

**Influence of root-knot nematode  
(*Meloidogyne incognita*) infection on crop  
physiology and biochemical parameters of  
ridge gourd (*Luffa acutangula*)**

**A**  
***Thesis submitted to the***  
***Odisha University of Agriculture and Technology***  
***in partial fulfilment of the requirement for the degree of***  
***Doctor of Philosophy in Agriculture***  
***(Nematology)***

***By***

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**2019**



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## **CERTIFICATE - I**

This is to certify that the thesis entitled “**Influence of root-knot nematode (*Meloidogyne incognita*) infection on crop physiology and biochemical parameters of ridge gourd (*Luffa acutangula*)**” submitted in partial fulfilment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY IN AGRICULTURE(NEMATOLOGY)** of the Odisha University of Agriculture and Technology, Bhubaneswar is a faithful record of bonafide research work carried out by **RITU KUMARI PANDEY** under my guidance and supervision. No part of the thesis has been submitted for the award of any other degree or diploma.

It is further certified that the assistance and help availed by her from various sources during the course of investigation has been duly acknowledged.

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**CERTIFICATE-II**

This is to certify that the thesis entitled “**Influence of root-knot nematode (*Meloidogyne incognita*) infection on crop physiology and biochemical parameters of ridge gourd (*Luffa acutangula*)**” submitted by **RITU KUMARI PANDEY** to the Odisha University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirement for the degree of **DOCTOR OF PHILOSOPHY IN AGRICULTURE (NEMATOLOGY)** has been approved by the students’ advisory committee and the external examiner.

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## **ACKNOWLEDGEMENT**

I fall in short of words to acknowledge those who were with me for the successful completion of my dissertation. First and foremost, I bow down before “GOD” with solicity and imploreness to express my deepest sense of gratitude for his blessings, mercy and love, which enable me to accomplish this venture.

I would like to express my deep and sincere gratitude to my research advisor Dr. D. K. Nayak, Professor and Head, Department of Nematology, College of Agriculture, OUAT, Bhubaneswar, the chairman of my Advisory Committee, for giving me the opportunity to do my work and providing invaluable guidance throughout this research. He has taught me the methodology to carry out the research work and to present the research work as clearly as possible. It was a great privilege and honour to work and study under his guidance. I am extremely grateful for what he has offered me. I am extending my heartfelt thanks for his guidance, constant supervision, steady encouragement, whole-hearted co-operation and painstaking efforts during the entire period of research work and preparation of this manuscript.

I wish to express profound sense of gratitude to my advisory committee members Dr. Surjya Kanta Beura, Professor and Head, Department of Plant Pathology, OUAT, Bhubaneswar for their sustained and valuable guidance, constructive suggestions, friendly approach, constant support and encouragement throughout the period of my research work.

I also wish to acknowledge from the core of my heart Dr. I.C. Mohanty, Assoc. Professor, Department of Biotechnology, Dr. S. K. Patnaik, Professor and Head, Dr. A. K. Mishra, Associate Professor, Department of Soil Science and Agricultural Chemistry, Mrs Rajkumari Bholra, Asst. Professor, Department of Plant Physiology, OUAT, Bhubaneswar for providing necessary laboratory facility during the biochemical analysis of my plant materials.

I also express my heartfelt gratitude to Dr.S.Sahoo, Ex-Assoc. Professor, Dr. B. K. Dash, Associate Professor, Dr. N. Das, Asst. Nematologist and Mr. J. K. Mahalik, Asst. Nematologist, Department of Nematology, Dr. S. Saren, Asst. Professor Department of Soil Science and Agricultural Chemistry, College of Agriculture, OUAT,

Bhubaneswar, for their proper guidance, needful encouragement and wholehearted support throughout the period of my analytical work and research work.

I am highly thankful to the Central Laboratory, OUAT and Institute of Life Science, Bhubaneswar for providing all facilities, for their guidance in completion of molecular work.

I am privileged to express my indebtedness to all field assistants for their much needed help during my research work.

Though this thesis is the result of patient survey and persistent effort of myself, it could not have been possible without a few who've ungrudgingly took pains and spared both time and energy for it.

I owe all my success to the special persons in my life, my parents and my brother whose love and affection have always been a source of inspiration and I owe thanks from depth of my heart to my dearest friends and seniors for their help and suggestions throughout my research work.

I also specially thank Mr. A. Kar Sir, for his keen interest and timely preparation of the manuscript.

I thank all those who helped me directly or indirectly during the period of my stay in the OUAT campus, Bhubaneswar.

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## ABSTRACT

Out of fifty-two varieties of ridge gourd screened against root-knot nematode, only Priya, Challenger, Jaipur long, BSS-1009, Tauri and Pallishree varieties showed resistant reaction with minimum number (7-10) of galls/plant. Highest decrease for dry shoot weight was observed for variety NHRG-1001(2.5g) & for dry root weight in Hybrid Patal, Tarai (1.1g) respectively. Root knot nematode caused overall reduction of total chlorophyll, chlorophyll 'a', chlorophyll 'b', content in the inoculated samples than healthy counterparts. This may be due to interference in the photosynthetic pathway and partitioning of the photosynthates. Overall reduction of total chlorophyll was 60.67, 73.78, 1.32, 3.68 and 30.12 per cent in Priya, BSS-1009, Aneeta, Aarti and Harsha respectively over their healthy counterparts. Overall reduction of chlorophyll 'a' was 40.47, 49.87, 48.71, 19.33 and 42.69 per cent in Priya, BSS-1009, Aneeta, Aarti and Harsha respectively and that of chlorophyll 'b' was 24.50, 33.75, 25.73, 34.64 and 30.81 per cent in Priya, BSS-1009, Aneeta, Aarti and Harsha respectively. The total nitrogen content was decreased in the shoot system of infected varieties of Priya, BSS-1009, Aneeta, Aarti & Harsha by 0.36, 0.46, 0.44, 0.35 & 0.28 percent respectively over healthy counterpart on dry weight basis. In the roots nitrogen content was increased by 0.49, 0.44, 0.47, 0.67 & 0.48 per cent in Priya, BSS-1009, Aneeta, Aarti & Harsha respectively. Reduced percentage of crude protein was observed in all the varieties studied than the healthy ones. Phosphorus content was decreased in the shoot system of infected varieties of Priya, BSS-1009, Aneeta, and Harsha respectively except Aarti over the healthy counterparts on dry weight basis. Potassium content was increased by 208.33, 175, 27.27 per cent in the shoots of varieties Aneeta, BSS-1009 & Priya respectively while decreased by 75 per cent in Harsha and 72.09 per cent in Aarti. The accumulation of proline in nematode infected samples is associated with the stress condition of the host plants induced by the nematodes. The enhancement of phenolic compound in the inoculated samples was due to rapid liberation of conjugated phenol by nematode feeding. The enhanced activity of PAL and TAL enzymes and appearance of aromatic amino acids like tyrosine and phenylalanine in the inoculated samples appeared interesting and significant. Increased peroxidase activity was observed in both shoot and root of inoculated varieties. By HPTLC analysis it was found that L-proline amino acid content increased in both shoots and roots of all inoculated varieties than their healthy ones. Decrease of aromatic amino acids like L-tyrosine was found in inoculated varieties than their healthy one. Reduction of free tryptophan was observed in inoculated varieties over healthy. The amount of L-tryptophan was less in resistant variety as compared to susceptible variety. Present study revealed that preventable yield losses due to root-knot nematode in ridge gourd variety Harsha was up to 74.52 per cent under field conditions with application of Furadon 3G @ 2 kg a.i./ha. The phylogenetic tree constructed by UPGMA method generated two main clusters i.e. cluster – I (Two sub cluster - varieties Aneeta, Aarti and BSS – 1009) and cluster – II ( varieties Harsha and Priya).

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## ABBREVIATIONS USED

%	Percentage
m	Meter
cm	Centimetre
g	Gram
mg	Milligram
dia	Diameter
<i>et al.</i>	Co-workers
spp.	Species
eg.	Example
etc	Etcetra
<i>i.e.</i>	That is
<i>viz.</i>	Namely
ha	Hectare
h	Hour
p.p.m	parts per million
S.E.(m)	Standard error mean
CD	Critical difference

# INTRODUCTION

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Ridge gourd, *Luffa acutangula* (L) Roxb. is an important Cucurbitaceous vegetable and susceptible host crop facing considerable yield loss due to pest and diseases. Ridge gourd, *Luffa acutangula* (L) Roxb. is more popular vegetable in the south and east India. It is known by different names in different regions as Torai, Mongitorai, Araturai, Dorkavidarbha, Turi, Hirekayi, Beera, Peerkankai, Peecha kam, Cheruppee rum and Ribbed leefa. Ridge gourd commonly known as Turai or Turiya is a native of India and also seen grown as ornamental plant in many parts of the world. Composition of saturated fats as well as calories is quite low in ridge gourd. Ridge gourd is quite lower in saturated fats as well as calories. It really is abundant with dietary fiber, vitamin C, riboflavin, zinc, thiamin, iron, as well as magnesium. It has got excellent health benefits as: excellent blood purifier, possessing laxative properties, cure for jaundice, beneficial for diabetes, aiding weight loss, anti-inflammatory and anti-biotic, fortifying the immune system, skin care, etc.

Among the production constraints, plant parasitic nematodes are one of the major limiting factors. Plant-parasitic nematodes are now recognized as potentially serious constraint to crop productivity. One of the most wide spread nematode limiting world agriculture productivity is the root-knot nematode, *Meloidogyne* species. It has a very wide host range, more than 3000 species. The host range of Root-knot nematode is extensive with more than two thousands of plant species. Four common *Meloidogyne* species (*Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne hapla* and *Meloidogyne arenaria*) comprise up to 95% of all root-knot nematode populations. *M. incognita* is the most common species accounting for 64 per cent of total population and infecting almost all cultivated plants.

Among the various plant parasitic nematodes, the root-knot nematode, *Meloidogyne spp.* are the most prevalent economical crop pests worldwide and they interfere with anchorage and absorption of crop plants. Root-knot nematode causes a quite different morphological and anatomical variations in different plants and even in various parts of a particular plant and different species can cause different responses in the same plant. The morphological variations of plants due to nematode infection resulted in severe stunting, chlorosis, wilting and drooping of leaves, delay in flowering, fruit

formation and yield, aggregation of nutrient deficiencies and retardation of growing point of shoot and root system. The root exudates containing sugars, amino acids and electrolytes influence the hatching of eggs. Enzymes observed in the amphidal pouches, nerves and body opening of the juveniles guide in chemotaxy and also in their final spatial regulation with respect to the vascular elements within roots. A shift from the sugars to the nitrogenous components of the roots exudates seem to be a predisposing factor for other secondary infection. The importance of studies on the biochemistry of plant and nematode relationship has been fairly well documented in recent reviews (Nayak and Mohanty, 2010). It was also reported that galled tissues contained decreased carbohydrates, Proteins, cellulose and lignins but increased hemicelluloses, organic acids, free amino acids and minerals. Formation of various gall sizes, tissue damage etc., are associated with various degree of susceptibility or resistance can be correlated with varied chemical phenomena. The survival of the susceptible host during pathogenesis reflects the combat capabilities, especially when lysis is initiated by the pathogens. Nematode pests in general, affect host plants quantitatively and qualitatively. Quantitatively by reducing yield about 20.6% worldwide and qualitatively by devaluating the produced crops as a result of some malformations as well as decreasing the organic and mineral components of produced fruit.

Various workers have reported the role of phenolic compounds in the defence mechanism of plants and consequently their accumulation in the cell damage by nematode feeding. The importance of production, rate of production and liberation of phenolic compounds from the plant tissue after nematode infection was emphasized by. Increase in the concentration of free phenols following nematode infection was reported by. The increase in phenol compounds helps in the formation of hypersensitive reaction towards nematode infection. Phenolic compounds play an important role for root-knot nematode through larval mortality and by rendering host roots less attractive to nematode. The increase in phenolic concentration coupled with increase in the activity of polyphenol oxidase in the diseased tissue. The infestation of plant parasitic nematode caused increased in sugar content and it was also observed that increased sugar content is helpful for survival of nematode. They have also suggested the increase in content of sugar in the infected leaves may be due to the degradation of starch or inhibition of starch synthesis from sugar and this was confirmed in root-knot infected tomato plants. Nematode species decrease the content of total carbohydrates, total protein, total soluble sugars and the

minerals Ca, Fe, Mg, N, P, K and Zn in the infected plant; however total phenol and tannins are increased. The rates of chemical reductions are generally higher in roots than in shoots which depend on nematode species, nematode density and host plant. The root-knot nematodes achieve higher rates of chemical reduction on infected plants. Total proteins, total soluble sugars, Zn, Mg, Fe, and P are most affected. Nayak and Mohanty, (2010) also confirmed the increase of sugar and protein levels in diseased tissues for high metabolic activity. The giant cells contain greater amount of DNA, RNA, and photosynthates especially three to four weeks after infection. The concentration of sugars, hemicelluloses, organic acids, free amino acids, proteins and lipids is greater during the late stage of infection.

The current restrictions on the indiscriminate use of chemical nematicides have contributed to the increased root knot nematode problems in horticultural crops. In context, Plant resistance (R) appears as the most effective method of control but due to restricted no. of cultivated species with nematode resistant 'R' genes available and possible occurrence of virulent nematodes are able to reproduce on 'R' plants. So it is necessary to have an adequate knowledge about the biochemistry and physiology of the host as well as parasites in order to understand the actual mechanism of resistance. Imbalance of amino acids composition and production of phenolic substances are marked as positive reaction of pest resistance and hence donor selection depends upon this characters..

Considering the importance of this object detailed characterization of this biochemical and physiological processes is essential to advance our understanding of plant nematode interaction and following investigations were undertaken to gather information on the mechanism of resistance and biochemistry and physiology of pathogenesis.

The biochemical analysis was carried out in resistant, moderately resistant, susceptible and highly susceptible varieties of both healthy and nematode infested roots to know the accumulation and variations in biochemical constituents with regard to total phenols, total amino acids, reducing sugar and total sugar. In this context, the use of resistant varieties to manage the abnoxious nematode is an ideal means and important component of overall integrated nematode management system. Traditional approaches for the development of nematode resistant varieties are time consuming and often limited by intraspecific barriers. Molecular biology and genetic engineering

provide potential knowledge for efficient development of nematode resistant plant cultivars which may be enhanced by elucidation of mechanisms limiting nematode development.

Following investigations were undertaken to gather information on the mechanism of resistance and biochemistry and physiology of pathogenesis.

- i) To characterize the phytomorphometric characters in ridge gourd varieties as influenced by root-knot nematode, *M. incognita*
- ii) To assess the physiological changes in resistant and susceptible ridge gourd varieties due to root-knot nematode, *M. incognita* infection
- iii) To establish the biochemical changes in resistant and susceptible varieties of ridge gourd due to root-knot nematode, *M. incognita* infection.



# REVIEW OF LITERATURE

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Plant parasitic nematodes during their feeding action change the metabolic processes of the affected cells, tissues and also the entire plant. The root-knot nematodes produce the galls in the root system, which are visible to the naked eye. Hence, on the basis of the gall numbers many workers have tried to classify different varieties of plants into resistant or susceptible ones. Many workers have estimated the physiological and biochemical changes brought about by root-knot nematodes in the plant systems to classify the plant variety into resistant and susceptible category depending on chemical composition of plants. Some of them are enumerated below.

## 2.1 Nematode association

Taylor and Sasser (1978) introduced the egg mass index (EI) which gave an approximate measurement of root-knot nematode reproduction based on numbers of egg masses per plant instead of actual numbers of eggs and juveniles/plant.

Bistline and Rhodes (1984) conducted a pot culture experiment on *Momordica charantia* inoculated with 10,000 J<sub>2</sub> of *Meloidogyne incognita* per pot and found there was presence of heavy root galls and reduced plant growth in bitter gourd seedlings.

Pankaj and Siyanand (1990) studied the effect of initial inoculum levels viz., 0, 10, 100, 1000 and 10,000 J<sub>2</sub> of *Meloidogyne incognita* /kg soil on bitter gourd (*Momordica charantia*) under pot conditions and they reported a significant reduction in the plant growth characters except fresh root weight, 1000 juveniles per plant about 2 months after inoculation. The final nematode population (soil + root) was found to be increasing with increase in inoculum level with maximum rate of multiplication of 145 times.

Gupta *et al.* (1995) studied the effect of initial inoculum levels viz. 0, 10, 100, 1000 and 10000 J<sub>2</sub> of *Meloidogyne javanica* per kg of sterilized soil on bitter gourd, *Momordica charantia* and reported a significant reduction of all growth parameters occurred at initial population level of 100 J<sub>2</sub> of *M. javanica*.

Ashraf *et al.* (2003) reported during survey that eleven species of plant parasitic nematodes were associated on cucurbitaceous vegetables including bitter gourd, bottle gourd, round gourd, sponge gourd, ridge gourd and cucumber in Aligarh

districts of Uttar Pradesh. Among all the nematodes, the highest frequency of infestation was recorded with *Meloidogyne spp.*

Premachandra *et al.* (2007) surveyed on infestations of root knot nematodes, *Meloidogyne spp.* on seven vegetable crops, including bitter melon at 13 localities in Matara District in Sri Lanka, considering 10 individual root systems of each vegetable crop per locality at the maturity stage for the assessment of root galls and root-gall severity.

Bhat *et al.* (2009) examined histologically *Meloidogyne incognita* infected bitter melon (*Momordica charantia*) roots and they found fully matured females were associated with giant cells.

Anwar and Mc Kenry (2010) conducted a survey in 16 major vegetable production areas of the Punjab, Pakistan with the purpose of determining the incidence and distribution of *Meloidogyne incognita* and its reproduction on vegetable crop genotypes in nematode infested fields and in the green house, and they found *Meloidogyne incognita* was the predominantly found species and detected in 13.6% of all the fields surveyed.

Fatima *et al.* (2015) conducted a greenhouse experiment to assess the damaging effect of root-knot nematode (*Meloidogyne incognita*) on different growth parameters of Cucumber (*Cucumis sativus*) by relating infectious plants with non-infectious plants at different inoculum levels and concluded that the effects of *M. incognita* on growth parameters correlate directly with inoculum and they observed that with the increase in inoculum level, the growth parameters were decreased, however, the nematode population was increased.

## **2.2 Screening and evaluation of ridge melon germplasm for resistance against root-knot nematode (*Meloidogyne incognita*)**

Darekar *et al.* (1988) screened thirty-nine cucumber cultivars for resistance against *M. incognita* race 3 in a greenhouse test taking 20 days old seedlings were inoculated with 1000 J2 and recorded no cultivar was found to be resistant except Gy-5937-587 which was moderately resistant with a root-knot index of 3.0 after 40 days of inoculation.

Khelu *et al.* (1989) tested cucumber varieties grown in greenhouses in central Lebanon for resistance to *M. arenaria* and *M. javanica*, using soil infested with

10,000-12,000 nematode eggs and larvae/kg soil and they found that 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 .

Walters and Wehners (1996) tested few root-knot nematode resistant germplasm of cucumber and among them, NC-42 and NC-43 showed high level of resistance to *M. arenaria*, *M. javanica* and *M. hapla*.

Singh *et al.* (2012) screened germplasm of bottle gourd and reported, IC-338867, IC-342078, IC-342079, IC-342081 and IC-427608 lines were moderately resistant to *M. javanica*.

Sharma *et al.* (1995) tested eighteen cucumber varieties for their reaction to *M. incognita*, and found only Hoe-707 and EC-173929 were resistant and moderately resistant reaction respectively while other sixteen varieties were susceptible with varying infestation levels (root gall-index 3.3 to 5.6) to the test nematode.

Mahapatra(2017) obtained 17 bitter gourd germplasm/lines from local market and farmers field to test in the greenhouse condition against root-knot nematodes, out of 17 bitter gourd germplasms or lines one was found to be resistant, 4 moderately resistant and rest 12 susceptible to highly susceptible to the test nematode. For proving the authenticity of resistance through biochemical analysis, three moderately resistant varieties, sundargarh local-1, Amatalla beej ghar karala long green, ankur hybrid and three highly susceptible check variety, Nakhara local, Indojapane Hybrid, Rajsunakhala local-1 were studied.

Nayak (2006) reported that plant height of brinjal varieties Pusa Kranti, Kantabaigan and Pusa Purple Long due to root-knot nematode infection were decreased by 9.04, 18.48, 23.50 per cent over uninoculated counterparts. Further, he marked decrease of shoot weight (34.08%) and root weight (31.67%) of the susceptible brinjal cultivars, Pusa Purple Long than other two resistant varieties.

Giribabu *et al.* in (2008) analysed the pathogenicity of *M. incognita* on two cultivars of ridge gourd (*L. acutangula* (L.) Roxb.) and sponge gourd (*L. cylindrica*). They marked that at 55 days of inoculation there was a progressive decrease in the growth parameters with the increase in inoculum levels from 100 to 10000 J2s/Kg soil and, there was no significant difference in growth parameters between inoculum levels 0 and 10 J2s/Kg soil but marked difference was found between treatments 10 and 100 J2s/kg soil.

Chandra *et al.* (2010) studied the effect of population density on the pathogenic potential of *M. incognita* in green house in pot culture experiments with four cucurbits: *L. ciceraria*, *C. sativa* L., *M. charantia* and *C. pepo* L. Second stage juveniles were inoculated at inoculum levels of 10, 100 and 1000 J2 into the root zone of 15 days old seedlings. All the four cucurbits were found to be highly or moderately susceptible to *M. incognita* but an inverse relationship was found between population density, population growth and severity of root galls.

Singh *et al.* (2010) conducted a survey for estimation of losses by rice root-knot nematode, *M. graminicola* Golden and Birchfield in wheat on number of galls/seedling, shoot and root length, shoot and root weight for two years (2004-05 and 2005-06). It was reported that during 2004-05, shoot length, root length, shoot weight and root weight were significantly reduced as compared to healthy uninfected seedlings while the number of knots/plant varied between 0.5-8.3.

Hussain *et al.* (2011) assessed the damage caused by *M. incognita* on okra cv. Punjab. Selection. The plants were inoculated with 500, 1000, 2000, 4000 and 8000 freshly hatched second stage juveniles and observations were recorded 45 days after nematode inoculation. It was found that all inocula levels reduced shoot and root lengths, as well as fresh and dry weights. Increasing the nematode inoculum level increased the number of galls, egg masses and nematode population build up.

Kim Sung Heun *et al.* (2012) conducted a pot experiment with seven cucumber cultivars viz. Green long, Sambar cucumber, Hassan local, Green short, White long, Portobello and Poinsette to screen against root-knot nematode (*M. incognita*) and Poinsette cultivar recorded least number of galls and root-knot index.

Kaur & Pathak (2011) screened 14 varieties of karela lines against root-knot nematode and found the Punjab-14 was heavily galled. Six genotypes, MB-3, 4, 5, 11, 13, 16 were found resistant & other 6 lines – MB-6,1014,15,30,35 were moderately resistant to RKNs.

Pavithra (2013) conducted pot culture experiment with seven cucumber cultivars viz. Green long, Sambar cucumber, Hassan local, Green short, White long, Portobello and Poinsette to screen against root-knot nematode (*M. incognita*) and he recorded least number of galls and root-knot index with Poinsette cultivars while the variety greenlong was highly susceptible to root-knot nematode having higher number of galls and root-knot index and none was found resistant to *Meloidogyne incognita*.

Pandey and Nayak (2016) observed that out of thirty-eight green gram varieties/cultivars screened against root-knot nematode, only thirty-four varieties have shown resistant reactions with 3-6 numbers of galls per plants, while four varieties/cultivars showed moderately resistant reaction with 10-11 numbers of galls per plants. The decrease in shoot length was more pronounced with 22.03 cm in 5 DGG-5 varieties, which was different from other resistant varieties.

NA Tamilselvi *et al.* (2016) conducted a pot culture experiments under *in vitro* to understand the biochemical transformations taking place during nematode attack in order to make out resistant rootstocks for grafting with bitter gourd scions. The results showed that, three genotypes viz., Kumatikai (*Citrulus colocynthis*), African horned cucumber (*Cucumis metuliferus*) and pumpkin (*Cucurbita moschata*) had lowest value for number of galls per gram root, egg masses per gram root and numbers of females per gram of root and showed resistant reaction followed by two rootstocks viz., Sponge gourd (*Luffa cylindrica*) and mithipakal (*Momordica charantia* var. *muricata*) with least values and were moderately resistant. This study revealed that there was brisk increase in total phenol and OD phenols activities and these were detected in resistant rootstocks namely *C. colocynthis*, *C. metuliferus* and *C. moschata* and these begun to amplify at 24 hours after inoculation and these were seen to have reached maximum at 96 hours after inoculation and subsequently declined.

Mahalik (2017) observed that out of eight gladiolus varieties/cultivars screened against root-knot nematode, only two varieties have shown moderately

resistant reaction with 16-19 numbers of galls per plants, while four varieties/cultivars showed susceptible reaction with 51-67 numbers of galls per plants & two showed highly susceptible reaction with 107-129 numbers of galls per plant. Due to infection of root-knot nematode, the maximum average shoot length of gladiolus cultivars/varieties was 102.96 cm in variety Precilla.

Mahapatra (2017) observed that out of seventeen bitter gourd varieties screened, only one cultivar showed resistant reaction with 7.67 number of galls per plants, while four varieties were recorded as moderately resistant with 21.08 number galls per plants.

Mallik (2017) observed that out of fifteen cucumber cultivars, screened against root-knot nematode, only one varieties had shown resistant reactions with 7.66 number of galls per plants and two show moderately resistant reaction with 14.66-27 no. of galls per plant. While rest varieties had shown susceptible and highly susceptible reaction with 30.66-84.66 and 103.33-162.66 no. of galls per plant respectively. Due to root-knot nematode infection, the maximum average shoot length of cucumber cultivars was recorded as 51.33 cm in variety Malini.

Baa (2018) found that out of seven gerbera varieties screened only one variety had shown resistant reaction with 8.66 numbers of galls per plants while five cultivars had shown moderately resistant reaction with minimum 22.82 to 29.10 no. of galls per plant and one was susceptible with 67.66 no. of galls per plant.

### **2.3 Effects on Chlorophyll content in nematode infected leaves**

Williamson and Gleason (2003) observed that root-knot nematodes cause measurable changes in the morphology and physiology of the host plants. Different nematologists namely Vashishth *et al.* (1994), Poornima and Vadivelu (1998), Ramakrishnan and Rajendran (1998) and Parveen *et al.* (2006) reported about reduction in chlorophyll content due to nematode infection.

The response of *Vigna radiata* regarding the biochemical attributes was significant at various nematode treatments. The growth of the plant responded negatively towards the lower as well as higher inoculum levels of *M. incognita*. Decrease in all these parameters including plant growth, leaf area, chlorophyll, seed protein, nitrogenase activity and leghaemoglobin due to increased in number of the

nematode. The root knot nematode infection often reduces plant growth and yield (Sasser and Freckman, 1987; Williamson, 1998) and decreases nutrient uptake (Patel *et al.*, 1988) and infested plants showed deficiencies of N, Mg, Fe, B, Cu and Zn (Good, 1968) due to RKN damage to the roots and subsequent prevention of water and nutrient uptake by the roots (Gaillaud *et al.*, 2008). Nematodes may reduce the effective life of rhizobia nodules as found by Taha and Raski (1969) with legumes infested with *M. javanica*. *Meloidogyne incognita* causes reduction in the number of nodules, dry weight of nodules, dry weight of shoot, total nitrogen uptake and chlorophyll contents of leaves of mungbean (*Vigna radiata* L). The functioning of nodules was adversely affected by nematode infection which could be attributed to reduction in the bacteroids, leghaemoglobin contents of nodules and reduced supply of photosynthate to the nodules.

Chahal and Chahal (1987a) studied the effect of different levels of *M. incognita* on *Vigna radiata* cv. G-65 and found that nematode caused reduction in the chlorophyll content of leaves which adversely affected the functioning of nodules. Increase of total chlorophyll 'a' and 'b' content was observed by Verma *et al.* (1996) on mungbean at all inoculation levels of root-knot nematode with growth period up to 30 days whereas decrease of the same was noted again on 45<sup>th</sup> day of inoculation .

Jaiswal and Sing (2008) observed that chlorophyll a, b and total chlorophyll contents were significantly decreased with an increase in nematode inoculum levels and was minimum at 10,000 and 5000 nematodes/plant followed by 1000 and 100 nematodes/plant.

Melakeberhan *et al.* (1985) reported that total chlorophyll and chlorophyll- a content in *Phaseolus vulgaris* plants infected with different levels of *M. incognita*, decreased significantly with increased levels of infestation at 45 days after inoculation.

Jain and Goswami (2002) reported that the inoculation of either *M. incognita* or *Fusarium oxysporum* in tomato cv. Pusa Ruby resulted reduction in chlorophyll content. The content was observed to be lowest when the nematode and fungus were simultaneously inoculated and also when the nematode was inoculated 10 days prior to inoculation of the fungus.

Nayak (2006) reported chlorophyll 'a' content of infected leaves of Pusa Kranti variety was reduced from 2.70 mg/g to 2.010 mg/g. Similarly, in other varieties like Kantabaigan and Pusa Purple Long, chlorophyll 'a' content also reduced. Further, he reported that chlorophyll 'b' and total chlorophyll content was reduced in the infected leaves of brinjal varieties.

Joshi *et al.* (2012) observed that the number of galls, egg masses/plant and final population of nematode increased with the increase in the primary inoculum level and maximum increase in various nematode factors was recorded at 5000 J<sub>2</sub>/500 g of soil. They also observed that there was less uptake of N, P and K in the roots due to parasitization by nematodes. They confirmed that there was considerable decline in N, P, K uptake in the plant and total chlorophyll content in the leaves at inoculum of 1000 J<sub>2</sub>/500 g soil and above.

Pandey *et al.* (2016) investigated on the biochemical alterations in black gram varieties inoculated with root-knot nematode, *Meloidogyne incognita*. Various parameters like total chlorophyll, total sugar contents, protein, and proline content were taken into observation during post infection period. At 45 days after inoculation, the deviation in total chlorophyll, total protein, proline and total sugar content in six cultivars *i.e.* PU 09-36(S), MU-44(S), VBG 11-031(R) VBG 11-016(R), KUG-715(R) and NUL-205(R) were studied. It was found that the percentage of total chlorophyll contents decreased in the inoculated samples than the healthy counterparts. They also observed that the amount of total protein, proline and total sugar contents was increased in the diseased tissues

Behera *et al.* (2016) studied the effects of root-knot nematode infection on the chlorophyll "a", "b" and "total contents of both susceptible and resistant rice bean varieties under infected and healthy condition & noted a decrease in chlorophyll content as 84.18, 2.93, 15.27 and 30.79% in the varieties BBSR-19-2-1, BRB-26-1, BRB-25-1 and BRB-7-1 respectively. Moreover, chlorophyll 'b' content was also reduced as 0.92%, 0.23%, 14.62% and 53.75% in the varieties BBSR-19-2-1, BRB-26-1, BRB-25-1 and BRB-7-1 respectively. Due to the nematode infection, total chlorophyll content of rice bean varieties was reduced as 10.39, 2.92, 15.16 and 38.75% in the varieties BBSR-19-2-1, BRB-26-1, BRB-25-1 and BRB-7-1 respectively.

Pandey *et al.* (2016) while investigating on the effects of chlorophyll content on resistant and susceptible greengram cultivars due to the root-knot nematode infection, it was observed that Chlorophyll 'a' was maximum decreased (96.36%) in resistant variety 7GGG 10-14 followed by 93.98% in susceptible check variety 28 PM 10-12 but increasing trend was observed from 7.31 to 28.30% in 24ML-233 and 17 IPM 9901-6 varieties respectively. Further, he reported that chlorophyll 'b' and total chlorophyll content was reduced in the infected leaves of greengram varieties.

Mahapatra *et al.* (2017) recorded that chlorophyll 'a' contents in the inoculated plants decreased by 0.336, 0.342, 0.336, 0.320, 0.322 and 0.355 mg/g in varieties Sundargarh local-1, Amatalla Beej ghar Karala long green, Ankur hybrid, Nakhara local, Indojapane hybrid and Rajsunakhala local-1 respectively. Further, he observed an overall reduction in chlorophyll 'b' & total in root knot nematode infected bitter gourd varieties.

Mallik *et al.* (2017) observed that the chlorophyll 'a' content reduced from 1.961 mg/g to 1.770 mg/g and 1.643 mg/g to 1.480 mg/g due to nematode infection in case of Greenstar and Greenlong variety. Similarly in the varieties Malavi, Hiramoti, 12 Patra and Kulak chlorophyll 'a' reduced from 1.752 to 1.320, 1.679 to 1.430, 1.780 to 1.222 and 1.887 to 1.212 mg/g respectively. Further, he observed an overall reduction in chlorophyll 'b' & total in root knot nematode infected cucumber varieties.

Mahalik *et al.* (2017) recorded that the chlorophyll 'a' content reduced from 2.46 mg/g to 2.35 mg/g and 2.63 mg/g to 2.46 mg/g due to nematode infection in case of Princess Margata Rosa and Forta Rosa variety, similarly in the varieties Magma, Yellow Goddess and Plum Tart chlorophyll 'a' reduced from 2.74 to 2.56, 2.36 to 2.07 and 2.55 to 2.24 mg/g respectively. Further, he observed an overall reduction in chlorophyll 'b' & total in root knot nematode infected gladiolus varieties.

#### **2.4 Effects on Macronutrients and Micronutrients content in nematode infected leaves**

Plant parasitic nematodes parasitize the plants by changing the nutrient contents and cause disturbance in water and nutrient relations, necessary for optimal plant growth as reported by Hussey and Williamson, (1996). The changes in nutrient

profile in plants inoculated with nematodes cause reduction in chlorophyll contents that ultimately leads to reduction of photosynthetic efficiency. The decrease in potassium concentration may affect CO<sub>2</sub> uptake or, by altering osmotic potential, lead to decreased photosynthesis (Ferguson, 1984). Nematode infection interrupts biological processes in plants manifested as nutritional deficiencies or excesses, stunting, wilting, and reduced yields. Singh *et al.* (1977) while investigating the effects of different levels of *M. incognita* in the presence or absence of *Rhizobium phaseoli* on *Phaseolus aureus* showed that the quantity of nitrogen was reduced at all level of nematode inoculation irrespective of presence of Rhizobium, indicating plants with Rhizobium nodules had significantly more N/shoot.

Oteifa (1952) conducted experiments on lima bean plants infected with nematodes and observed the plants had lesser amounts of nitrogen, phosphorus, calcium, magnesium and potassium against the control plants after chemical analysis.

Jacob *et al.* (1996) assessed the shoot and root samples of resistant tomato (Mangla) and susceptible tomato (Pusa ruby) varieties inoculated with *Meloidogyne incognita* and they observed the percentage boost in the concentrations of calcium and magnesium, sodium, manganese and iron over check in the roots of susceptible and resistant plant found to be 11.76 and 17.24, 8.51 and 33.33, 25.0 and 27.77, 6.14 and 9.15, 20.0 and 1. correspondingly. They also observed shoots of resistant cultivar, the concentration of calcium elevated slightly (3%) where as 13.76% decrease was recorded in susceptible cultivar following the inoculation of *M. incognita*.

Mohanty *et al.* (1990) studied on chemical changes in horsegram plant variety DS 2-1 being infected by *M. incognita* and found that there was an increase in percentage of nitrogen, crude protein, phosphorus and potassium content in infected roots than respective control plant. Whereas decrease in the various nutrients except phosphorus in infected shoots as compared to healthy counterpart.

Nayak (2006) reported that nitrogen composition of the brinjal varieties, Pusa Kranti, Kantabaigan and Pusa Purple Long. The infected shoots of brinjal plant content was higher in comparison to healthy one, ultimately all the varieties of brinjal contain increased percentage of nitrogen which was highest i.e., 62.09% in susceptible variety i.e., Pusa Purple Long. The phosphorous content of brinjal

varieties Pusa Kranti, Kantabaigan, Pusa Purple Long was lower on dry weight basis whereas, it was increased more than double after nematode infection and it was higher in susceptible varieties. He also reported that there was an increase in the potassium content in the dried healthy shoots of brinjal varieties, Pusa Kranti, Kantabaigan, and Pusa Purple Long, while reduction in  $K_2O$  was noticed in nematode inoculated plants.

Rao *et al.* (1988) observed that the root knot nematode reduced nitrogen, phosphorus, iron, reducing sugars and phenols in the rice root and shoot. They reported an increase in Mn, K and Mg in the root and a decrease of RNA in the shoot with increase in the protein content in both parts of the samples. They also observed the infection of *H. oryzae* infection caused a reduction in the concentration of nitrogen, phosphorus, iron and reducing sugars both in root and shoot along with total sugars, protein, IAA, cytokinin and thiamine were also reduced. There was increase in calcium, sodium, soluble amino acids, ABA and starch in both root and shoot of the plants.

Sharma and Trivedi (1996) noticed increase in nitrogen, decrease in phosphorus and potassium in *M. incognita* diseased roots of the highly susceptible okra cv. Pusa Sawani and the less susceptible Punjab-7 in pot tests.

Pandey *et al.* (2017) reported the nutrient content of resistant and susceptible green gram varieties against root knot nematode. Four resistant cultivars, 24 ML-233, 7 GGG 10-14, 17 IPM 9901 susceptible cultivars 28 PM 10-12 and 29 PUSA 0672 were selected to assess for change in leaf nitrogen, phosphorus and potassium contents of both infected and healthy plants. Maximum nitrogen content of 196.61% was noted in variety 7 GGG 10-14 while minimum as 88.57% in variety 28 PM 12. Similarly Phosphorous was maximum (46.49 %) noted in varieties 29 PUSA 0672 and minimum 52.26% in varieties 28 PM 10-12. The crude protein content was maximum (-4.10%) in the variety 8 GM 04-02.

## **2.5 Effects on Protein content on nematode infected plants**

Masood and Hussain (1975) reported that the root-knot nematode infested tomato cultivar possessed higher concentrations of amino acids and protein as compared to susceptible one.

Ganguly and Dasgupta (1981) observed the increased protein levels in the diseased tissues of Pusa Ruby, SL-120 & Nematex, 28 days after inoculation by

*M.incognita*. Ganguly and Dasgupta (1987) found that the protein content was lower in the galled roots but the peroxidase activity was higher in the non-galled roots.

Gopinath *et al.* (2002) found that moderately resistant tomato cultivars Vivek and Radha recorded maximum concentration of proteins, which confer resistance to root-knot nematode whereas in susceptible cultivars Pusa Ruby the protein concentration is less as compared to the present tested cultivars.

Nayak (2006) reported that crude protein content of infected shoots reached the highest in Pusa Purple Long (62%) than that of Pusa Kranti and Kantabaigan as tested against root-knot nematode, *M.incognita* under pot culture condition.

Pandey *et al.* (2017) found four resistant cultivars, 24 ML-233, 7 GGG 10-14, 17 IPM 9901 susceptible cultivars 28 PM 10-12 and 29 PUSA 0672 were selected to assess for change found the crude protein content was maximum (-4.10%) in the variety 8 GM 04-02.

## **2.6 Effects on Sugar content in nematode infected plants**

Farooqi *et al.* (1980) observed that both reducing and total sugar increased in *Meloidogyne incognita* infected tomato plants.

Tayal and Agarwal (1982) indicated low contents of starch, non-reducing sugar, total sugar and high level of reducing sugar, soluble protein and total free amino acids in *Meloidogyne incognita* infected brinjal plants.

Agarwal *et al.*, (1985) reported the decrease level of non-reducing and total sugars with increased level of reducing sugar in root-knot nematode, *Meloidogyne incognita* infected okra plants.

Upadhyay and Banerjee in the year (1986) observed reducing sugar decreased from 7.3 to 13.1 per cent in the root and 9.3 to 32.2 per cent in the stem; non-reducing sugar from 78.4 to 87.2 per cent in root and 24.4 per cent in the stem and the total sugar from 13.3 to 19.9 per cent in the root and 10.7 to 22.1 per cent in the stem of chickpea plants infected with *M.javanica*.

Mohanty *et al.* (1995) found a higher concentration of sugar in inoculated sample than healthy sample of Pusa Purple Long (susceptible) and Ghatika white (resistant) against *M.incognita*.

Mohanty *et al.* (1997) while investigating on post-infection bio-chemical changes in susceptible (Annapurna) and resistant (Manika, OR 624-46) rice cultivars inoculated with root-knot nematode *M. graminicola* they found higher accumulation of sugar in both the cultivars, as compared to their healthy counterparts.

Devaranjan and Rajendran (2002) found that the amount of total carbohydrates and sugars were more in the susceptible clone Matti than other clones in banana clones under pot culture condition. Amount of total CHO level decreased where as there is increase in sugar level was observed in the nematode infested roots.

Nayak (2006) reported an increase in the amount of total sugar content in the inoculated plants of brinjal varieties ,Pusa Kranti,Kantabaigan and Pusa Purple Long on fresh weight basis,but,the amount of sugar present was decreased in all cases than healthy plants,i.e.,49.76,50.09,and 46.98 % in the shoot portion of these varieties when infected by root-knot nematode.

Pandey *et al.* (2017) reported the variations in total sugar and starch contents in six greengram cultivars i.e 24 ML-233 (R), 7 GGG 10-14 (R), 17 IPM 9901-6 (R) ,8 GM 04-02 (R), 28 PM 10-12(S) and 29 PUSA 0672 (S) which was studied 45 days after inoculation of *Meloidogyne incognita* . Total sugar content was maximum decreased (61.78%) in susceptible variety 28 PM 10-12 followed by 44.45% in susceptible variety 29 PUSA 0672 but increasing trend was observed in the variety 8 GM 04-02 (39%). Total starch content was maximum decreased in resistant variety 24 ML-233(93.55 %) and least decrease in the resistant variety 17 IPM 9901-6(9.67 %). The total sugar contents in the shoots of both healthy and resistant cultivars were higher compared to the susceptible cultivars.

## **2.7 Effects on Phenol content in nematode infected plants**

Ganguly and Dasgupta (1982) suggested that the polyphenol oxidase enzyme in resistant variety was different from that of the susceptible variety and enzymes in the resistant variety were more stable in wide range of temperature and pH conditions than that of any susceptible variety.

Thakur and Yadav (1985) reported that higher levels of phenol per gram fresh root were associated with greater resistance. There was no increase in phenol content in inoculated susceptible varieties.

Agrawal *et al.* (1985) revealed that the infected okra plants by *M.incognita* had increased levels of phenolic compounds over the healthy plants.

Zacheo (1988) observed that browning in hypersensitive reaction is mainly caused by the enzyme polyphenol oxidase which catalyses the oxidation of various o-dihydroxy phenols to quinines or free radicals, and which can react with several biological molecules to create an unfavourable environmental condition for growth of potential pathogens.

Pankaj *et al.* (1992) observed that the phenol content of the resistant varieties was higher than that of the susceptible varieties even before nematode infection.

Nayak (2015) studied the effects of nematode infestation on phenolic substances as influenced by root-knot nematodes in both susceptible and resistance brinjal and he found that the total phenolic substance in the roots of both healthy and resistance brinjal cultivars was higher compared to that of susceptible cultivars.

Pandey *et al.* (2016) observed the effects of nematode infestation on phenolic substances as influenced by root-knot nematodes in both susceptible and resistance green gram and he found that the total phenolic substance of both healthy and resistance green gram cultivars was higher compared to that of susceptible cultivars.

## **2.8 Effects on Proline content in nematode infected plants**

The high metabolic activity in roots associated with giant cells and gall formation as well as egg-production exerts a requirement for energy which is supplied by a free proline manufactured in the leaves and translocated to the site of nematode

infection. Besides, the ratio of Lproline and hydroxyl proline play key role in imparting susceptible and resistant reactions and always associated with the stress condition of the host plant. Decrease in arginine content in both the varieties during post-infection period possibly due to conversion of L arginine to L-proline through ornithine cycle.

Okie *et al.* (1984) reported reduced free amino acids in shoots and roots as measured by levels of ninhydrin reactive compounds Peach root stock due to *Criconemella xenoplax* infection. They also found that the proportions of specific amino acids were changed and the molar percentage of proline, glycine and alanine increased, but arginine content was decreased in roots of both seedlings and herbaceous cuttings in the presence of nematode.

Trivedi and Sharma (1996) while analyzing quantitatively for different metabolites in both highly susceptible (Pusa Sawani) and less susceptible (Punjab 7) cultivars. They observed a reduction in sugar, proteins, total free amino acids, proline, phenols, ascorbic acid, enzyme, nitrogen and sodium excepting total sugars, non-reducing sugar, phosphorus and potassium in diseased roots of both the okra cultivars over their healthy counterparts.

Pandey *et al.* (2016) reported the variation in total proline content in six greengram cultivars i.e., 24 ML-233 (R), 7 GGG 10-14 (R), 17 IPM 9901-6 (R), 8 GM 04-02 (R), 28 PM 10-12(S) and 29 PUSA 0672 (S) which were studied 45 days after inoculation of *Meloidogyne incognita*. The total proline content in the shoots of both healthy and resistant cultivars was higher compared to the susceptible cultivars. However, in the inoculated plants the total proline content in four resistant variety varied from 100 mg/g to 150 mg/g as compared to two susceptible variety (90 mg/g to 140 mg/g) . An increasing trend was also observed in the proline contents in the shoots of inoculated susceptible and resistant cultivars.

## **2.9 Effects on enzymes (Catalase, Peroxidase, Superoxide Dismutase , Phenylalanine Ammonia Lyase, Tyrosine Ammonia Lyase) content in nematode infected plants**

Fogain (1996) reported enhanced peroxidase activity and phenolic compounds associated with resistant cultivar of *Musa spp.* to nematodes infection.

Indu Rani *et al.* (2008) studied the biochemical basis of root-knot nematode, *M. incognita* resistance in tomato hybrids CLN 2026C x SL 120, CLN 2026E x SL 120, LE 812 x SL 120 and CLN 1464A x SL 120. They also reported that among the hybrids, CLN 2026 C x SL 120 had the highest level of peroxidase and polyphenol oxidase enzyme activity with high level of total phenols while the susceptible check (COTH-1) indicated the lowest value for all the biochemical constituents.

Sundharaiya *et al.* (2011) reported that among F1 and hybrids of tomato cross lines CLN 2123A X HN2 and HN2 X CLN 2123A and their parents along with resistant and susceptible check varieties or hybrids only HN2 X CLN 2123A, nematode resistant check, Hissar Lalit and the parents, HN2 registered higher root phenol content along with ascorbic acid content. These genotypes also registered higher peroxides, polyphenol oxidase, IAA oxidase and acid phosphatase activity and lower root knot index than susceptible genotypes, CO3 and LCR-2.

Umamaheswari *et al.* (2005) found that higher activity of defense enzymes PAL and chitinase in *T. viridae* & *M. incognita* treated plants compared to untreated plants.

Chawla and Pankaj (2007) reported the peroxidase activity in the shoots of different cultivars moderately resistant chickpea varieties had higher degree of peroxidase enzyme activity in Pusa 256 and also minimum activity was observed in susceptible cultivars, Pusa 362.

Nayak and Pandey (2016) reported Phenylalanine Ammonia Lyase as mg of *p*-cinnamic acid formed per gram of fresh root extract. These were 24.03, 27.52 and 30.45 mg/g in Pusa Kranti, Kantabaigan and Pusa Purple Long respectively. In the inoculated roots these values increased to 29.75, 42.85 and 50.25 mg/g respectively, registering 23.30, 55.70 and 65.00 per cent enhancement over healthy counterparts.

Nayak and Pandey (2016) reported Tyrosine Ammonia Lyase was calculated as mg of *p*-coumaric acid formed per gram of fresh roots. In the normal roots the activities were recorded as 18.2, 22.06 and 26.20 mg/g in Pusa Kranti, Kantabaigan and Pusa Purple Long respectively but in the nematode infected roots the amounts increased by 8.24, 18.80 and 61.10 per cent respectively over healthy counterparts.

Nayak and Pandey (2016) observed the effect of root-knot nematode infection on peroxidase activity in brinjal varieties. The increased peroxidase activity was

observed in nematode inoculated samples of both susceptible and resistant brinjal varieties but higher enzymatic activity was recorded in resistant varieties and registered 45.18, 32.09 and 11.30 per cent increase over healthy counterparts.

Nayak and Pandey (2016) recorded the effect of root-knot nematode infection on catalase activity in brinjal varieties. The catalase activity was decreased (15.68 %) in case of nematode inoculated susceptible cultivar, Pusa Purple Long whereas its activity was increased considerably in resistant varieties inoculated with root-knot nematode over healthy counterparts.

### **2.10 Effects on amino acid content in nematode infected plants**

Nayak and Mohanty (2010) observed higher concentration of various amino acids and amides in brinjal cv. Pusa Purple Long inoculated with *M.incognita*.

Mohanty and Biswas (2000) found 10 amino acids like L-alanine, L-glutamine, L-glutamic acid, L-leucine, L-isoleucine, L-methionine, L-tyrosine, L-threonine, L-valine and L-tryptophan in susceptible chilly (Utkal Ava) and resistant (Pusa Jwala) varieties which were in higher concentration except L-tryptophan, by root-knot nematode infection.

Tripathy and Mohanty (2008) found 6 free amino acids such as L-serine, L-alanine, L-phenylalanine, L-tryptophan, L-tyrosine and L-glutamic acid in healthy and inoculated samples of susceptible and resistant varieties. They also found decreased percentage of phenylalanine and tyrosine of banana (Grand Naine & Dwarf Cavendish) infected with *M.incognita* in resistant variety during post infection period.

### **2.11 Assessment of avoidable yield losses due to root-knot nematode, *Meloidogyne* spp. infesting ridge gourd**

Reddy (1986) reported 28.08, 33.68, 43.48 and 28.60 per cent yield losses in okra, brinjal, french bean and cow pea, respectively due to root-knot nematode, *M. incognita* infection when the pots were treated with aldicarb (10 G) at 2 kg a.i./ha.

Darekar and Mhase (1988) recorded 46.92, 32.73 and 36.72 per cent loss in yield of tomato, brinjal and bitter gourd, respectively due to root-knot nematode, *M. incognita* when the pots were treated with aldicarb 10 G or carbofuran 3 G at 6 kg a.i./ha.

Jonathan *et al.* (1990) observed after application of carbofuran 3G @ 4 kg a.i./ha to fields of Piper betle infested with *M. incognita* reduced nematode populations over control. The leaf yield of untreated plants showed 38 per cent loss over treated plants.

Jain *et al.* (1994) reported that the avoidable yield loss of 71.9 per cent in the yield of tomato due to *M. incognita* and 43.3, 41.8 and 29.9 per cent in the yields of tomato, brinjal and okra, respectively in sick plot infested with *M. javanica*.

Ray *et al.* (1995) observed the soil application of carbofuran 3G @ 3 kg a.i./ha, 3 weeks after planting resulted in reduction of avoidable yield losses due to *M. incognita*, to the extent of 33.61 per cent in turmeric and 26.30 per cent in ginger with high gall index values in control plots compared to treated ones in both ginger and turmeric.

Makhnotra *et al.* (1997) conducted experiment on ginger plants that inoculated with *M. incognita* at an initial population level of 200 larvae/200 cc of soil in pot culture experiments and observed that carbofuran (1 kg a.i./ha) treated plots showed 20 per cent yield increase and decreased in nematode populations at harvest.

Mote and Mhase (1997) reported 36.45 per cent avoidable loss in yield of bitter gourd due to root knot nematode when the crop was treated with carbofuran 3 G @ 2 kg a.i./ha.

Yadav and Mathur (1999) reported an average yield loss of 53.36 and 39.76 per cent from Carbofuran 3G and Phorate 10G @ 2 Kg a.i./ha treated plots respectively from green chillies, (*Capsicum annum* cv. Jaipur local) infested with *M. incognita*. (>2J<sub>2</sub>/g soil).

Nagesh and Reddy (2000) observed that application of phenamiphos at 2.5kg a.i./ha to carnation and gerbera flower crops, recorded higher values for plant height, spike length and number of flowers compared with untreated plants, and increased flower yield by 26.6 per cent and stem height by 30 per cent compared with untreated plants in carnation flower yield by 31.1 per cent and spike length by 38.8 per cent in gerbera with yield loss of 26.6% and 31.1% in carnation and gerbera respectively due to nematode infestation.

Bhargava and Sharma (2001) observed that the avoidable loss in chilli (cv. RCH-1) ranged between 11 and 33 per cent with an average of 23 per cent with the application of carbofuran 3G at 2 kg a.i./ha, while in the susceptible tomato cultivar (Pusa Early Dwarf) avoidable yield losses ranged from 19 to 29 per cent with an average of 26 per cent with the application of carbofuran 3G at 2 kg a.i./ha. In the resistant tomato cultivar (PNR-7) avoidable yield losses ranged between 13 and 18 per cent with an average of 25 per cent with the application of carbofuran 3G at 2 kg a.i./ha.

Khanna and kumar (2003) recorded maximum increase in yield, 5825 kg of fruits from nematicide-treated beds compared to 3333.3 kg/ha from the untreated control beds and yield losses ranging between 22.9 to 42.8 per cent from *Momordica charantia* infested by *M.incognita*.

Anwar and Mckenry (2010) reported that *M. incognita* causes 40 per cent yield losses in vegetable crops in Punjab.

Shendge *et al.* (2010a) reported that the loss in yield of okra was 27.02 per cent, when the crop was treated with carbofuran 3 G @ 2 kg a.i./ha. infested by *M. incognita*.

## **2.12 Molecular characterisation using ISSR markers**

Chang *et al.* (2003) classified twenty pumpkin cultivars into three large categories and identified genetic distance of cluster ranging from 38% and 100%.

Resmi and Sreelathakumary (2011) estimated Jaccard's similarity coefficients and constructed dendrogram by using UPGMA revealed the presence and extent of genetic similarities among the twenty-five landraces of ashgourd. Pair-wise genetic similarities among the landraces determined using Jaccard's coefficient ranged from 0.14 to 1.00.

Song *et al.* (2010) recorded that genetic similarity between wax gourd and chieh-qua germplasm was in the range of 60% to 99%.

Latha (2012) studied RAPD analysis in cucumber and reported that OPA-19 primer recorded the highest polymorphism (86.6%). Out of 130 bands, 115 bands were polymorphic for a specific primer and can be used as differential markers for varietal differentiation.

A yaf (2014) studied RAPD profile in squash gourd and generated high level of polymorphism. OPA-03 and OPC-19 primers showed high value for number of main, amplified and unique bands and success in giving a distinct fingerprint for all genotypes. The highest value for primer efficiency and discriminatory value were produced by the primers OPD-13 and OPN 06, respectively.

Sikadar *et al.* (2010) observed that out of the ten (ISSR) primers, eight produced informative data for phylo-genetic analysis in *Cucurbita* species. These eight primers produced 139 ISSR fragments, an average of 17.37 bands per primer. Amplified product sizes ranged from 599 to 2399 bp.

Huang *et al.* (2010) observed that size of amplification ranged from 150 bp to 2700 bp with six ISSR primers in 38 diverse bitter gourd accessions.

Bhawna *et al.* (2014) studied 209 amplified bands from 20 ISSR primers in which 186 were polymorphic (89.00%) bands. Jaccard's similarity coefficient matrix was generated for pair-wise comparisons between individual ISSR profiles and UPGMA cluster analysis based on this matrix showed clustering into six groups. Jaccard's coefficient of similarity values ranged from 0.409 to 0.847, with a mean of 0.628 revealing a moderate level of genetic diversity in bottle gourd.

Yildiz *et al.* (2014) characterized 24 accessions covering different groups of *Cucumis melo*L. collected from Eastern and South-eastern Anatolian regions of Turkey by using 43 morphological traits and 207 markers obtained from 31 ISSR and 16 SSR primers. The findings indicated wide range of variations for investigated characteristics in Turkish gene pool that provides a good source of diversity to use in melon improvement program for better yield.

De *et al.* (2015) studied phylo-genetic patterns and relatedness among selected species of cucurbits using ISSR markers. Total of 117 bands, of which 57 were polymorphic, were amplified by five primers. The phylogram generated on the basis of Jaccard's similarity coefficient revealed a close genetic relationship between *C. maderaspatanus* and *C. melo*, while *C. sativus*, a member of the same genus, was placed as a distant relative from both species, thereby demonstrating remarkable diversification among members of the same genus.



# MATERIALS AND METHODS

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## 3.1 Collection of nematodes

### 3.1.1 Stock culture

The experimental stock cultures of the root-knot nematode, *Meloidogyne incognita* (Race-2) for this study was originally obtained from a single egg mass progeny, maintained and multiplied on susceptible brinjal variety, Pusa Purple Long. The seeds of mung bean plants were sown in 30 cm pots containing sterilized soil, autoclaved at 15 lbs/ sq. inch. pressure for 20 minutes. The populations of root-knot nematode were sub-cultured periodically inoculating with infective J<sub>2</sub> in sterile water suspension to the root zone of three week old seedlings grown in the pots.

## 3.2 Experimental procedure

As and when required plants were taken out from cultured pots, washed the roots free of soil, dissected out of the egg-masses from the galls under a stereoscopic microscope, treated with sodium hypochlorite solution, washed with sterile water and put over tissue paper supported by aluminium wire gauge in petridishes with water. J<sub>2</sub>-that hatched out were collected daily in beakers, surface sterilized by treating with 0.5 per cent streptomycin sulphate solution for 12 hours. Water from the upper portion of the beaker was drained off without disturbing the nematodes at the bottom. These larvae were utilized to conduct all the experiments. The larvae that could not be inoculated just after collection were stored in refrigerator at 8 to 10°C for use within the next 3 to 4 days.

Earthen pots of 15 cm dia, were sterilized with formaldehyde solution (1.0 %) and filled with autoclaved soil (15 lbs/20min). These pots were arranged on green house benches according to the treatments and replications. The water used for irrigation purpose was passed through a 500 mesh sieve before use.

For all chemical analysis purposes sterilized oven dried glassware and double distilled water were used throughout the experiment.

### 3.2.1 Collection of ridge gourd germplasm/cultivars

Seeds of 52 ridge gourd germplasms/cultivars each 2 to 3 g , were collected from local market and nearby villages of Dhenkanal . The seeds were surface sterilized by treating them with 0.1 % HgCl<sub>2</sub> for 5 minutes, washed thoroughly with sterile water and air dried. These seeds were sown in the pots to conduct screening and evaluation of seeds against test nematode, *M.incognita* (race-2)

### **3.2.2 Sowing of seeds and inoculation of nematodes**

The soil to be filled in the pots were pulverised, mixed with N, P, K fertilizers @ 250:100:100 per hectare on soil weight basis and filled into the pots @ 1 kg/pot. The surface sterilized seeds were sown @ 4 to 5 seeds per pot. Each variety was replicated 3 times. Moisture was maintained regularly after the emergence of seedlings. At 15 days after sowing the seedlings were thinned keeping one healthy seedling per pot at the centre. A small glass tube (2 cm long, 0.5 cm bore) was inserted into the soil near the root zone of each of the seedlings. Two weeks after seedling emergence axenised nematodes were counted under a stereoscopic microscope and released into the holes near the root zones @ 1000 J<sub>2</sub> ± 20 per seedling in 10 ml sterile water. For chemical analysis two sets of plants were maintained, one for the uninoculated control (Healthy) and the other infected with the nematode. Each set was arranged on separate platform in the green house in order to avoid cross infection.

### **3.3 Screening of ridge gourd germplasm/ cultivars against root-knot nematode**

Root-knot nematodes are polyphagus in nature. Fifty two number of genotypes (varieties) procured were used for testing and scoring against test nematode for resistance/ susceptibility.

Pots containing soil were arranged on greenhouse benches in complete randomized design with three replications. Seeds were sown and sprinkled with water.

After 2 weeks of sowing J<sub>2</sub> of root-knot were released into four holes near the root zone of the plant of each pot. Watering was done just to drench the soil avoiding over flooding. Forty five days after sowing the pots were washed under water tap. Water was allowed to pass upon the pot soil with sufficient pressure so that the soil particles were flooded away. Whole of the root system was obtained by this method. Roots were observed under a stereoscopic microscope and the numbers of galls produced on each plant roots were counted. The average number of galls of all replications are presented in Table 1. Subsequently the root system was fixed in 4 % formalin and stored in small plastic containers with proper label, for observation of egg-masses.

The root system of each plant was chopped and out of this, one gram was stained with lacto-phenol acid fuchsine solution. The egg masses present in it were counted through a stereoscopic microscope. Ridge gourd varieties or germplasm were categorized as per the 1-5 gall index given below (Taylor and Sasser, 1978).

### Gall index (1 to 5 scale)

Scale	No. of galls/eggs/eggmass	Reactions
1	0	Highly resistant (HR)
2	1-10	Resistant (R)
3	11-30	Moderately resistant (MR)
4	31-100	Susceptible (S)
5	More than 100	Highly susceptible (HS)

#### 3.3.1 Evaluation of Ridge gourd varieties against root-knot nematode

Fifty-two ridge gourd varieties were sown in the sterilized earthen pots containing 1 kg of autoclaved soil/pot in the green house as described earlier. The plants were washed 45 days after sowing of seeds and observations were made on number of galls/plant, plant height (cm), fresh shoot weight (g), fresh root weight (g) dry root weight (g), number of eggmass/plant and final nematode population on each variety and reactions of the varieties to the test nematode, *Meloidogyne incognita*.

#### 3.4 Biochemical analysis of the plant samples

In order to understand the basis of nematode resistance five varieties namely Priya, BSS-1009 (Resistant), Aneeta (Moderately Resistant) and Aarti, Harsha (Susceptible) were sown in earthen pots in the greenhouse as described earlier maintaining five replications in Completely Randomised Design. These plants were washed 45 days after sowing of seeds or 30 days after inoculation of J<sub>2</sub> juveniles/c.c. soil and the following chemical compositions were estimated in both resistant and susceptible plants under controlled and inoculated condition.

Biochemical analysis of different parameters

1. Estimation of chlorophyll content of leaves
2. Estimation of change of macronutrients due to root-knot nematode infection (Nitrogen & crude protein, Phosphorus, Potassium)
3. Estimation of change of micronutrients due to root-knot nematode infection (Zinc, Copper, Calcium, Sulphur, Iron, Manganese, Magnesium)
4. Estimation of Protein content
5. Estimation of Total sugar & starch content
6. Estimation of phenolic substances

7. Estimation of proline substances
8. Estimation of enzymes (Catalase, peroxidase, Superoxide Dismutase, Phenylalanine Ammonia Lyase & Tyrosine ammonia Lyase) content
9. Estimation of free amino acid contents & amino acid separation by **HPTLC Method**
10. Assessment of avoidable yield loss due to root-knot nematode, *Meloidogyne incognita* infesting ridge gourd
11. Molecular characterisation using ISSR markers

### 3.4.1 Chlorophyll estimation of leaf (mg/g) on fresh weight basis

One hundred fifty mg leaf portion of each treatment were cut from the composite leaves and were immersed in 50 ml of 80 % acetone in a conical flask and kept in dark for 24 hours for extraction of chlorophyll from the leaf samples. Thereafter, the chlorophyll extracts were filtered through Whatman No.1 filter paper. Absorbance of the chlorophyll extract was measured at 645 nm and 663 nm using a colorimeter. The amount of chlorophyll-a, chlorophyll-b and total chlorophyll were calculated in mg/g fresh weight according to the following equations.

i) Chlorophyll -a (mg/g fresh weight of leaf )

$$= [12.7 \times (D-663) - 2.69 \times (D-645)] \times \frac{V}{1000 \times W}$$

ii) Chlorophyll -b (mg/g fresh weight of leaf)

$$= [22.9 \times (D-645) - 4.68 \times (D-663)] \times \frac{V}{1000 \times W}$$

iii) Total chlorophyll (mg/g fresh wt. of leaf)

$$= [20.2 \times (D-645) + 8.02 \times (D-663)] \times \frac{V}{1000 \times W}$$

Where

D -645	=	optical density at 645 nm
D-663	=	optical density at 663 nm
V	=	final volume of 80 % acetone chlorophyll extract in ml
W	=	Fresh weight in g of corresponding amount of fresh leaves used in the extraction of chlorophyll

### 3.4.2 Estimation of nitrogen and crude protein content of plant samples

Crude protein and nitrogen content of shoot were estimated by following the procedure of Mahadevan and Sridhar (1986). Two hundred mg of powdered plant parts were taken in 100 ml micro Kjeldahl digestion flasks. About 200 mg of digestion mixture ( $K_2SO_4$ :  $CuSO_4 = 5:1$ ) and 4 ml of concentrated  $H_2SO_4$  were added. These flasks were kept as such for about one hour and then heated slowly till frothing occurred. To check the frothing, two crystals of sodium thiosulphate were added to each digestion flask. Thereafter, digestion was continued until the contents of the flask became completely clear blue syrupy liquid without any bubbling. The flask was cooled and content was diluted to 25 ml with distilled water. Then 10 ml of diluted sample extract was transferred into micro Kjeldahl distillation unit. Thereafter, 10 ml of 40 % NaOH was added and distillation was continued for 10 minutes. During distillation period, liberated ammonia was absorbed by 150 ml conical flask containing 2 drops of mixed indicator. After completion of distillation, distillate was titrated against 0.05  $NH_2SO_4$ .

#### Calculation

$$\begin{aligned} & \text{Per cent } N_2 \text{ in sample} \\ &= \frac{(\text{Sample titer} - \text{blank titer}) \times N_2 \text{ of } H_2SO_4 \times 14 \times 100 \times 2.5}{\text{Sample weight (g)} \times 1000} \end{aligned}$$

### 3.4.3 Crude protein

Percentage of protein present in shoots was determined by multiplying the per cent  $N_2$  with 6.25. This protein is called crude protein.

### 3.4.4 Estimation of phosphorus and potassium content as influenced by root-knot nematode in ridge gourd

#### Digestion of samples

Powdered plant samples (0.5 g) were taken in 100 ml conical flasks. To each flask 15 ml of concentrated  $HNO_3$  was added. The flask were kept as such over night. Then the flasks containing samples were heated on a hot plate till brown fumes evolved. Five ml of di-acid mixture ( $HNO_3$  :  $HClO_4$  (70 %) = 3:2) was added to each flask. Again the flasks were heated till white fumes evolved reducing the volume of content to about 2 ml. Thereafter, conical flasks were taken out from hot plate and allowed to cool. One ml of 6N HCL was added and flasks were heated gently for one minute. Then 15 ml of warm distilled water was added to each flask. The content of

the conical flask was transferred to a 50 ml volumetric flask followed by twice rinsing with distilled water. Then the volume was made up to 50 ml with distilled water and the aliquot was filtered through Whatman No.42 filter paper. The filtered extract was kept for the estimation of phosphorus and potassium.

### **Estimation of phosphorus present in plant samples.**

Phosphorus present in plant samples was estimated by adopting the procedure of Jackson (1973).

### **Chemical reagents**

1. Molybdate – Vanadate solution
  - a) Dissolve 6.250 g ammonium molybdate in 125 ml of distilled water.
  - b) Dissolve 313 mg ammonium Vanadate in 125 ml of 1(N) HNO<sub>3</sub>Then mix the reagents (a) and (b) in a 250 ml volumetric flask  
The resulting solution is called molybdate – vanadate solution.
2. 2(N) HNO<sub>3</sub> : Dilute the 60 ml concentrated HNO<sub>3</sub> to 480 ml with distilled water.
3. The standard phosphorus solution (25 ppm) : Dissolve 55 mg monobasic potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) in distilled water and dilute to 500 ml.

### **Sample analysis**

Standards of 0, 2.5, 5.0, 7.5 and 10.0 ml of 25 ppm phosphorus solution and 2 ml of digested sample extracts were taken in 25 ml volumetric flasks. Five ml of 2(N) HNO<sub>3</sub> solution was added to each flask. Then required amount of distilled water was added to each flask to make the final volume 15 ml. Thereafter, 2.5 ml molybdate vanadate solution was added. Final volume was made up to 25 ml with distilled water and flasks were shaken well. Absorbance was measured by a colorimeter at 420 nm after 20 minutes of shaking. The phosphorus content of plant samples was calculated in percentage by using the standard curve.

### **3.4.5 Estimation of potassium content as influenced by root-knot nematode in ridge gourd**

One ml digested sample extract of shoot were taken in 25 ml volumetric flasks and the volume was adjusted to 25 ml with distilled water. Similarly 1, 2, 3, 4 and 5 ppm standard K solution (i.e. 0.1907 g KCL/lit) were taken in 100 ml volumetric flasks with water. The readings for standards and samples were taken in a digital flame photometer. As

per the standard curve, the ppm of potassium present in extracting solution was calculated. Then the percentages of potassium present in shoot samples were calculated.

#### **3.4.6 Estimation of total sugar content as influenced by root-knot nematode in ridge gourd**

The total sugar content of shoot were determined by adopting the following procedure.

##### **Extraction of shoot sugar**

One hundred mg of ground seed samples were taken in 15 ml centrifuge tubes and 10 ml of 80 % ethanol was added to it. The mouth of the centrifuge tube was covered with polythene paper and kept in a water bath at 80-85°C for 30 minutes. Then it was cooled and centrifuged for 15 minutes at 2000 rpm. After centrifugation, the supernatant was decanted into a 25 ml. Volumetric flask. This extraction procedure was repeated once again and the supernatant was collected in the previous 25 ml. Volumetric flask. The final volume was made up to 25 ml with distilled water and was filtered through Whatman No.1 filter paper. This was the sugar extract kept for sugar estimation.

##### **Estimation of total sugar**

Two ml of sugar extract was transferred into a 50 ml volumetric flask and volume was made up to 50 ml volumetric flask and volume was made up to 50 ml with distilled water. Five ml of this extract was taken in a 25 ml volumetric flask. Simultaneously standards of 0 ml, 1 ml, 1.5 ml and 2 ml. Of 100 ppm glucose solution were taken in 25 ml volumetric flasks. Volume of these standards was made up to 5 ml with addition of distilled water and 2 drops of 80 per cent ethanol. Volumetric flasks containing samples and standards were kept in an ice-bath. To each volumetric flask, 10 ml of anthrone reagent (2 gm of anthrone in one litre of 95 % H<sub>2</sub>SO<sub>4</sub>) was added allowing it to run down the side of the volumetric flask. The contents of the flasks were shaken slowly by swirling the flask and then shaken thoroughly. The volumetric flasks were kept in boiling water bath for exactly 7.5 minutes. Then immediately the flasks were cooled in ice. After cooling, absorbance was measured at 630 nm and sugar content was calculated by the help of standard curve.

#### **3.4.7 Estimation of total starch content as influenced by root-knot nematode in ridge gourd**

1 ml of starch extract was taken in a 100 ml volumetric flask and diluted to 100 ml with distilled water. Five ml of the above extract was transferred in a 50 ml

test tube. Then all the standards and sample test tubes were kept in ice bath for cooling ,and 10 ml of anthrone reagent was added to each test tube, allowing the reagent to run down the side of the flask. It was stirred slowly with a glass rod and then shaken thoroughly. The flask was kept in boiling water bath exactly for 7.5 minutes. Then the test tube was immediately cooled in ice-bath. After cooling ,the O.D. at 630 nm was measured and the starch content was calculated by the help of standard curve, which was multiplied by 0.91 to get the exact value of the same.

#### **3.4.8 Estimation of total protein content as influenced by root-knot nematode in ridge gourd**

Two ml of the sample extract was pipette into the test tubes, and the volume make up was done upto 2ml in all test tubes with water.Then 10ml of reagent C was added to each test tube including blank.It was mixed well and allowed to stand for 10 min. Then 1 ml of reagent D was added,mixed well and was incubated at room temperature in the dark for 30 min. Then blue colour was developed.Finally, absorbence was measured at 660 nm and protein content was calculated by the help of standard curve.

#### **3.4.9 Estimation of total free amino acid content as influenced by root-knot nematode in ridge gourd**

Five hundred mg of the plant sample was weighed and macerated in a pestle and mortar with small quantity of acid washed sand.Then all 500 mg finely ground plant sample was taken in a centrifuge tube.The residue was washed which was left in the pestle twice with little amount of 80% ethanol and transfer to the centrifuge tube.Then the volume was made up upto the centrifuge mark of 10 ml.The tubes were centrifused for 10 min. & the supernatant was decanted in to a 50 ml vol. flask and the extraction was repeated with 5 ml of 80% ethanol twice & supernatant was transferred to the above 50 ml vol. flask.The volume was reduced ,& the extract was used for the quantitative estimation of total free amino acids. Finally standards and samples were prepared,& after taking the above reagents in standards and sample test tubes,it was heated in boiling water bath for 20 mins.Quickly,15 ml diluent was added just after removing the test tubes from water bath and was shaken vigorously & allowed to cool at room temperature for 15 min.Finally, absorbance was measured at 570 nm by a spectro-colourimeter.

#### **3.4.10 Estimation of total phenol content as influenced by root-knot nematode in ridge gourd**

Exactly 0.5 g shoot and root sample was ground with a pestle and mortar in 10 ml of 80 per cent ethanol until it became a pulp.

The homogenate was centrifuged at 5000 rpm for 20 minutes. The process was repeated with another 5 ml of 80 per cent ethanol. Both the supernatants were pooled and evaporated to dryness. The residue was dissolved in 10 ml distilled water. The aliquot was pipetted into test tubes with 0.5 ml each. The volume was made up to 3 ml with distilled water. Exactly 0.5 ml of folin-ciocalteu reagent was added into it. After 3 minutes 2ml of 20 per cent  $\text{Na}_2\text{CO}_3$  solution was added into each tube. The contents were mixed thoroughly, placed in boiling water for 1 minute and then cooled. Absorbance was measured at 650 nm in a colorimeter and compared with a blank. A standard curve was prepared using different concentrations of catechol.

### **Calculation**

The concentrations of the phenol in test samples was calculated by comparing with the standard curve and expressed as mg/g material (catechol).

#### **3.4.11 Estimation of total proline substance as influenced by root-knot nematode in ridge gourd**

Exactly 200mgs each of shoot and root was macerated with 5 ml of sulfo-salicylic acid.

The residue was centrifused at 4000 r.p.m. for 15 minutes. The supernatant liquid was decanted to a 50 ml test tube. 5 ml of glacial acetic acid and 5ml of acid ninhydrin was added to it. The mouth of the test tube was closed by polythene paper and rubber band. It was boiled for 1hr. in water bath at 100°C. After boiling of standards and sample, the reaction mixture was transferred to 60 ml separating funnels. 20ml of toluene was added and shaken vigorously. It was then allowed to settle. The chromophore containing toluene was separated out through the bottom hole of the separating funnels. Absorbance was measured at 520 nm. By the help of standard curve data, the amount of proline present in plant sample was calculated and expressed as mg proline/gram or fresh sample.

#### **3.4.12 Estimation of micro-nutrients as influenced by root-knot nematode in Ridge gourd plant samples**

##### **Mineral Nutrient analysis of extrudate product (ribbon noodle)**

Mineral acids like of diacid ( $\text{HNO}_3$  -  $\text{HClO}_4$ ) digestion (Jackson, 1973) by SS method through model GCB Awanta.

## Method

Powdered samples (0.5 g) were taken in a 100 ml conical flask. To each flask 10 ml of concentrated HNO<sub>3</sub> was added. The flasks were kept undisturbed overnight. Then the flask containing samples were heated on a hot plate till brown fumes evolved. Five ml. Of di-acid mixture (HNO<sub>3</sub>: HClO<sub>4</sub> (70%)):: 3:2 by volume) was added to each flask. Again the flasks were heated till white fumes evolved reducing the volume of content to about 2 ml. Thereafter, conical flasks were taken out from hot plate and allowed to cool. One ml. Then 15 ml warm distilled water was added to each flask. The content of conical flask was transferred to a 50 ml volumetric flask followed by twice rinsing with distilled water. Then the volume was made up to 50 ml with distilled water and the aliquot was filtered through Whatman No.42 filter paper. The filtered extract was kept for estimation of mineral nutrients like potassium, sodium, phosphorus, Calcium, Magnesium and micronutrients like Fe, Zn, Cu by adopting the procedure of Jackson (1973).

### Micronutrients (Fe, Zn, Cu, Mn) Estimation

Digested sample was introduced to AAS for Fe, Zn and Cu analysis after standardizing the AAS with respective standards.

$$(\text{Fe, Zn, Cu, Mn})\text{mg}/100 \text{ g} = \frac{\text{AAS R} \times 50}{\text{sample wt}(g) \times 10}$$

#### 3.4.13 Estimation of Phenylalanine Ammonia Lyase (PAL) content as influenced by root-knot nematode in ridge gourd

0.5 ml borate buffer, 0.2 ml enzyme solution and 1.3 ml water was pipetted out in a test tube. The reaction was initiated by the addition of 1 ml L-Phenylalanine solution. It was then incubated for 30-60 min at 32°C. The reaction was stopped by the addition of 1 M trichloroacetic acid. Then a control was run in which phenylalanine was added after trichloroacetic acid. The absorbance was measured at 290 nm. Finally a standard graph was prepared for trans-cinnamic acid.

#### 3.4.14 Estimation of Tyrosine Ammonia Lyase (TAL) content as influenced by root-knot nematode in ridge gourd

Leaf samples (200 mg) were homogenized in 2 ml of 25 mM borate buffer, pH 8.8 containing 2 µl β mercaptoethanol and a pinch of polyvinyl polypyrrolidone (PVP). The homogenate was filtered through the cheese cloth, centrifuged at 12,000 ×

g for 10 min and the supernatant was used for enzyme activity assay according to Sadasivam and Manickam (1992). One unit of the enzyme was defined as increase in absorbance of one unit per min. The activity of the enzyme was expressed as units per mg of soluble protein.

All the steps described under PAL can be used to measure the TAL except that the phenylalanine replaced by tyrosine. The p- coumeric acid is extracted from reaction mixture with ether and its concentration measured at 333nm or alternatively at 650nm using FCR.

#### **3.4.15 Estimation of Superoxide dismutase (SOD) content as influenced by root-knot nematode in ridge gourd**

##### **Extraction of enzyme**

0.2 g of fresh and cleaned leaf sample was homogenized in 10 ml of 0.5 M phosphate buffer (pH 7.5) containing 1% NEDD (1-naphthyl ethylenediamine dihydrochloride). The homogenate was then centrifused at 4°C for 30 min at 30,000 ×g. After centrifugation, the supernatant was stored for the enzyme assay and the pellet was discarded. SOD activity in the supernatant was assayed by measuring its ability to inhibit the photochemical reduction of NBT.

##### **Enzyme Assay**

1.5 ml buffer (pH 7.5), 0.1 ml of enzyme extract, 0.1 ml of sodium carbonate solution, 0.1 ml of NBT solution, 0.2 ml of methionine solution, 0.1 ml of EDTA solution and 1 ml of double distilled water were taken in test tubes. 0.1 ml of riboflavin solution was added just before illumination of reaction mixture. Blank 'A' with enzyme should be kept in dark whereas blank 'B' without enzyme should be placed with sample tubes 30 cm below a light source of two 15 watt inflorescence lamps for 10 min. Reaction was stopped by switching off the light and tubes were covered with black cloth. The non-irradiated reaction mixture containing enzyme extract did not develop light blue colour. Absorbance of samples alongwith blank 'B' was measured at 560 nm against the blank 'A'. Then the difference of per cent reduction in the colour between blank 'B' and the sample was worked out.

#### **3.4.16 Estimation of Catalase content as influenced by root-knot nematode in ridge gourd**

The assay mixture contained 0.5 ml of hydrogen peroxide, 1 ml of buffer and 0.4 ml of water. 0.2 ml of the enzyme was added to initiate the reaction. 2 ml of the

dichromate /acetic acid reagent was added after 0,30,60,90 seconds of incubation. To the control tube the enzyme was added after the addition of the acid reagent Aebi (1984). The tubes were then heated for 10 min. and then colour developed was read at 610 nm. The activity of catalase was expressed as umoles of H<sub>2</sub>O<sub>2</sub> decomposed / min/mg protein.

#### **3.4.17 Estimation of Peroxidase content as influenced by root-knot nematode in ridge gourd**

Peroxidase was assayed following method of Hammerschmidt *et al.* (1982). Enzyme activity was determined by following decrease in A<sub>420</sub> for 1.5 ml of a reaction mixture containing 0.45 mL of 0.1 M phosphate buffer (pH7.0), 0.75 mL of guaiacol (8 mM), 0.25 mL solution H<sub>2</sub>O<sub>2</sub> (18 mM) and 50 µl crude enzyme extract. Enzyme activity was calculated using extinction coefficient of 25 mM<sup>-1</sup> cm<sup>-1</sup>.

#### **3.4.18 Estimation of amino acids by HPTLC influenced by root-knot nematode in ridge gourd**

In order to understand the basis of nematode resistance the ridge gourd plant was again grown in earthen pots in the net house. After 45 days of nematode inoculation the plants are uprooted and the roots were thoroughly washed and the free amino acid and amide content were estimated as follows.

#### **Preparation of test samples for analysis of amino acids and amides :**

Five grams of root sample was taken, ground separately with 80 percent ethyl alcohol, added at the rate of 20ml for each sample, in a sterile mortar pestle till the roots were macerated to pulp. These materials after grinding were boiled for 5 to 10 minutes in a hot water bath. After boiling, the alcoholic root extracts were filtered through a double layered fine cheese cloth so as to remove the floating plant debris. The extracts were centrifuged at 5000 rpm for 10 minutes. All the suspended materials were settled at the bottom and top portion became very clear. The supernatant liquid in the test tubes were carefully poured into separate watch glasses and pellets were discarded. The supernatant liquid was allowed to evaporate till complete dryness by keeping them at room temperature and the process was speeded up by means of hot air blower. Then finally a syrupy material was obtained in the watch glass, which was slight yellowish in colour. This syrupy material was dissolved in 1ml of 10% isopropyl alcohol and transferred to separate glass vials representing for a single lot which were stored in the freezer compartment of refrigerator for subsequent use in HPTLC.

### **Preparation of standard amino acids**

Eighteen amino acids and two amides were used for the preparation of standard maps. The amino acids were L-cystine, L-threonine, L-lysine, L-histidine, L-alanine, L-arginine, L-glycine, L-alanine, L-proline, L-tryptophan, L-tyrosine, L-methionine, L-valine L-leucine, L-isoleucine, L-phenylalanine, L-aspartic acid and L-glutamic acid and the amides were L-glutamine and L-asparagine. The standard solution of each amino acid and amide were made by dissolving 0.5mg of each in 1ml of 10% isopropyl alcohol solution. The amino acids namely, L-cystine, L-phenylalanine, L-tryptophan and L-glutamic acid could not be dissolved very easily. Consequently, a few drops of 100 % hypochloric acid were added to dissolve them.

### **Instrumentation and chromatographic conditions:**

HPTLC was performed on 20 x 10 cm aluminium backed plates coated with silica gel 60F 254 (Merck, Germany). 8 µL of Standard solution and 10 µL of sample solution were applied to the plates as bands 6 mm wide and 10 mm from bottom edge of the same chromatographic plate by use of a Camag (Switzerland) Linomat V sample applicator equipped with a 100 µL Hamilton (USA) Syringe. Ascending development was performed at room temperature with n-butanol: acetic acid:water (160:40:40) as mobile phase, followed with phenol: water: ammonia (180:20:1) solvent by perpendicularly placing the plate opposite direction of the first solvent in a Camag glass twin-trough chamber previously saturated with mobile phase vapor. After development, the plates were air dried and then dipped in ninhydrin solution (0.25 %) and the plates were dried in hot air oven. Quantitative analyses of the compounds were done by scanning the plates at 385 nm with a Camag TLC scanner with WINCAT software.

### **3.4.19 Estimation of Molecular characterisation in susceptible and resistant ridge gourd cultivars against induced population of root-knot nematode, *Meloidogyne incognita* through ISSR based molecular markers**

#### **Total Genomic DNA extraction**

Total Genomic DNA extraction was done by using CTAB method developed by Rogers and Bendich (1994). Purification of DNA was by RNase treatment & subsequently precipitated. Assessing the quality & quantity of DNA was done by using NanoDrop (Ratio OD260/ OD280). ISSR PCR programme: (35 cycle). Electrophoresis of ISSR PCR Product was done. Gel Documentation was done. Scoring of bands was done. Finally, data analysis following FreeTree software.

### 3.5 Assessment of yield loss due to root-knot nematode in ridge gourd

An experiment was laid out in the sick plot, Nematology Department, College of Agriculture, OUAT, naturally infested with root-knot nematode (Race-2) using pair plot technique to assess the avoidable yield loss of ridge gourd due to root-knot nematode, *Meloidogyne incognita*.(Race-2)

The field was thoroughly cultivated and pulverized. Soil samples (200c.c) were taken with the help of a hoe upto a depth of 15 cm and mixed thoroughly to prepare composite sample, one sample of 200 c.c was taken and processed for nematode extraction by Cobb's sieving and decanting method (Cobb,1918) to know initial nematode population.

The sick plot was divided into two equal blocks i.e., treated and untreated each having 10 sub plots each of size 3mx2m serving as replication and seeds were sown in pits in the sub plots. For treated plots, Carbofuran (Furadon 3G) @ 2 kg a.i/ha was applied before sowing of ridge gourd seeds followed by light irrigation. The crop was grown following agronomic package and practices. 100 days after sowing, the experiment was terminated and observations were recorded on plant growth parameters as well as on the nematode population growth in both treated and untreated sub-plots.

<b>Treated</b>	<b>Healthy</b>
R-1	R-10
R-2	R-7
R-3	R-9
R-4	R-8
R-5	R-4
R-6	R-1
R-7	R-5
R-8	R-6
R-9	R-3
R-10	R-2

#### 3.5.1 Recording of observations

##### Shoot length

Shoot length of each plant was measured from the base of the plant upto the top most portion in meter scale.

##### Root length

Root portion of each plant was cut, labelled and knots, if any, were opened followed by straightening of roots. The root length of each plant was measured upto tip in the meter scale.

### **Fresh weight of shoots and roots**

Fresh weights of shoot and root of individual plant were recorded in grams. These were further labeled and kept for recording of dry weights.

### **Dry weight of shoot and root**

Shoot and root of individual plants were air dried and kept in separate paper packets. Such packets were then placed in hot air oven at 70 °C temperature for 48 hrs after which, the dry weights were recorded in gram.

### **Number of galls**

Total number of galls on roots of each plant was counted with the help of hand tally counter before drying and recorded as per root knot index in 1 to 5 scale.

### **3.5.2 Estimation of *M.incognita* population in roots**

At the time of harvest, roots of ridge gourd plants inoculated with *M.incognita* were lifted carefully. Infected root measuring 1 g from each replication of different treatments were tied separately with cotton threads and labeled accordingly. Then nematode in roots are stained by Byrd / acid-fuchsin method.

### **3.5.3 Nematode population in soil**

Soil from each sub plot was mixed thoroughly and 200ml sample from each sub plot was collected and screened by Cobb's sieving and decanting technique (Cobb, 1918) and modified Baermann funnel technique (Schindler, 1916) for estimation of nematode population in different treatments.

### **3.5.4 Yield**

Yield from each plot was recorded at the time of termination of experiment. Data obtained were analysed according to 't' test for paired comparison at 5% level of probability.

### **3.5.5 Statistical analysis**

$$\text{'t' at error degree of freedom} = \frac{\bar{X}_1 - \bar{X}_2}{S_d}$$

Where  $\bar{X}_1$  = Mean yield of treated plot

$\bar{X}_2$  = Mean yield of un treated plot

$$S_d = \frac{s}{\sqrt{n}}$$

Where  $S$  = Standard deviation =  $\sqrt{\frac{\sum d^2}{n} - \frac{(\sum d)^2}{n^2}}$

$n$  = number of paired plots.

$d$  = differential value between two paired plots.

The avoidable yield loss and percent increase in yield over control (untreated) by following formulae. (Pradhan 1964).

$$\text{Avoidable yield loss (\%)} = \frac{\text{Mean yield of treated plot} - \text{Mean yield of untreated plot}}{\text{Mean yield of treated plot}} \times 100$$

$$\text{Increase in yield (\%)} = \frac{\text{Mean yield of treated plot} - \text{Mean yield of untreated plot}}{\text{Mean yield of untreated plot}} \times 100$$



## RESULTS

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Root knot nematodes are polyphagous in nature, for which it is very difficult to find resistant varieties/lines for them. Injuries caused by them are often insidious. It also increases the susceptibility of plants to different diseases such as cotton wilt, tomato wilt, blank shank of tobacco and rhizoctonia rot of Peanuts. The root-knot nematode is much more specialised in its selection of host plants than other species of plant parasitic nematodes. In this context, an intimate knowledge of physiology and biochemistry of plants as well as nematodes are absolutely essential to understand the complicated areas of host-nematode interactions. Hence, 52 ridge gourd varieties/lines were collected from different sources for testing their resistance against Root-knot nematode, *Meloidogyne incognita* (Race-2) under pot culture condition in the greenhouse conditions.

### 4.1 Screening of ridge gourd germplasm/cultivars

The varieties were categorised into highly resistant(HR), resistant (R), moderately resistant (MR), susceptible (S) and highly susceptible (HS) types on the basis of their egg mass, root-knot indices following 1-5 scale (Taylor and Sasser, 1978).

Scale	No. of galls/eggs/eggmass	Reactions
1	0	Highly resistant (HR)
2	1-10	Resistant (R)
3	11-30	Moderately resistant (MR)
4	31-100	Susceptible (S)
5	More than 100	Highly susceptible (HS)

Ridge gourd (*Luffa acutangula* (L)Roxb.) is an important vegetable crop grown in almost all parts of India and is affected by numerous biotic factors including plant parasitic nematodes.

Out of the fifty two tested varieties, only 6 showed resistant, 7 showed moderately resistant, 32 susceptible & 5 highly susceptible to the test nematode, *M. incognita* under greenhouse condition (Table 1).

**Table 1. Screening of ridge gourd germplasm/cultivars against root-knot nematode (*Meloidogyne incognita*)**

Sl. No.	Varieties	No.of galls	Reaction	Final population*
1	Priya	8.66	Resistant	239.33 (2.37)
2	Challenger	10	Resistant	244.33 (2.38)
3	Narayana	26	Moderately Resistant	293.66 (2.46)
4	Aneeta	24.33	Moderately Resistant	458.33 (2.66)
5	NHRG-1001	27	Moderately Resistant	352.66 (2.54)
6	Maharashtra 16pata jhinga	61.66	Susceptible	762.66 (2.88)
7	Aarti	112.66	Highly Susceptible	1426 (3.15)
8	Hybrid Patal Tarai	47.33	Susceptible	985 (2.99)
9	Jaipur long	9	Resistant	271.66 (2.43)
10	Ridge gourd 12 pata	30.33	Moderately Resistant	455 (2.65)
11	12 Pata Jhinga	27	Moderately Resistant	471.66 (2.67)
12	Hybrid Jhinga	52.33	Susceptible	968.33 (2.98)
13	Harsha	116.33	Highly Susceptible	1226.66 (3.08)
14	SS-Ramu	47	Susceptible	975 (2.98)
15	Sumitra	91.33	Susceptible	762.66 (2.88)
16	BSS-1009	9.66	Resistant	241.66 (2.38)
17	Machar Jhinga	52.66	Suseptible	961 (2.98)
18	Debsundari	64.33	Suseptible	970 (2.99)
19	Ramu F1	46.66	Suseptible	761.66 (2.88)
20	Cluster	38.66	Suseptibe	971.66 (2.98)
21	Seventstar	32.33	Suseptible	772.66 (2.88)

<b>Sl. No.</b>	<b>Varieties</b>	<b>No.of galls</b>	<b>Reaction</b>	<b>Final population*</b>
22	Dhenkanal local	23	Moderately Resistant	428 (2.63)
23	Nayagarh local	31	Moderately Resistant	255 (2.40)
24	Athgarh local	58.66	Suseptible	935 (2.97)
25	Sankar pur local	61.33	Suseptible	941 (2.97)
26	Mira	49	Suseptible	935 (2.97)
27	Tauri	8.66	Resistant	941 (2.97)
28	Yeshvi-38	29.33	Moderately Resistant	775 (2.88)
29	Vaishali	51.66	Suseptible	265 (2.42)
30	Devika-776	47.33	Suseptible	420 (2.62)
31	Indo –US-216	26.33	Moderately Resistant	985 (2.99)
32	F1 Stella	115	Highly Susceptible	978.33 (2.99)
33	MHRG 7	45.66	Susceptible	435 (2.63)
34	Estilo	60.66	Susceptible	1615 (3.20)
35	SS-Chandrani	52.66	Susceptible	970 (2.98)
36	SE-19	47	Susceptible	767 (2.88)
37	Marwari	47	Susceptible	968.33 (2.98)
38	Krishna -51	61.33	Susceptible	975 (2.98)
39	DEB-2404	89.66	Susceptible	985 (2.99)
40	SC-18	60.66	Susceptible	967 (2.98)
41	Rohini	113.33	Highly Susceptible	746 (2.87)
42	Kaveri	57.33	Susceptible	961.66 (2.98)
43	Lavanya	31.33	Susceptible	1295 (3.11)

Sl. No.	Varieties	No.of galls	Reaction	Final population*
44	Avanti	45.66	Susceptible	772.66 (2.88)
45	BSS-1036	91	Susceptible	785 (2.89)
46	Saniya-4	51.33	Susceptible	985 (2.99)
47	Arun	46.33	Susceptible	978.33 (2.99)
48	Laila	114.33	Highly Susceptible	1472.33 (3.16)
49	NS-474	59.66	Susceptible	771.66 (2.88)
50	Lumbini	63	Susceptible	818.33 (2.91)
51	Pallishree	7.33	Resistant	763.33 (2.88)
52	Ankur Latika	31.33	Susceptible	651.66 (2.81)
	<b>SEM(±)</b>	<b>4.69</b>	-	<b>20.878</b>
	<b>CD(0.05)</b>	<b>13.13</b>	-	<b>58.521</b>
	<b>CV%</b>	<b>16.15</b>	-	<b>45.55</b>

\*Figures in parentheses are log transformed values

## 4.2 Evaluation of Ridge gourd varieties against root-knot nematode, *Meloidogyne incognita*

### 4.2.1 Effect of varietal reaction on nematode infection

Fifty two ridge gourd varieties/cultivars were screened and evaluated against root-knot nematode, *Meloidogyne incognita*. Out of which 6 showed resistant reaction with 7-10 number of galls per plant, 7 showed moderately resistant reaction with 23-31 number of galls per plant, 32 susceptible reaction with 31-91 number of galls per plants & 5 highly susceptible with 112-116 number of galls per plants.

**Table 2. Evaluation of ridge gourd varieties /cultivars against root-knot nematode, *Meloidogne incognita* (mean of three replications)**

S.I.No.	Varieties	Fresh shoot wt.(g)	Fresh root wt.(g)	Shoot length (cm)	Root length (cm)	No.of galls	Reactions	Root Knot index (1-5 scale)	Dry shoot wt.(g)	Dry root wt.(g)	Final population*
1	Priya	24.43	0.26	112.33	24.26	8.66	Resistant	2	8	0.13	239.33 (2.37)
2	Challenger	10.86	0.6	95.66	15.23	10	Resistant	1.66	6.03	0.26	244.33 (2.38)
3	Narayana	21.2	0.3	85.33	18.43	26	Moderately Resistant	2.66	4.06	0.13	293.66 (2.46)
4	Aneeta	10.3	2.53	83	20.16	24.33	Moderately Resistant	2.66	3.16	2.2	458.33 (2.66)
5	NHRG-1001	12.66	0.53	118	25.03	27	Moderately Resistant	3	2.56	0.33	352.66 (2.54)
6	Maharastra 16 pata jhinga	15.43	1.33	159	18.16	61.66	Susceptible	3.66	4.03	0.76	762.66 (2.88)
7	Aarti	15.9	2.4	99.66	24.83	112.66	Highly Susceptible	4.66	3.86	1.86	1426 (3.15)
8	Hybrid Patal Tarai	8.5	1.46	77.5	18.66	47.33	Susceptible	4	3.43	1.1	985 (2.99)
9	Jaipur long	12.66	0.5	84.66	14.5	9	Resistant	2	5.16	0.3	271.66 (2.43)
10	Ridge gourd 12 pata	8.46	0.83	72	38.66	30.33	Moderately Resistant	3	3	0.63	455 (2.65)
11	12 Pata Jhinga	11.93	1.13	71.33	28	27	Moderately Resistant	2.66	3.26	0.83	471.66 (2.67)

S.I.No.	Varieties	Fresh shoot wt.(g)	Fresh root wt.(g)	Shoot length (cm)	Root length (cm)	No.of galls	Reactions	Root Knot index (1-5 scale)	Dry shoot wt.(g)	Dry root wt.(g)	Final population*
12	Hybrid Jhinga	11.7	1.63	43	19.16	52.33	Susceptible	4.33	3.13	0.4	968.33 (2.98)
13	Harsha	15.33	1.36	113.33	23.56	116.33	Highly Susceptible	4.66	2.4	0.93	1226.66 (3.08)
14	SS-Ramu	22.96	1.8	16.83	16.2	47	Susceptible	3.66	5.46	1.43	975 (2.98)
15	Sumitra	11.73	1.63	56	32.33	91.33	Susceptible	4	2.56	1.3	762.66 (2.88)
16	BSS-1009	12.36	0.23	55.5	25.96	9.66	Resistant	2	2.76	0.13	241.66 (2.38)
17	Machar Jhinga	10.56	1.9	107.5	24.93	52.66	Susceptible	3.66	3.43	1.53	961 (2.98)
18	Debsundari	26.46	1.76	115	17.43	64.33	Susceptible	4.33	5.23	1.3	970 (2.99)
19	Ramu F1	14.76	2.26	100	11.36	46.66	Susceptible	3.66	3.66	1.6	761.66 (2.88)
20	Cluster	14.46	0.63	107.33	14.7	38.66	Susceptible	3.66	4.03	0.4	971.66 (2.98)
21	Seventstar	14.2	2.03	100.33	30.66	32.33	Susceptible	4.33	4.6	1.26	772.66 (2.88)
22	Dhenkanal local	6.53	0.56	62	13.83	23	Moderately Resistant	3	2.6	0.23	428 (2.63)
23	Nayagarh local	5.53	0.8	53.5	17	31	Moderately Resistant	3	1.96	0.63	255 (2.40)

S.I.No.	Varieties	Fresh shoot wt.(g)	Fresh root wt.(g)	Shoot length (cm)	Root length (cm)	No.of galls	Reactions	Root Knot index (1-5 scale)	Dry shoot wt.(g)	Dry root wt.(g)	Final population*
24	Athgarh local	11.53	1.16	125.66	18.03	58.66	Susceptible	4.33	4.26	0.43	935 (2.97)
25	Sankar pur local	11.46	1.4	155.66	16.66	61.33	Susceptible	4.33	4.33	3.83	941 (2.97)
26	Mira	22.03	1.16	78.66	19.33	49	Susceptible	4.33	33.2	3.46	935 (2.97)
27	Tauri	23.66	0.23	109.66	23.66	8.66	Resistant	2	4.9	6.36	941 (2.97)
28	Yeshvi-38	7.66	2.4	115	18.66	29.33	Moderately Resistant	2.66	3.23	2.76	775 ( 2.88)
29	Vaishali	10.63	0.66	59.33	17.46	51.66	Susceptible	4.33	5.43	4.06	265 (2.42)
30	Devika-776	11.96	0.6	59.33	18.66	47.33	Susceptible	2	3.33	3.43	420 (2.62)
31	Indo –US-216	11.7	0.4	61.66	38.33	26.33	Moderately Resistant	2.66	1.86	2.66	985 (2.99)
32	F1 Stella	14.06	1.3	110.66	23.66	115	Highly Susceptible	4.66	2.3	3.66	978.33 (2.99)
33	MHRG 7	11.1	1.4	154.66	17	45.66	Susceptible	3.66	4.06	3.13	435 (2.63)
34	Estilo	10.9	1.5	59	16.53	60.66	Susceptible	3.66	5.33	2.86	1615 (3.20)
35	SS-Chandrani	11.43	2.2	17.66	29.66	52.66	Susceptible	4	3.4	3.36	970 (2.98)

S.I.No.	Varieties	Fresh shoot wt.(g)	Fresh root wt.(g)	Shoot length (cm)	Root length (cm)	No.of galls	Reactions	Root Knot index (1-5 scale)	Dry shoot wt.(g)	Dry root wt.(g)	Final population*
36	SE-19	10.53	2.2	114.33	17.33	47	Susceptible	4.33	5.36	5.46	767 (2.88)
37	Marwari	10.53	1.23	99	18.66	47	Susceptible	4.33	3.4	3.26	968.33 (2.98)
38	Krishna -51	10.46	1.5	90.33	32	61.33	Susceptible	4.33	5.2	3.9	975 (2.98)
39	DEB-2404	10.53	1.2	102.33	29.66	89.66	Susceptible	4	5.4	3.46	985 (2.99)
40	SC-18	10.56	0.7	107	15	60.66	Susceptible	4.33	2.83	2.93	967 (2.98)
41	Rohini	13.03	1.23	109.33	23	113.33	Highly Susceptible	4.66	2.43	2.36	746 (2.87)
42	Kaveri	10.8	1.16	112.33	15	57.33	Susceptible	4.33	3.03	3.9	961.66 (2.98)
43	Lavanya	11.23	0.66	101.66	24.66	31.33	Susceptible	3.66	3.5	5.66	1295 (3.11)
44	Avanti	10.76	1.86	80.33	16	45.66	Susceptible	4	5.6	3.4	772.66 (2.88)
45	BSS-1036	11.43	0.6	160	29.66	91	Susceptible	4	3.36	4.6	785 (2.89)
46	Saniya-4	10.76	2.1	57.33	16.36	51.33	Susceptible	4.33	3.56	5.4	985 (2.99)
47	Arun	10.6	1.43	103.66	29.66	46.33	Susceptible	3.66	3	2.53	978.33 (2.99)

S.I.No.	Varieties	Fresh shoot wt.(g)	Fresh root wt.(g)	Shoot length (cm)	Root length (cm)	No.of galls	Reactions	Root Knot index (1-5 scale)	Dry shoot wt.(g)	Dry root wt.(g)	Final population*
48	Laila	14.66	2.06	114.66	22.33	114.33	Highly Susceptible	4.66	2.46	2.46	1472.33 (3.16)
49	NS-474	25.13	1.2	157.66	18.33	59.66	Susceptible	3.66	5.46	5.13	771.66 (2.88)
50	Lumbini	14.43	1.23	60	16.66	63	Susceptible	3.66	2.36	2.83	818.33 (2.91)
51	Pallishree	23.6	0.36	112.66	25.33	7.33	Resistant	2.33	5.16	2.86	763.33 (2.88)
52	Ankur Latika	13.53	1.23	60	29.66	31.33	Susceptible	4.33	3.4	4.03	651.66 (2.81)
	<b>SE(m)±</b>	<b>1.15</b>	<b>0.29</b>	<b>9.66</b>	<b>1.45</b>	<b>4.69</b>	-	<b>0.49</b>	<b>0.65</b>	<b>0.53</b>	<b>20.87</b>
	<b>CD(0.05)</b>	<b>3.22</b>	<b>8.23</b>	<b>27.04</b>	<b>4.06</b>	<b>13.13</b>	-	<b>1.39</b>	<b>1.84</b>	<b>1.49</b>	<b>58.52</b>
	<b>CV%</b>	<b>14.86</b>	<b>41.12</b>	<b>17.99</b>	<b>11.59</b>	<b>16.15</b>	-	<b>23.84</b>	<b>29.68</b>	<b>24.11</b>	<b>45.55</b>

\* Figures in parentheses are log transformed values

### **4.3 Biochemical compositions**

Many kinds of chemicals are present in the plant system that influence the metabolism of the pests, fed upon them. The chemicals may consist of simple nitrogen, phosphorus, potassium, carbohydrates and fats to complex proteins, enzymes, phenolic compounds etc., which may nourish, starve or kill the feeding organisms upon them. In the course of feeding some chemicals may be depleted or some others may be synthesized, de novo, that may be detrimental to the pest. In order to know the chemical and genetic basis of resistance, five varieties were chosen for the detailed analysis. These varieties were Priya and BSS-1009 (Resistant check), Aneeta, (Moderately Resistant) Aarti and Harsha (Susceptible check) included for detailed study. These plants were grown with utmost care, both in inoculated and controlled conditions. One set of each uninoculated (healthy) and inoculated (infected) plants were analysed to test the effect of root-knot nematode infection on the chemical composition, of the plants and their root-system and their effects on plant growth & vigour, nematode population, root-knot index, etc.

#### **4.3.1 Effect of the nematode infection on chlorophyll content**

Chlorophyll content was reduced from 0.42 mg/g to 0.25 mg/g due to nematode infection in the variety Priya. Similarly, other varieties BSS-1009, Aneeta, Aarti and Harsha. Chlorophyll 'a' was reduced from 0.40 to 0.20 mg/g, 0.42 to 0.22 mg/g, 0.33 to 0.26 mg/g and 0.26 to 0.15 mg/g respectively. The test nematode caused reduction of chlorophyll 'a' content of Priya by 40.47 per cent, 49.87 per cent in BSS-1009, 48.71 per cent in Aneeta, 19.33 per cent in Aarti and 42.69 per cent in Harsha over their healthy counterparts.

Chlorophyll 'b' content reduction from healthy to infected plants recorded were 0.08 to 0.06, 0.16 to 0.10, 0.13 to 0.10, 0.25 to 0.16 and 0.15 to 0.11 mg/g in varieties Priya, BSS-1009, Aneeta, Aarti and Harsha respectively. So the test nematode caused 24.50 per cent reduction of chlorophyll 'b' content of Priya, 33.75 per cent in BSS-1009, 25.73 per cent in Aneeta, 34.64 per cent in Aarti and 30.81 per cent reduction in Harsha over their healthy counterparts.

Similarly the total chlorophyll content was decreased from 0.59 to 0.23 mg/g in Priya, 0.59 to 0.15 mg/g in BSS-1009, 0.38 to 0.37 mg/g in Aneeta, 0.35 to 0.34 mg/g in Aarti and 0.31 to 0.21 mg/g in Harsha. The above decrease trend by nematode infection caused 60.67 per cent reduction in Priya, 73.78 per cent in BSS-1009, 1.32 per cent in Aneeta, 3.68 per cent in Aarti and 30.12 per cent in Harsha. (Table 3) over their healthy counterparts. Overall there was considerable reduction of chlorophyll 'a', chlorophyll 'b' and total chlorophyll due to nematode infection.

**Table 3. Variations in chlorophyll contents (a, b, total) in resistant and susceptible varieties as influenced by root-knot nematode, *M.incognita***

Sl. No.	Variety	Chlorophyll 'a' content mg/g leaf				Chlorophyll 'b' content mg/g leaf				Total chlorophyll content mg/g leaf			
		Infected (I)	Healthy (H)	Mean	% decrease (-) over healthy	Infected (I)	Healthy (H)	Mean	% decrease (-) over healthy	Infected (I)	Healthy (H)	Mean	% decrease (-) over healthy
1	Priya	0.25	0.42	0.33	-40.47	0.06	0.08	0.07	-24.70	0.23	0.59	0.41	-60.67
2	BSS-1009	0.20	0.40	0.30	-49.87	0.10	0.16	0.13	-33.75	0.15	0.59	0.37	-73.78
3	Aneeta	0.22	0.42	0.32	-48.71	0.10	0.13	0.11	-25.73	0.37	0.38	0.37	-2.63
4	Aarti	0.26	0.33	0.29	-19.33	0.16	0.25	0.21	-34.64	0.34	0.35	0.34	-3.68
5	Harsha	0.15	0.26	0.21	-42.69	0.11	0.15	0.13	-30.81	0.21	0.31	0.26	-30.12
	<b>SE(m) ±</b>	<b>0.02</b>	<b>0.20</b>			<b>0.03</b>	<b>0.02</b>			<b>0.18</b>	<b>0.14</b>		
	<b>CD(0.05)</b>	<b>0.07</b>	<b>0.65</b>			<b>0.10</b>	<b>0.07</b>			<b>0.59</b>	<b>0.46</b>		

#### **4.3.2 Variations in Nitrogen Contents in the resistant/susceptible ridge gourd varieties as influenced by root knot nematode, *M.incognita***

Root-knot nematodes are known to reduce the chlorophyll contents of the plants by disrupting its nutrient uptake and partitioning of the photosynthates.

The total Nitrogen content was decreased in the shoot system of infected varieties of ridge gourd ,Priya,BSS-1009,Aneeta,Aarti &Harsha by 0.36, 0.46, 0.44, 0.35 & 0.28 percent respectively over their healthy counterparts on dry weight basis,whereas nitrogen content in the roots was increased by 0.49, 0.44, 0.47, 0.67 & 0.48 percent in all the above mentioned varieties accordingly due to nematode infestation over healthy counterparts.

The total Nitrogen content was decreased in all shoot system by 20% in resistant variety and 40.42% in susceptible variety but the same was increased by 88.46% in the root system of resistant variety as compared to susceptible one (2.12%) (Table 4).

The present results of increase of total Nitrogen content of nematode infected root samples is in confirmation with the findings of the earlier workers (Zaki and Bhatti,1986,Nayak,2006)

The nutrient accumulation in infected roots maybe due to impaired translocation to the aerial parts or the immobilization of nutrients from shoot to root.The percentage increase in nitrogen content in shoots of resistant varieties may be due to higher absorption capacity or higher requirement for the resistance mechanisms.

#### **4.3.3 Estimation of Crude Protein Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M.incognita***

The percentage of shoot crude protein content was low as 20.35% in variety Priya,6.20% in BSS-1009,14.59% in Aneeta,5.57% in Aarti &40.27% in Harsha,whereas crude protein content in root was highest in Priya (86.66%) followed by BSS-1009(58.28%),Aneeta(0.68%),Aarti(61.06%) and Harsha (1.01%) (Table 5).

The crude protein of both the infected shoot decreased and infected root increased in the similar trend like Nitrogen content of the infected shoot.The increased crude protein content in roots of nematode infected plants was reported by various workers(Ganguly and Dasgupta, 1983; Devarajan and Rajendran, 2002; Vaitheeswaran *et al.*, 2005, Nayak, 2006)

**Table 4. Estimation of Nitrogen Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M. incognita***

Sl. No.	Varieties	Nitrogen content % on dry weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)
01	Priya	0.36	0.45	0.40	-20	0.49	0.26	0.37	88.46
02	BSS-1009	0.46	0.49	0.47	-6.12	0.44	0.28	0.36	57.14
03	Aneeta	0.44	0.51	0.47	-13.72	0.47	0.46	0.46	2.17
04	Aarti	0.35	0.37	0.36	-5.40	0.67	0.42	0.54	59.52
05	Harsha	0.28	0.47	0.37	-40.42	0.48	0.47	0.47	2.12
	SE(m)±	0.28	0.15			0.2	0.02		
	CD(0.05)	0.92	0.51			0.65	0.07		

**Table 5. Estimation of Crude Protein Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M. incognita***

Sl. No.	Varieties	Crude Protein Content % on dry weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)
01	Priya	2.27	2.85	2.56	-20.35	3.08	1.65	2.36	86.66
02	BSS-1009	2.87	3.06	2.96	-6.20	2.77	1.75	2.26	58.28
03	Aneeta	2.75	3.22	2.98	-14.59	2.93	2.91	2.92	0.68
04	Aarti	2.20	2.33	2.26	-5.57	4.22	2.62	3.42	61.06
05	Harsha	1.75	2.93	2.34	-40.27	3.00	2.97	2.98	1.01
	SE(m)±	1.78	0.99			1.25	0.13		
	CD(0.05)	5.82	3.22			4.07	0.44		

#### **4.3.4 Influence of nematode infection on the Phosphorous content of resistant/susceptible ridge gourd varieties/cultivars.**

The Phosphorus content was decreased in the shoot system of infected varieties of ridge gourd, Priya, BSS-1009, Aneeta, and Harsha by 92%, 11.42%, 62.74% and 50.87% respectively except Aarti (113.33%) and increased in the infected roots by 0.57, 0.26, 0.44, 0.49, 0.75 percent in ridge gourd varieties Priya, BSS-1009, Aneeta, Aarti and Harsha respectively over their healthy counterparts on dry weight basis.

Phosphorus content was recorded highest as 850% in infected roots of variety Priya followed by 420% in BSS-1009, 57.14% in Aneeta, 50% in Harsha and 8.88% in Aarti (Table 6). The results of present investigation revealed that the infected plants had decreased percent of phosphorus content in shoots of crop variety irrespective to their reaction to the test nematode. Similar trend was also observed by Hunter, 1958; Chakraborty and Mishra, 2002 in root-knot nematode infected plants. The amino acid, protein, Sugar and absorbed  $N_2$ ,  $P_2O_5$  and  $K_2O$  and other elements accumulate in the roots of infected plants and remain unutilised by the plant systems which ultimately find the path into the growth and production of nematode. This may be the possible reasons for the accumulation of phosphorus in nematode infected plants (Table 6).

#### **4.3.5 Estimation of Potassium Contents in the resistant/susceptible ridgegourd varieties influenced by root knot nematode, *M.incognita***

Potassium content of root-knot nematode infected ridge gourd varieties measured an increase, to the tune of 208.33, 175, 27.27 percent in shoots of varieties Aneeta, BSS-1009, & Priya respectively while decrease was 75 percent in Harsha and 72.09 percent in Aarti. In the roots of inoculated plants potassium content was increased by 850, 420, 67.85, 8.88 and 50 percent in varieties Priya, BSS-1009, Aneeta, Aarti, and Harsha respectively. Table 4 revealed that there was an increase of potassium content in both susceptible and resistant infected plants which was more pronounced in both shoots and roots of resistant varieties as compared to the susceptible varieties (Table 7).

In the nematode infected plant tissues various compounds relating to ion-exchange may be decreased which is reflected by the reduction of 'K' content in nematode infected plant samples.

**Table 6. Estimation of Phosphorus Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M.incognita***

Sl. No.	Varieties	Phosphorous content % on dry weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)
01	Priya	0.06	0.75	0.40	-92.00	0.57	0.06	0.31	850
02	BSS-1009	0.31	0.35	0.33	-11.42	0.26	0.05	0.15	420
03	Aneeta	0.19	0.51	0.35	-62.74	0.44	0.28	0.36	57.14
04	Aarti	0.32	0.15	0.23	113.33	0.49	0.45	0.47	8.88
05	Harsha	0.28	0.57	0.42	-50.87	0.75	0.50	0.62	50
	SE(m)±	0.07	0.19			0.15	0.15		
	CD(0.05)	0.25	0.62			0.49	0.51		

**Table 7. Estimation of potassium contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M.incognita***

Sl. No.	Varieties	Potassium content % on dry weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)
01	Priya	0.42	0.33	0.37	27.27	0.57	0.06	0.31	850
02	BSS-1009	0.33	0.12	0.22	175	0.26	0.05	0.15	420
03	Aneeta	0.37	0.12	0.24	208.33	0.47	0.28	0.37	67.85
04	Aarti	0.12	0.43	0.27	-72.09	0.49	0.45	0.47	8.88
05	Harsha	0.19	0.76	0.95	-75	0.75	0.50	0.62	50
	SE(m)±	0.05	0.07			0.11	0.15		
	CD(0.05)	0.17	0.22			0.37	0.51		

#### 4.3.6 Action of nematode infection on total sugar content

##### Total sugar content in shoots

The amount of sugar present in the shoot portion of the inoculated plants were recorded as 2.22, 2.21, 1.82, 1.42, 1.76 mg/g of the varieties Priya, BSS-1009, Aneeta, Aarti and Harsha respectively on fresh weight basis (Table 8). In the

healthy shoots of these varieties sugar contents were 2.00, 1.83, 1.58, 1.05 and 1.68 mg/g respectively. The percentage of increase in total sugar content due to rootknot nematode infection over healthy counterparts was noted to be 11.00, 20.76, 15.18, 35.23 and 4.76 per cent respectively.

### Total sugar contents in roots

The total sugar content in the healthy roots of ridge gourd varieties was 2.85, 2.22, 2.02, 1.59 and 1.05 mg/g in the varieties Priya, BSS-1009, Aneeta, Aarti and Harsha respectively (Table 8) on fresh weight basis. In the infected roots of these varieties sugar contents were 2.98, 2.34, 2.21, 1.99 and 1.49 mg/g respectively. The percentage of increase in total sugar content over control was noted to be 4.56, 5.41, 9.41, 25.15 and 41.90 per cent respectively.

**Table 8 Estimation of Percentage increase/decrease of total sugar content in the root and shoot of ridge gourd varieties as influenced by *M.incognita* infection**

Sl. No.	Varieties	Total sugar content mg/g on fresh weight basis							
		Shoot (leaf)				Root			
		Infected (I)	Healthy (H)	Mean	%increase(+)/decrease (-) over healthy	Infected (I)	Healthy (H)	Mean	%increase(+)/decrease (-) over healthy
01	Priya	2.22	2.00	2.11	11.00	2.98	2.85	2.92	4.56
02	BSS-1009	2.21	1.83	2.02	20.76	2.34	2.22	2.28	5.41
03	Aneeta	1.82	1.58	1.7	15.18	2.21	2.02	2.12	9.41
04	Aarti	1.42	1.05	1.24	35.23	1.99	1.59	1.79	25.15
05	Harsha	1.76	1.68	1.72	4.76	1.49	1.05	1.27	41.90
	<b>SE(m)±</b>	<b>0.03</b>	<b>0.09</b>			<b>0.28</b>	<b>0.12</b>		
	<b>CD(0.05)</b>	<b>0.10</b>	<b>0.29</b>			<b>0.91</b>	<b>0.39</b>		

### 4.3.7 Effect of nematode infection on total starch content:

#### Total starch content in shoots

The amount of starch present in the shoots of the inoculated plants were recorded as 1.48, 2.12, 2.24, 2.59 & 3.05 mg/g in the varieties Priya, BSS-1009, Aneeta, Aarti and Harsha respectively on fresh weight basis (Table 9). Conversely this amount was increased in all cases than healthy plants i.e., 8.71, 9.80, 5.53 per cent, in varieties BSS-1009, Aneeta, Harsha except decrease as 22.10 percent in variety Priya & 8.80 per cent in variety Aarti in the leaf portion of these varieties by the root-knot infection.

### Total starch content in roots

The amount of starch contained in the healthy roots of varieties was 1.41, 1.46, 1.64, 1.56, 1.59 mg/g in Priya, BSS-1009, Aneeta, Aarti and Harsha respectively (Table 9) on fresh weight basis. In the infected roots of these varieties starch contents were 1.27, 1.41, 1.51, 1.45 and 1.55 mg/g respectively. The percentage of decrease in starch content by rootknot nematode infection over control was noted to be 9.92, 3.42, 7.92, 7.05 and 2.51 per cent respectively.

**Table 9 Percentage change in total starch content in healthy (H) and root-knot infected (I) plant**

Sl. No.	Varieties	Starch content mg/g on fresh weight basis							
		Shoot (leaf)				Root			
		Infected (I)	Healthy (H)	Mean	%increase(+)/decrease (-) over healthy	Infected (I)	Healthy (H)	Mean	%increase(+)/decrease (-) over healthy
01	Priya	1.48	1.90	1.69	-22.10	1.27	1.41	1.34	-9.92
02	BSS-1009	2.12	1.95	2.03	8.71	1.41	1.46	1.43	-3.42
03	Aneeta	2.24	2.04	2.14	9.80	1.51	1.64	1.57	-7.92
04	Aarti	2.59	2.84	2.71	-8.80	1.45	1.56	1.50	-7.05
05	Harsha	3.05	2.89	2.97	5.53	1.55	1.59	1.57	-2.51
	<b>SE(m)±</b>	<b>2.30</b>	<b>0.17</b>			<b>1.31</b>	<b>0.12</b>		
	<b>CD(0.05)</b>	<b>7.49</b>	<b>0.57</b>			<b>4.27</b>	<b>0.39</b>		

### 4.3.8 Effect of nematode infection on contents of phenolic substances

#### Total phenol content in shoots

The proline content of infected shoots of ridge gourd cultivars were 0.12, 0.13, 0.08, 0.07 and 0.08 µg/g in Priya, BSS-1009, Aneeta, Aarti and Harsha respectively. Moreover, the phenol contents of these varieties increased by 21.15, 1.55, 10.95, 54.90 and 3.89 percent in shoots and 32.97, 7.57, 27.27, 2.94 and 3.33 percent in roots respectively in the above said varieties due to the root-knot infection (Table 10).

The phenol content of infected roots of ridge gourd cultivars were 0.12, 0.07, 0.05, 0.07 and 0.13 µg/g in Priya, BSS-1009, Aneeta, Aarti and Harsha respectively. Moreover, phenol contents of these varieties increased by 32.97, 7.57, 27.27, 2.94 and 3.33 percent in roots, due to the root-knot infection. (Table 10)

**Table 10 Estimation of %increase /decrease in Phenol content in Healthy (H) and root-knot infected (I) plant**

**Total phenol content in roots**

S.I. No.	Varieties	Phenol content µg/g on fresh weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)
01	Priya	0.12	0.10	0.11	21.15	0.12	0.09	0.10	32.97
02	BSS-1009	0.13	0.12	0.13	1.55	0.07	0.06	0.06	7.57
03	Aneeta	0.08	0.07	0.07	10.95	0.05	0.04	0.05	27.27
04	Aarti	0.07	0.05	0.06	54.90	0.07	0.06	0.06	2.94
05	Harsha	0.08	0.07	0.07	3.89	0.13	0.12	0.12	3.33
	SE(m)±	1.02	0.64			0.91	1.44		
	CD(0.05)	3.35	2.09			2.99	4.69		

**4.3.9 Effect of nematode infection on the contents of proline substances**

**Total proline content in shoots**

The proline contents of infected shoots of ridge gourd cultivars were 5.59, 5.87, 6.77, 7.29 and 7.62 mg/g in Priya, BSS-1009, Aneeta, Aarti and Harsha respectively. Moreover, the proline contents of these varieties increased by 5.87, 13.98, 6.44, 14.08 and 0.39 percent in shoots and 8.82, 6.37, 4.78, 18.09 and 15.72 percent in roots respectively in the above said varieties by the root-knot infection (Table 11). The amount of proline content was more in both shoots and roots of resistant cultivars than susceptible ridge gourd cultivars.

**Total proline content in roots**

The proline content of infected roots of ridge gourd cultivars were 5.67, 5.51, 7.23, 7.57 and 7.36 mg/g in Priya, BSS-1009, Aneeta, Aarti and Harsha respectively. Moreover, the phenol contents of these varieties increased by 8.82, 6.37, 4.78, 18.09 and 15.72 percent in roots, by the root-knot infestation (Table 11).

**Table 11 Percentage change in proline content in healthy (H) and root-knot infected (I) plant**

Sl. No.	Varieties	Proline content mg/g on fresh weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)
01	Priya	5.59	5.28	5.43	5.87	5.67	5.21	5.44	8.82
02	BSS-1009	5.87	5.15	5.51	13.98	5.51	5.18	5.34	6.37
03	Aneeta	6.77	6.36	6.56	6.44	7.23	6.90	7.06	4.78
04	Aarti	7.29	6.39	6.84	14.08	7.57	6.41	6.99	18.09
05	Harsha	7.62	7.59	7.61	0.39	7.36	6.36	6.86	15.72
	SE(m)±	0.20	0.24			0.18	0.16		
	CD(0.05)	0.65	0.78			0.61	0.52		

#### **4.3.10 Variation in Micro-nutrient Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M.incognita***

##### **Effect of nematode infection on calcium content**

The calcium content was decreased in the shoot system of infected varieties of ridge gourd Priya, BSS-1009, Aneeta, Aarti and Harsha by 17.33, 26.38, 24 and 32.66 mg/100g and in root system by 14.66, 24.34, 21.33 and 28.66 mg/100g respectively. It was depicted from Table 12 that the calcium content was recorded highest in a decreased trend as 40.55% in infected roots of variety Priya followed by 29.51% in Harsha, 23.39% in BSS-1009, 12.05% in Aneeta and 5.86% in Aarti over control on dry weight basis. The results of the present investigation revealed that the infected plants had decreased percentage of calcium content in both shoots and roots of susceptible and resistant varieties. More accurately, there was an increase trend of calcium content from susceptible varieties to resistant one. The above data is in confirmation with the research findings of Mohanty *et al.* (1999) indicating there was reduction of micro nutrients viz. Zn, Cu, Fe, Mn, Mo and B on cowpea when inoculated with *R. reniformis*.

**Table 12. Estimation of Calcium Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M.incognita***

Sl. No.	Varieties	Calcium content ppm on dry weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy(%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy(%)
01	Priya	17.33	27.33	22.33	-36.58	14.66	24.66	19.66	-40.55
02	BSS-1009	26.00	40.66	33.33	-36.05	24	31.33	27.66	-23.39
03	Aneeta	38.00	38.66	38.33	-1.70	34	38.66	36.33	-12.05
04	Aarti	24.00	28.66	26.33	-16.25	21.33	22.66	21.99	-5.86
05	Harsha	32.66	42.66	37.66	-23.44	28.66	40.66	34.66	-29.51
	SE(m)±	1.42	1.9			1.59	0.72		
	CD(0.05)	4.62	6.19			5.18	2.34		

#### 4.3.11 Effect of nematode infection on magnesium content

The magnesium content was decreased in the shoot of infected varieties of ridge gourd Priya, BSS-1009, Aneeta, Aarti and Harsha by 14, 16, 21.33, 12 and 24 ppm and in root system by 10.66, 12, 12.66, 9.33 and 15.33 ppm respectively. It was observed that magnesium content was highest in a decreasing order as 35.69% in infected roots of variety BSS-1009 followed by 32.15% in Aneeta, 28.12% in Harsha, 17.65% in Aarti and 5.91% in Priya over control on dry weight basis. (Table 13) The results of present investigation revealed that the infected plants had decreased percentage of magnesium content in both shoots and roots of susceptible and resistant varieties. The above data is in confirmation with the research findings of Mohanty *et al.* (1999) indicating that there was reduction of micro nutrients viz. Zn, Cu, Fe, Mn, Mo and B on cowpea inoculated with *R.reniformis*.

**Table 13 Estimation of Magnesium Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M.incognita***

Sl. No.	Varieties	Magnesium content ppm on dry weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy(%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy(%)
01	Priya	14	17.33	15.66	-19.21	10.66	11.33	10.99	-5.91
02	BSS-1009	16	20.66	18.33	-22.55	12	18.66	15.33	-35.69
03	Aneeta	21.33	25.33	23.33	-15.79	12.66	18.66	15.66	-32.15
04	Aarti	12	14.66	13.33	-18.14	9.33	11.33	10.33	-17.65
05	Harsha	24	30.66	27.33	-21.72	15.33	21.33	18.33	-28.12
	SE(m)±	1.51	2.02			1.27	1.85		
	CD(0.05)	4.92	6.58			4.15	6.03		

#### 4.3.12 Effect of nematode infection on sulphur content

The sulphur content was decreased in the shoot system of infected varieties of ridge gourd Priya, BSS-1009, Aneeta, Aarti and Harsha by 0.29, 0.36, 0.02, 0.02 and 0.02 % and in root system by 0.02, 0.03, 0.03, 0.02 and 0.02 % respectively. It was depicted that sulphur content was recorded highest as 93.58% in infected roots of variety BSS-1009, followed by 93.35% in Aarti, 92.94% in Harsha, 91.02% in Aneeta and 17.85% in Priya over control on dry weight basis. (Table 14) The results of present investigation revealed that the infected plants had decreased percentage of sulphur content in both shoots and roots of susceptible and resistant varieties .

**Table 14. Estimation of Sulphur Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M.incognita***

Sl. No.	Varieties	Sulphur content % on dry weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy(%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy(%)
01	Priya	0.29	0.36	0.32	-18.45	0.02	0.03	0.02	-17.85
02	BSS-1009	0.36	0.42	0.39	-15.49	0.03	0.48	0.25	-93.58
03	Aneeta	0.02	0.33	0.18	-91.66	0.03	0.32	0.17	-91.02
04	Aarti	0.02	0.03	0.02	-13.33	0.02	0.34	0.18	-93.35
05	Harsha	0.02	0.35	0.191	-91.78	0.02	0.34	0.18	-92.94
	SE(m)±	0.02	0.01			0.72	0.38		
	CD(0.05)	0.05	0.06			2.37	0.12		

#### 4.3.13 Effect of nematode infection on copper content

The copper content was decreased in shoot of infected varieties Priya, BSS-1009, Aneeta, Aarti and Harsha by 6.56, 4.3, 5.56, 5.93 and 3.6 ppm and in root by 5.93, 3.06, 1.63, 6.56 and 2.63 ppm respectively, but copper content was recorded highest percentage in a decreasing trend as 61.46 in infected roots of variety Aneeta, followed by 44.66 in BSS-1009, 29.49 in Harsha, 20.29 in Aarti and 10.55 in Priya over healthy counterparts on dry weight basis. The results of present investigation revealed that the infected plants had decreased percentage of copper content in both shoots and roots of susceptible and resistant varieties. More accurately, there was an increase trend of decrease of copper content from susceptible varieties to resistant one, Table 15. The above data is in confirmation with the investigation of Nasar *et al.* (1980) who reported increase in concentration of calcium, magnesium, copper, in both leaves and roots of ridge gourd infested with root-knot nematode.

**Table 15. Estimation of Copper Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M. incognita***

Sl. No.	Varieties	Copper content ppm on dry weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)
01	Priya	6.56	8.23	7.39	-20.29	5.93	6.63	6.28	-10.55
02	BSS-1009	4.3	6.7	5.5	-35.82	3.06	5.53	4.29	-44.66
03	Aneeta	5.56	8	6.78	-30.5	1.63	4.23	2.93	-61.46
04	Aarti	5.93	7.5	6.71	-20.93	6.56	8.23	7.39	-20.29
05	Harsha	3.6	6	6.6	-40	2.63	3.73	3.18	-29.49
	SE(m)±	0.70	0.87			0.49	0.38		
	CD(0.05)	2.30	2.85			1.60	1.26		

#### 4.3.14 Effect of nematode infection on iron content

The iron content was decreased in the shoot system of infected varieties Priya, BSS-1009, Aneeta, Aarti and Harsha by 18.30, 22.10, 17.74, 14.33 and 18.25 ppm and in root system by 11.22, 22.07, 22.11, 14.32 and 24.15 ppm respectively, but the iron content was recorded highest percentage in a decreasing order as 38.65% in infected roots of variety Priya followed by 31.15% in Aarti, 15.18% in Aneeta, 13.11% in BSS-1009 and 2.34% in Harsha over healthy counterparts on dry weight basis. The present investigation revealed that the infected plants had decreased percentage of iron content in both shoots and roots of susceptible and resistant

varieties .More accurately,there was a negatively increase trend of iron content from susceptible varieties to resistant one(Table 16).Present findings is in confirmation with the findings of Mohanty *et al.* (1999) indicating there was reduction of micro nutrients viz. Zn,Cu,Fe,Mn,Mo and B on cowpea when inoculated by *R.reniformis*.

**Table 16. Estimation of Iron Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode,*M.incognita***

Sl. No.	Varieties	Iron content ppm on dry weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy(%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy(%)
01	Priya	18.30	15.34	16.82	19.29	11.22	18.29	14.75	-38.65
02	BSS-1009	22.10	26.10	24.1	-15.32	22.07	25.40	23.73	-13.11
03	Aneeta	17.74	20.80	19.27	-14.71	22.11	26.07	24.09	-15.18
04	Aarti	14.33	15.53	14.93	-7.72	14.32	20.8	17.56	-31.15
05	Harsha	18.25	24.47	21.36	-25.41	24.15	24.73	24.44	-2.34
	SE(m)(±)	3.05	1.51			1.92	1.83		
	CD(0.05)	9.95	4.92			6.25	5.99		

#### 4.3.15 Effect of nematode infection on zinc content

The zinc content was decreased in the shoot system of infected varieties Priya,BSS-1009,Aneeta,Aarti and Harsha by 20.76,16.66,35.56,22.56 and 20.13 ppm and in root system by 18.73,9.43,5.36,19.33 and 4.13 ppm respectively,but the zinc content was recorded highest percentage in a decreasing order as 47.91% in infected roots of variety Harsha followed by 39.09% in Aneeta,31.39 % in Priya,16.98% in BSS-1009 and 3.63% in Aarti over healthy counterparts on dry weight basis.Present investigation revealed that the infected plants had decreased percentage of zinc content in both shoots and roots of susceptible and resistant varieties (Table 17) .More accurately,there was a negatively increase trend of zinc content from susceptible varieties to resistant one.The present findings is in confirmation with the findings of Mohanty *et al.* (1999) indicating there was reduction of micro nutrients viz. Zn,Cu,Fe,Mn,Mo and B on cowpea when inoculated with *R.reniformis*.

**Table 17 Estimation of Zinc Contents in the resistant/susceptible ridgegourd varieties influenced by root knot nematode, *M.incognita***

S.I. No.	Varieties	Zinc content ppm on dry weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)
01	Priya	20.76	36.83	28.79	-43.63	18.73	27.3	23.01	-31.39
02	BSS-1009	16.66	44.53	30.59	-62.58	9.43	11.36	10.39	-16.98
03	Aneeta	35.56	37.13	36.34	-4.22	5.36	8.80	7.08	-39.09
04	Aarti	22.56	26.96	24.76	-16.32	19.33	20.06	19.69	-3.63
05	Harsha	20.13	20.32	20.21	-0.83	4.13	7.93	6.03	-47.91
	SE(m)±	2.37	5.92			2.57	1.35		
	CD(0.05)	7.72	19.29			8.37	4.40		

#### 4.3.16 Effect of nematode infection on manganese content

The manganese content was decreased in the shoot of infected varieties Priya, BSS-1009, Aneeta, Aarti and Harsha by 42.5, 47, 32.7, 56.4 and 69.9 ppm and in root system by 111.6, 85.6, 184.4, 149.6, and 38.7 ppm respectively, but the zinc content was highest in a decreasing order as 31.38% in the roots of variety Harsha followed by 15.66% in BSS-1009, 13.62% in Priya, 13.38% in Aneeta and 2.73% in Aarti over healthy counterpart on dry weight basis. The present investigation revealed that the infected plants had decreased percentage of magnesium content in both shoots and roots of susceptible and resistant varieties (Table 18) during post infection period.

**Table 18 Estimation of Manganese Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M. incognita***

Sl. No.	Varieties	Manganese content ppm on dry weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)
01	Priya	42.5	46.7	44.6	-8.99	111.6	129.2	120.4	-13.62
02	BSS-1009	47	52.8	49.9	-10.98	85.6	101.5	93.55	-15.66
03	Aneeta	32.7	36.9	34.8	-11.38	184.4	212.9	198.65	-13.38
04	Aarti	56.4	72.4	64.4	-22.09	149.6	153.8	151.7	-2.73
05	Harsha	69.9	77	73.45	-9.22	38.7	56.4	47.55	-31.38
	SE(m)±	2.78	2.70			2.93	1.44		
	CD(0.05)	9.06	8.80			9.55	4.69		

#### 4.3.17 Effect of nematode infection on protein content

##### Total Protein in shoots

The total protein contents in the shoots portion of the inoculated plants were recorded as 5.21,5.39,7.11,4.50,3.90 mg/g of the varieties Priya,BSS-1009,Aneeta,Aarti and Harsha respectively on fresh weight basis. In the healthy shoots of these varieties total protein contents were 5.31,7.54,7.32,4.52 and 4.12 mg/g respectively. The percentage of decrease in total protein content over healthy counterpart was recorded as 1.88,28.51,2.86,0.44,5.33 per cent respectively during post infection period.(Table 19)

##### Total Protein in roots

Total protein contents in the healthy roots of varieties were 8.77, 4.64, 7.76, 7.76 and 7.11 mg/g of the varieties Priya,BSS-1009,Aneeta,Aarti and Harsha respectively on fresh weight basis. In the infected roots of these varieties protein contents were 9.98, 6.09, 8.05, 9.33 and 8.12 mg/g respectively. The percentage of increase in total protein contents over healthy counterpart recorded as 13.79, 31.25, 3.73, 20.23 and 14.20 per cent respectively,during post infection period. (Table 19)

**Table 19 Estimation of percentage changes in total protein content in shoots & shoots of healthy (H) and root-knot infected (I) ridge gourd plants**

Sl. No.	Varieties	Protein content mg/g on fresh weight basis							
		Shoot (leaf)				Root			
		Infected (I)	Healthy (H)	Mean	%increase(+)/decrease (-) over healthy	Infected (I)	Healthy (H)	Mean	%increase(+)/decrease (-) over healthy
01	Priya	5.21	5.31	5.26	-1.88	9.98	8.77	9.37	13.79
02	BSS-1009	5.39	7.54	6.46	-28.51	6.09	4.64	5.36	31.25
03	Aneeta	7.11	7.32	7.21	-2.86	8.05	7.76	7.9	3.73
04	Aarti	4.50	4.52	4.51	-0.44	9.33	7.76	8.54	20.23
05	Harsha	3.90	4.12	4.01	-5.33	8.12	7.11	7.61	14.20
	<b>SE(m)±</b>	<b>0.27</b>	<b>0.33</b>			<b>0.18</b>	<b>0.30</b>		
	<b>CD(0.05)</b>	<b>0.88</b>	<b>1.07</b>			<b>0.58</b>	<b>0.99</b>		

#### 4.3.18 Effect of nematode infection on free amino acid contents

##### Total free amino acid in shoots

The free amino acid present in the shoot portion of the infected plants were recorded as 2.92,3.08,4.36,6.01 and 4.84  $\mu\text{g/g}$  of the varieties Priya,BSS-1009,Aneeta,Aarti and Harsha respectively on fresh weight basis. In the healthy shoots of these varieties free amino acid contents were 1.04, 2.99, 4.10, 5.39 and 3.16  $\mu\text{g/g}$  respectively. The percentage of decrease in free amino acid contents over healthy counterpart were recorded as 180.76, 3.01, 6.34, 11.50 and 53.16 per cent respectively,during post infection period.(Table 20)

##### Total free amino acid in roots

The amount of free amino acid present in the root portion of the inoculated plants were recorded as 7.13,6.80,7.11,7.94 and 8.08  $\mu\text{g/g}$  of the varieties Priya,BSS-1009,Aneeta,Aarti and Harsha respectively on fresh weight basis .In the healthy roots of these varieties free amino acid contents were 6.03,6.61,6.03,6.76 and 7.15  $\mu\text{g/g}$  respectively. The percentage of decrease in free amino acid content due to root knot nematode infection over healthy counterpart was observed as 18.24,2.87,17.91,17.45 and 13.01 per cent respectively, during post infection period(Table 20).

**Table 20. Estimation of Total free amino acid Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M. incognita***

Sl. No.	Varieties	Total free amino acid content in $\mu\text{g/g}$							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	Increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)
01	Priya	2.92	1.04	1.98	180.76	7.13	6.03	6.58	18.24
02	BSS-1009	3.08	2.99	3.03	3.01	6.8	6.61	6.70	2.87
03	Aneeta	4.36	4.1	4.23	6.34	7.11	6.03	6.57	17.91
04	Aarti	6.01	5.39	5.70	11.50	7.94	6.76	7.35	17.45
05	Harsha	4.84	3.16	4.00	53.16	8.08	7.15	7.61	13.01
	SE(m) $\pm$	0.236	0.346			0.540	0.334		
	CD(0.05)	0.646	0.948			1.480	0.915		

#### 4.3.19 Activity of phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) during post infection period

##### The activity of PAL in shoots

The Phenylalanine Ammonia Lyase was measured as  $\mu$ moles of t-cinnamic acid formed per mg protein per minute of fresh shoot extract. These were 0.98, 0.83, 0.82, 0.81 and 0.83  $\mu$ moles t-cinnamic acid/mg protein/min in the healthy varieties Priya, BSS-1009, Aneeta, Aarti and Harsha respectively. In the inoculated shoots these values increased to 2.77, 1.03, 1.04, 1.01 and 0.95  $\mu$ moles t-cinnamic acid/mg protein/min respectively, registering 182.65, 24.09, 26.82, 24.69 and 14.45 per cent enhancement over healthy counterpart (Table 21).

##### The activity of PAL in roots

It was measured as  $\mu$ moles of t-cinnamic acid formed per mg protein per minute of fresh root extract and these were 1.93, 2.05, 1.71, 1.82 and 0.92  $\mu$ moles t-cinnamic acid/mg protein/min in Priya, BSS-1009, Aneeta, Aarti and Harsha, respectively in healthy varieties. In the inoculated roots these values increased to 2.20, 2.56, 2.50, 2.07 and 1.80  $\mu$ moles t-cinnamic acid/mg protein/min respectively, registering 13.98, 24.87, 46.19, 13.73 and 95.65 per cent enhancement over healthy counterparts during post infection periods of the crop by root-knot nematode, *M. incognita* (Table 21).

**Table 21. Estimation of phenylalanine ammonia Lyase (PAL) Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M. incognita***

Sl. No.	Varieties	phenylalanine ammonia Lyase (PAL) content in $\mu$ moles t-cinnamic acid/mg protein/min							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)
01	Priya	2.77	0.98	1.87	182.65	2.2	1.93	2.06	13.98
02	BSS-1009	1.03	0.83	0.93	24.09	2.56	2.05	2.30	24.87
03	Aneeta	1.04	0.82	0.93	26.82	2.5	1.71	2.10	46.19
04	Aarti	1.01	0.81	0.91	24.69	2.07	1.82	1.94	13.73
05	Harsha	0.95	0.83	0.89	14.45	1.8	0.92	1.36	95.65
	SE(m) $\pm$	0.2	0.077			0.204	0.267		
	CD(0.05)	0.548	0.211			0.559	0.074		

#### 4.3.20 The activity of TAL in shoots

The activity of TAL was calculated as  $\mu$ moles of p-coumaric acid formed per mg protein/min of fresh shoots. The activities of TAL in the healthy shoots were recorded as 0.93, 0.97, 0.93, 0.81 and 0.81  $\mu$ moles t-cinnamic acid/mg protein/min in Priya, BSS-1009, Aneeta, Aarti and Harsha respectively but during post infection period the amounts increased by 5.10, 87.62, 73.11, 90.12 and 13.58 per cent respectively over healthy counterparts in the shoots.

#### The activity of TAL in roots

The activity of TAL was calculated as  $\mu$ moles of p-coumaric acid formed per mg protein/min of fresh roots (Table 22) The activities were recorded as 1.71, 1.17, 0.95, 0.82 and 0.91  $\mu$ moles t-cinnamic acid/mg protein/min in Priya, BSS-1009, Aneeta, Aarti and Harsha respectively but during post infection period in the roots, the amounts increased by 31.57, 103.41, 107.36, 121.95 and 78.02 per cent respectively over healthy counterparts in the roots. (Table 22)

**Table 22. Estimation of Tyrosine Ammonia Lyase (TAL) Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M. incognita***

Sl. No.	Varieties	Tyrosine Ammonia Lyase (TAL) content $\mu$ moles of p-coumaric acid formed per mg protein/min							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	Increase/decrease over healthy(%)	Infected (I)	Healthy (H)	Mean	Increase/decrease over healthy(%)
01	Priya	1.03	0.93	0.98	5.10	2.25	1.71	1.98	31.57
02	BSS-1009	1.82	0.97	1.39	87.62	2.38	1.17	1.98	103.41
03	Aneeta	1.61	0.93	1.27	73.11	1.97	0.95	1.46	107.36
04	Aarti	1.54	0.81	1.17	90.12	1.82	0.82	1.32	121.95
05	Harsha	0.92	0.81	0.86	13.58	1.62	0.91	1.26	78.02
	SE(m) $\pm$	0.334	0.026			0.197	0.288		
	CD(0.05)	0.915	0.071			0.539	0.789		

#### 4.3.21 Effect of root-knot nematode infection on peroxidase activity in shoots & roots of ridge gourd varieties

##### The activity of Peroxidase in shoots

The increased peroxidase activity was observed in nematode inoculated shoot samples of both susceptible and resistant varieties but higher enzymatic activity was

observed the in resistant varieties which were recorded as 35.34,20.58,19.83,8.92 and 50.47 per cent increase over healthy counterpart in the varieties Priya,BSS-1009,Aneeta,Aarti and Harsha respectively.(Table 23)

### The activity of Peroxidase in roots

The increased peroxidase activity was observed in nematode inoculated root samples of both susceptible and resistant varieties but higher enzymatic activity was recorded in resistant varieties which registered as 67.58,86.26,77.21,53.01 and 54.91 per cent increase over healthy counterparts Priya,BSS-1009,Aneeta,Aarti and Harsha respectively,during post infestation periods.

**Table 23. Estimation of Peroxidase Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode,*M.incognita***

Sl. No.	Varieties	Peroxidase content in units/mg protein/min							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)
01	Priya	63.99	47.28	55.63	35.34	126.81	75.67	101.24	67.58
02	BSS-1009	76.69	63.60	70.14	20.58	117.16	62.9	96.41	86.26
03	Aneeta	82.36	68.73	75.54	19.83	103.3	58.29	80.79	77.21
04	Aarti	54.46	50.00	52.23	8.92	122.64	80.15	101.39	53.01
05	Harsha	60.61	40.28	50.44	50.47	115.47	74.54	95.01	54.91
	SE(m)±	0.311	4.938			0.35	0.493		
	CD(0.05)	0.852	13.535			0.959	1.351		

### 4.3.22 Effect of root-knot nematode infection on catalase activity in shoots & roots of ridge gourd varieties

#### The activity of Catalase in shoots

The catalase activity was increased considerably in the infected resistant varieties by 25.42,32.74,24.25,33.90 and 53.34 percent in case of nematode inoculated cultivars, Priya,BSS-1009,Aneeta,Aarti and Harsha respectively,over healthy counterparts.The formation of giant cells and galls in plants, susceptible to the endoparasitic nematode was due to increased level of auxin (Giebel 1973) (Table 24).

### The activity of Catalase in roots

The catalase activity was increased considerably in resistant varieties inoculated with root-knot nematode over healthy counterparts by 7.76,14.39, 46.51, 57.44 and 52.83 percent in case of nematode inoculated cultivar, Priya,BSS-1009,Aneeta,Aarti and Harsha respectively.The catalase activity in the inoculated varieties was increased by 18.87,14.54,8.19,8.14 and 9.17 units/mg protein/min and it was decreased in healthy cultivars.(Table 24).

**Table 24. Estimation of Catalase Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode,*M.incognita***

Sl. No.	Varieties	Catalase content in units/mg protein/min							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)
01	Priya	9.62	7.67	8.64	25.42	18.87	17.51	18.19	7.76
02	BSS-1009	9.73	7.33	8.53	32.74	14.54	12.71	13.62	14.39
03	Aneeta	13.01	10.47	11.74	24.25	8.19	5.59	6.89	46.51
04	Aarti	7.03	5.25	6.14	33.90	8.14	5.17	6.65	57.44
05	Harsha	6.18	4.03	5.10	53.34	9.17	6.00	7.58	52.83
	SE(m)±	0.176	0.298			0.204	0.221		
	CD(0.05)	0.482	0.816			0.559	0.605		

### 4.3.23 Effects of nematode infection on Superoxide dismutase (SOD) content

#### Total Superoxide dismutase (SOD) content in shoots

The superoxide dismutase (SOD) contents of infected shoots of ridge gourd cultivars were 2.75,2.45,2.60,3.70 and 4.65 mg/g in Priya,BSS-1009,Aneeta,Aarti and Harsha respectively, and these were increased by 37.50,6.52,20.93,15.62 and 5.68 percent in shoots during post infection period (Table 25).

#### Total Superoxide dismutase (SOD) content in roots

The superoxide dismutase (SOD) contents of infected roots of ridge gourd cultivars were 7.30,6.40,6.35,6.10 and 5.25 mg/g in Priya,BSS-1009,Aneeta,Aarti and Harsha respectively, and these were increased by 5.79,21.90,11.40,18.44 and 19.31 percent in roots,during post infection period. (Table 25)

**Table 25. Estimation of Superoxide dismutase (SOD) Contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M.incognita***

Sl. No.	Varieties	Superoxide dismutase (SOD) content in unit/mg protein/min							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	increase/decrease over healthy (%)
01	Priya	2.75	2	2.37	37.5	7.3	6.9	7.1	5.79
02	BSS-1009	2.45	2.3	2.37	6.52	6.4	5.25	5.82	21.90
03	Aneeta	2.6	2.15	2.37	20.93	6.35	5.7	6.02	11.4
04	Aarti	3.7	3.2	3.45	15.62	6.1	5.15	5.62	18.44
05	Harsha	4.65	4.4	4.52	5.68	5.25	4.4	4.82	19.31
	SE(m)±	0.402	0.134			0.452	0.450		
	CD(0.05)	1.101	0.367			1.238	1.233		

#### 4.3.24 Effect of nematode infection on amino acid by HPTLC

Generally, with few exceptions, there was both an increase in L-glycine content and L-methionine content in the roots of inoculated resistant Priya variety than the healthy ones. The increase of sulphur-containing amino acid L-methionine in the inoculated sample of resistant variety appears to be interesting and significant. L-methionine is widely involved in trans-methylation reactions in the synthesis of methylated compounds like thiamine, methylated phenols, lignin etc. and play a vital role imparting resistant reaction.

There was an increase in L-serine content in inoculated shoots of highly susceptible Harsha variety than the healthy samples. But, there was a decrease in L-arginine content in shoots of inoculated highly susceptible Harsha than healthy counterparts.

A decrease in arginine content in the variety during post-infection period possibly due to conversion of L-arginine to L-proline through the ornithine cycle. Unique therapeutic quality of L-proline as a defense mechanism against nematode infection. There was an increase in amino acid L-proline in roots of inoculated moderately resistant Aneeta variety than the healthy ones, while there was a decrease in amino acid i.e., L-leucine in roots of inoculated moderately resistant Aneeta variety than the healthy ones. The accumulation of L-proline in nematode-infected samples might be associated with the stress condition of the host plants induced by the nematodes.

The high metabolic activity in roots associated with giant cells and gall formation as well as egg-production exerts a requirement for energy which is supplied by a free proline manufactured in the leaves and translocated to the site of nematode infection.

Besides, the ratio of L-proline and hydroxyl proline play key role in imparting susceptible and resistant reactions and always associated with the stress condition of the host plant.

There was increase in L-isoleucine content in roots of inoculated highly susceptible harsha than healthy counterparts.

There was decrease in L-tyrosine contents in roots of healthy highly susceptible harsha variety than the inoculated samples. Decrease of aromatic amino acids like L-tyrosine was known to have significant role in imparting resistant reaction through lignification.

The aromatic deamination of both the amino acids play significant role for providing the phenyl propanoid skeleton necessary for the biosynthesis of aromatic compounds.

There was increase in L-tryptophan in healthy roots of highly susceptible Aarti variety than the inoculated counterparts, whereas there was decrease in L-phenylalanine content in roots of inoculated highly susceptible Aarti variety than healthy ones.

In the present study the reduction of free tryptophan as infection progressed might be concern with initiation and progress of the galling activity.

The rate of decrease of L-tryptophan in resistant variety was less as compared to susceptible check, which clearly indicates the restricted release of growth regulator IAA in resistant variety.

Quantitative decrease of aromatic amino acids like L-phenylalanine was known to have significant role in imparting resistant reaction through lignifications.

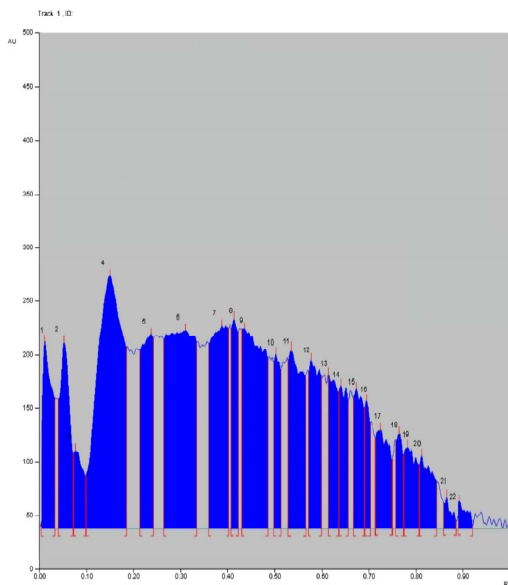
The aromatic deamination of both the amino acids play significant role for providing the phenyl propanoid skeleton necessary for the biosynthesis of aromatic compounds.

**Table 26. Presence and absence of different amino acid and amide in shoot as well as root of different ridge gourd varieties**

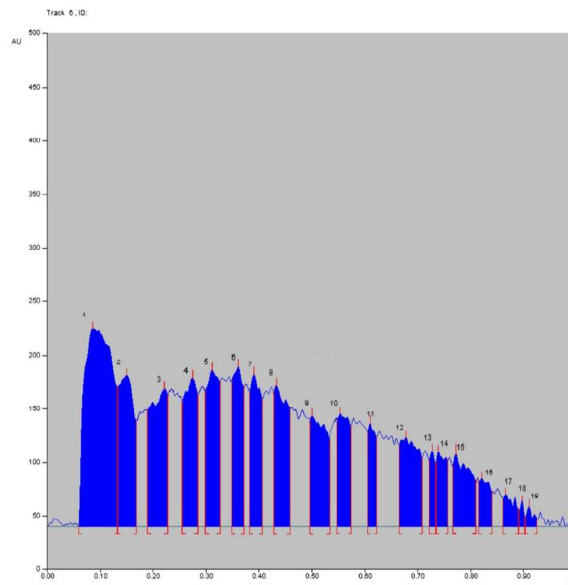
Sample no Amino acid	Track 1	Track 2	Track 3	Track 4	Track 5	Track 6	Track 7	Track 8	Track 9	Track 10	Track 11	Track 12	Track 13	Track 14	Track 15	Track 16	Track 17	Track 18	Track 19	Track 20
L- cystine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L- glutamic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L- threonine	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+
L- aspartic acid	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	+	+	+	+
L- serine	+	+	+	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+
L- glutamine	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-	-	+	+	+
L- asparagine	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+
L- lysine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L- histidine	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-	-	+	-	+
L- arginine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
L- glycine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
L- alanine	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
L- proline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L- tryptophan	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-
L- tyrosine	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L- methionine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-
L- valine	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
L- leucine	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
L- phenylalanine	+	+	+	+	+	-	-	+	+	-	+	+	+	-	+	+	+	+	+	+
L- isoleucine	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-

**Table 27. Changes in area (Infected – Healthy) of different amino acid and amide in shoot as well as root of different ridge gourd varieties**

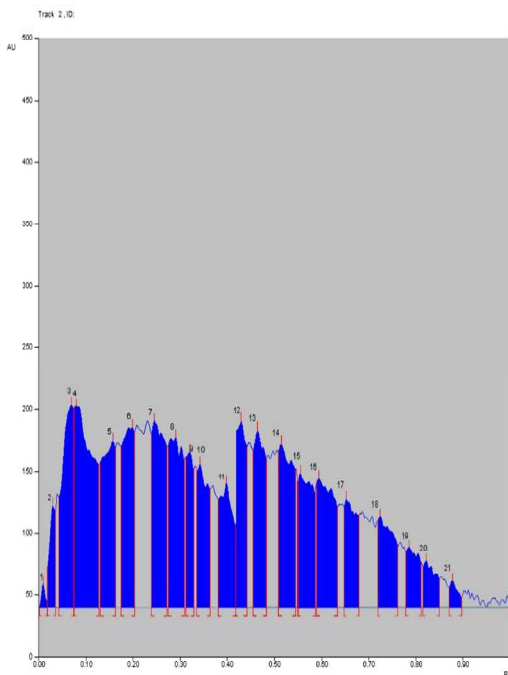
<b>Sample</b>	<b>Change in area(AU)(I-H) Track (6-1)</b>	<b>Change in area (AU) (I-H)Track (7-2)</b>	<b>Change in area(AU)(I-H) Track (8-3)</b>	<b>Change in area(AU)(I-H) Track (9-4)</b>	<b>Change in area (AU) (I-H) Track (10-5)</b>	<b>Change in area(AU)(I-H) Track (16-11)</b>	<b>Change in area(AU)(I-H) Track (17-12)</b>	<b>Change in area(AU)(I-H) Track (18-13)</b>	<b>Change in area(AU)(I-H) Track (19-14)</b>	<b>Change in area(AU)(I-H) Track (20-15)</b>
<b>Amino acid</b>										
L- cystine	+8065.90	-	+ 159.00	- 2556.00	-	-	+ 465.60	-	-	-
L- glutamic acid	-	-	+ 207.80	+ 155.00	-	-	+ 754.60	-	-	-
L- threonine	-	+744.80	+1355.60	-	+ 1318.00	+ 417.90	-	-	-	+1482.30
L- aspartic acid	-	-	-	-	+ 100.20	-	-	- 1376.90	+ 177.60	+126.50
L- serine	-	-	-	-	-	-	-	+ 914.70	+ 124.20	-
L- glutamine	-	- 205.00	-	- 183.60	- 849.30	-	-	- 87.80	- 312.00	- 73.50
L- asparagine	-	+ 36.70	-	-	-	+ 1264.70	-	+ 1797.10	-	+ 840.90
L- lysine	-	- 267.30	- 618.90	-	-	-	- 1314.50	+ 18.80	- 116.30	-1499.40
L- histidine	-	- 118.90	-1643.50	-896.90	-	-	-	-	-	-
L- arginine	-	-	-1217.90	-1819.70	-145.60	-	-74.20	-	-	-1372.90
L- glycine	+ 41.10	-	+ 49.00	+ 33.70	+ 70.20	-	+ 65.70	+ 2961.2	+ 634.20	-
L- alanine	+ 20.00	- 1707.90	-	+ 87.50	+ 795.00	+ 1007.70	+ 948.20	-1025.10	+ 267.00	+ 3026.10
L- proline	+ 179.00	+ 301.90	+ 186.30	+ 456.70	+ 253.90	+ 693.00	+1151.70	+ 819.80	+217.60	+2273.80
L- tryptophan	-	- 1038.5	- 1811.80	- 537.40	-1334.30	-	-2556.00	-	-1203.30	-
L- tyrosine	+1779.40	-	+ 561.10	+ 203.90	-	+ 236.80	+ 81.20	-	+ 147.70	+ 653.20
L- methionine	-	-	-	+ 595.30	-	-	-	+ 293.40	-	-
L- valine	-	-	-	+ 141.80	-	-	+ 71.10	-	-	-
L- leucine	+ 106.10	- 502.30	+ 55.20	+ 25.10	+ 4.50	- 98.90	-	-	+ 238.10	+ 677.30
L- phenylalanine	-	-	+ 2.00	+ 461.00	-	+ 50.60	+ 361.40	+ 738.60	-	+1190.90
L- isoleucine	+ 245.70	-	+ 152.70	+ 7.10	+ 24.30	+ 127.60	+ 11.40	-	+ 237.20	-



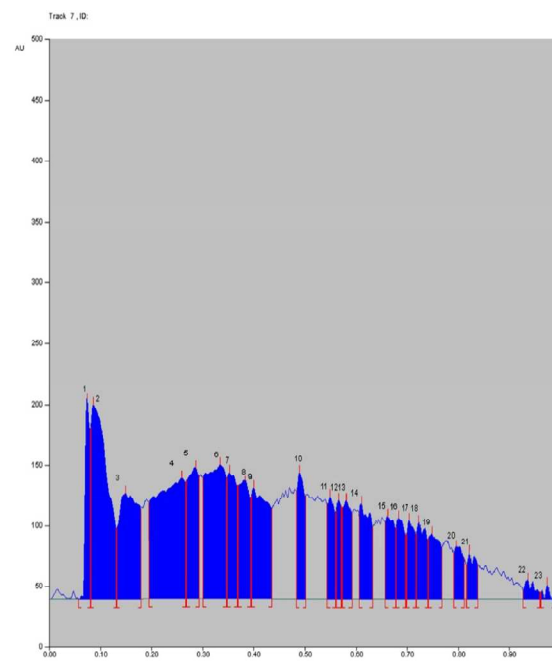
**Fig. 1** Presence and absence of different amino acids and amides in shoot of the healthy ridge gourd variety Priya(R)



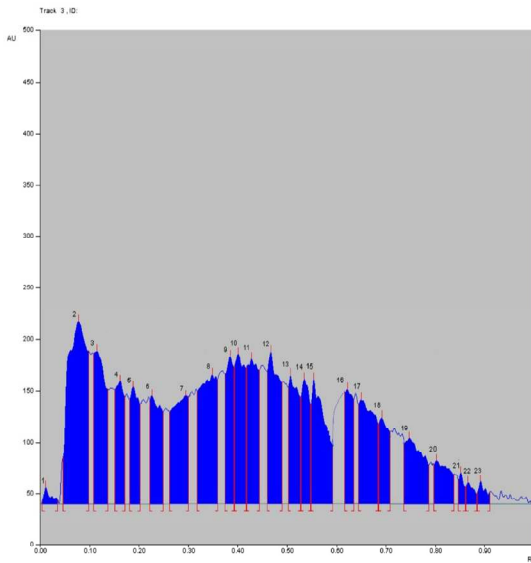
**Fig. 2** Presence and absence of different amino acids and amides in shoot of the inoculated ridge gourd variety Priya(R)



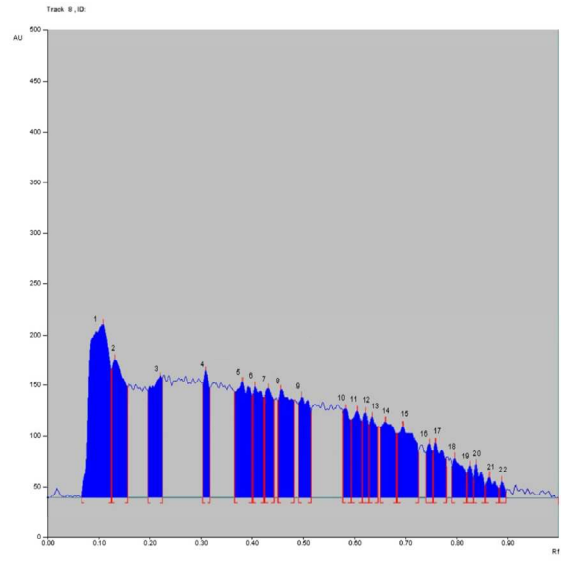
**Fig. 3** Presence and absence of different amino acids and amides in shoot of the healthy ridge gourd variety BSS-1009(R)



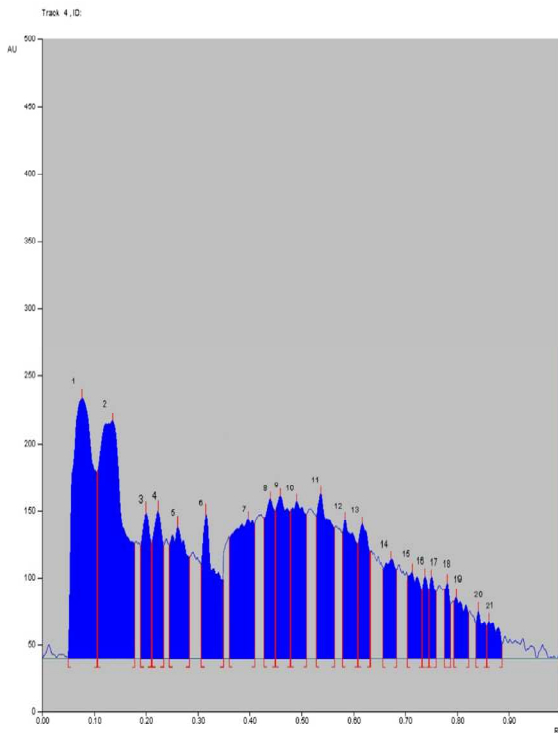
**Fig. 4** Presence and absence of different amino acids and amides in shoot of the inoculated ridge gourd variety BSS-1009(R)



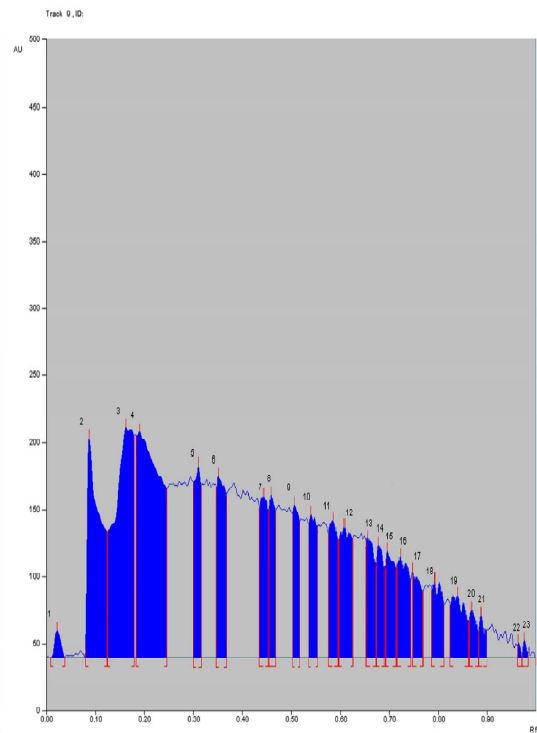
**Fig. 5** Presence and absence of different amino acids and amides in shoot of the healthy ridge gourd variety Aneeta (MR)



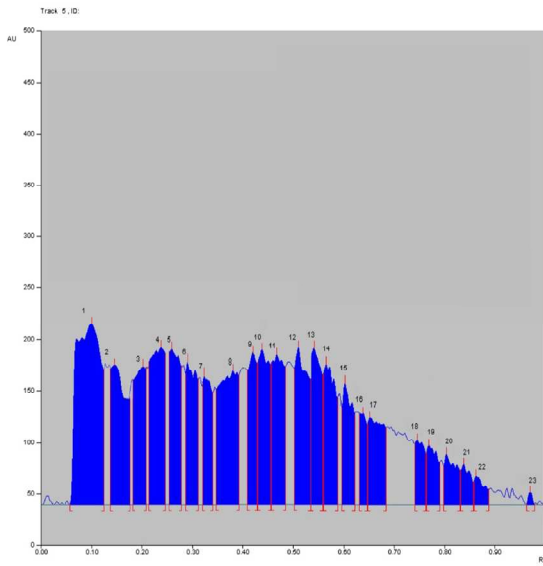
**Fig. 6** Presence and absence of different amino acids and amides in shoot of the inoculated ridge gourd variety Aneeta (MR)



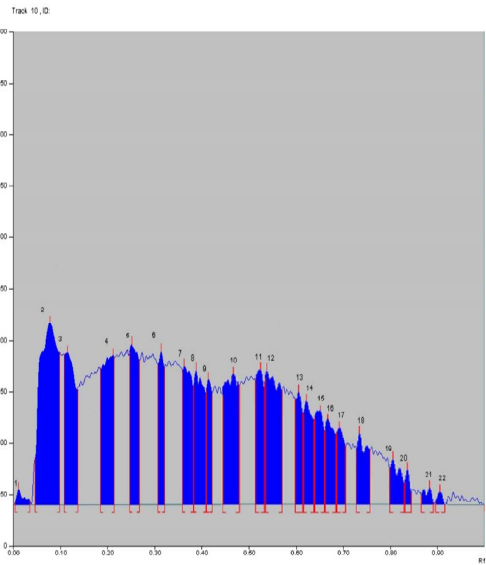
**Fig. 7** Presence and absence of different amino acids and amides in shoot of the healthy ridge gourd variety Aarti (HS)



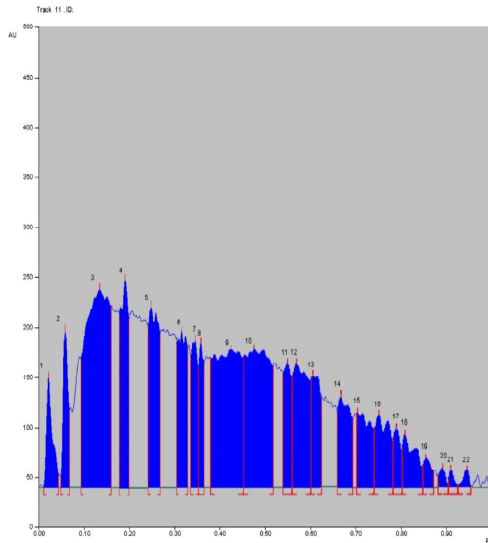
**Fig. 8** Presence and absence of different amino acids and amides in shoot of the inoculated ridge gourd variety Aarti (HS)



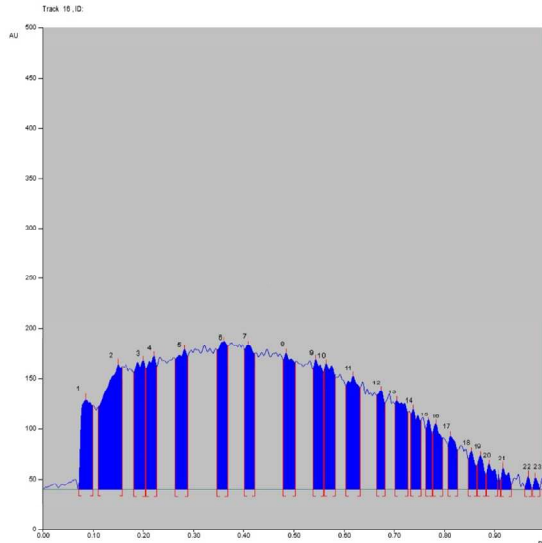
**Fig. 9** Presence and absence of different amino acids and amides in shoot of the healthy ridge gourd variety Harsha (HS)



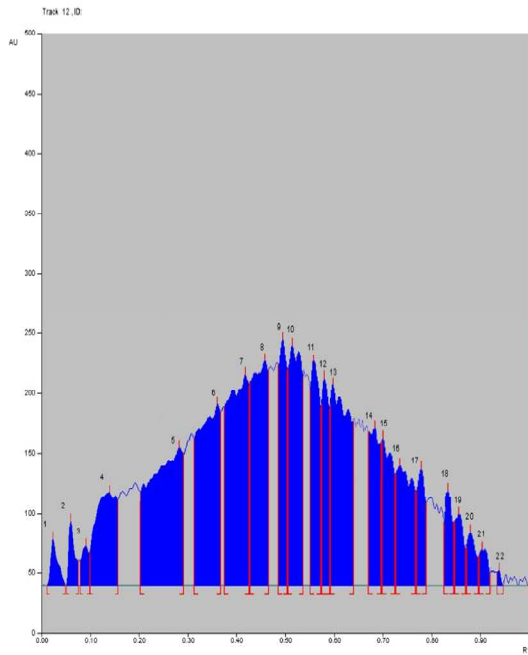
**Fig. 10** Presence and absence of different amino acids and amides in shoot of the inoculated ridge gourd variety Harsha (HS)



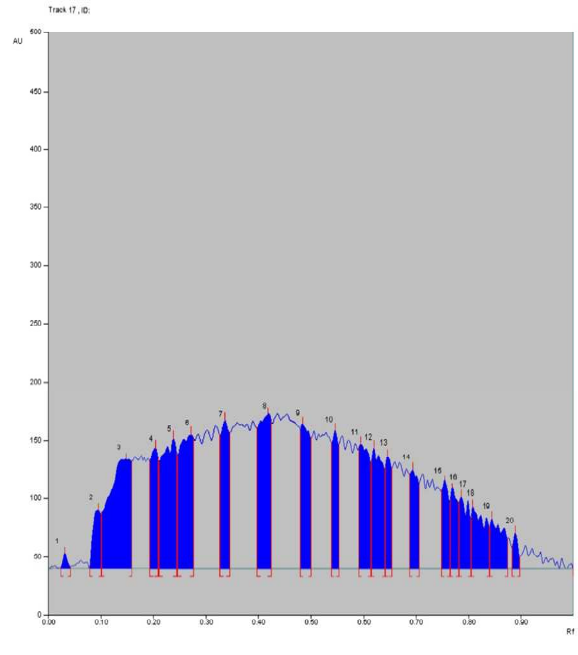
**Fig. 9** Presence and absence of different amino acids and amides in root of the healthy ridge gourd variety Priya(R)



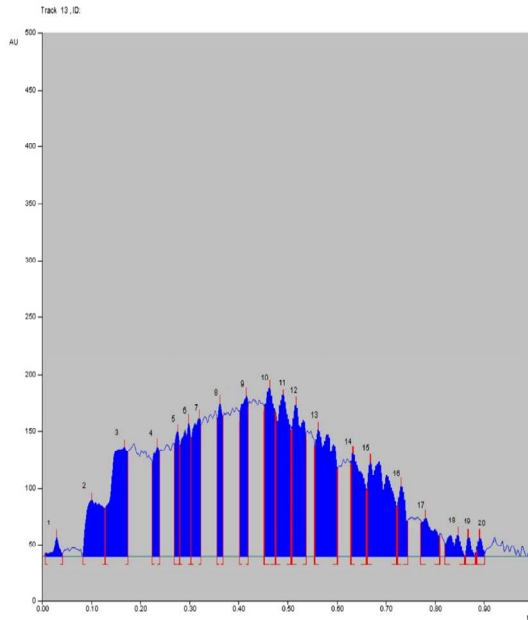
**Fig. 10** Presence and absence of different amino acids and amides in root of the inoculated ridge gourd variety Priya(R)



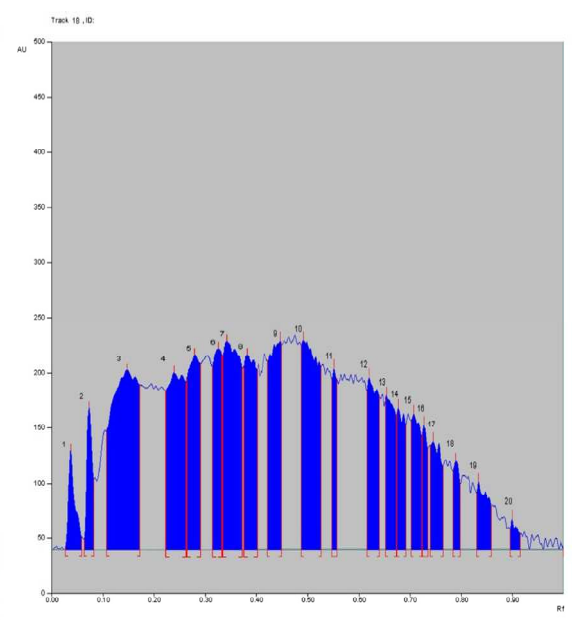
**Fig. 13** Presence and absence of different amino acids and amides in root of the healthy ridge gourd variety BSS-1009(R)



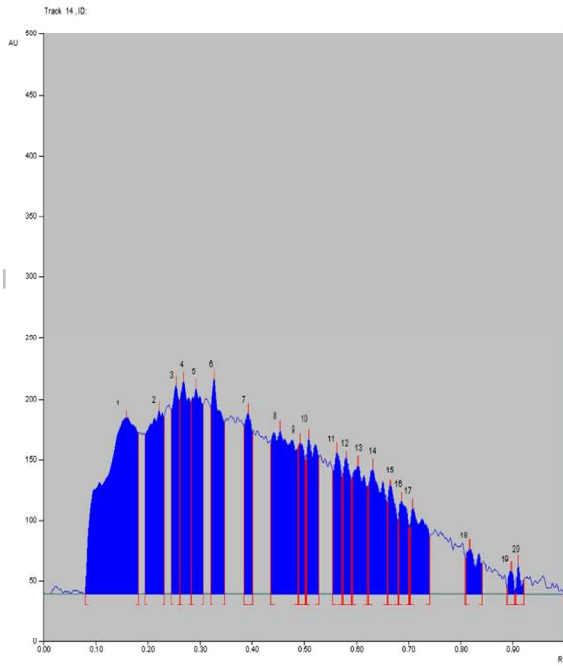
**Fig.14** Presence and absence of different amino acids and amides in root of the inoculated ridge gourd variety BSS-1009(R)



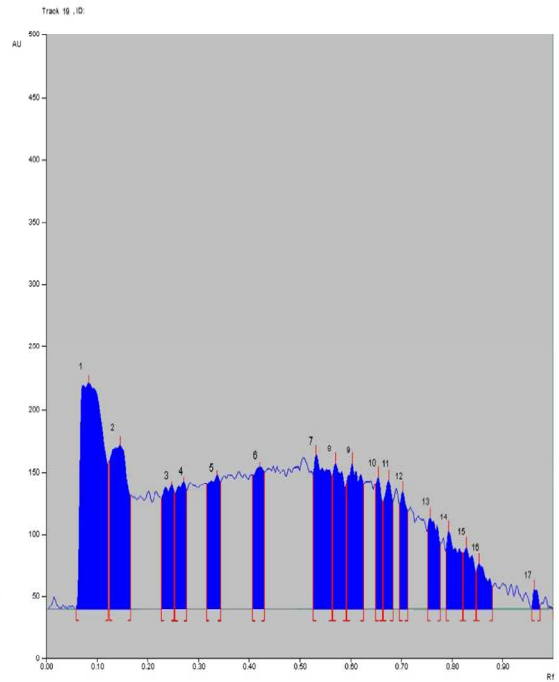
**Fig. 15** Presence and absence of different amino acids and amides in root of the healthy ridge gourd variety Aneeta (MR)



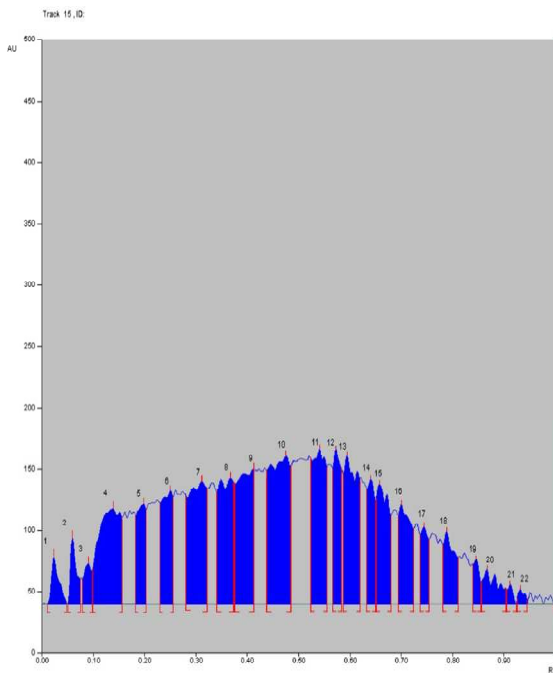
**Fig.16** Presence and absence of different amino acids and amides in root of the inoculated ridge gourd variety Aneeta (MR)



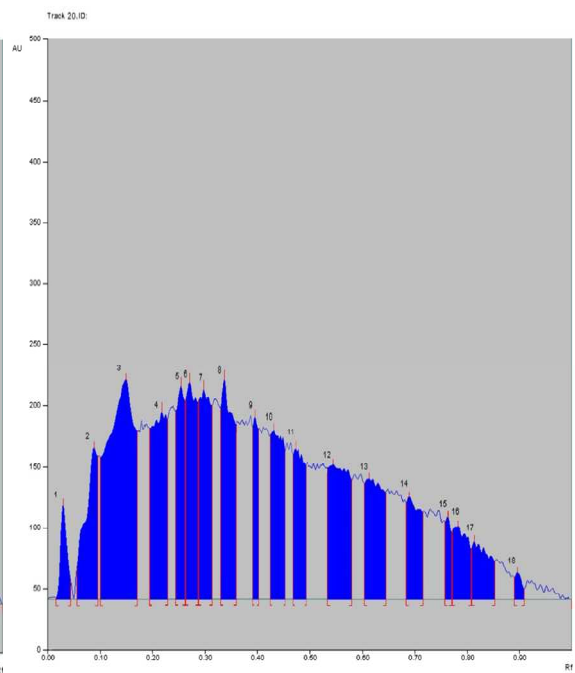
**Fig. 17** Presence and absence of different amino acids and amides in root of the healthy ridge gourd variety Aarti (HS)



**Fig.18** Presence and absence of different amino acids and amides in root of the inoculated ridge gourd variety Aarti (HS)



**Fig. 19** Presence and absence of different amino acids and amides in root of the healthy ridge gourd variety Harsha (HS)



**Fig.20** Presence and absence of different amino acids and amides in root of the inoculated ridge gourd variety Harsha (HS)

#### **4.3.25 Assessment of avoidable yield loss due to root-knot nematode, *M.incognita* infesting ridge gourd under sick plot condition**

The crop losses due to phytonematodes ranged from 5 to 10 per cent of crop value annually. The information on monetary losses due to phytonematodes is essential for understanding the control measures. The experiment was conducted under field conditions to evaluate the preventable yield losses due to root knot nematode on ridge gourd using paired plot technique with soil application of carbofuran 3 G at 2 kg a.i./ha over untreated control. The indicated preventable yield loss in ridge gourd cv. Harsha was recorded to be 74.52 per cent in the treated plot with application of carbofuran 3 G at 2 kg a.i./ha.

It is clearly evident from the Table 28 that ,there was an increase in shoot length in different replications of treated plants over untreated parts and maximum increase was observed in R-10 (7.86%) & least per cent was observed in R-3 (1.04%).While, there was maximum increase in root length in R-6 (25.87%) and least increase was in R-7 (7.74%).

It is clearly evident from the Table 29 that ,there was a increase in fresh root weight in different replication in treated plants over untreated counterparts and maximum increase was found in R-9 (39.38%) & least per cent was observed in replication no.3 (18.02%).While, there was a maximum increase in fresh shoot weight in replication no.6 (15.56%) and least increase was in R-3 (1.97%).

It is clearly evident from the Table 30 that, there was a increase in dry root weight in different replication in treated plants over untreated plot and maximum increase was found in R-4 (74.48%) & least per cent was observed in R-3 (45.52%).While, there was maximum increase in dry shoot weight in R-8 (13.70%) and least increase was in R-4 (12.14%).

Darekar and Mhase (1988) they estimated the yield loss in different crops infected by root-knot nematode such as brinjal,bitter gourd and tomato as 32.73 per cent,36.72 per cent and 46.92 per cent respectively.Further ,it was also observed yield loss due to root-knot nematode in bitter gourd was 22.9 to 42.8 per cent by (Khanna and Kumar,2003).

It is clearly evident from the Table 32 that ,there was a reduction in number of root galls per egg masses per plant in different replications in treated plants over untreated plots and maximum decrease was found in R-5 & R-8 (16.66%) & least per cent was observed in replication no.4 (3.92%).While, there was maximum decrease in gall index per plant in replication no.3 (36%) and least increase was in replication no.4 &10 (20%).Furthermore, there was maximum reduction in reproduction factor which was found highest decrease in R-2 (72.72%) and least in R-10 (26.49%).

**Table 28.** Effect of nematicidal treatment on root and shoot length of ridge gourd infested with root-knot nematode, *M. incognita*

Replications	Root Length(cm)			Shoot Length(cm)		
	Treated	Untreated control	%increase over control	Treated	Untreated control	% increase over control
1	45.30	38.51	17.63	310.29	298.21	4.05
2	44.31	36.23	22.30	302.59	297.52	1.70
3	42.21	35.03	20.49	301.65	298.53	<b>1.04</b>
4	41.23	33.89	21.65	305.28	297.55	2.59
5	43.12	34.52	24.91	311.80	298.82	4.34
6	45.88	36.45	<b>25.87</b>	302.66	297.65	1.68
7	40.32	37.42	7.74	303.45	287.45	5.56
8	40.20	32.44	23.92	306.48	285.25	7.44
9	38.20	31.43	21.53	304.42	282.23	<b>7.86</b>
10	35.21	32.48	<b>8.40</b>	309.45	281.14	
SE(m)±	0.706			2.682		
t value	9.567			4.987		
CD(0.05)	8.03			15.903		

**Table 29.** Effect of nematicidal treatment on fresh weight of root and shoot of ridge gourd infested with root-knot nematode, *M. incognita*

Replications	Fresh weight of root/plant (g)			Fresh weight of shoot/plant (g)		
	Treated	Untreated Control	%increase over control	Treated	Untreated control	%increase over control
1	50.70	42.75	18.59	125.21	98.2	<b>27.5</b>
2	51.24	41.55	23.32	125.35	118.21	6.04
3	50.10	42.45	<b>18.02</b>	123.75	121.35	<b>1.97</b>
4	48.75	36.45	33.74	124.81	120.85	3.27
5	45.23	38.23	18.31	128.72	125.75	2.36
6	42.21	31.31	34.81	141.51	122.45	15.56
7	40.55	30.45	33.16	123.58	119.21	3.66
8	40.23	31.25	28.73	119.83	99.35	20.61
9	45.23	32.45	<b>39.38</b>	112.86	98.25	14.87
10	41.11	32.35	27.07	102.92	94.48	8.93
SE(m)±	0.612			2.744		
t value	15.701			4.025		
CD(0.05)	11.425			13.132		

**Table 30. Effect of nematicidal treatment on dry weight of root and shoot of ridge gourd infested with root-knot nematode, *M. incognita***

Replications	Dry weight of root/plant (g)			Dry weight of shoot/plant (g)		
	Treated	Untreated control	%increase over control	Treated	Untreated control	%increase over control
1	19.51	11.95	63.26	81.75	72.75	12.42
2	20.75	12.93	60.47	82.35	72.45	13.66
3	20.01	13.75	<b>45.52</b>	83.78	73.85	13.44
4	17.85	10.23	<b>74.48</b>	81.25	72.45	<b>12.14</b>
5	20.07	12.30	63.17	80.35	71.23	12.80
6	17.02	10.75	58.32	84.25	74.25	13.46
7	18.25	11.00	65.90	85.31	75.35	13.21
8	17.75	10.88	63.14	83.48	73.42	<b>13.70</b>
9	17.25	11.09	55.54	84.45	74.41	13.49
10	16.85	10.85	55.29	82.23	72.41	13.56
SE(m)±	0.23			0.154		
t value	30.09			62.771		
CD(0.05)	9.78			11.493		

**Table 31. Effect of nematicidal treatment on yield of ridge gourd infested with root-knot nematode, *M. incognita***

Replications	Yield in kg/m <sup>2</sup> at termination		
	Treated	Untreated control	% increase over control
1	4.10	3.80	7.89
2	4.07	3.91	<b>4.09</b>
3	3.82	2.80	36.42
4	3.90	2.24	74.10
5	3.74	2.60	43.84
6	4.00	3.82	4.71
7	3.81	2.65	43.77
8	3.90	2.42	61.15
9	3.70	2.12	<b>74.52</b>
10	4.00	3.72	7.52
SE(m)±	0.192		
t value	4.661		
Average yield loss	22.951		
% yield loss	29.787		
CD(0.05)	1.064		

**Table 32. Effect of nematicidal treatment on number of root galls/egg masses, gall index and Rf(reproduction factor) of root-knot nematode, *M. incognita* infesting ridge gourd**

Replications	Number of root galls/egg masses/plant at termination			Gall index/plant at termination			*Rf (reproduction factor)		
	Treated	Untreated Control	%decrease over control	Treated	Untreated control	%decrease over control	Treated	untreated control	%decrease over control
1	47	54	12.96	3.8	5	24	0.57	1.19	52.10
2	38	43	11.62	3.3	5	34	0.39	1.43	<b>72.72</b>
3	34	40	15	3.2	5	<b>36</b>	0.36	1.14	68.42
4	49	51	<b>3.92</b>	4	5	<b>20</b>	0.61	1.18	48.30
5	35	42	16.66	3.4	5	32	0.53	1.11	52.25
6	44	52	15.38	3.7	5	26	0.49	1.10	55.45
7	47	51	7.84	3.9	5	22	0.64	1.19	46.21
8	45	54	<b>16.66</b>	3.3	5	34	0.43	1.23	65.04
9	37	44	15.90	3.6	5	28	0.60	1.05	42.85
10	48	56	14.28	4	5	20	0.86	1.17	<b>26.49</b>
SE(m)±	0.667			0.09			0.06		
t value	9.438			14.31			9.87		
CD(0.05)	7.484			1.82			0.83		

\* Rf (reproduction factor)=final population/initial population

#### 4.3.26 Molecular characterisation using ISSR markers

Four ISSR primers generated total of 46 bands/alleles in which 23 bands were polymorphic. Primer B3 produced maximum 15 bands, while primer UBC-855 produced minimum 8 bands.

The average bands per primer were 11.50. Maximum polymorphism observed for the primer UBC – 856 (66.66 %) and lowest for UBC – 808 (Table 33 and Figure 21).

Polymorphic % ranges from 13.16% (between BSS – 1009 and Aneeta ) to 43.18 % (between Aarti and Harsha).

The phylogenetic tree constructed by UPGMA method generated two main clusters i.e. cluster – I ( Two sub cluster with varieties Aneeta, Aarti and BSS – 1009) and cluster – II ( with varieties Harsha and Priya) .

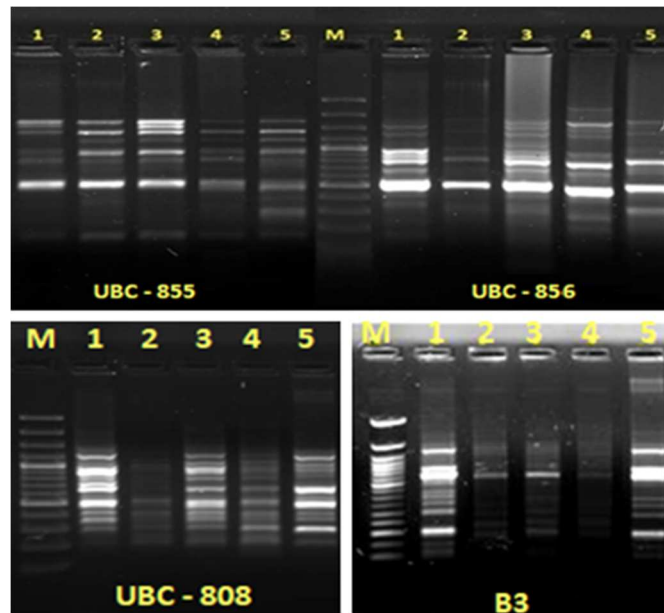
This study showed that ISSR markers produced specific DNA fragments i.e., 100bp for identification of ridge gourd varieties.

**Table 33 List of ISSR primers used, total number of bands, number of polymorphic bands, & percentage of polymorphism obtained by analyzing five ridge gourd varieties**

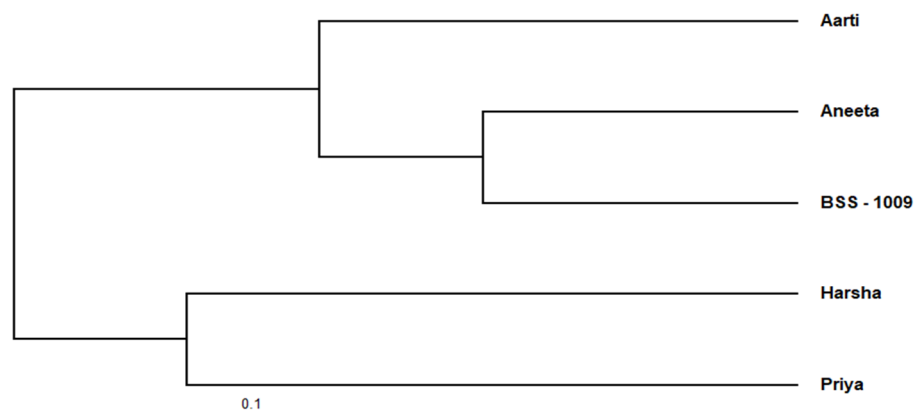
Sl. No.	ISSR Primers	Sequence (5' 3')	Number of bands(A)	Polymorphic bands (B)	Polymorphic % $\{(B/A) \times 100\}$
1.	UBC-808	(AG) <sub>8</sub> C	14	5	35.71
2.	UBC-855	(AC) <sub>8</sub> CTT	8	3	37.50
3.	UBC-856	((AC) <sub>8</sub> CTA	9	6	66.66
4.	B3	(GA) <sub>8</sub> A	15	9	60.00

**Table 34 Polymorphic % of ridge gourd varieties considering four ISSR markers**

	Priya	BSS - 1009	Aneeta	Aarti
BSS - 1009	32.56			
Aneeta	25.58	13.16		
Aarti	37.21	21.62	18.42	
Harsha	25.58	26.83	31.82	43.18



**Fig.21** ISSR marker profile and frequency of alleles amplified by four ISSR markers in five Ridge gourd varieties



**Fig.22.** Dendrogram depicting the genetic relationship among five ridge gourd varieties based on four ISSR markers



## DISCUSSION

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Root-knot nematode *Meloidogyne* spp. is one of the most important pest that affect agricultural production and productivity. Their polyphagous nature has become a great hindrance in controlling them. For their efficient control we have to rely upon resistant varieties along with careful management strategies. Use of resistant varieties would be an economical approach as compared to the use of chemicals because the chemicals nematicides tend to be costly. Though systemic nematicides have been used earlier to control these nematodes but their adverse effect on human health and environment has become a limiting factor for their efficient use in India.

No single plant cultivar has all the desired agronomic traits as well as resistance to pests. Moreover the excessive use of resistant cultivars may lead to adaptation of resistance factors by the nematodes and cause resurgence in them. So, the different genotypes like resistant, tolerant and susceptible genotypes are equally important. Both tolerant and susceptible varieties are suitable hosts for the nematodes but in contrary to the tolerant host it is the susceptible host which suffers a yield loss.

Complementing resistance with other methods and strategies is the most important point to ponder upon. The physiology and metabolism of the plant is greatly affected due to the disruption of root system and obstruction in nutrient uptake. This is caused due to the destructive, adaptive and neoplastic feeding behavior of the nematode. The above ground symptoms include yellowing, stunting and poor growth of the plant. The characteristic underground symptoms are formation of galls. There are accounts of the effect of nematode infection and host-parasite interaction on the amount of macro and micro nutrients present. Increase in phenol levels during post infection site might be due to the involvement of different mechanisms of plant to counteract the metabolic activities of the pathogen.

The biochemical resistance of a plant is attributed to the various biochemical allied with it. Pertaining to non-preference and antibiosis, the biochemical factors are more important than the morphological and physiological parameters. And among the non chemical methods available to control the nematodes, the best alternative is the use of resistant varieties because it is the most effective and environmentally safe alternatives. So it had become essential to study the effect of root-knot nematode on

gerbera. The establishment of biochemical mechanism due to nematode infection was done in present investigation by estimating the total phenols, proteins at 45 days after planting of plants which are artificially inoculated with root-knot nematode, *Meloidogyne incognita*.

Nematode infection seriously affected the plant growth parameters, like shoot length and shoot weight were decreased but root length increased. In the subsequent stages level of chlorophyll decreased. Photosynthetic process is affected due to the morphological and physiological changes occurring in plants as a result of nematode invasion. Root- knot nematodes cause the formation of giant cells in the roots which disrupts the root vascular system, hamper water uptake and nutrient transportation from roots to shoots (Abad *et al.*, 2003). The formation of giant cells causes galling of roots which is possible reason of reduction in length and weight of the shoots and root weight in the infected plant.

The present study intended at determining the change in the nutrient content of the different resistant and susceptible varieties or cultivars of ridge gourd against root-knot nematode, *Meloidogyne* infection. Out of the seven varieties/ cultivars screened, five varieties were selected out of which one was resistant, three were moderately resistant and one was susceptible.

### **5.1 Screening and evaluation of ridge gourd varieties**

For screening purpose fifty two ridge gourd germplasms were collected from the locality of Bhubaneswar and nearby villages of Dhenkanal and were tested in green house condition. Out of the fifty two ridge gourd varieties, only 6 showed resistant reaction with 7-10 number of galls per plant, 7 showed moderately resistant reaction with 23-31 number of galls per plant, 32 susceptible reaction with 31-91 number of galls per plants & 5 highly susceptible with 112-116 number of galls per plants. (Table1).

To substantiate, about the resistance occurring in the plants, biochemical analysis of one moderately resistant, two resistant and two highly susceptible variety i.e. Priya, BSS-1009, Aneeta, Aarti and Harsha was done.

### **5.2 Effects of nematode infection on plant growth parameters**

#### **Shoot length and root length (cm)**

Due to infection of root-knot nematode, the maximum average shoot length of ridgegourd varieties was 160cm in BSS-1036, and root length was 38.66cm in Ridge

gourd 12 pata. The decrease in shoot length & root length were more pronounced i.e., in SS-Ramu (16.83cm) and Ramu F1(11.36) respectively. The decrease was possibly due to improper uptake and transport of elements, nutrients and water resulted from nematode infection. (Table 3)

After infection of root-knot nematode, the maximum average shoot length of ridgegourd varieties was 160cm in BSS-1036, and root length was 38.66cm in Ridge gourd 12 pata. The decrease in shoot length & root length were more pronounced i.e., in SS-Ramu (16.83cm) and Ramu F1(11.36) respectively. The decrease in shoot weight (5.53g) and root weight (0.23) of the ridgegourd variety, Nayagarh Local and Tauri, BSS-1009 respectively.

Pandey (2016) studied due to infection of root-knot nematode, the maximum average shoot length of greengram cultivars/varieties was 40.66 cm in 20 MH-175. The decrease in shoot length was more pronounced 22.03 cm in 5 DGG-5 variety, which was statistically different from other resistant varieties. The decrease is possibly due to improper uptake and transport of elements, nutrients and water resulted from nematode infection.

#### **Shoot weight and root weight (g)**

The decrease in fresh shoot weight (5.53g) and fresh root weight (0.23) of the ridgegourd variety, Nayagarh Local and Tauri, BSS-1009 respectively. These varieties were significantly different from the rest varieties showing susceptible and moderately resistant reaction to the test nematode. The same trend was observed for fresh root weight in both the varieties. The decrease in dry shoot weight (2.56g) in variety NHRG-1001 & dry root weight (1.10g) in Hybrid Patal Tarai was observed. Possible reasons for low shoot weight and root weight in infected plants may be due to improper supply of nutrients resulting from nematode infection for which it is compensated to some extent in resistant varieties (Table 3).

Nayak (2006) reported that plant height of brinjal varieties due to root-knot nematode infection were decreased by 9.04, 18.48, 23.50 per cent in varieties Pusa Kranti, Kantabaigan and Pusa Purple Long, respectively over uninoculated control. Further, he had shown decrease of shoot weight (34.08%) and root weight (31.67%) of the susceptible brinjal cultivars, Pusa Purple Long which was different from other two resistant varieties showing resistance reaction.

Pandey (2016) observed the decrease in shoot weight (22.70 g) and dry root weight (0.3 g) of the greengram variety, 9 GM 11-02 and 15 IPM 2K 15-4 was significantly different from the rest varieties showing resistant and moderately resistant reaction to the test nematode.

### **5.3 Chemical analysis of plant samples**

#### **5.3.1 Effect of the nematode infection on chlorophyll contents**

Chlorophyll content is the most important constituent of the plants as it manufactures the food, which is necessary for the growth and development of all the parts of plant. It is directly correlated with the yield of the crops. Root-knot nematode are known to reduce the chlorophyll content of the plants by disrupting its nutrient uptake and partitioning of the photosynthates.

Chlorophyll 'a' is recognized as the main pigment which convert light energy into chemical energy. Chlorophyll 'b', as accessory pigment acts indirectly in photosynthesis by transferring the light it absorbs to chlorophyll 'a'. Chlorophyll molecule has  $Mg^{2+}$  at the centre which makes it ionic and hydrophilic and the ring is hydrophobic in nature, with a Carbonyl group as its tail.

In the experiment it was observed that the chlorophyll content reduced from 0.42 mg/g to 0.25 mg/g due to nematode infection in case of Priya variety. Similarly in the varieties BSS-1009, Aneeta, Aarti and Harsha chlorophyll 'a' reduced from 0.40 to 0.20 mg/g, 0.42 to 0.22 mg/g, 0.33 to 0.26 mg/g and 0.26 to 0.15 mg/g respectively. Hence nematode caused 40.47 per cent reduction in chlorophyll 'a' content of Priya, 49.87 per cent reduction in BSS-1009, 48.71 per cent reduction in Aneeta, 19.33 per cent reduction in Aarti and 42.69 per cent reduction in Harsha.

In case of chlorophyll 'b' content reduction from healthy to infected plants recorded were 0.08 to 0.06, 0.16 to 0.10, 0.13 to 0.10, 0.25 to 0.16 and 0.15 to 0.11 mg/g in case of varieties Priya, BSS-1009, Aneeta, Aarti and Harsha respectively. Hence nematode caused 24.50 per cent reduction in chlorophyll 'b' content of Priya, 33.75 per cent reduction in BSS-1009, 25.73 per cent in Aneeta, 34.64 per cent reduction in Aarti and 30.81 per cent reduction in Harsha.

Similarly the total chlorophyll content was decreased from 0.59 to 0.23 mg/g in Priya, 0.59 to 0.15 mg/g in BSS-1009, 0.38 to 0.37 mg/g in Aneeta, 0.35 to 0.34 mg/g in Aarti and 0.31 to 0.21 mg/g in Harsha. Hence nematode caused 60.67 per

cent reduction in total chlorophyll content of Priya, 73.78 per cent reduction in BSS-1009, 1.32 per cent reduction in Aneeta, 3.68 per cent reduction in Aarti and 30.12 per cent reduction in Harsha.(Table 1 and Fig. 1)

Nevertheless, the decline was insignificant in resistant varieties which evidently specified that the reduction of chlorophyll is limited to inoculated resistant varieties as compared to the susceptible variety. Similar results were also reported by Vaishnab *et al.*, 1985; Tyagi and Alam, 1990. Reduction in chlorophyll content may probably be due to the alteration of host nutrition and bodily processes by nematode infectivity.

### **5.3.2 Effects of nematode infection on nitrogen content**

The total Nitrogen content was decreased in the shoot system of infected varieties of ridge gourd ,Priya,BSS-1009,Aneeta,Aarti &Harsha by 0.36, 0.46, 0.44,0.35 & 0.28 percent respectively over control on dry weight basis.Whereas in the roots Nitrogen content was increased by 0.49,0.44,0.47,0.67 & 0.48 percent in all the above mentioned varieties accordingly.

The total Nitrogen content was decreased in all shoot system of resistant variety by 20% and 40.42% in susceptible variety but increased by 88.46% in the root system of resistant variety as compared to susceptible one (2.12%) (Table 1).While the Nitrogen content in the roots increased in all the varieties. The Nitrogen content (%) was significantly higher both in root and shoot in the susceptible varieties as compared to that of resistant variety. The increment in the total Nitrogen content of nematode infected root samples is substantiated with the findings of the earlier workers (Zaki and Bhatti, 1986; Mohanty *et al.*, 1990). The nutrient accumulation in infected roots maybe attributed to their improper translocation to the aerial parts or the mobilization of nutrition from shoot to root. The higher absorption capacity or higher requirement for the resistance mechanisms and lower translocation rate may be the possible reason of increase in percentage of N<sub>2</sub>-content in the shoots of resistant varieties.

### **5.3.3 Effect on crude protein content**

The infected shoots showed a decline in the crude protein content in a similar manner like the Nitrogen content in the infected shoot. The decrease in shoot crude protein content of different varieties may be due to the action of proteolytic enzymes produced as a result of host plant and the invading nematode

pathogen interaction. The defensive action of the host plant against nematode infection may be attributed to the changes brought about in the protein content during post infection period and this is more evident in the resistant varieties. The increased crude protein content in nematode infected plants was documented by various workers (Ganguly and Dasgupta, 1983; Devarajan and Rajendran, 2002) (Table 5).

#### **5.3.4 Effects of nematode infection on phosphorus content**

The result of present study revealed that the infected plants had decreased percentage of phosphorus content in shoots but with increased percentage in roots of susceptible and resistant varieties. Similar findings were reported by Hunter, 1958; Chakraborty and Mishra, 2002 in root-knot nematode infected plants, which showed a decrease in percentage of phosphorus content in shoots with an increase in percentage in the roots of infected susceptible and resistant varieties. The accumulation of the amino acid, protein, Sugar and absorbed  $N_2$ ,  $P_2O_5$  and  $K_2O$  and other elements in the roots of nematode infected plants which stay under-utilised by the plant system eventually aid in the growth and reproduction of nematode. This can be attributed as the possible reasons for the buildup of phosphorus in nematode infected plants (Table 6).

#### **5.3.5 Effects of nematode infection on potassium content**

The present investigation revealed there was significant increase of potassium content of both susceptible and resistant infected plants which increase was more evident in both shoots and roots of resistant varieties as compared to the susceptible varieties (Table 7). The present findings are in confirmation with the findings of Chakraborty and Mishra (2002) who have also reported a gradual decrease in potassium content due to infection of *Xiphinema americanum* and *M. incognita* in sour cherry and chickpea. The turgor pressure of the tissues of the plant is retained by Potassium and the various compounds linked with ion exchange may be decreased in the nematode infected plant tissues which is a sign of reduction of 'K' content in nematode infected plant samples.

#### **5.3.6 Effects of nematode infection on total sugar content**

All living organisms require sugar as it is the primary source of metabolic energy. Examination of the present data clearly envisioned a significant increase in quantity of total sugars in both shoots & roots of nematode infected plants (Table 8). Increase in sugar

content post nematode infection is in validated with the findings of earlier workers (Ganguly and Dasgupta, 1983). The movement of various metabolites from other parts of plants towards the infection site might have resulted in increased sugar content in infected samples. On the other hand more of these metabolites are manufactured by cell at the site of infection resulting in the requirement of more carbohydrates for respiration and metabolism (Wood, 1968).

### **5.3.7 Effects of nematode infection on starch content**

Starch content in roots of root knot nematodes inoculated ridge gourd varieties showed a decrease in percentage (Table 9). The starch content (%) both in root and shoot were notably higher in the susceptible varieties as compared to that of moderately resistant varieties. These findings are in confirmation with the observation of Devranjan and Rajendran (2002) who envisioned the decrease in starch content in nematode infested roots.

### **5.3.8 Effects of nematode infection on phenolic substances**

A noteworthy increase of phenolic compounds was observed in moderately resistant varieties due to nematode infection as compared to the susceptible varieties, the rise of phenolic compound in the moderately resistant varieties was significant. The increase in phenolic content was evident with the findings of Chakraborty and Mishra, 2002, in chickpea plant infected by *M. incognita*. The phenolic compounds are attributed as the best factors of responses and there is discrete association between the degree of plant resistance imparted by the phenolic compounds in nematode inoculated samples. There is a rapid liberation of conjugated phenols from the glycosidic compounds manufactured by the action of hydrolytic enzymes during feeding process of the nematodes (Whilski and Giebel, 1966). Further, the raise in phenolic compounds during the infection period might be an element to the rapid breakdown of bound phenols and switching over of phenols to different pathways which may lead to the formation of various compounds like lignin which plays important role in resistant reaction.

### **5.3.9 Effects of nematode infection on proline contents**

Similar to phenol content of infected moderately resistant germplasms/lines the proline content was found to have increased which were evident to the findings of Lewis and Mc Clure, 1975; Mohanty and Pradhan, 1990. With the due course of time it was observed that proline content of

infected susceptible cultivars increased except that highest proline content was found in nematode infected (healthy leaf) of resistant cultivars.

#### **5.3.10 Effects of nematode infection on micro-nutrient contents**

The consequences of root knot nematode infections are reduced plant growth, loss in yield and decrease in nutrient uptake in the infested plants showing the deficiencies of iron, calcium, magnesium, sulphur, manganese due to disruption of root and subsequent obstacle in water & nutrient uptake by the roots and their translocation.

**Root:** Data revealed that there was a decrease in trend of micronutrients i.e. in iron by 2.34 -38.65% (Table 10.a), magnesium by 5.91- 35.69% (Table 10.b), Calcium by 5.86-40.55% (Table 10.c), manganese by 2.73- 31.38% (Table 10.e) except in sulphur it increased by 17.85-93.58% (Table 10.d) in root knot infected plants ridge gourd varieties over their healthy counterparts.

#### **5.3.11 Effects of nematode infection on peroxidase and catalase contents**

The result presented in this investigation indicated an elevated level of peroxidase and catalase activities during post-infection period. The increase level of peroxidase activities was reported by (Mohanty *et al.*, 1988, Kalirasan *et al.*, 2006, Chawla and Pankaj, 2007). This increase in peroxidase activity possible due to synthesis of new iso enzymes as a response of nematode infection. Peroxidase is often cited as one of the key enzyme require for lignin bio-synthesis and phenol metabolism. Futher superoxide radicals and singlet O<sub>2</sub> can be generated from NADH and H<sub>2</sub>O<sub>2</sub> through the action of catalase and peroxidase respectively, which are linked with resistant reaction of host plant.

The present study that provides same basic information relating to host-pathogen interaction and bio-chemical mechanism of resistance. The above knowledge of physiological and bio-chemical events during the post-infection period have initiated search for molecules, which can trigger the function of enzymes involve in hyper sensitive response, production of phytoalexins lignin or other secondary metabolites which are detrimental to nematode feeding and development.

#### **5.3.12 Effects of nematode infection on PAL and TAL contents**

In this present study higher activity of enzymes PAL and TAL were recorded in ridge gourd plants infected by root knot nematode. The enhanced activity of both the enzymes and appearance of aromatic aminoacids like tyrosine and phenylalanine

in inoculated samples appears to be interesting and significant. These amino acids are known to have significant role in lignifications. Both t-cinnamic acid and p-coumaric acids the product of TAL and PAL activities respectively are lignin precursors and play significant role in defense mechanism.

It can be inferred from the present study that the root knot nematode *Meloidogyne incognita* induce majorable biochemical changes in plants. Understanding the biochemical and molecular basis of plant nematode interaction will help us in identifying new targets to intervene with nematode parasitism and develop novel strategies to combat them. The information generated from this investigation can be manipulated through advanced biotechnological research for planning suitable management strategies.

### **5.3.13 Effect of assessment of avoidable yield loss due to root-knot nematode, *M. incognita* infesting ridge gourd**

The evaluation of avoidable yield losses due to root-knot nematode in ridge gourd indicated the loss in yield of ridge gourd upto 74.52 per cent under field conditions, when the crop was treated with carbofuran 3 G at 2 kg a.i./ha. It can be inferred that root - Knot nematodes are major obligate parasites which disturb host metabolism. Understanding the biochemical and molecular basis of plant nematode interaction will help us in identifying new targets to intervene with nematode parasitism and develop novel strategies to combat them. The information generated from this investigation can be manipulated through advanced biotechnological research for planning suitable management strategies.

The present study that provides same basic information relating to host-pathogen interaction and bio-chemical mechanism of resistance. The above knowledge of physiological and bio-chemical events during the post-infection period have initiated search for molecules, which can trigger the function of enzymes involve in hyper sensitive response, production of phytoalexins lignin or other secondary metabolites which are detrimental to nematode feeding and development.

### **5.3.14 Molecular characterisation using ISSR markers**

Four ISSR primers generated total of 46 bands/alleles in which 23 bands were polymorphic. Primer B3 produced maximum 15 bands, while primer UBC-855 produced minimum 8 bands.

The average bands per primer were 11.50. Maximum polymorphism observed for the primer UBC – 856 (66.66 %) and lowest for UBC – 808 (Table 1 and Figure 1).

Polymorphic % ranges from 13.16% (between BSS – 1009 and Aneeta ) to 43.18 % (between Aarti and Harsha).

The phylogenetic tree constructed by UPGMA method generated two main clusters i.e. cluster – I ( Two sub cluster with varieties Aneeta, Aarti and BSS – 1009) and cluster – II ( with varieties Harsha and Priya) .

This study showed that ISSR markers produced specific DNA fragments varies from 100bp to 1500bp for identification of ridge gourd varieties.

### **5.3.15 Effect of nematode infection on amino acid by HPTLC**

Generally,with few exception, there was both increase in L-glycine content and L-mehionine content in the roots of inoculated resistant priya variety than the healthy ones.The increase of sulphur containing amino acid L-methionine in the inoculated sample of resistant variety appears to be interesting and significant.L-methionine is widely involved in trans-methylation reaction in the synthesis of methylated compounds like thiamine, methylated phenols,lignin etc. and play vital role imparting resistant reaction.

There was increase in L-serine content in inoculated shoots of highly susceptible harsha variety than the healthy samples. But ,there was decrease in L-arginine content in shoots of inoculated highly susceptible harsha than healthy counterparts.

Decrease in arginine content in the variety during post-infection period possibly due to conversion of L-arginine to L-proline through ornithine cycle.Unique therapeutic quality of L-proline as defense mechanism against nematode infection.

There was increase in amino acid L-proline in roots of inoculated moderately resistant Aneeta variety than the healthy ones, while there was decrease in amino acid i.e.,L-leucine in roots of inoculated moderately resistant Aneeta variety than the healthy ones.

The accumulation of L-proline in nematode infected samples might be associated with the stress condition of the host plants induced by the nematodes.

The high metabolic activity in roots associated with giant cells and gall formation as well as egg-production exerts a requirement for energy which is supplied by a free proline manufactured in the leaves and translocated to the site of nematode infection.

Besides, the ratio of L-proline and hydroxyl proline play key role in imparting susceptible and resistant reactions and always associated with the stress condition of the host plant.

There was increase in L-isoleucine content in roots of inoculated highly susceptible harsha than healthy counterparts.

There was decrease in L-tyrosine contents in roots of healthy highly susceptible harsha variety than the inoculated samples. Decrease of aromatic amino acids like L-tyrosine was known to have significant role in imparting resistant reaction through lignification.

The aromatic deamination of both the amino acids play significant role for providing the phenyl propanoid skeleton necessary for the biosynthesis of aromatic compounds.

There was increase in L-tryptophan in healthy roots of highly susceptible Aarti variety than the inoculated counterparts, whereas there was decrease in L-phenylalanine content in roots of inoculated highly susceptible Aarti variety than healthy ones.

In the present study the reduction of free tryptophan as infection progressed might be concern with initiation and progress of the galling activity.

The rate of decrease of L-tryptophan in resistant variety was less as compared to susceptible check, which clearly indicates the restricted release of growth regulator IAA in resistant variety.

Quantitative decrease of aromatic amino acids like L-phenylalanine was known to have significant role in imparting resistant reaction through lignifications.

The aromatic deamination of both the amino acids play significant role for providing the phenyl propanoid skeleton necessary for the biosynthesis of aromatic compounds.



## SUMMARY AND CONCLUSION

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Out of fiftytwo varieties screened against root-knot nematode, only 6 varieties i.e. Priya, Challenger , Jaipur long ,BSS-1009, Tauri and Pallishree showed resistant reaction with minimum number (7-10 ) of galls/plant. There was a decrease in shoot length & root length in inoculated samples than the healthy counterparts. The decrease was possibly due to improper uptake and transport of elements, nutrients and water resulted from nematode infection. Highest decrease for dry shoot weight & dry root weight was observed for variety NHRG-1001(2.56g) & dry root weight in Hybrid Patal, Tarai(1.10g) respectively. Possible reasons for low shoot weight and root weight in infected plants may be due to improper supply of nutrients.

Root knot nematode caused overall reduction of total chlorophyll, chlorophyll 'a', chlorophyll 'b', content in inoculated samples than the healthy counterparts. It may be due to interference in the photosynthetic pathway and partitioning of the photosynthates. Overall reduction of total chlorophyll was 60.67, 73.78 , 1.32 , 3.68 and 30.12 per cent in Priya, BSS-1009, Aneeta, Aarti and Harsha respectively than the healthy counterparts. Overall reduction of chlorophyll 'a' was 40.47, 49.87, 48.71, 19.33 and 42.69 per cent in Priya, BSS-1009, Aneeta, Aarti and Harsha respectively. Overall reduction of chlorophyll 'b' was 24.50 , 33.75 , 25.73 , 34.64 and 30.81 per cent in Priya, BSS-1009, Aneeta, Aarti and Harsha respectively.

The total Nitrogen content was decreased in the shoot system and increase in the root system of infected varieties of ridge gourd than the healthy counterparts. The Nitrogen content (%) in shoots were significantly higher in the resistant varieties as compared to susceptible variety and vice-versa. The nutrient accumulation in infected roots maybe due to impaired translocation to the aerial parts or the mobilization of nutrition from shoot to root. The percentage increase in nitrogen content in shoots of resistant varieties may be due to higher absorption capacity or higher requirement for the resistance mechanisms. The total Nitrogen content was decreased in the shoot system of infected varieties of ridge gourd ,Priya,BSS-1009,Aneeta,Aarti & Harsha by 0.36,0.46,0.44,0.35 & 0.28 percent respectively over control on dry weight basis. In the roots Nitrogen content was increased by 0.49,0.44,0.47,0.67 & 0.48 percent in Priya,BSS-1009,Aneeta,Aarti & Harsha respectively. The total crude protein content was decreased in the shoot system and increase in the root system of infected varieties

of ridge gourd than the healthy counterparts. The crude proteins were reduced in shoots of infected plants because of the enzymatic degradation of plant protein and reduced photosynthesis causes accumulation of soluble amino acids in the infected plants. Due to infection of root-knot nematode, the percentage of shoot crude protein content was low in varieties Priya (20.35%), BSS-1009(6.20%) , Aneeta(14.59%) , Aarti(5.57%) & Harsha(40.27%) than the healthy counterparts. Crude protein content in root was high in varieties Priya (86.66%) than that of BSS 1009(58.28%), Aneeta (0.68%), Aarti(61.06%) and Harsha (1.01%) than the healthy counterparts.

The total Phosphorus content was decreased in the shoot system and increase in the root system of infected varieties of ridge gourd than the healthy counterparts.

The amino acid, protein, Sugar and absorbed N<sub>2</sub>, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O and other elements accumulate in the roots of nematode infected plants and remain under-utilised by the plant systems which ultimately find the path into the growth and production of nematode. This may be the possible reasons for the accumulation of phosphorus in nematode infected plants

The Phosphorus content was decreased in the shoot system of infected varieties of ridge gourd ,Priya,BSS-1009,Aneeta, and Harsha by 92%,11.42%,62.74% and 50.87% respectively except Aarti (113.33%) than the healthy counterparts on dry weight basis. Increased in infected root by 0.57,0.26,0.44,0.49,0.75 percent in ridge gourd varieties Priya ,BSS-1009,Aneeta,Aarti and Harsha respectively than the healthy counterparts on dry weight basis.

Potassium content was significantly increased in both susceptible and resistant infected plants which increase was more pronounced in both shoots and roots of resistant varieties as compared to the susceptible varieties. The change in potassium concentration seems to be important because of its effect on physiological processes such as osmotic potential. Potassium content was increased to 208.33,175,27.27 per cent in shoots of varieties Aneeta,BSS-1009 & Priya respectively while decrease as 75 percent in Harsha and 72.09 percent in Aarti. The inoculated plants in roots was increased by 850, 420, 67.85, 8.88 and 50 percent in varieties Priya, BSS-1009, Aneeta, Aarti & Harsha respectively.

The percentage of Calcium, Magnesium, Sulphur, Iron, Zinc, Copper and Manganese were found lower in inoculated shoot and root sample than the healthy

counterpart in most cases. Root-knot nematode infection often reduces plant growth and yield. The decreases nutrient uptake in the infested plants showing the deficiencies of Calcium, Magnesium, Sulphur, Iron, Zinc, Copper and Manganese due to root damage and subsequent prevention of water and nutrient uptake by the roots.

Variation in protein content in infected tissue may be due to impair protein metabolism due to infection of root knot nematode.

Starch the complex carbohydrate ultimately get converted in to sugar. Reduce percentage of starch in root may be due to high conversion ratio to sugar in infected plant. Increased sugar content in infected samples in both shoot and root might be due to movement of various metabolites towards the infection site from other parts of plants. This changes in total free amino acid in infected shoot and root may be due to impair in protein metabolism in infected tissue.

The accumulation of proline in nematode infected samples might be associated with the stress condition of the host plants induced by the nematodes.

The enhancement of phenolic compound in inoculated samples might be due to rapid liberation of conjugated phenol from the glycosidic compounds by the action of hydrolytic enzymes release by nematode during feeding process.

The enhanced activity of PAL and TAL enzymes and appearance of aromatic amino acids like tyrosine and phenylalanine in inoculated samples appears to be interesting and significant.

These amino acids are known to have significant role in lignifications. Both *p*-coumaric acid and *p*-cinnamic acid the product of TAL and PAL activities respectively are lignin precursors and play significant role in defense mechanism.

This increase peroxidase activity in both shoot and root may be due to synthesis of new iso enzymes as a response of nematode infection. Peroxidase is often cited as one of the key enzyme require for lignin bio-synthesis and phenol metabolism.

Superoxide radicals and singlet O<sub>2</sub> can be generated from NADH and H<sub>2</sub>O<sub>2</sub> through the action of catalase and peroxidase respectively, which are linked with resistant reaction of host plant. Due to root knot nematode infection super oxide (O<sub>2</sub><sup>-</sup>) amount increases in both shoot and root of infected tissue. As a result enzymatic activity of Superoxide dismutase (SOD) increases in infected part to neutralize the super oxide (O<sub>2</sub><sup>-</sup>).

By HPTLC analysis it was found that L-proline amino acid content increased in shoot as well as roots of all the inoculated varieties than the healthy ones. The accumulation of L-proline in nematode infected samples might be associated with the stress condition of the host plants induced by the nematodes. Decrease of aromatic amino acids like L-tyrosine was known to have significant role in imparting resistant reaction through lignification. In the present study the reduction of free tryptophan as infection progressed might be concern with initiation and progress of the galling activity. The rate of decrease of L-tryptophan in resistant variety was less as compared to susceptible variety.

Result of present study revealed that preventable yield losses due to root-knot nematode in ridge gourd variety Harsha was up to 74.52 per cent under field conditions with application of Furadon 3G at 2 kg a.i./ha.

The phylogenetic tree constructed by UPGMA method generated two main clusters i.e. cluster – I ( Two sub cluster with varieties Aneeta, Aarti and BSS – 1009) and cluster – II ( with varieties Harsha and Priya) .

Six varieties i.e. Priya, Challenger , Jaipur long ,BSS-1009, Tauri and Pallishree showed resistant reaction and can be directly grown as resistant varieties against root knot nematode, *M.incognita* in Odisha condition. These resistant varieties can a boon to ridge gourd growers for maximizing production without much residual chemicals through use of nematicides. Chlorophyll, macro nutrient, potassium, total proline, Phenol content, activity of enzyme PAL, TAL, SOD, Amino acid L- proline, L- tyrosine, L- tryptophan content were the important physiological and biochemical changes can be consider for selection against root knot nematode along with morphological character. Morphological along with physiological and biochemical character will definitely increase the selection precision and development of good resistant varieties against root-knot nematode, *M. incognita*.



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## APENDIX

**APPENDIX Track 1 to 20 represents presence and absence of different amino acids and amides in the roots and shoots of the resistant and susceptible ridge gourd varieties**

**Track 1 Presence and absence of different amino acids and amides in shoot of the healthy ridge gourd variety Priya(R)**

Peak	Start Rf	End Rf	Rf Range	Area(AU)	Assigned substance
1	0.00	0.04	0.00 - 0.04	2673.5	Unknown
2	0.04	0.08	0.04 - 0.08	2693.2	Unknown
3	0.08	0.10	0.08 - 0.10	1067.1	L- cystine
4	0.10	0.19	0.10 - 0.19	9521.9	L- glutamic acid, L- threonine
5	0.22	0.25	0.22 - 0.25	3502.0	L- aspartic acid
6	0.27	0.34	0.27 - 0.34	8268.7	L- serine, L- glutamine
7	0.36	0.41	0.36 - 0.41	4996.1	L- asparagine, L- lysine
8	0.42	0.44	0.42 - 0.44	1961.6	L- histidine
9	0.44	0.49	0.44 - 0.49	6275.0	L- arginine
10	0.50	0.52	0.50 - 0.52	1747.7	L- glycine
11	0.54	0.58	0.54 - 0.58	3320.8	L- alanine
12	0.58	0.60	0.58 - 0.60	2743.4	Unknown
13	0.62	0.64	0.62 - 0.64	1966.4	L- proline
14	0.64	0.66	0.64 - 0.66	1735.3	L- tryptophan
15	0.67	0.69	0.67 - 0.69	1860.8	Unknown
16	0.70	0.71	0.70 - 0.71	974.3	L- tyrosine
17	0.72	0.75	0.72 - 0.75	3500.4	L- methionine
18	0.76	0.78	0.76 - 0.78	977.8	L- valine
19	0.78	0.81	0.78 - 0.81	1424.4	L- leucine
20	0.81	0.85	0.81 - 0.85	1325.5	L- phenylalanine
21	0.86	0.88	0.86 - 0.88	321.5	L- isoleucine
22	0.88	0.93	0.88 - 0.93	536.2	Unknown

**Track 2 Presence and absence of different amino acids and amides in shoot of the inoculated ridge gourd variety Priya(R)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.00	0.02	0.00 - 0.02	140.9	Unknown
2	0.02	0.04	0.02 - 0.04	1061.4	Unknown
3	0.04	0.07	0.04 - 0.07	3508.8	Unknown
4	0.07	0.13	0.07 - 0.13	5894.9	L- cystine
5	0.13	0.17	0.13 - 0.17	2253.9	L- glutamic acid
6	0.18	0.21	0.18 - 0.21	2276.1	L- threonine
7	0.24	0.27	0.24 - 0.27	2746.4	L- aspartic acid, L- serine
8	0.27	0.32	0.27 - 0.32	2841.1	L- glutamine
9	0.32	0.34	0.32 - 0.34	1168.7	Unknown
10	0.34	0.37	0.34 - 0.37	1500.3	L- asparagine
11	0.38	0.42	0.38 - 0.42	1790.4	L- lysine
12	0.42	0.44	0.42 - 0.44	1836.1	L- histidine
13	0.45	0.48	0.45 - 0.48	1215.4	L- arginine
14	0.51	0.54	0.51 - 0.54	1411.0	L- glycine
15	0.54	0.59	0.54 - 0.59	2738.2	L- alanine
16	0.59	0.63	0.59 - 0.63	1288.8	L- proline
17	0.65	0.68	0.65 - 0.68	2092.1	L- tryptophan
18	0.72	0.76	0.72 - 0.76	2222.9	L- methionine, L- valine
19	0.78	0.81	0.78 - 0.81	1173.9	L- leucine
20	0.81	0.85	0.81 - 0.85	887.3	L- phenylalanine
21	0.87	0.90	0.87 - 0.90	357.4	L- isoleucine

**Track 3 Presence and absence of different amino acids and amides in shoot of the healthy ridge gourd variety BSS-1009(R)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.00	0.04	0.00 - 0.04	202.5	Unknown
2	0.05	0.10	0.05 - 0.10	5487.7	L- cystine
3	0.11	0.14	0.11 - 0.14	3048.2	L- glutamic acid
4	0.16	0.17	0.16 - 0.17	1236.1	Unknown
5	0.18	0.20	0.18 - 0.20	1215.4	L- threonine
6	0.22	0.25	0.22 - 0.25	1411.0	L- aspartic acid
7	0.27	0.30	0.27 - 0.30	4254.4	L- serine , L- glutamine
8	0.32	0.35	0.32 - 0.35	4538.5	Unknown
9	0.37	0.39	0.37 - 0.39	2391.6	L- asparagine
10	0.39	0.42	0.39 - 0.42	2972.2	L- lysine
11	0.42	0.45	0.42 - 0.45	3184.4	L- histidine
12	0.47	0.49	0.47 - 0.49	3560.3	L- arginine
13	0.50	0.53	0.50 - 0.53	1866.9	L- glycine
14	0.53	0.56	0.53 - 0.56	1958.7	Unknown
15	0.56	0.59	0.56 - 0.59	3547.1	L- alanine
16	0.62	0.64	0.62 - 0.64	1217.2	L- proline
17	0.65	0.69	0.65 - 0.69	2765.1	L- tryptophan
18	0.69	0.71	0.69 - 0.71	1374.3	L- tyrosine
19	0.74	0.79	0.74 - 0.79	2246.1	L- methionine, L- valine
20	0.81	0.84	0.81 - 0.84	1064.8	L- leucine
21	0.85	0.86	0.85 - 0.86	290.4	L- phenylalanine
22	0.86	0.88	0.86 - 0.88	279.9	L- isoleucine
23	0.89	0.91	0.89 - 0.91	296.8	Unknown

**Track 4 Presence and absence of different amino acids and amides in shoot of the inoculated ridge gourd variety BSS-1009(R)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.05	0.11	0.05 - 0.11	5902.5	L- cystine
2	0.11	0.18	0.11 - 0.18	6128.8	L- glutamic acid
3	0.19	0.21	0.19 - 0.21	1253.8	L- threonine
4	0.21	0.23	0.21 - 0.23	1323.8	Unknown
5	0.24	0.28	0.24 - 0.28	1942.0	L- aspartic acid, L- serine
6	0.31	0.34	0.31 - 0.34	1750.1	L- glutamine
7	0.36	0.41	0.36 - 0.41	4538.5	L- asparagine, L- lysine
8	0.43	0.45	0.43 - 0.45	2391.6	L- histidine
9	0.45	0.49	0.45 - 0.49	2972.2	L- arginine
10	0.49	0.51	0.49 - 0.51	1684.4	L- glycine
11	0.53	0.56	0.53 - 0.56	3560.3	Unknown
12	0.58	0.61	0.58 - 0.61	1666.9	L- alanine
13	0.61	0.63	0.61 - 0.63	1558.7	L- proline
14	0.66	0.68	0.66 - 0.68	1530.3	L- tryptophan
15	0.70	0.73	0.70 - 0.73	1160.8	L- tyrosine
16	0.73	0.75	0.73 - 0.75	616.9	L- methionine
17	0.75	0.76	0.75 - 0.76	597.3	L- valine
18	0.78	0.79	0.78 - 0.79	550.5	Unknown
19	0.79	0.82	0.79 - 0.82	900.1	L- leucine
20	0.83	0.86	0.83 - 0.86	501.4	L- phenylalanine
21	0.86	0.89	0.86 - 0.89	534.9	L- isoleucine

**Track 5 Presence and absence of different amino acids and amides in shoot of the healthy ridge gourd variety Aneeta(MR)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.06	0.13	0.06 - 0.13	7933.5	L- cystine. L- glutamic acid
2	0.14	0.18	0.14 - 0.18	4014.5	Unknown
3	0.19	0.22	0.19 - 0.22	2130.2	L- threonine
4	0.23	0.25	0.23 - 0.25	2600.6	L- aspartic acid
5	0.26	0.28	0.26 - 0.28	2418.8	L- serine
6	0.29	0.31	0.29 - 0.31	2305.3	L- glutamine
7	0.32	0.34	0.32 - 0.34	1681.5	Unknown
8	0.35	0.39	0.35 - 0.39	3954.3	L- asparagine , L- lysine
9	0.41	0.43	0.41 - 0.43	1967.1	Unknown
10	0.43	0.45	0.43 - 0.45	2257.5	L- histidine
11	0.45	0.48	0.45 - 0.48	2887.7	L- arginine
12	0.51	0.54	0.51 - 0.54	2054.4	L- glycine
13	0.54	0.56	0.54 - 0.56	2247.9	Unknown
14	0.56	0.59	0.56 - 0.59	2056.4	L- alanine
15	0.60	0.62	0.60 - 0.62	1924.1	Unknown
16	0.63	0.65	0.63 - 0.65	1110.8	L- proline
17	0.65	0.68	0.65 - 0.68	2384.1	L- tryptophan
18	0.74	0.76	0.74 - 0.76	1051.3	L- methionine
19	0.76	0.79	0.76 - 0.79	1127.4	L- valine
20	0.80	0.83	0.80 - 0.83	1076.1	L- leucine
21	0.83	0.86	0.83 - 0.86	691.2	L- phenylalanine
22	0.86	0.89	0.86 - 0.89	329.6	L- isoleucine
23	0.96	0.98	0.96 - 0.98	89.6	Unknown

**Track 6 Presence and absence of different amino acids and amides in shoot of the inoculated ridge gourd variety Aneeta(MR)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.06	0.13	0.06 - 0.13	9133.0	L- cystine
2	0.13	0.17	0.13 - 0.17	3717.5	L- glutamic acid
3	0.19	0.23	0.19 - 0.23	2222.9	L- threonine
4	0.26	0.29	0.26 - 0.29	2092.1	L- serine
5	0.30	0.33	0.30 - 0.33	2079.9	L- glutamine
6	0.35	0.37	0.35 - 0.37	1236.1	L- asparagine
7	0.38	0.41	0.38 - 0.41	1215.4	L- lysine
8	0.44	0.47	0.44 - 0.47	1411.0	L- histidine, L- arginine
9	0.50	0.54	0.50 - 0.54	1478.8	L- glycine
10	0.55	0.58	0.55 - 0.58	2720.0	L- alanine
11	0.60	0.62	0.60 - 0.62	1545.4	L- proline
12	0.67	0.71	0.67 - 0.71	2753.7	L- tyrosine
13	0.72	0.73	0.72 - 0.73	717.5	Unknown
14	0.73	0.76	0.73 - 0.76	1169.8	L- methionine, L- valine
15	0.77	0.81	0.77 - 0.81	1530.5	L- leucine
16	0.81	0.84	0.81 - 0.84	848.3	Unknown
17	0.86	0.89	0.86 - 0.89	567.2	L- isoleucine
18	0.89	0.90	0.89 - 0.90	171.1	Unknown
19	0.90	0.93	0.90 - 0.93	218.0	Unknown

**Track 7 Presence and absence of different amino acids and amides in shoot of the healthy ridge gourd variety Aarti(HS)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.06	0.08	0.06 - 0.08	1409.6	Unknown
2	0.08	0.13	0.08 - 0.13	4796.6	L- cystine, L- glutamic acid
3	0.13	0.18	0.13 - 0.18	3020.9	L- threonine
4	0.20	0.27	0.20 - 0.27	4071.1	L- aspartic acid
5	0.27	0.30	0.27 - 0.30	1488.4	L- serine
6	0.31	0.34	0.31 - 0.34	2636.1	L- glutamine
7	0.34	0.36	0.34 - 0.36	1536.7	L- asparagine
8	0.36	0.40	0.36 - 0.40	1523.1	L- lysine
9	0.40	0.44	0.40 - 0.44	1717.2	L- histidine
10	0.48	0.51	0.48 - 0.51	1511.7	L- arginine, L- glycine
11	0.54	0.56	0.54 - 0.56	430.3	Unknown
12	0.56	0.57	0.56 - 0.57	657.9	Unknown
13	0.57	0.59	0.57 - 0.59	1030.3	L- alanine
14	0.61	0.63	0.61 - 0.63	1590.7	L- proline
15	0.66	0.68	0.66 - 0.68	1053.6	L- tryptophan
16	0.68	0.70	0.68 - 0.70	926.6	Unknown
17	0.70	0.72	0.70 - 0.72	821.8	L- tyrosine
18	0.72	0.74	0.72 - 0.74	1009.3	L- methionine
19	0.74	0.77	0.74 - 0.77	925.6	L- valine
20	0.79	0.81	0.79 - 0.81	671.6	L- leucine
21	0.81	0.83	0.81 - 0.83	699.3	Unknown
22	0.93	0.96	0.93 - 0.96	286.5	Unknown
23	0.96	0.99	0.96 - 0.99	102.5	Unknown

**Track 8 Presence and absence of different amino acids and amides in shoot of the inoculated ridge gourd variety Aarti(HS)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.08	0.12	0.08 - 0.12	5646.7	L- cystine
2	0.12	0.15	0.12 - 0.15	3156.0	L- glutamic acid
3	0.21	0.23	0.21 - 0.23	2571.0	L- threonine
4	0.32	0.33	0.32 - 0.33	836.4	Unknown
5	0.38	0.41	0.38 - 0.41	2353.3	L- lysine
6	0.41	0.43	0.41 - 0.43	1672.1	Unknown
7	0.43	0.45	0.43 - 0.45	1540.9	L- histidine
8	0.45	0.49	0.45 - 0.49	2342.4	L- arginine
9	0.51	0.53	0.51 - 0.53	1915.9	L- glycine
10	0.59	0.61	0.59 - 0.61	1074.1	Unknown
11	0.61	0.63	0.61 - 0.63	1403.5	L- proline
12	0.63	0.64	0.63 - 0.64	761.7	Unknown
13	0.64	0.67	0.64 - 0.67	953.3	L- tryptophan
14	0.67	0.70	0.67 - 0.70	1935.4	L- tyrosine
15	0.70	0.74	0.70 - 0.74	2137.9	L- methionine
16	0.76	0.77	0.76 - 0.77	618.2	L- valine
17	0.77	0.80	0.77 - 0.80	1120.0	L- leucine
18	0.81	0.84	0.81 - 0.84	720.2	Unknown
19	0.84	0.85	0.84 - 0.85	292.4	L- phenylalanine
20	0.85	0.88	0.85 - 0.88	432.6	L- isoleucine
21	0.88	0.90	0.88 - 0.90	323.2	Unknown
22	0.90	0.91	0.90 - 0.91	123.1	Unknown

**Track 9 Presence and absence of different amino acids and amides in shoot of the healthy ridge gourd variety Harsha(HS)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.01	0.03	0.01 - 0.03	213.4	Unknown
2	0.08	0.13	0.08 - 0.13	3346.5	L- cystine
3	0.13	0.19	0.13 - 0.19	6283.8	L- glutamic acid
4	0.19	0.25	0.19 - 0.25	6698.6	L- threonine, L- aspartic acid
5	0.30	0.32	0.30 - 0.32	1566.5	L- glutamine
6	0.36	0.38	0.36 - 0.38	1817.8	L- asparagine, L- lysine
7	0.44	0.46	0.44 - 0.46	1494.7	L- histidine,
8	0.46	0.48	0.46 - 0.48	1152.5	L- arginine
9	0.51	0.53	0.51 - 0.53	1718.1	L- glycine
10	0.54	0.56	0.54 - 0.56	1143.4	Unknown
11	0.58	0.60	0.58 - 0.60	1754.4	L- alanine
12	0.60	0.62	0.60 - 0.62	2015.4	L- proline
13	0.66	0.68	0.66 - 0.68	992.9	L- tryptophan
14	0.68	0.70	0.68 - 0.70	918.1	Unknown
15	0.70	0.72	0.70 - 0.72	1364.7	L- tyrosine
16	0.72	0.75	0.72 - 0.75	1212.2	L- methionine
17	0.75	0.77	0.75 - 0.77	739.1	L- valine
18	0.79	0.81	0.79 - 0.81	925.2	L- leucine
19	0.82	0.86	0.82 - 0.86	962.4	L- phenylalanine
20	0.86	0.88	0.86 - 0.88	542.0	L- isoleucine
21	0.88	0.90	0.88 - 0.90	286.7	Unknown
22	0.97	0.98	0.97 - 0.98	59.8	Unknown
23	0.98	0.99	0.98 - 0.99	76.4	Unknown

**Track 10 Presence and absence of different amino acids and amides in shoot of the inoculated ridge gourd variety Aneeta(HS)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.00	0.04	0.00 - 0.04	202.5	Unknown
2	0.05	0.10	0.05 - 0.10	6087.7	L- cystine
3	0.11	0.14	0.11 - 0.14	3248.2	L- glutamic acid
4	0.19	0.21	0.19 - 0.21	3448.2	L- threonine
5	0.24	0.26	0.24 - 0.26	2717.8	L- aspartic acid
6	0.31	0.32	0.31 - 0.32	1456.0	L- glutamine
7	0.37	0.39	0.37 - 0.39	1930.3	L- asparagine
8	0.39	0.41	0.39 - 0.41	1983.2	L- lysine
9	0.41	0.42	0.41 - 0.42	1012.1	Unknown
10	0.45	0.49	0.45 - 0.49	2742.1	L- arginine
11	0.51	0.53	0.51 - 0.53	2124.6	L- glycine
12	0.53	0.57	0.53 - 0.57	2851.4	L- alanine
13	0.60	0.61	0.60 - 0.61	1296.0	Unknown
14	0.61	0.63	0.61 - 0.63	1364.7	L- proline
15	0.63	0.66	0.63 - 0.66	1102.6	Unknown
16	0.66	0.69	0.66 - 0.69	1049.8	L- tryptophan
17	0.69	0.71	0.69 - 0.71	865.9	L- tyrosine
18	0.74	0.77	0.74 - 0.77	968.3	L- methionine, L- valine
19	0.80	0.83	0.80 - 0.83	1080.6	L- leucine
20	0.83	0.84	0.83 - 0.84	396.4	Unknown
21	0.87	0.90	0.87 - 0.90	353.9	L- isoleucine
22	0.90	0.92	0.90 - 0.92	105.0	Unknown

**Track 11 Presence and absence of different amino acids and amides in root of the healthy ridge gourd variety Priya(R)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.01	0.04	0.01 - 0.04	760.7	Unknown
2	0.05	0.07	0.05 - 0.07	895.3	Unknown
3	0.10	0.16	0.10 - 0.16	5779.3	L- cystine, L- glutamic acid
4	0.18	0.20	0.18 - 0.20	1551.3	L- threonine
5	0.25	0.28	0.25 - 0.28	1618.1	L- aspartic acid, L- serine
6	0.31	0.33	0.31 - 0.33	1609.5	L- glutamine
7	0.34	0.36	0.34 - 0.36	1351.1	Unknown
8	0.36	0.37	0.36 - 0.37	908.2	L- asparagine
9	0.38	0.45	0.38 - 0.45	3571.1	L- lysine, L- histidine
10	0.45	0.52	0.45 - 0.52	3363.9	L- arginine, L- glycine
11	0.55	0.57	0.55 - 0.57	961.5	L- alanine
12	0.57	0.60	0.57 - 0.60	1933.4	Unknown
13	0.60	0.62	0.60 - 0.62	1441.8	L- proline
14	0.67	0.70	0.67 - 0.70	1322.3	L- tryptophan
15	0.70	0.73	0.70 - 0.73	1300.7	L- tyrosine
16	0.73	0.78	0.73 - 0.78	1050.6	L- methionine, L- valine
17	0.78	0.80	0.78 - 0.80	776.9	L- leucine
18	0.80	0.84	0.80 - 0.84	849.9	Unknown
19	0.84	0.87	0.84 - 0.87	333.3	L- phenylalanine
20	0.88	0.90	0.88 - 0.90	167.5	L- isoleucine
21	0.90	0.92	0.90 - 0.92	123.9	Unknown
22	0.92	0.94	0.92 - 0.94	101.3	Unknown

**Track 12 Presence and absence of different amino acids and amides in root of the inoculated ridge gourd variety Priya(R)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.01	0.05	0.01 - 0.05	491.2	Unknown
2	0.05	0.08	0.05 - 0.08	567.1	Unknown
3	0.08	0.10	0.08 - 0.10	453.7	L- cystine
4	0.10	0.15	0.10 - 0.15	2307.4	L- glutamic acid
5	0.21	0.28	0.21 - 0.28	4582.9	L- threonine, L- aspartic acid, L- serine
6	0.32	0.37	0.32 - 0.37	3046.4	L- asparagine
7	0.38	0.42	0.38 - 0.42	3332.3	L- lysine
8	0.42	0.46	0.42 - 0.46	2909.3	L- histidine
9	0.49	0.51	0.49 - 0.51	1692.0	L- arginine
10	0.51	0.54	0.51 - 0.54	1779.3	L- glycine
11	0.56	0.58	0.56 - 0.58	982.1	L- alanine
12	0.58	0.62	0.58 - 0.62	831.5	L- proline
13	0.62	0.65	0.62 - 0.65	3568.1	L- tryptophan
14	0.67	0.70	0.67 - 0.70	2120.2	Unknown
15	0.70	0.73	0.70 - 0.73	1600.4	L- tyrosine
16	0.73	0.77	0.73 - 0.77	2223.6	L- methionine
17	0.77	0.79	0.77 - 0.79	900.7	L- valine
18	0.83	0.85	0.83 - 0.85	721.2	Unknown
19	0.85	0.87	0.85 - 0.87	605.2	L- phenylalanine
20	0.87	0.90	0.87 - 0.90	389.3	L- isoleucine
21	0.90	0.92	0.90 - 0.92	458.8	Unknown
22	0.94	0.96	0.94 - 0.96	85.2	Unknown

**Track 13 Presence and absence of different amino acids and amides in root of the healthy ridge gourd variety BSS-1009(R)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.01	0.04	0.01 - 0.04	175.0	Unknown
2	0.08	0.13	0.08 - 0.13	1493.6	L- cystine
3	0.13	0.17	0.13 - 0.17	2935.0	L- glutamic acid
4	0.22	0.24	0.22 - 0.24	1081.2	L- aspartic acid
5	0.27	0.28	0.27 - 0.28	812.1	L- serine
6	0.28	0.30	0.28 - 0.30	1183.2	Unknown
7	0.30	0.32	0.30 - 0.32	1330.3	L- glutamine
8	0.35	0.36	0.35 - 0.36	956.0	L- asparagine
9	0.40	0.42	0.40 - 0.42	1517.8	L- lysine
10	0.46	0.48	0.46 - 0.48	1821.9	L- arginine
11	0.48	0.51	0.48 - 0.51	1891.4	Unknown
12	0.51	0.54	0.51 - 0.54	1857.7	L- glycine
13	0.56	0.60	0.56 - 0.60	2245.3	L- alanine
14	0.63	0.67	0.63 - 0.67	1675.0	L- proline
15	0.67	0.72	0.67 - 0.72	2441.7	L- tryptophan, L- tyrosine
16	0.72	0.74	0.72 - 0.74	586.1	L- methionine
17	0.77	0.81	0.77 - 0.81	460.2	L- valine, L- leucine
18	0.82	0.86	0.82 - 0.86	270.8	L- phenylalanine
19	0.86	0.89	0.86 - 0.89	155.9	L- isoleucine
20	0.89	0.91	0.89 - 0.91	165.1	Unknown

**Track 14 Presence and absence of different amino acids and amides in root of the inoculated ridge gourd variety BSS-1009(R)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.08	0.18	0.08 - 0.18	8746.5	L- cystine, L- glutamic acid
2	0.20	0.23	0.20 - 0.23	3538.5	L- threonine
3	0.24	0.26	0.24 - 0.26	1991.6	L- aspartic acid
4	0.26	0.29	0.26 - 0.29	1872.2	L- serine
5	0.29	0.31	0.29 - 0.31	2684.4	L- glutamine
6	0.33	0.36	0.33 - 0.36	2860.3	L- asparagine
7	0.39	0.40	0.39 - 0.40	1389.2	L- lysine
8	0.44	0.49	0.44 - 0.49	3285.5	L- histidine, L- arginine
9	0.49	0.51	0.49 - 0.51	823.1	Unknown
10	0.51	0.53	0.51 - 0.53	1648.7	L- glycine
11	0.56	0.58	0.56 - 0.58	1279.4	L- alanine
12	0.58	0.60	0.58 - 0.60	1003.7	Unknown
13	0.60	0.63	0.60 - 0.63	1714.7	L- proline
14	0.63	0.67	0.63 - 0.67	2012.6	L- tryptophan
15	0.67	0.69	0.67 - 0.69	1060.7	Unknown
16	0.69	0.71	0.69 - 0.71	731.6	L- tyrosine
17	0.71	0.74	0.71 - 0.74	1386.9	L- methionine
18	0.81	0.84	0.81 - 0.84	765.8	L- leucine
19	0.89	0.91	0.88 - 0.91	287.6	L- isoleucine
20	0.91	0.93	0.91 - 0.93	399.5	Unknown

**Track 15 Presence and absence of different amino acids and amides in root of the healthy ridge gourd variety Aneeta(MR)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.01	0.05	0.01 - 0.05	491.2	Unknown
2	0.05	0.07	0.05 - 0.07	567.1	Unknown
3	0.08	0.10	0.08 - 0.10	453.7	Unknown
4	0.10	0.16	0.10 - 0.16	3107.4	L- cystine, L- glutamic acid
5	0.19	0.21	0.19 - 0.21	1478.8	L- threonine
6	0.23	0.26	0.23 - 0.26	1471.8	L- aspartic acid
7	0.28	0.32	0.28 - 0.32	2260.8	L- glutamine
8	0.35	0.38	0.35 - 0.38	2135.3	L- asparagine
9	0.38	0.42	0.38 - 0.42	2366.4	L- lysine
10	0.45	0.49	0.45 - 0.49	3243.4	L- arginine
11	0.52	0.55	0.52 - 0.55	2573.4	L- glycine
12	0.57	0.59	0.57 - 0.59	1392.8	L- alanine
13	0.59	0.62	0.59 - 0.62	2573.7	Unknown
14	0.63	0.65	0.63 - 0.65	1226.6	L- proline
15	0.65	0.69	0.65 - 0.69	1712.1	L- tryptophan
16	0.69	0.73	0.69 - 0.73	1546.6	L- tyrosine
17	0.74	0.76	0.74 - 0.76	756.2	L- methionine, L- valine
18	0.79	0.81	0.79 - 0.81	1026.4	L- leucine
19	0.84	0.86	0.84 - 0.86	362.2	L- phenylalanine
20	0.86	0.91	0.86 - 0.91	605.3	L- isoleucine
21	0.91	0.93	0.91 - 0.93	135.6	Unknown
22	0.93	0.95	0.93 - 0.95	105.6	Unknown

**Track 16 Presence and absence of different amino acids and amides in root of the inoculated ridge gourd variety Aneeta(MR)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.08	0.11	0.08 - 0.11	1923.3	L- cystine
2	0.12	0.17	0.12 - 0.17	4659.0	L- glutamic acid
3	0.19	0.21	0.19 - 0.21	1969.2	L- threonine
4	0.21	0.23	0.21 - 0.23	1796.4	Unknown
5	0.27	0.29	0.27 - 0.29	2372.4	L- serine
6	0.35	0.37	0.35 - 0.37	2172.9	L- asparagine
7	0.40	0.42	0.40 - 0.42	2172.9	L- lysine
8	0.48	0.51	0.48 - 0.51	2372.4	L- arginine, L- glycine
9	0.54	0.56	0.54 - 0.56	1796.4	Unknown
10	0.57	0.59	0.57 - 0.59	1969.2	L- alanine
11	0.60	0.63	0.60 - 0.63	2134.8	L- proline
12	0.68	0.69	0.68 - 0.69	1107.9	Unknown
13	0.70	0.73	0.70 - 0.73	1537.5	L- tyrosine
14	0.74	0.76	0.74 - 0.76	1066.6	L- methionine
15	0.77	0.78	0.77 - 0.78	625.8	L- valine
16	0.78	0.80	0.78 - 0.80	786.3	Unknown
17	0.81	0.83	0.81 - 0.83	678.0	L- leucine
18	0.85	0.87	0.85 - 0.87	383.9	L- phenylalanine
19	0.87	0.89	0.87 - 0.89	295.1	L- isoleucine
20	0.89	0.91	0.89 - 0.91	295.3	Unknown
21	0.91	0.93	0.91 - 0.93	258.4	Unknown
22	0.96	0.97	0.96 - 0.97	87.8	Unknown
23	0.98	0.99	0.98 - 0.99	72.2	Unknown

**Track 17 Presence and absence of different amino acids and amides in root of the healthy ridge gourd variety Aarti (HS)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.02	0.04	0.02 - 0.04	142.2	Unknown
2	0.08	0.11	0.08 - 0.11	919.3	L- cystine
3	0.11	0.17	0.11 - 0.17	3062.0	L- glutamic acid
4	0.19	0.21	0.19 - 0.21	1012.1	L- threonine
5	0.21	0.25	0.21 - 0.25	1983.2	L- aspartic acid
6	0.25	0.28	0.25 - 0.28	1930.3	L- serine
7	0.32	0.34	0.32 - 0.34	1456.0	Unknown
8	0.40	0.43	0.40 - 0.43	2017.8	L- lysine
9	0.48	0.50	0.48 - 0.50	1617.8	L- arginine
10	0.54	0.55	0.54 - 0.55	1856.0	L- glycine
11	0.58	0.61	0.58 - 0.61	1930.3	L- alanine
12	0.61	0.64	0.61 - 0.64	1983.2	L- proline
13	0.64	0.65	0.64 - 0.65	1012.1	L- tryptophan
14	0.69	0.71	0.69 - 0.71	1681.2	L- tyrosine
15	0.75	0.76	0.75 - 0.76	882.2	L- methionine
16	0.77	0.78	0.77 - 0.78	971.8	L- valine
17	0.78	0.80	0.78 - 0.80	956.0	L- leucine
18	0.81	0.84	0.81 - 0.84	1221.3	Unknown
19	0.84	0.87	0.84 - 0.87	966.6	L- phenylalanine
20	0.88	0.90	0.88 - 0.90	400.7	L- isoleucine

**Track 18 Presence and absence of different amino acids and amides in root of the inoculated ridge gourd variety Aarti(HS)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.01	0.04	0.01 - 0.04	760.7	Unknown
2	0.05	0.07	0.05 - 0.07	895.3	Unknown
3	0.10	0.16	0.10 - 0.16	4779.3	L- cystine, L- glutamic acid
4	0.22	0.26	0.22 - 0.26	2458.1	L- aspartic acid
5	0.26	0.29	0.26 - 0.29	1726.8	L- serine
6	0.31	0.32	0.31 - 0.32	1242.5	L- glutamine
7	0.34	0.38	0.34 - 0.38	2753.1	L- asparagine
8	0.38	0.41	0.38 - 0.41	1835.6	L- lysine
9	0.43	0.46	0.43 - 0.46	3508.9	L- histidine
10	0.49	0.53	0.49 - 0.53	4818.9	L- glycine
11	0.55	0.56	0.55 - 0.56	1220.2	L- alanine
12	0.62	0.64	0.62 - 0.64	2494.8	L- proline
13	0.66	0.68	0.66 - 0.68	1938.7	L- tryptophan
14	0.68	0.70	0.68 - 0.70	1584.4	Unknown
15	0.70	0.72	0.70 - 0.72	1680.9	L- tyrosine
16	0.72	0.74	0.72 - 0.74	879.5	L- methionine
17	0.74	0.77	0.74 - 0.77	1659.8	L- valine
18	0.79	0.80	0.79 - 0.80	793.5	L- leucine
19	0.83	0.86	0.83 - 0.86	1009.4	L- phenylalanine
20	0.90	0.92	0.90 - 0.92	278.9	Unknown

**Track 19 Presence and absence of different amino acids and amides in root of the healthy ridge gourd variety Harsha(HS)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.06	0.12	0.06 - 0.12	6968.3	L- cystine
2	0.12	0.17	0.12 - 0.17	3815.7	L- glutamic acid
3	0.22	0.25	0.22 - 0.25	2169.2	L- aspartic acid
4	0.25	0.28	0.25 - 0.28	1996.4	L- serine
5	0.31	0.34	0.31 - 0.34	2372.4	L- glutamine
6	0.40	0.42	0.40 - 0.42	1772.9	L- lysine
7	0.52	0.56	0.52 - 0.56	2282.9	L- glycine
8	0.57	0.60	0.57 - 0.60	1546.4	L- alanine
9	0.60	0.63	0.60 - 0.63	1932.3	L- proline
10	0.66	0.67	0.66 - 0.67	809.3	L- tryptophan
11	0.67	0.69	0.67 - 0.69	992.0	Unknown
12	0.70	0.72	0.70 - 0.72	879.3	L- tyrosine
13	0.75	0.78	0.75 - 0.78	1044.9	L- valine
14	0.79	0.82	0.79 - 0.82	1003.9	L- leucine
15	0.82	0.85	0.82 - 0.85	667.5	L- phenylalanine
16	0.85	0.89	0.85 - 0.89	524.8	L- isoleucine
17	0.97	0.98	0.97 - 0.98	100.6	Unknown

**Track 20 Presence and absence of different amino acids and amides in root of the inoculated ridge gourd variety Harsha(HS)**

Peak	Start Rf	End Rf	Rf Range	Area (AU)	Assigned substance
1	0.02	0.04	0.02 - 0.04	898.3	Unknown
2	0.06	0.10	0.06 - 0.10	2394.4	L- cystine
3	0.10	0.18	0.10 - 0.18	9090.0	L- glutamic acid
4	0.20	0.23	0.20 - 0.23	2961.1	L- threonine
5	0.24	0.26	0.24 - 0.26	1598.3	L- aspartic acid
6	0.26	0.29	0.26 - 0.29	2023.7	L- serine
7	0.29	0.31	0.29 - 0.31	2187.3	L- glutamine
8	0.33	0.36	0.33 - 0.36	2976.2	L- asparagine
9	0.40	0.41	0.40 - 0.41	867.0	L- lysine
10	0.43	0.46	0.43 - 0.46	2214.3	L- histidine
11	0.48	0.50	0.48 - 0.50	1870.5	L- arginine
12	0.54	0.58	0.54 - 0.58	4408.9	L- alanine
13	0.61	0.65	0.61 - 0.65	3500.4	L- proline
14	0.69	0.72	0.69 - 0.72	2199.8	L- tyrosine
15	0.77	0.78	0.77 - 0.78	796.6	L- valine
16	0.78	0.81	0.78 - 0.81	1703.7	L- leucine
17	0.81	0.85	0.81 - 0.85	1553.1	L- phenylalanine
18	0.89	0.91	0.89 - 0.91	286.5	Unknown





## Physiological and biochemical alteration in susceptible and resistant ridge gourd cultivars against induced population of root-knot nematode, *Meloidogyne incognita*

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Received: 16 March 2018 / Revised: 26 October 2018 / Accepted: 29 October 2018 / Published online: 9 November 2018  
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### Abstract

Biochemical changes in Ridge gourd varieties inoculated with root-knot nematode, *Meloidogyne incognita* were investigated. Observations were recorded on biochemical modifications relating to various parameters like chlorophyll 'a', chlorophyll 'b', total chlorophyll, total sugar and starch and total protein content during post infection periods. The variation in chlorophyll 'a', chlorophyll 'b', total chlorophyll, total protein, total sugar and starch content in five cultivars, i.e. Priya, BSS-1009, Aneeta, Aarti and Harsha were studied 30 days after inoculation. Reduced percentage of chlorophyll 'a', chlorophyll 'b', total chlorophyll contents were observed in inoculated samples than the healthy counterparts. However, an increase in amount of total sugar, starch and total protein contents was observed in the diseased tissues.

**Keywords** Chlorophyll content · *Meloidogyne incognita* · Protein content · Ridge gourd cultivars · Starch content · Sugar content

### Introduction

Root-knot nematodes like many other obligate parasites are capable of disturbing the host metabolism. The changes in the physiological and biochemical processes of infected host as a consequence of disturbed metabolism decide whether the host becomes susceptible or resistant to nematode attack (Krusberg 1963). 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 the 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; Howell and Krusberg 1966; Mohanty et al. 1995; Nayak 2015). Considering the importance of the subject, the present investigation was undertaken to find the changes if any, in total sugar content, chlorophyll contents, total protein and starch

contents in relation to ridgegourd inoculated with root-knot nematode, *Meloidogyne incognita*. Cultivars of ridgegourd were sown in 15 cm diameter earthen pots filled with steam sterilized soil. A week after germination, one seedling per pot was retained and inoculated with axenised suspension of approximately 1000 J2 of *M. incognita* per seedling per pot. A set of plants was left uninoculated to serve as control. Healthy and inoculated plants were harvested at 45 days after planting. The harvested roots were washed thoroughly under running tap water to remove the adhering soil particles. Then the shoots were surface sterilized in 1% HgCl<sub>2</sub> solution followed by repeated washing with distilled water. The harvested shoots were dried on blotting paper and kept separately for biochemical analysis. The chlorophyll content was estimated by Spectro-colourimeter method. Total sugar and starch and total protein contents were estimated by following Spectrocolourimeter methods.

### Materials and methods

In order to understand the basis of nematode resistance, five varieties namely Priya, BSS-1009, Aneeta, Aarti and Harsha were grown in earthen pots in the greenhouse. These plants were washed 30 days after nematode inoculation and the

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following chemical compositions were estimated by different methods, i.e.

- Chlorophyll content (Spectro-colourimeter Method) (Lichtenthaler & Welburn 1983)
- Total protein content (Lowry Method) (Lowry et al. 1951)
- Total sugar content (Spectro-colourimeter Method) (Yoshida et al. 1972)
- Total starch content (Spectro-colourimeter Method) (Yoshida et al. 1972).

### Chlorophyll estimation of leaf

One hundred fifty mg leaf portion of each treatment were cut from the composite leaves and were immersed in 50 ml of 80% acetone in a conical flask and kept in dark for 24 h for extraction of chlorophyll from the leaf samples. Thereafter, the chlorophyll extracts were filtered through Whatman No. 1 filter paper. Absorbance of the chlorophyll extract was measured at 645 nm and 663 nm using a colorimeter. The amount of chlorophyll-a, chlorophyll-b and total chlorophyll were calculated in mg/g fresh weight according to the following equations. (i) Chlorophyll-a (mg/g fresh weight of leaf) =  $12.7 \times (D-663) - 2.69 \times (D-645)$  (ii) Chlorophyll-b (mg/g fresh weight of leaf) =  $22.9 \times (D-645) - 4.68 \times (D-663)$  (iii) Total chlorophyll (mg/g fresh wt. of leaf) =  $20.2 \times (D-645) + 8.02 \times (D-663) \times$  where D-645 = optical density at 645 nm D-663 = optical density at 663 nm V = final volume of 80% acetone chlorophyll extract in ml W = Fresh weight in g of corresponding amount of fresh leaves used in the extraction of chlorophyll.

### Estimation of total sugar content

The total sugar content of shoot were determined by adopting the following procedure.

### Extraction of shoot sugar

One hundred mg of ground seed samples were taken in 15 ml centrifuge tubes and 10 ml of 80% ethanol was added to it. The mouth of the centrifuge tube was covered with polythene paper and kept in a water bath at 80–85 °C for 30 min. Then it was cooled and centrifuged for 15 min at 2000 rpm. After centrifugation, the supernatant was decanted into a 25 ml volumetric flask. This extraction procedure was repeated once again and the supernatant was collected in the previous 25 ml volumetric flask. The final volume was made up to 25 ml with distilled water and was filtered through Whatman No. 1 filter paper. This was the sugar extract kept for sugar estimation.

### Estimation of total sugar

Two ml of sugar extract was transferred into a 50 ml volumetric flask and volume was made up to 50 ml with distilled water. Five ml of this extract was taken in a 25 ml volumetric flask. Simultaneously standards of 0 ml, 1 ml, 1.5 ml and 2 ml. Of 100 ppm glucose solution were taken in 25 ml volumetric flasks. Volume of these standards was made up to 5 ml with addition of distilled water and 2 drops of 80% ethanol. Volumetric flasks containing samples and standards were kept in an ice-bath. To each volumetric flask, 10 ml of anthrone reagent (2 gm of anthrone in one litre of 95% H<sub>2</sub>SO<sub>4</sub>) was added allowing it to run down the side of the volumetric flask. The contents of the flasks were shaken slowly by swirling the flask and then shaken thoroughly. The volumetric flasks were kept in boiling water bath for exactly 7.5 min. Then immediately the flasks were cooled in ice. After cooling, absorbance was measured at 630 nm and sugar content was calculated by the help of standard curve.

### Estimation of total starch content

1 ml of starch extract was taken in a 100 ml volumetric flask and diluted to 100 ml with distilled water. Five ml of the above extract was transferred in a 50 ml test tube. Then all the standards and sample test tubes were kept in ice bath for cooling, and 10 ml of anthrone reagent was added to each test tube, allowing the reagent to run down the side of the flask. It was stirred slowly with a glass rod and then shaken thoroughly. The flask was kept in boiling water bath exactly for 7.5 min. Then the test tube was immediately cooled in ice-bath. After cooling, the O.D. at 630 nm was measured and the starch content was calculated by the help of standard curve, which was multiplied by 0.91 to get the exact value of the same.

### Estimation of total protein content

Two ml of the sample extract was pipette into the test tubes, and the volume make up was done upto 2 ml in all test tubes with water. Then 10 ml of reagent C was added to each test tube including blank. It was mixed well and allowed to stand for 10 min. Then 1 ml of reagent D was added, mixed well and was incubated at room temperature in the dark for 30 min. Then blue colour was developed. Finally, absorbance was measured at 660 nm and protein content was calculated by the help of standard curve.

## Results and discussion

Many kinds of elements and enzymes are present in the plant system that influences the metabolism of the pests fed upon them. The chemicals may consist of simple nitrogen, phosphorus, potassium, carbohydrates and fats to complex proteins, enzymes, phenolic compounds etc., which may nourish, starve or kill the feeding organisms upon them. In the course of feeding some chemicals may be depleted or some others may be de novo synthesized, that may be detrimental to the pest. In order to know the chemical and genetic basis of resistance, five varieties were chosen for a detailed analysis. These plants were grown with utmost care, both in inoculated and control conditions. One set of each uninoculated (healthy) and inoculated (infected) plants were analysed to test the effects of root-knot nematode infection on the growth and vigour of the plants and their root-system.

### Effect of nematode infection on chlorophyll content

#### Estimation of chlorophyll of leaves

Chlorophyll content is the most important constituent of the plants as it manufactures the food, which is necessary for the growth and development of all the parts of plant. It is directly correlated with the yield of the crops. Root-knot nematodes are known to reduce the chlorophyll contents of the plants by disrupting its nutrient uptake and partitioning of the photosynthates.

In the experiment it was observed that the chlorophyll content reduced from 0.42 to 0.25 mg/g due to nematode infection in case of Priya variety. Similarly in the varieties BSS-1009, Aneeta, Aarti and Harsha chlorophyll 'a' reduced from 0.40 to 0.20 mg/g, 0.42 to 0.22 mg/g, 0.33 to 0.26 mg/g and 0.26 to 0.15 mg/g respectively. Hence nematode caused 40.47% reduction in chlorophyll 'a' content of Priya, 49.87% reduction in BSS-1009, 48.71% reduction in Aneeta, 19.33% reduction in Aarti and 42.69% reduction in Harsha.

In case of chlorophyll 'b' content reduction from healthy to infected plants recorded were 0.08–0.06, 0.16–0.10, 0.13–0.10, 0.25–0.16 and 0.15–0.11 mg/g in case of varieties Priya, BSS-1009, Aneeta, Aarti and Harsha respectively. Hence nematode caused 24.50% reduction in chlorophyll 'b' content of Priya, 33.75% reduction in BSS-1009, 25.73% in Aneeta, 34.64% reduction in Aarti and 30.81% reduction in Harsha.

Similarly the total chlorophyll content was decreased from 0.59 to 0.23 mg/g in Priya, 0.59 to 0.15 mg/g in BSS-1009, 0.38 to 0.37 mg/g in Aneeta, 0.35 to 0.34 mg/g in

Aarti and 0.31 to 0.21 mg/g in Harsha. Hence nematode caused 60.67% reduction in total chlorophyll content of Priya, 73.78% reduction in BSS-1009, 1.32% reduction in Aneeta, 3.68% reduction in Aarti and 30.12% reduction in Harsha (Table 1; Fig. 1a, b, c).

### Effect of nematode infection on protein content

#### Total Protein in shoots

The amount of total protein present in the shoot portion of the inoculated plants were recorded as 5.21, 5.39, 7.11, 4.50, 3.90 mg/g of the varieties Priya, BSS-1009, Aneeta, Aarti and Harsha respectively on fresh weight basis (Table 2). In the healthy shoots of these varieties total protein contents were 5.31, 7.54, 7.32, 4.52 and 4.12 mg/g respectively. The percentage of decrease in total protein content due to root-knot nematode infection over control was noted to be 1.88, 28.51, 2.86, 0.44, 5.33% respectively (Fig. 2a).

#### Total protein in roots

The amount of total protein contained in the healthy roots of varieties was 8.77, 4.64, 7.76, 7.76 and 7.11 mg/g of the varieties Priya, BSS-1009, Aneeta, Aarti and Harsha respectively (Table 2) on fresh weight basis. In the infected roots of these varieties sugar contents were 9.98, 6.09, 8.05, 9.33 and 8.12 mg/g respectively. The percentage of increase in total protein content due to rootknot nematode infection over control was noted to be 13.79, 31.25, 3.73, 20.23 and 14.20% respectively (Fig. 2b).

### Action of nematode infection on total sugar content

#### Total sugar in shoots

The amount of sugar present in the shoot portion of the inoculated plants were recorded as 2.22, 2.21, 1.82, 1.42, 1.76 mg/g of the varieties Priya, BSS-1009, Aneeta, Aarti and Harsha respectively on fresh weight basis (Table 3). In the healthy shoots of these varieties sugar contents were 2.00, 1.83, 1.58, 1.05 and 1.68 mg/g respectively. The percentage of increase in total sugar content due to rootknot nematode infection over control was noted to be 11.00, 20.76, 15.18, 35.23 and 4.76% respectively (Fig. 3b).

#### Total sugar in roots

The amount of total sugars contained in the healthy roots of varieties was 2.85, 2.22, 2.02, 1.59 and 1.05 mg/g of the varieties Priya, BSS-1009, Aneeta, Aarti and Harsha respectively (Table 3) on fresh weight basis. In the infected roots of these varieties sugar contents were 2.98, 2.34, 2.21, 1.99

**Table 1** Estimation of chlorophyll content (a, b, total) in resistant and susceptible variety due to the infection of root-knot nematode

S. no.	Variety	Chlorophyll 'a' content mg/g leaf			Chlorophyll 'b' content mg/g leaf			Total chlorophyll content mg/g leaf					
		Infected (I)	Healthy (H)	Mean	% decrease (-) over control	Infected (I)	Healthy (H)	Mean	% decrease (-) over control	Infected (I)	Healthy (H)	Mean	% decrease (-) over control
1	Priya	0.25	0.42	0.33	-40.47	0.06	0.08	0.07	-24.70	0.23	0.59	0.41	-60.67
2	BSS-1009	0.20	0.40	0.30	-49.87	0.10	0.16	0.13	-33.75	0.15	0.59	0.37	-73.78
3	Aneeta	0.22	0.42	0.32	-48.71	0.10	0.13	0.11	-25.73	0.37	0.38	0.37	-2.63
4	Aarti	0.26	0.33	0.29	-19.33	0.16	0.25	0.21	-34.64	0.34	0.35	0.34	-3.68
5	Harsha	0.15	0.26	0.21	-42.69	0.11	0.15	0.13	-30.81	0.21	0.31	0.26	-30.12
	SE (m) ±	0.02	0.20			0.03	0.02			0.18	0.14		
	CD (0.05)	0.07	0.65			0.10	0.07			0.59	0.46		

and 1.49 mg/g respectively. The percentage of increase in total sugar content due to root-knot nematode infection over control was noted to be 4.56, 5.41, 9.41, 25.15 and 41.90% respectively (Fig. 3a; Table 3).

**Effect of nematode infection on total starch content**

**Total starch in shoots**

The amount of starch present in the shoots of the inoculated plants were recorded as 1.48, 2.12, 2.24, 2.59 and 3.05 mg/g in the varieties Priya, BSS-1009, Aneeta, Aarti and Harsha respectively on fresh weight basis (Table 4). Conversely this amount was increased in all cases than healthy plants i.e., 8.71, 9.80, 5.53%, in varieties BSS-1009, Aneeta, Harsha except decrease as 22.10% in variety Priya and 8.80% in variety Aarti in the leaf portion of these varieties due to root-knot infection (Fig. 4b).

**Total starch in roots**

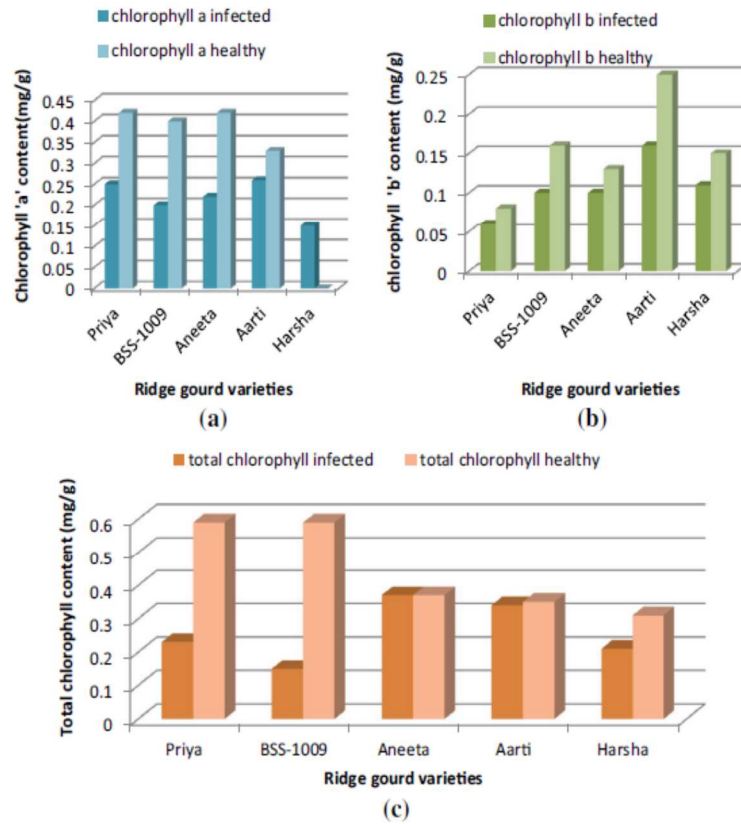
The amount of starch contained in the healthy roots of varieties was 1.41, 1.46, 1.64, 1.56, 1.59 mg/g in Priya, BSS-1009, Aneeta, Aarti and Harsha respectively (Table 4) on fresh weight basis. In the infected roots of these varieties starch contents were 1.27, 1.41, 1.51, 1.45 and 1.55 mg/g respectively. The percentage of decrease in starch content due to rootknot nematode infection over control was noted to be 9.92, 3.42, 7.92, 7.05 and 2.51% respectively (Fig. 4).

**Conclusion**

Chlorophyll content is the most important constituent of the plants as it manufactures the food, which is necessary for the growth and development of all the parts of plant. It is directly correlated with the yield of the crops. Root-knot nematodes are known to reduce the chlorophyll content of the plants by disrupting its nutrient uptake and partitioning of the photosynthates.

Sugar is the prime source of metabolic energy in all living organisms. Perusal of data clearly envisaged significant increase quantity of total sugars in the roots of nematode inoculated plants but on the other hand the sugar content decreases in the shoots of nematode infected plants. Increase in sugar content following nematode infection is in confirmative with the findings of earlier workers [Ganguly and Dasgupta 1983 (4); Mohanty et al. 2001 (7)]. Increased sugar content in infected samples might be due to movement of various metabolites towards the infection site from other parts of plants. Alternatively more of these metabolites are produced by cell at the infection site as a result more of carbohydrates are required for respiration and metabolism.

**Fig. 1** Chlorophyll 'a' (a) and Chlorophyll 'b' (b) contents of inoculated and healthy ridge gourd cultivars/varieties. c Total Chlorophyll contents of inoculated and healthy ridge gourd cultivars/varieties



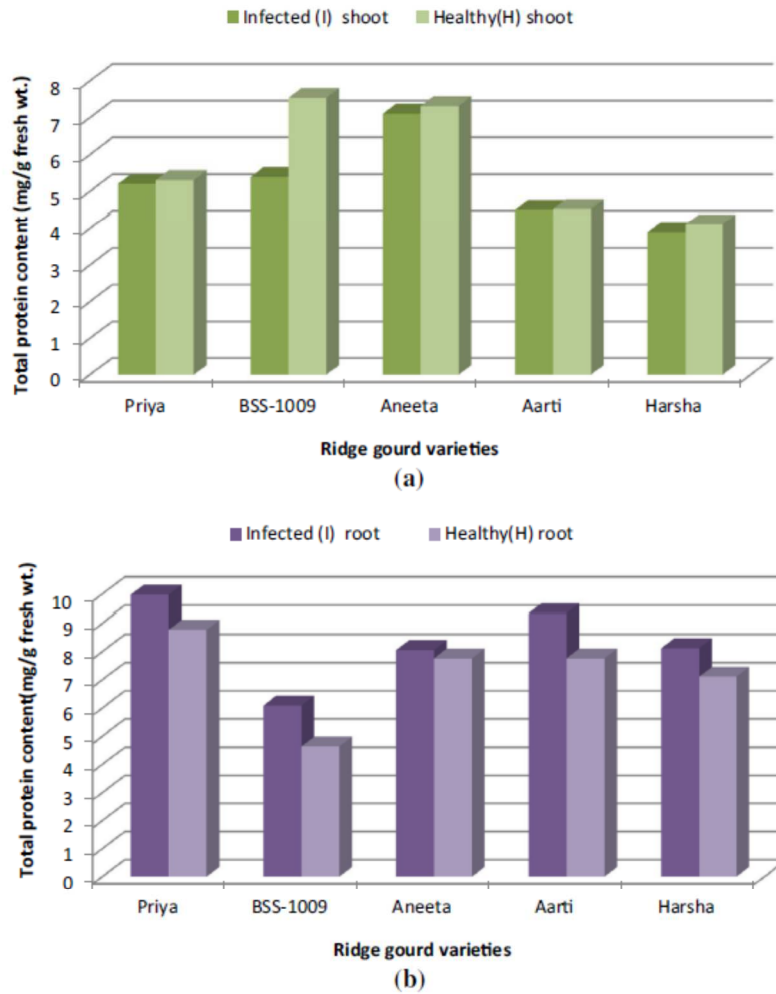
**Table 2** Estimation of percentage increase/decrease of total protein content in roots and shoots of healthy (H) and root-knot infected (I) ridge gourd plants

S. no.	Varieties	Protein content mg/g on fresh weight basis							
		Shoot (leaf)				Root			
		Infected (I)	Healthy (H)	Mean	%increase(+)/decrease (-) over control	Infected (I)	Healthy (H)	Mean	%increase(+)/decrease (-) over control
01	Priya	5.21	5.31	5.26	- 1.88	9.98	8.77	9.37	13.79
02	BSS-1009	5.39	7.54	6.46	- 28.51	6.09	4.64	5.36	31.25
03	Aneeta	7.11	7.32	7.21	- 2.86	8.05	7.76	7.9	3.73
04	Aarti	4.50	4.52	4.51	- 0.44	9.33	7.76	8.54	20.23
05	Harsha	3.90	4.12	4.01	- 5.33	8.12	7.11	7.61	14.20
	SE (m) ±	0.27	0.33			0.18	0.30		
	CD (0.05)	0.88	1.07			0.58	0.99		

The present investigation clearly indicated that *Meloidogyne incognita* played key role in altering the normal physiology and biochemical processes of the tested host plant. Further, it is opinion that the basic information provided in

this investigation will certainly be helpful to understand the complicated areas of the biochemical mechanisms of plant nematode-interaction in relating to root-knot and other plant parasitic nematodes.

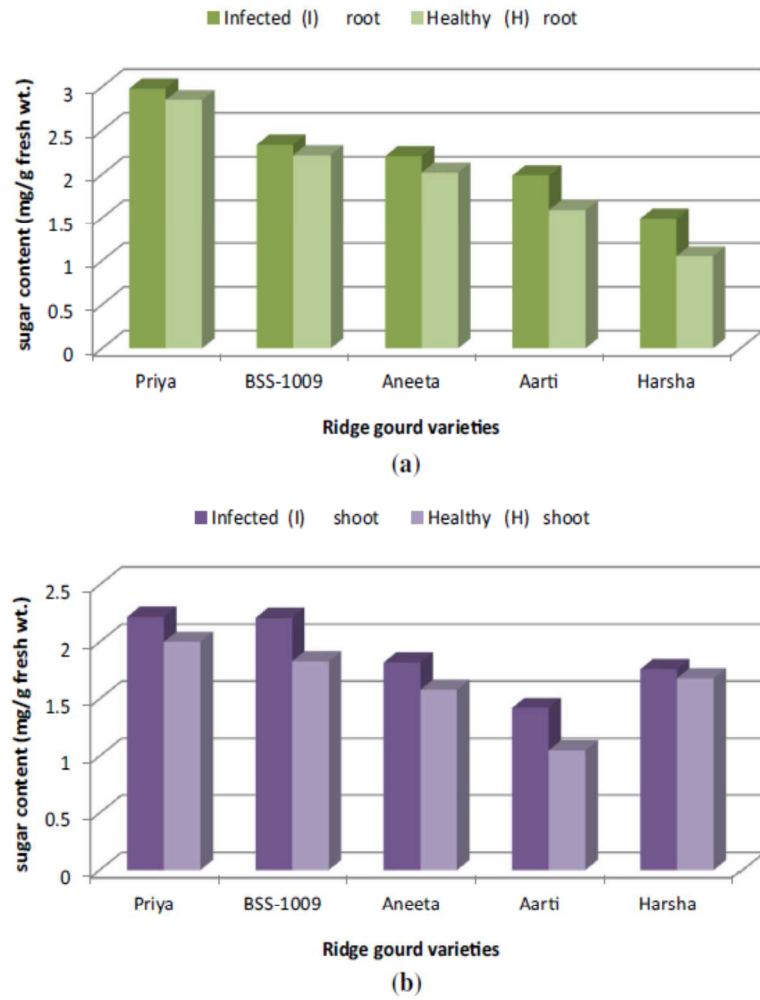
**Fig. 2** Total protein content of shoot (a) and root (b) in control and infected ridge gourd plants



**Table 3** Percentage increase/decrease of total sugar content in the root and shoot of ridge gourd varieties as influenced by *M.incognita* infection

S. no	Varieties	Total sugar content mg/g on fresh weight basis							
		Shoot (leaf)				Root			
		Infected (I)	Healthy (H)	Mean	%increase(+)/decrease (-) over control	Infected (I)	Healthy (H)	Mean	%increase(+)/decrease (-) over control
01	Priya	2.22	2.00	2.11	11.00	2.98	2.85	2.92	4.56
02	BSS-1009	2.21	1.83	2.02	20.76	2.34	2.22	2.28	5.41
03	Aneeta	1.82	1.58	1.7	15.18	2.21	2.02	2.12	9.41
04	Aarti	1.42	1.05	1.24	35.23	1.99	1.59	1.79	25.15
05	Harsha	1.76	1.68	1.72	4.76	1.49	1.05	1.27	41.90
	SE (m) ±	0.03	0.09			0.28	0.12		
	CD (0.05)	0.10	0.29			0.91	0.39		

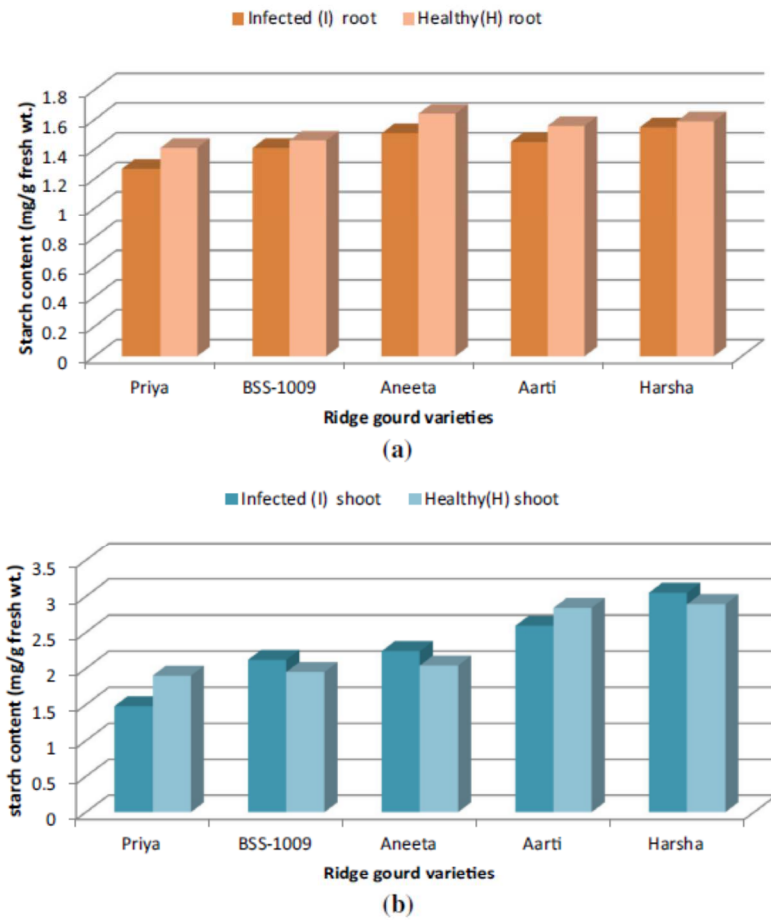
**Fig. 3** Total sugar content of root (a) and shoot (b) in control and infected plants



**Table 4** Effects of starch content in the roots and shoots of ridge gourd varieties as influenced by *M. incognita* infection

S. no.	Varieties	Starch content mg/g on fresh weight basis							
		Shoot (leaf)				Root			
		Infected (I)	Healthy (H)	Mean	%increase(+)/decrease (-) over control	Infected (I)	Healthy (H)	Mean	%increase(+)/decrease (-) over control
01	Priya	1.48	1.90	1.69	- 22.10	1.27	1.41	1.34	- 9.92
02	BSS-1009	2.12	1.95	2.03	8.71	1.41	1.46	1.43	- 3.42
03	Aneeta	2.24	2.04	2.14	9.80	1.51	1.64	1.57	- 7.92
04	Aarti	2.59	2.84	2.71	- 8.80	1.45	1.56	1.50	- 7.05
05	Harsha	3.05	2.89	2.97	5.53	1.55	1.59	1.57	- 2.51
	SE (m)±	2.30	0.17			1.31	0.12		
	CD (0.05)	7.49	0.57			4.27	0.39		

**Fig. 4** Total starch content of root (a) and shoot (b) in control and infected ridge gourd plants



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## Effects on macronutrient contents in susceptible and resistant ridge gourd cultivars against induced population of root-knot nematode, *Meloidogyne incognita*

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Received: 11 June 2018 / Revised: 8 December 2018 / Accepted: 21 December 2018 / Published online: 18 January 2019  
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### Abstract

Macronutrient changes in Ridge gourd varieties inoculated with root-knot nematode, *Meloidogyne incognita* were investigated. Observations were recorded on macronutrient modifications relating to various parameters like nitrogen, crude protein, phosphorus and potassium content during post infection periods. The variation in nitrogen, crude protein, phosphorus and potassium content in five cultivars i.e. Priya, BSS-1009, Aneeta, Aarti and Harsha were studied 30 days after inoculation. Reduced percentage of nitrogen, crude protein and phosphorus contents were observed in inoculated shoot samples than the healthy counterparts, whereas it increased in root samples. However, an increase in amount of potassium contents was observed in the diseased tissues.

**Keywords** Macronutrient contents · *Meloidogyne incognita* · Ridge gourd cultivars

### Introduction

The root-knot nematode, *Meloidogyne incognita* (Chitwood, 1949) are the most important nematode pests worldwide due to their great damage resulted in the very wide host range which include more than 3000 plant species. Nematode pests, in general, affect host plants quantitatively and qualitatively. Quantitatively by reducing the total production causing about 20.6% worldwide yield loss, and qualitatively by devaluating the produced crops as a result of some malformations as well as decreasing the organic and mineral components of the produced fruits. Plant parasitic nematode, *Meloidogyne incognita* alters the metabolic processes of the host plant which are manifested in the form of cellular, physiological and biochemical changes in the infected host. The root-knot nematodes cause measurable changes in the morphology and physiology of the tomato plant (Williamson and Gleason 2003). Root-knot nematodes like many other obligate parasites are capable of disturbing

the host metabolism. The changes in the physiological and biochemical processes of infected host as a consequence of disturbed metabolism decide whether the host becomes susceptible or resistant to nematode attack (Krusberg 1963). In the 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; Howell and Krusberg 1966; Mohanty et al. 1995; Nayak 2015). Considering the importance of the subject, the present investigation was undertaken to find the changes if any, in nitrogen content, crude protein, phosphorus and potassium content in relation to ridge gourd inoculated with root-knot nematode, *M. incognita*. Cultivars of ridge gourd were sown in 15 cm diameter earthen pots filled with steam sterilized soil. A week after germination, one seedling per pot was retained and inoculated with axenised suspension of approximately 1000 J2 of *M. incognita* per seedling per pot. A set of plants was left uninoculated to serve as control. Healthy and inoculated plants were harvested at 45 days after planting. The harvested roots were washed thoroughly under running tap water to remove the adhering soil particles. Then the shoots were surface sterilized in 1% HgCl<sub>2</sub> solution followed by repeated washing with distilled water. The harvested shoots were dried on blotting paper and kept separately for chemical analysis. The nitrogen content and crude protein was estimated by

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micro Kjeldahl method. Phosphorus content was estimated by Vanadomolybdo-phosphoric acid yellow colour method and potassium content were estimated by flame photometer methods.

## Materials and methods

In order to understand the basis of nematode resistance, five varieties namely Priya, BSS-1009, Aneeta, Aarti and Harsha were grown in earthen pots in the greenhouse. These plants were washed 30 days after nematode inoculation and the following chemical compositions were estimated by different methods i.e.

Nitrogen content and crude protein content (Kjeldahl method).

Phosphorus content (Vanadomolybdo-phosphoric acid yellow colour method).

Potassium content (flame photometer method).

## Results and discussion

Many kinds of elements and enzymes are present in the plant system that influences the metabolism of the pests fed upon them. The chemicals may consist of simple nitrogen, phosphorus, potassium, carbohydrates and fats to complex proteins, enzymes, phenolic compounds etc., which may nourish, starve or kill the feeding organisms upon them. In the course of feeding some chemicals may be depleted or some others may be de novo synthesized, that may be detrimental to the pest. In order to know the chemical and genetic basis of resistance, five varieties were chosen for a detailed analysis. These plants were grown with utmost care, both in inoculated and control conditions. One set of each uninoculated (healthy) and inoculated (infected) plants were

analysed to test the effects of root-knot nematode infection on the growth and vigour of the plants and their root-system.

### Estimation of nitrogen contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M. incognita*

Chlorophyll content is approximately proportional to leaf nitrogen content, too (EVANS 1983). Chlorophyll content is the most important constituent of the plants as it manufactures the food, which is necessary for the growth and development of all the parts of plant. It is directly correlated with the yield of the crops. Root-knot nematodes are known to reduce the chlorophyll contents of the plants by disrupting its nutrient uptake and partitioning of the photosynthates. The nitrogen plays main role in ridge gourd nutrition because of its importance in protein and nucleic acid synthesis as well plant species and cultivars.

The total nitrogen content was decreased in the shoot system of infected varieties of ridge gourd, Priya, BSS-1009, Aneeta, Aarti and Harsha by 0.36, 0.46, 0.44, 0.35 and 0.28% respectively over control on dry weight basis. Whereas in the roots nitrogen content was increased by 0.49, 0.44, 0.47, 0.67 and 0.48% in all the above mentioned varieties accordingly.

The total nitrogen content was decreased in all shoot system of resistant variety by 20% and 40.42% in susceptible variety but increased by 88.46% in the root system of resistant variety as compared to susceptible one (2.12%) (Table 1).

The nitrogen content (%) in shoots were significantly higher in the resistant varieties as compared to susceptible variety and vice versa. The present results of increase of total nitrogen content of nematode infected root samples is in confirmation with the findings of the earlier workers (Zaki and Bhatti 1986; Nayak 2006)

The nutrient accumulation in infected roots maybe due to impaired translocation to the aerial parts or the mobilization

**Table 1** Estimation of nitrogen contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M. incognita*

S.I. no.	Varieties	Nitrogen content % on dry weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	Increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	Increase/decrease over healthy (%)
01	Priya	0.36	0.45	0.40	-20	0.49	0.26	0.37	88.46
02	BSS-1009	0.46	0.49	0.47	-6.12	0.44	0.28	0.36	57.14
03	Aneeta	0.44	0.51	0.47	-13.72	0.47	0.46	0.46	2.17
04	Aarti	0.35	0.37	0.36	-5.40	0.67	0.42	0.54	59.52
05	Harsha	0.28	0.47	0.37	-40.42	0.48	0.47	0.47	2.12
	SE (m) ±	0.28	0.15			0.2	0.02		
	CD (0.05)	0.92	0.51			0.65	0.07		

of nutrition from shoot to root. The percentage increase in nitrogen content in shoots of resistant varieties may be due to higher absorption capacity or higher requirement for the resistance mechanisms. This may be due to lower translocation rate.

**Estimation of crude protein contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M. incognita***

Due to infection of root-knot nematode, the percentage of shoot crude protein content was low 20.35% in variety Priya, 6.20% in BSS-1009, 14.59% in Aneeta, 5.57% in Aarti and 40.27% in Harsha, whereas crude protein content in root was high in Priya (86.66%) than that of BSS-1009 (58.28%), Aneeta (0.68%), Aarti (61.06%) and Harsha (1.01%) (Table 2).

The crude protein of both the infected shoot decreased and infected root increased in the similar trend like Nitrogen content of the infected shoot. The increased crude protein content in roots of nematode infected plants was reported by various workers (Ganguly and Dasgupta 1983; Devaranjan

and Rajendran 2002; Vaitheeswaran et al. 2005; Nayak 2006)

**Estimation of phosphorus contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M. incognita***

The phosphorus content was decreased in the shoot system of infected varieties of ridge gourd, Priya, BSS-1009, Aneeta, and Harsha by 92, 11.42, 62.74 and 50.87% respectively except Aarti (113.33%) and increased in infected root by 0.57, 0.26, 0.44, 0.49, 0.75% in ridge gourd varieties Priya, BSS-1009, Aneeta, Aarti and Harsha respectively over control on dry weight basis.

Whereas phosphorus content was recorded highest as 850% in infected roots of variety Priya followed by 420% in BSS-1009, 57.14% in Aneeta, 50% in Harsha and 8.88% in Aarti (Table 3). The result of present investigation revealed that the infected plants had decreased percent of phosphorus content in shoots of highly susceptible and moderately resistant and resistant varieties. Similar trend was also observed by Hunter (1958), Chakraborti and Mishra (2002) in root-knot nematode infected plants. The amino acid, protein,

**Table 2** Estimation of crude protein contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M. incognita*

S.I. no.	Varieties	Crude protein content % on dry weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	Increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	Increase/decrease over healthy (%)
01	Priya	2.27	2.85	2.56	-20.35	3.08	1.65	2.36	86.66
02	BSS-1009	2.87	3.06	2.96	-6.20	2.77	1.75	2.26	58.28
03	Aneeta	2.75	3.22	2.98	-14.59	2.93	2.91	2.92	0.68
04	Aarti	2.20	2.33	2.26	-5.57	4.22	2.62	3.42	61.06
05	Harsha	1.75	2.93	2.34	-40.27	3.00	2.97	2.98	1.01
	SE (m) ±	1.78	0.99			1.25	0.13		
	CD (0.05)	5.82	3.22			4.07	0.44		

**Table 3** Estimation of phosphorus contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M. incognita*

S.I. no.	Varieties	Phosphorous content % on dry weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	Increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	Increase/decrease over healthy (%)
01	Priya	0.06	0.75	0.40	-92.00	0.57	0.06	0.31	850
02	BSS-1009	0.31	0.35	0.33	-11.42	0.26	0.05	0.15	420
03	Aneeta	0.19	0.51	0.35	-62.74	0.44	0.28	0.36	57.14
04	Aarti	0.32	0.15	0.23	113.33	0.49	0.45	0.47	8.88
05	Harsha	0.28	0.57	0.42	-50.87	0.75	0.50	0.62	50
	SE(m) ±	0.07	0.19			0.15	0.15		
	CD(0.05)	0.25	0.62			0.49	0.51		

**Table 4** Estimation of potassium contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M. incognita*

S.I. no	Varieties	Potassium content % on dry weight basis							
		Shoot				Root			
		Infected (I)	Healthy (H)	Mean	Increase/decrease over healthy (%)	Infected (I)	Healthy (H)	Mean	Increase/decrease over healthy (%)
01	Priya	0.42	0.33	0.37	27.27	0.57	0.06	0.31	850
02	BSS-1009	0.33	0.12	0.22	175	0.26	0.05	0.15	420
03	Aneeta	0.37	0.12	0.24	208.33	0.47	0.28	0.37	67.85
04	Aarti	0.12	0.43	0.27	-72.09	0.49	0.45	0.47	8.88
05	Harsha	0.19	0.76	0.95	-75	0.75	0.50	0.62	50
	SE (m) ±	0.05	0.07			0.11	0.15		
	CD (0.05)	0.17	0.22			0.37	0.51		

**Table 5** Effect of root-knot nematode, *M. incognita* on nutrient contents in soil of five Ridge gourd cultivars as compared to Healthy plant (H)

Varieties	Nitrogen		% Increase (+) or % decrease (-) over Healthy	Phosphorous		% Increase (+) or % decrease (-) over Healthy	Potassium		% Increase (+) or % decrease (-) over Healthy
	Healthy	Inoculated		Healthy	Inoculated		Healthy	Inoculated	
Priya	240	213	-11.25	114	112	-1.75	111	98	-11.71
BSS-1009	156	126	-19.23	57	55	-3.50	103	87	-15.53
Aneeta	133	103	-22.55	85	83	-2.35	98	86	-12.24
Aarti	189	159	-15.87	100	96	-4	103	96	-6.79
Harsha	183	153	-16.39	116	113	-2.58	101	93	-7.92

Sugar and absorbed N<sub>2</sub>, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O and other elements accumulate in the roots of nematode infected plants and remain under-utilised by the plant systems which ultimately find the path into the growth and production of nematode. This may be the possible reasons for the accumulation of phosphorus in nematode infected plants (Table 3).

**Estimation of potassium contents in the resistant/susceptible ridge gourd varieties influenced by root knot nematode, *M. incognita***

The root-knot nematode inoculated plants measured an increase in potassium content to the tune of 208.33, 175, 27.27% in shoots of varieties Aneeta, BSS-1009 and Priya respectively while decrease as 75% in Harsha and 72.09% in Aarti. The inoculated plants in roots was increased by 850, 420, 67.85, 8.88 and 50% in varieties Priya, BSS-1009, Aneeta, Aarti, and Harsha respectively. Table 4 revealed that there was significant increase of potassium content of both susceptible and resistant infected plants which increase was more pronounced in both shoots and roots of resistant varieties as compared to the susceptible varieties.

Chakraborti and Mishra (2002) have also recorded decrease in potassium content due to infection of *Xiphinema americanum* and *M. incognita* in sour cherry and chickpea respectively. Potassium plays a significant role for maintaining turgor

**Table 6** Effect of root-knot nematode, *M. incognita* on final population in soil of five Ridge gourd cultivars

Varieties	Final population <sup>a</sup>
Priya	239.33 (2.37)
BSS-1009	241.66 (2.38)
Aneeta	458.33 (2.46)
Aarti	1426 (3.15)
Harsha	1226.66 (3.08)

<sup>a</sup>Figures in parentheses are log transformed values

pressure of tissues of plants. In the nematode infected plant tissues various compounds relating to ion-exchange may be decreased which is reflected by the reduction of 'K' content in nematode infected plant sample. Soil nutrient status was also studied (Table 5). In all the cases nitrogen, phosphorous and potassium content was decrease over healthy. Final nematode population in soil was also counted and presented in Table 6. It reflected the severity of root-knot nematode infection in different ridge gourd cultivars.

## Conclusion

The single generation of *M. incognita* showed the changes in both concentration and total content of different elements in the ridge gourd varieties to establish relationship between nutrients status and physiological processes. Chlorophyll content is approximately proportional to leaf nitrogen content, too (Evans 1983). Chlorophyll content is the most important constituent of the plants as it manufactures the food, which is necessary for the growth and development of all the parts of plant. It is directly correlated with the yield of the crops. Root-knot nematodes are known to reduce the chlorophyll content of the plants by disrupting its nutrient uptake and partitioning of the photosynthates. The nitrogen plays main role in ridge gourd nutrition because of its importance in protein and nucleic acid synthesis as well plant species and cultivars. The nutrient accumulation in infected roots maybe due to impaired translocation to the aerial parts or the mobilization of nutrition from shoot to root. The percentage increase in nitrogen content in shoots of resistant varieties may be due to higher absorption capacity or higher requirement for the resistance mechanisms. This may be due to lower translocation rate.

The crude proteins were reduced in shoots of infected plants because of the enzymatic degradation of plant protein and reduced photosynthesis causes accumulation of soluble amino acids in the infected plants.

The result of present investigation revealed that the infected plants had decreased percent of phosphorus content in shoots of highly susceptible and moderately resistant and resistant varieties. Similar trend was also observed by Hunter (1958), Chakraborti and Mishra (2002) in root-knot nematode infected plants. The amino acid, protein, Sugar and absorbed  $N_2$ ,  $P_2O_5$  and  $K_2O$  and other elements accumulate in the roots of nematode infected plants and remain under-utilised by the plant systems which ultimately find the path into the growth and production of nematode. This may be the possible reasons for the accumulation of phosphorus in nematode infected plants

There was significant increase of potassium content of both susceptible and resistant infected plants which increase was more pronounced in both shoots and roots of resistant varieties as compared to the susceptible varieties Potassium plays a significant role for maintaining turgor pressure of tissues of plants. In the nematode infected plant tissues various compounds relating to ion-exchange may be decreased which is reflected by the reduction of 'K' content in nematode infected plant samples.

The changes in concentration of these nutrient elements in the plant, small as they may be appear to have a profound effect on the host physiology. The data indicate that a change in the concentration of the nutrient elements in

plant is probably one of the first effects of the nematode on host physiology. These changes in nutrient concentration after host metabolism contributes directly or indirectly to the chlorosis of infected plants. These effects on the increase with level and duration of infection and, along with changes in other physiological problems such as photosynthesis appear to be the main cause of a lower yield in nematode infected plants. The change in potassium concentration seems to be important because of its effect on physiological processes such as osmotic potential

The present investigation clearly indicated that *M. incognita* played key role in altering the nutrient contents of the tested host plant. Further, it is opinion that the basic information provided in this investigation will certainly be helpful to understand the complicated areas of the chemical mechanisms of plant nematode-interaction in relating to root-knot and other plant parasitic nematodes.

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