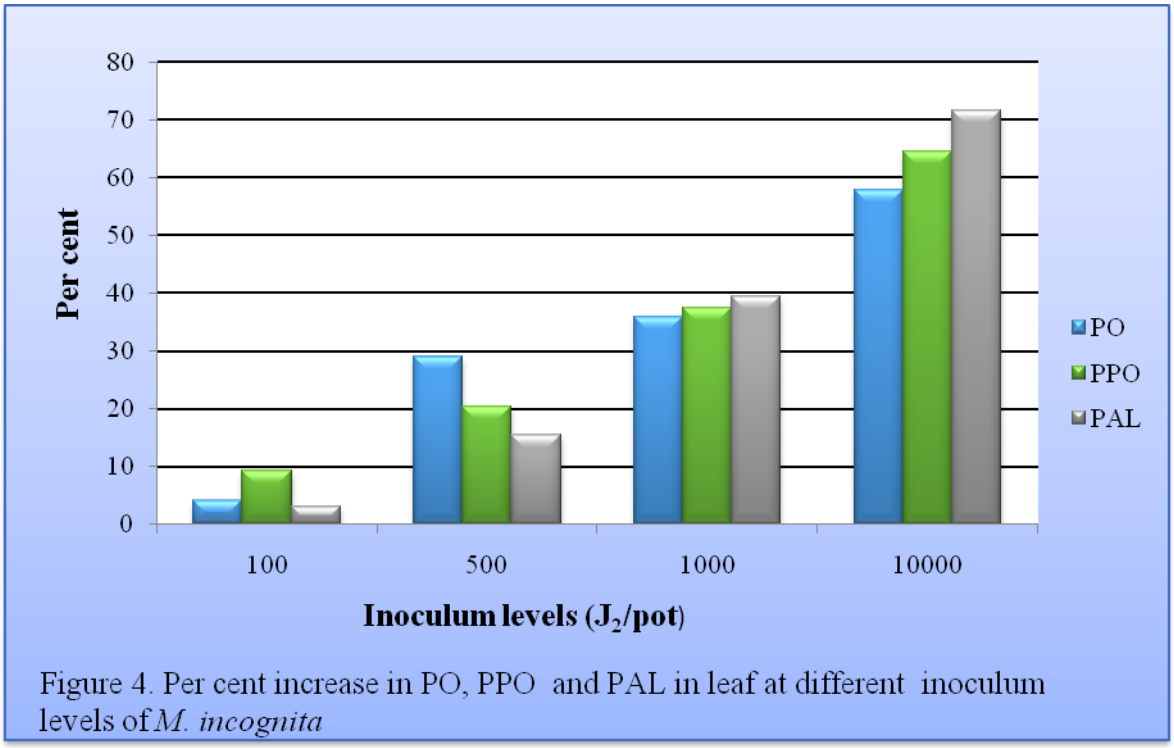



Figure 4. Per cent increase in PO, PPO and PAL in leaf at different inoculum levels of *M. incognita*

respectively. Higher phenol production was seen in rhizomes than in leaves (Figure 5 and 6.). Similar finding was reported by Mishra and Mohanty (2007), who found that *M. graminicola* infected rice varieties Annapurna, Manika and Ramakrishna produced greater amount of phenolics compared to the healthy plants. Bajaj and Mahajan (1977) reported that the oxidized forms of phenolic compounds occurring in high concentration in roots of resistant tomato plants and it contributes to the *M. incognita* resistance by creating a toxic environment for nematode penetration and multiplication. Taiz and Zeiger (2002) reported that the early increases in phenol caused by pathogen invasion triggered the transcription of messenger RNA that codes for phenylalanine ammonia lyase; increasing amounts of PAL in the plants brought about the synthesis of phenolic compounds. The phenolic accumulation was due to the activation of hexose monophosphate shunt pathway, acetate pathway and release of bound phenols by hydrolic enzymes (Goodman *et al*., 1967). The increased phenolic content in tomato roots indicated the degree of resistance to *M. incognita* (Rani *et al*., 2008).

Phenol production in plants helps to reduce the infection of *M. incognita.* Higher the infection of *M. incognita* leads to increased production of phenolic substances in both rhizome and leaf samples. The plants itself have the capacity to reduce the infection of *M. incognita* by producing the phenols.

### *5.1.3.3 pH and EC of Leaf and Rhizome Samples*

Inoculation of *M. incognita* in varying population levels resulted in a corresponding decrease in pH content of leaves and rhizome at six MAI. The lower pH content was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$. The per cent decrease in the pH content of leaf and rhizome in plants inoculated with 10,000 $J_2$ $pot^{-1}$ was 13.39 and 9.42 per cent respectively when compared to uninoculated plants (Figure 7 and 8). Similar findings was reported by Darsana (2015), who found that pH content of leaf in plants inoculated with 10,000 and

5000 $J_2$ $pot^{-1}$ decreased 9.91 and 4.78 per cent respectively in *M. graminicola* infected rice plants.

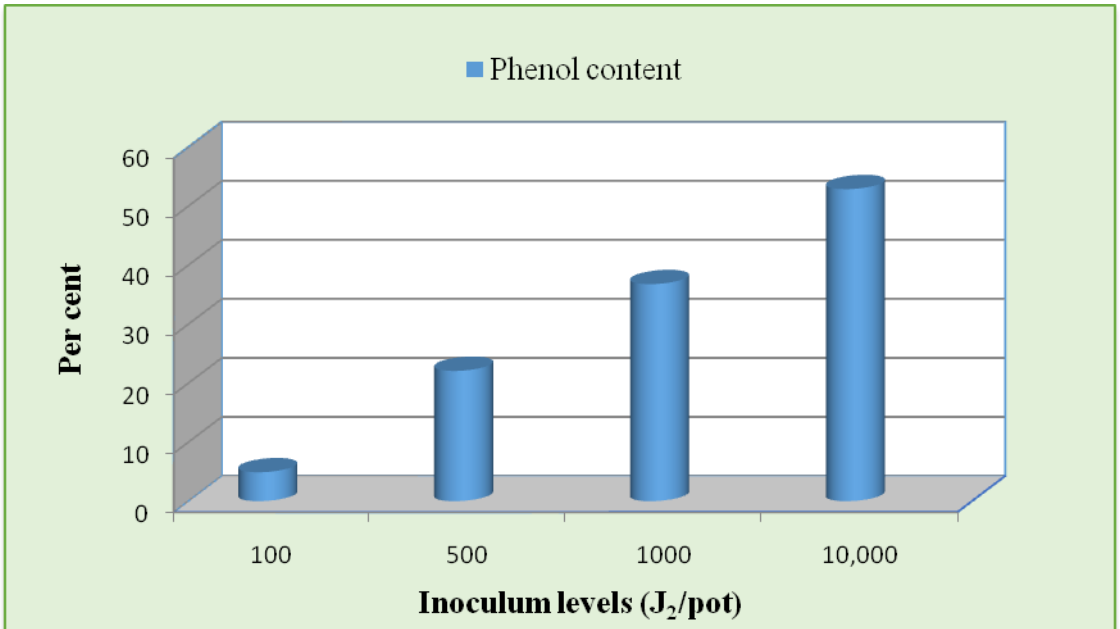



Figure 5. Per cent increase in phenol content of rhizome at different inoculum levels of *M. incognita*

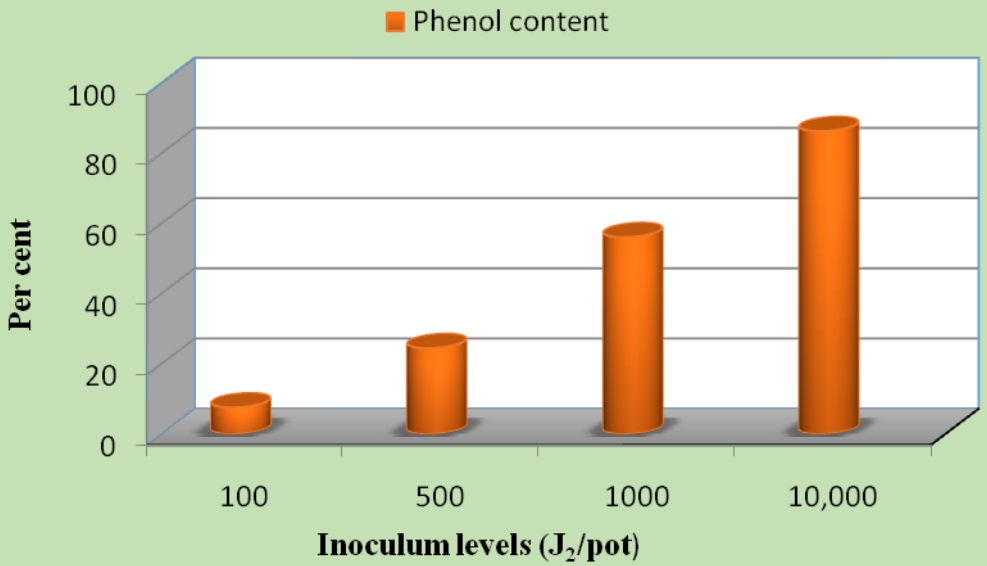



Figure 6. Per cent increase in phenol content of leaf at different inoculum levels of *M. incognita*

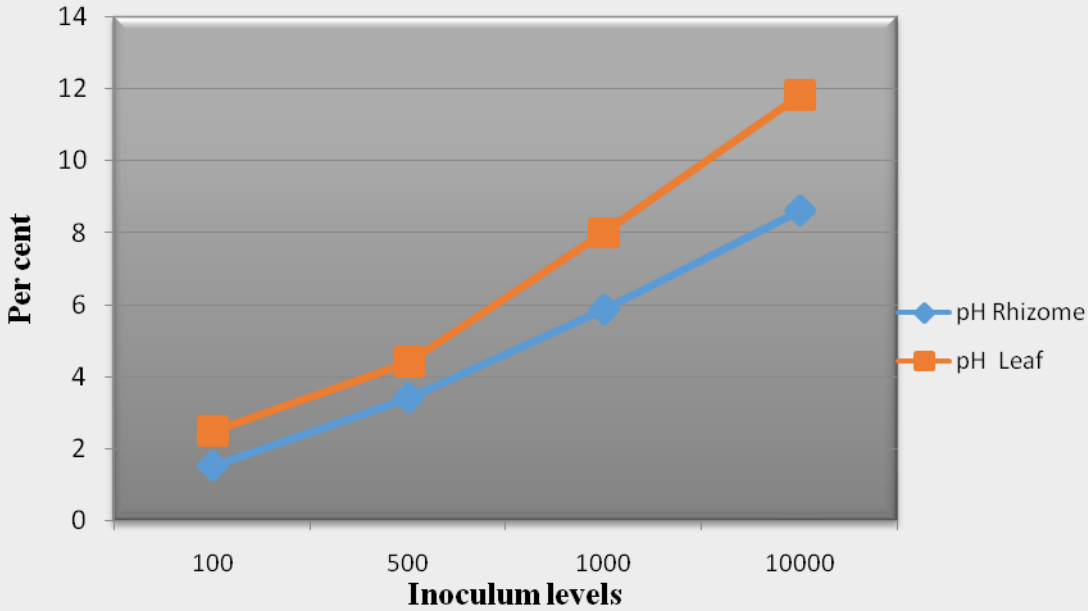



Figure 7. Per cent decrease in pH of rhizome and leaf at different inoculum levels of *M. incognita*

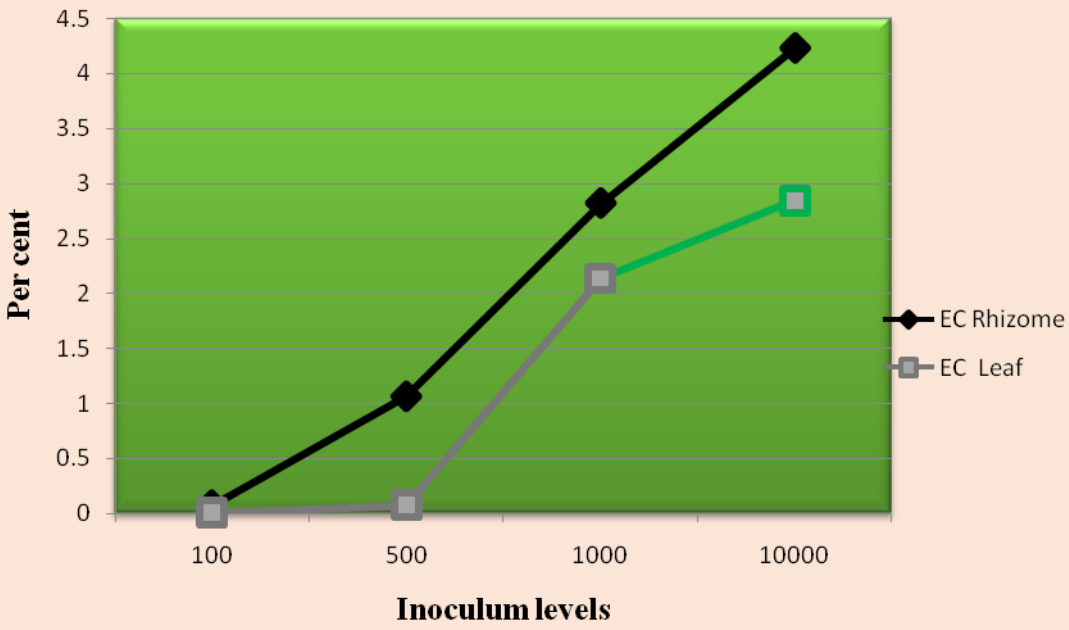



Figure 8. Per cent decrease in EC of rhizome and leaf at different inoculum levelsof*M. incognita*

Inoculation of *M. incognita* in varying population levels resulted in corresponding decrease in EC of leaves and rhizome at six MAI. The lower EC was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$. The per cent decrease in the EC of leaf and rhizome in plants inoculated with 10,000 $J_2$ $pot^{-1}$ was 2.14 and 4.23 per cent respectively. Early stage of fungi infection involves the production and the accumulation of a large amount of oxalic acid which appears to be one of the essential determinants of the pathogenecity (Dutton, M. V. and Evans, C. S. 1996; Zhou, T. and Boland, G. J. 1999). Mohanty and Pradhan (1989) reported that *M. incognita* infected green gram plants showed increase in amino acids and amides. Primary metabolites *viz.*, amino acids, nucleic acids were produced in plants during pathogen attack (Freeman and Beattie, 2008). The infection of *M. incognita* leads to increased production of acid compounds in plants. As a result there was an increase in $H^+$ ion concentration and decrease in cations viz., K+, Ca+, Mg+, which lead to decrease in EC of plants samples.

### *5.1.3.4 Starch and Protein Content of Rhizome*

The starch content of rhizomes exhibited reduction at different inoculum levels of *M. incognita* when compared to uninoculated plants. Highest decrease of starch content **(**31.15 per cent**)** was recorded in plants inoculated with 10,000 $J_2$ $pot^{-1}$. The varying inoculum levels of *M. incognita* resulted in corresponding decrease in the percentage of protein in the rhizomes compared to uninoculated plants. The highest reduction in protein content of the rhizome was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$. In 1000 and 10,000 $J_2$ inoculated plants, the protein content of the rhizome decreased significantly by 33.55 and 39.78 per cent respectively over uninoculated plants (Figure 9). These finding was in line with the reports of Abbasi and Hisamuddin (2014). They reported that the protein content of green gram exhibited reduction at all the *M. incognita* inoculum levels when compared with the protein content of uninoculated plants. Patil and Gaur (2014) also reported that the rice grains produced on plants inoculated with the nematode *M. graminicola* were lighter in weight and had poorer nutrient qualities, such as amylase and protein content.

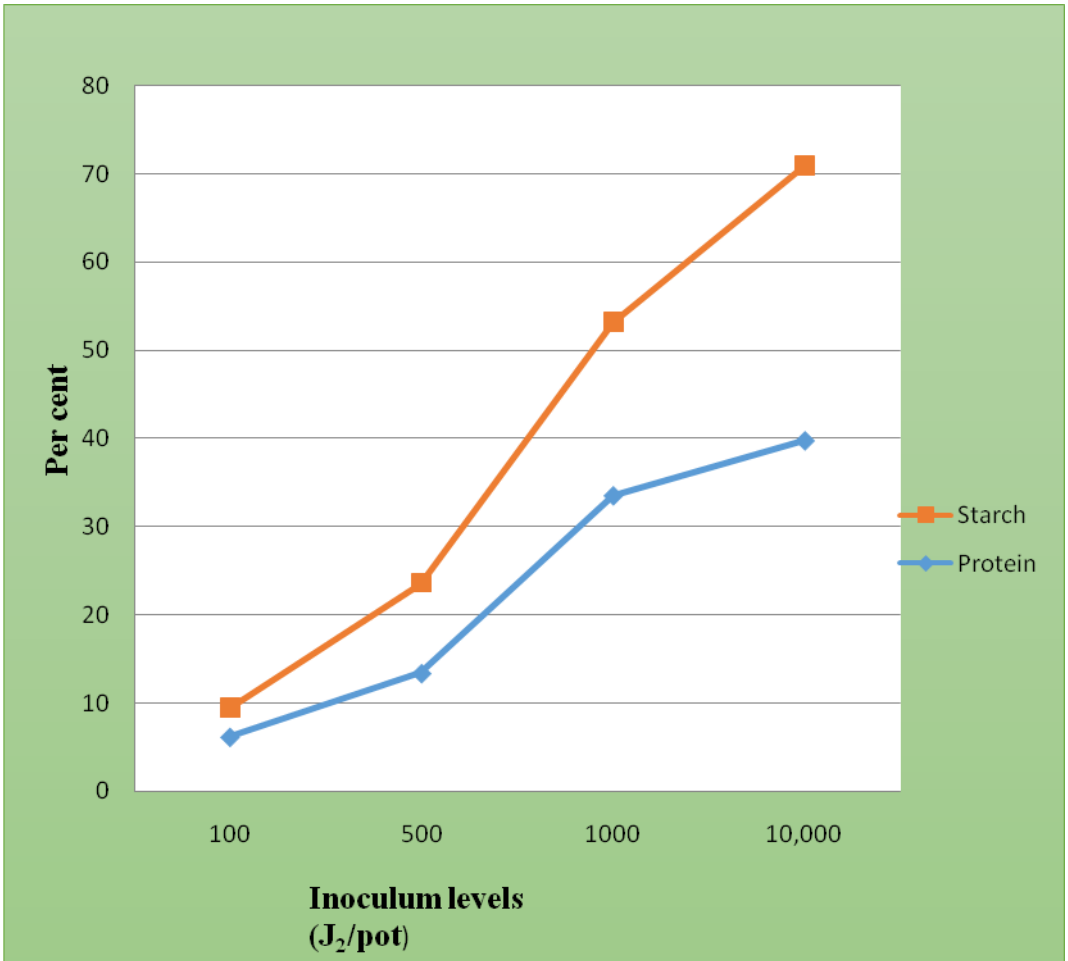



Figure 9. Per cent decrease in starch and protein content in ginger at different inoculum levels of *M. incognita*

Korayem *et al*. (2013) found that crude protein and fat contents decreased in peanut seeds infected by *M. arenaria*. Darsana *et al*. (2015) reported that reduction in protein and starch content of rice with increase in inoculum levels of *M. incognita*.

The lower level of proteins during the later stages of infection suggested that the developing *M. incognita* continuously withdraw large amounts of nutrients from the giant cells (Dorhout *et al.,* 1993).

### *5.1.3.5 Crude Fibre, Oleoresin, Total Ash, Gingerol and Shagaol Content in Rhizome*

The crude fibre content of rhizome exhibited reduction at different inoculum levels *M. incognita* when compared to uninoculated plants. Highest decrease of crude fibre content **(**19.01 per cent**)** was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$. The varying inoculum levels of *M. incognita* resulted in a corresponding decrease in the percentage of oleoresin in the rhizome compared to uninoculated plants. The highest reduction in oleoresin content of the rhizome was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$. In 1000 and 10,000 $J_2$ inoculated plants, the oleoresin content of the rhizome decreased by 20.79 and 29.41 per cent significantly over uninoculated plants. The total ash content of rhizome decreased with increase in inoculum levels of *M incognita* as compared to uninoculated plants. Highest decrease of total ash content (29.93 per cent) was recorded in plants inoculated with 10,000 $J_2$ $pot^{-1}$. The percentage reduction in gingerol and shagaol content of rhizome samples in plants inoculated with 1000 $J_2$ was 19.95 and 21.92 per cent respectively and with 10,000 $J_2$ was 31.01 & 34.24 per cent respectively over uninoculated plants (Figure 10). These results confirmed with the findings of Choudhari and Kareppa (2013), who reported that crude fibre, gingerol, shagaol, oleoresin, volatile oil and total ash content were reduced due to infection of *Fusarium* spp in ginger.

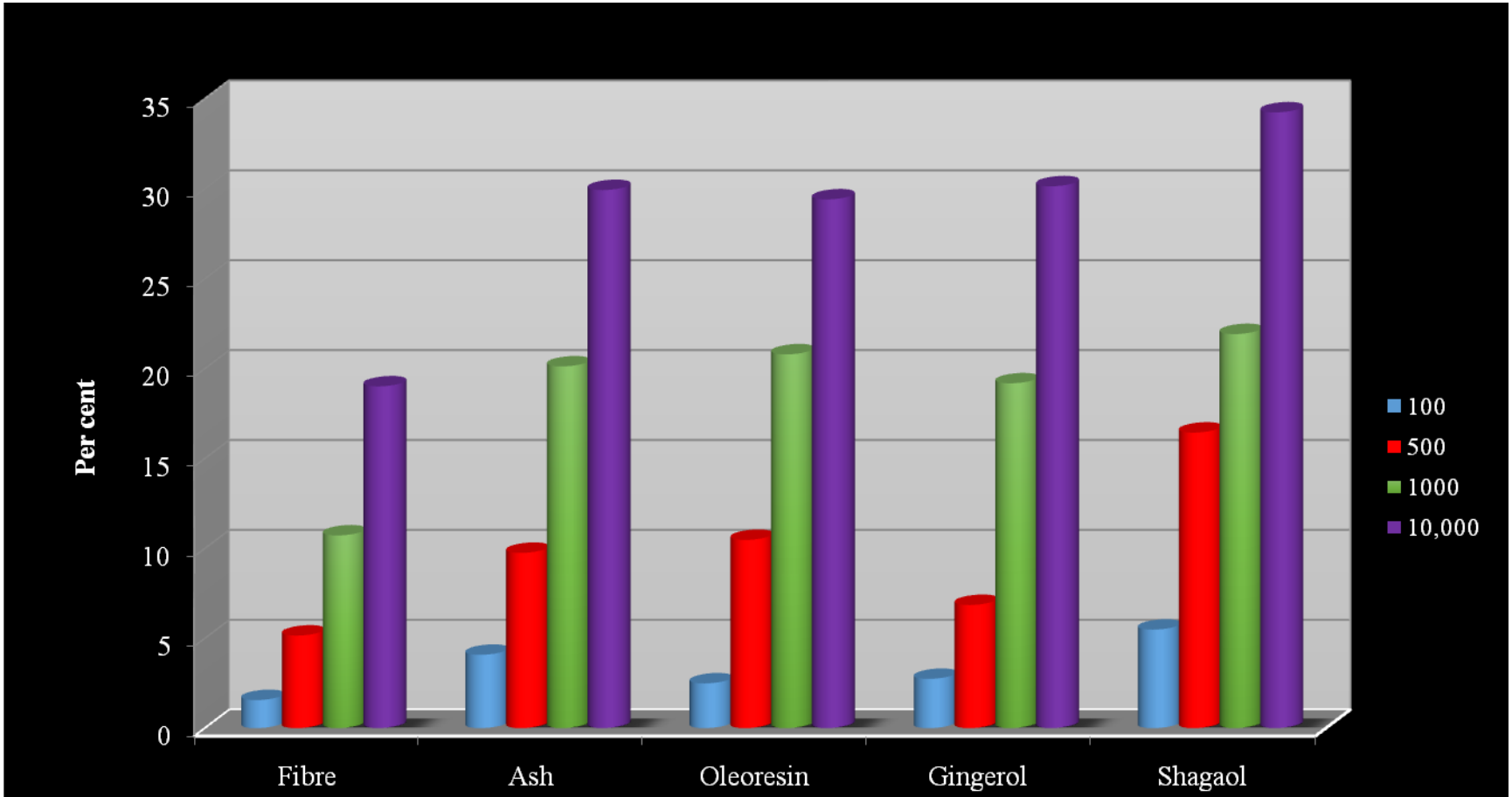



Figure 10. Per cent decrease in fibre, ash, oleoresin, gingerol and shagaol content in ginger at different inoculum levels of *M. incognita*

## 5.2 VARIETAL SCREENING

### 5.2.1 Reaction of Varieties

The ginger varieties *viz*., IISR Varada, IISR Rejatha and Karthika were susceptible to *M. incognita* infection. Highest number of galls and egg mass per plant was recorded in the variety Karthika (106.80 plant$^{-1}$ and 80.10 plant$^{-1}$ respectively) and lowest number was seen in variety IISR Mahima (28.6 plant$^{-1}$, 21.5 plant$^{-1}$ respectively).

The variety Karthika showed a gall index of 5 and was rated as highly susceptible to *M. incognita*. However, the varieties Athira, IISR Rejatha and IISR Varada were rated as susceptible with a gall index 4. The variety IISR Mahima showed a gall index of 3 and was moderately resistant to *M. incognita*. The varieties Karthika, IISR Rejatha and IISR Varada showed a egg mass index of 4, 3.18 and 3.51 respectively and were rated as highly susceptible to *M. incognita*. However, the varieties Athira and IISR Mahima (1.07) were rated as moderately resistant to *M. incognita* with a egg mass index 1.88 and 1.07 respectively. This was in accordance with the findings of Sasikumar *et al.,* 2003. They reported that the variety IISR Mahima was resistant to *M. incognita* in field condition.

There are several reports on the reaction of root-knot nematode to crop varieties. Okorocha *et al.* (2014) found that out of seven varieties screened, Maran, U.G I and Rio de Jenairo were resistant while U.G II, Himachall Pradesh and St. Vincent were susceptible and Wynad Local was tolerant in first year. In the second year, U.G II was tolerant while Maran was susceptible. Mohanta *et al.* (2015) reported that out of seventy turmeric cultivars screened, only five cultivars, Dugirala, PTS-31, Ansitapani, PTS-42, PTS-47 were found to be resistant. 361 Gorakhpur, 328 Sugandham and PTS-21 were rated as moderately resistant and remaining cultivars were susceptible to *M. incognita*. Berliner *et al*. (2014) reported that the rice cultivars were categorized as resistant or susceptible on the basis of root knot index. Out of 414 rice genotypes screened, only two cultivars,

127-28-1-1-1 and 183-6- 1-1-3 were found resistant based on root knot index. Nayak and Pandey (2015) reported that resistant brinjal varieties showed least gall index of 1.1 to 2.0, moderately resistant varieties with gall index of 2.1 to 3.0, susceptible varieties having gall index of 3.1 to 4.0 and highly susceptible varieties with highest gall index of 4.1 to 5.0 against the *M. incognita*.

### 5.2.2 Enzymatic Activity of Varieties

Significant variation was recorded in peroxidase, polyphenol oxidase, and phenyl alanine ammonia lyase activity of different ginger varieties due to *M. incognita*. Highest PO, PPO and PAL content was observed in the variety IISR Mahima (9.02 changes in absorbance $min^{-1}$ $g^{-1}$, 0.94 changes in absorbance $min^{-1}$ $g^{-1}$ and 17.78 changes in cinnamic acid $min^{-1}$ $g^{-1}$ respectively) and lowest PO, PPO and PAL activity was seen in variety Karthika (6.92 changes in absorbance $min^{-1}$ $g^{-1}$, 0.70 changes in absorbance $min^{-1}$ $g^{-1}$ and 14.59 changes in cinnamic acid $min^{-1}$ $g^{-1}$ respectively). Compared to all other varieties, the enzyme activity was more in variety IISR Mahima and thereby exhibited more defense action against *M. incognita.* This was in hormony with the findings of Darsana, (2015) who found that the peroxidase, polyphenol oxidase, and phenyl alanine ammonia lyase activity was more in resistant rice varieties than in susceptible varieties due to infection of *M. graminicola*. Shukla and Chakraborthy (1988) also reported that the *M. incognita* infected resistant varieties of tomato showed significantly higher peroxidase enzyme activity than the susceptible varieties. Devi *et al*. (2007) reported that PAL activity was more in resistant banana cultivars than in susceptible cultivars due to the infection of *Pratylenchus coffeae* Zimmermann.

### 5.2.3 Growth and Yield Parameters of Varieties

Changes in plant height, number of tillers, fresh weight and dry weight of shoot were observed due to *M. incognita* infection in different ginger varieties. The dry weight and fresh weight of shoot was highest in variety IISR Athira (34.4

g and 238.0 g respectively) and minimum in Karthika (21.4 g and 122.2 g respectively).

The highest fresh weight and dry weight of rhizome was observed in variety Athira (328.2 g and 63.8 g respectively) and lowest was recorded in variety Karthika (234.8 g and 45.38 g respectively).

These results are in good harmony with those obtained by Khan *et al*. (2012). They found that considerable reduction in plant growth was seen in different rice cultivars due to the infection of *M. graminicola*. The rice cultivars Pusa-44 and Sugandh-5 showed significantly lower plant height, shoot and root dry weight with increasing inoculum levels of *M. incognita*. Win *et al*. (2013) also reported that the root-knot nematode infected rice varieties *viz.,* Yatanartoe, MR 9 and Saytanar 1 showed significant reduction in plant height and root length eight weeks after inoculation.

### 5.2.4 Population of Nematode

Significant variation was seen in the nematode population obtained from the roots, rhizome and rhizosphere of the different ginger varieties. Among the varieties lowest population of *M. incognita* in rhizosphere, root and rhizome was recorded from the variety IISR Mahima (2994.2, 57.4 and 30.6 respectively) and highest population was observed in Karthika (4005.2, 164.6 and 83.6 respectively). This was in accordance with the findings of Khan *et al*. (2012) who reported that highest soil population of *M. graminicola* was recorded in the root zone of rice cvs. R-Dhan and Sugandh and lowest population was recorded from the rice cv. Abhishek. Devi, 2014 also found that out of 10 rice varieties screened against *M. graminicola***,** the variety Lamyanba showed maximum soil population (7500), root population (3500) and total population (11000) at harvest.

# SUMMARY

## 6. SUMMARY

Basic information on the biochemical alterations, growth and yield characters of ginger at different inoculum levels of *M. incognita* is essential for effective management of the nematode problem. In view of the wide spread occurrence of the nematode in ginger in the state, a pot culture study was conducted to determine the biochemical alterations occuring in ginger due to the infection of *M. incognita*. Five important ginger varieties *viz.,* Athira, Karthika, IISR Mahima, IISR Rejatha and IISR Varada were screened to identify sources of resistance to *M. incognita*. Another pot culture experiment was conducted to determine the enzymatic activity, growth characters and nematode population in these varieties. The salient results of the study are summarized below.

A progressive decrease in plant growth parameters *viz.,* plant height, fresh weight of shoot, dry weight of shoot and number of tillers were observed with an increase in population levels of nematode after six months of inoculation (MAI). The highest percentage reduction in plant height (38.38 per cent), fresh weight of shoot (43.03 per cent), dry weight of shoot (44.78 per cent) and number of tillers (41.10 per cent) were seen in plants inoculated with 10,000 $J_2$ over uninoculated plants. The progressive decrease in yield parameters *viz.,* fresh weight of rhizome and dry weight of rhizome was observed with increase in population levels of nematode after six MAI. The highest percentage reduction in fresh weight of rhizome (40.60) and dry weight of rhizome (47.18) was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ over uninoculated plants. There was no significant difference between the plants inoculated with 100 $J_2$ $pot^{-1}$ and uninoculated plants.

The various levels of nematode population showed reduction in pH and EC content in both leaf and rhizome samples compared to uninoculated plants. Plants inoculated with 10,000 $J_2$ $pot^{-1}$ showed low pH (6.37) and EC (272 d S $m^{-1}$) compared to that of the uninoculated plants. The plants inoculated with 100 $J_2$ $pot^{-1}$ was found to be non significant. A progressive increase in phenol content was observed with increase in inoculum levels of 500 $J_2$ onwards at six MAI.

Plants inoculated with 10,000 $J_2$ $pot^{-1}$ showed higher peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) activity compared to uninoculated plants. The PO, PPO and PAL activity increased 60.60, 58.29 and 58.61 per cent respectively in rhizome and 57.93, 64.64 and 71.64 per cent in leaf respectively compared to uninoculated plants. Higher phenol and defense enzyme production was seen in rhizome than in leaf samples. The phenol content increased 52.88 per cent in rhizome and 86.33 per cent in leaf compared to uninoculated plants.

The rhizome quality was also affected by the infection of *M. incognita*. The starch, protein, crude fibre, oleoresin and total ash content of dried rhizome decreased with increase in nematode inoculum levels after 6 months of inoculation. The highest reduction was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$. The starch and protein content decreased 31.15 and 39.78 per cent respectively in plants inoculated with 10,000 $J_2$ $pot^{-1}$compared to uninoculated plants. Lower crude fiber, oleoresin, total ash content was seen in plants inoculated with 10,000 $J_2$/pot was 4.69 %, 3.52 % and 1.24 % respectively. The gingerol content of rhizome in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (0.59 per cent) was significantly different from all other treatments. The percentage decrease in gingerol content of plants inoculated with 10,000 $J_2$ $pot^{-1}$ was 31.01 per cent over uninoculated plants. The different inoculum levels of *M. incognita* resulted in a corresponding decrease in the percentage of shagaol content of rhizome compared to uninoculated plants at 6 MAI. The plants inoculated with 10.000 $J_2$ $pot^{-1}$ (0.10 per cent) showed low shagaol content and it was significantly superior to other treatments. Highest nematode population in soil was observed in plants inoculated with 10,000 $J_2$ $pot^{-1}$ (3673.60). The plants inoculated with 100 $J_2$ $pot^{-1}$ (22.80) showed lowest nematode population in rhizome. Plants inoculated with 500 and 1000 $J_2$ $pot^{-1}$ showed population of 65.60 and 104.80 respectively in root samples.

The varietal reaction of five popular ginger varieties grown in Kerala *viz*., Athira, Karthika, IISR Mahima, IISR Rejatha and IISR Varada against

*M. incognita* was also studied. All the varieties were susceptible to the *M. incognita* except variety IISR Mahima (based on both gall index and egg mass index). Lower number of galls (28.60) and egg mass (21.45) per plant was observed in the variety IISR Mahima and it was on par with variety Athira (50.20 and 37.65 respectively). According to gall index, the variety Karthika was rated as highly susceptible to *M. incognita*. The varieties Athira, IISR Rejatha and IISR Varada were rated as susceptible. However, the variety IISR Mahima was rated as moderately resistant to *M. incognita*. The egg mass index was highest in Karthika (4) and lowest in case of variety IISR Mahima (1.07). Based on egg mass index, the varieties Karthika, IISR Rejatha and IISR Varada were rated as highly susceptible to *M. incognita* and the variety IISR Mahima and Athira rated as moderately resistant to *M. incognita*.

Significant variation was observed in PO, PPO and PAL activity of different ginger varieties due to nematode infection. Highest PO (9.02 changes in absorbance $min^{-1}$ $g^{-1}$), PPO (0.94 changes in absorbance $min^{-1}$ $g^{-1}$) and PAL (17.78 changes in cinnamic acid $min^{-1}$ $g^{-1}$) activity were seen in variety IISR Mahima and thereby exhibited more defense action against *M. incognita*. The minimum enzyme activity like PO, PPO and PAL activity was seen in variety Karthika (6.92 changes in absorbance $min^{-1}$ $g^{-1}$, 0.70 changes in absorbance $min^{-1}$ $g^{-1}$ and 14.59 changes in cinnamic acid $min^{-1}$ $g^{-1}$ respectively).

Changes in plant height, fresh and dry weight of shoot and number of tillers were seen in different ginger varieties due to infection of *M. incognita*. The variety Karthika recorded lowest fresh weight of shoot (122.20 g) and dry weight of shoot (21.40 g) due to infection of *M. incognita*. However, the variety Athira showed a highest fresh weight (238.00 g) and dry weight of shoot (40.20 g).

The nematode population in root, rhizome and soil were worked out at 6 MAI. The variety Karthika recorded highest nematode population in root, rhizome and soil (164.6, 83.6 and 4005.2 respectively) compared to other

varieties. However, variety IISR Mahima showed lowest nematode population (2994.2, 30.60 and 57.40 respectively). Based on the results of the study, the variety IISR Mahima is comparatively suitable for growing in *M. incognita* infected field for better yield.

Based on the results of the study, it is concluded that there was a progressive decrease in plant growth and yield parameters with increase in inoculum levels of *M. incognita*. Plants inoculated with 1000 and 10,000 $J_2$ $pot^{-1}$ showed more reduction in biochemical parameters compared to other treatments. Progressive increase in phenol content and defense enzymes *viz*., PO, PPO, PAL in plants were observed with increase in inoculum levels of *M. incognita*. The variety IISR Mahima showed better performance against *M. incognita* than all other varieties.

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# APPENDICES

## APPENDIX I

## BUFFER FOR ENZYME (PEROXIDISE AND POLYPHENOL OXIDASE) ANALYSIS

### 0.1 M Sodium Phosphate Buffer (pH 6.5)

Stock solutions

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

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

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

## APPENDIX II

## BUFFER FOR PHENYL AMMONIA LYASE ANALYSIS

### 0.1 M Sodium Borate Buffer (pH 8.8)

Stock solutions

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

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

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

## APPENDIX III

## ESTIMATION OF PROTEIN

1. **0.1 M Sodium acetate buffer (pH 4.7)**

   Stock solutions

   A. 0.2 M solution of acetic acid (11.55 ml in 1000 ml)

   B. 0.2 M solution of Sodium acetate (16.4 g of $C_2H_3O_2$ Na or 27.2 g of C2H3O2Na. 3H2O in 1000 ml)

   22.7 ml of A is mixed with 27 ml of B, diluted to total of 100 ml.

2. **Preparation of stock dye solution for estimation of protein**

   100 mg of Coomassie brilliant blue G-250 was dissolved in 50 ml of 95 % ethanol and 100 ml of concentrated Orthophosphoric acid was added. The volume was made up to 200 ml with water and kept at 4 $^{o}$C. The working dye was prepared just before use by diluting the stock solution to five times with water.

## APPENDIX IV

## ANTHRONE REAGENT FOR STARCH ANALYSIS

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

# BIOCHEMICAL CHANGES DUE TO ROOT- KNOT NEMATODE, *Meloidogyne incognita* (Kofoid and White) Chitwood IN GINGER (*Zingiber officinale* Roscoe.)

*by*

**SUNILKUMAR. B. C.**

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**Abstract of the thesis**

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**COLLEGE OF AGRICULTURE**
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# ABSTRACT

## 9. ABSTRACT

An experiment entitled "Biochemical changes due to root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood in ginger *(Zingiber officinale* Roscoe)" was undertaken at Department of Agricultural Entomology, College of Agriculture, Vellayani, Thiruvananthapuram, during 2014-16. The main objective of the study was to assess the biochemical changes in ginger due to the infestation of *M. incognita* and to screen five important ginger varieties *viz.*, IISR Mahima, IISR Rejatha, IISR Varada, Athira and Karthika against *M. incognita.*

The study involved two pot culture experiments, both laid out in completely randomized design. First pot culture experiment was carried out in 30 cm diameter earthen pots by introducing four inoculum levels of *M. incognita* $J_2$ (each replicated five times) ten days after planting. Six months after inoculation (MAI), biochemical analysis of rhizome and leaf samples were done.

The result of the first pot culture experiment revealed that there was a progressive decrease in plant growth and yield parameters were observed with increase in inoculum levels of *M. incognita* at six MAI. The maximum reduction in plant height (38.38 per cent), fresh weight of shoot (43.03 per cent), dry weight of shoot (44.78 per cent), number of tillers (41.10 per cent) and rhizome yield (40.6 per cent) were observed in plants inoculated with 10,000 $J_2$. The plant height, dry weight of shoot, number of tillers and the yield parameters decreased significantly in plants inoculated with 10,000 $J_2$ compared to other levels and uninoculated plants.

The plants inoculated with 10,000 $J_2$ showed low pH (6.37) and EC (0.27 d S $m^{-1}$) compared to the uninoculated plants and was significantly different from all other treatments. Defense enzymes *viz*., peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) of rhizome increased with increase in inoculum levels of *M. incognita* at six MAI. The plants inoculated with 10,000 $J_2$

showed high phenol content (3.932 mg $g^{-1}$ tissue), PO (8.82 changes in absorbance $min^{-1}$ $g^{-1}$ tissue), PPO (0.74 changes in absorbance $min^{-1}$ $g^{-1}$ tissue) and PAL (16.70 changes in cinnamic acid $min^{-1}$ $g^{-1}$ tissue) in rhizome compared to other treatments. Similar results were noticed in leaf samples as well.

After harvest, the starch, protein, crude fibre, total ash and oleoresin content of rhizome decreased in plants inoculated with 500 $J_2$ $pot^{-1}$ onwards. The plants inoculated with 10,000 $J_2$ showed low starch (12.78 per cent), protein (2.52 per cent), crude fibre (4.69 per cent), total ash (1.24 per cent), oleoresin content (3.518 per cent) compared to the uninoculated plants.

The second pot culture experiment was carried out to screen five important ginger varieties against *M. incognita*. Minimum number of galls (28.6 $plant^{-1}$) and gall index (3) was observed in the variety IISR Mahima and it was significantly different from other varieties. The variety Karthika was highly susceptible with a gall index 5 and varieties IISR Varada, IISR Rejatha and Athira were found to be susceptible (gall index 4) to *M. incognita*. However, variety IISR Mahima was found to be moderately resistant to *M. incognita* on the basis of gall index (3). The nematode population in soil, rhizome and root were minimum in IISR Mahima and maximum in case of variety Karthika. The lowest PO, PPO and PAL activity was observed in the case of variety Karthika.

Based on the results of the study, it is concluded that there was a progressive increase in phenol content and defense enzymes *viz*. PO, PPO, PAL in both leaf and rhizome with increase in inoculum levels of *M. incognita*. Starch, protein, crude fibre, total ash, oleoresin content, pH and EC of rhizome decreased with increase in inoculum levels of *M. incognita*. The variety IISR Mahima showed more defense against *M. incognita* compared to other varieties due to higher PO, PPO, and PAL activity.