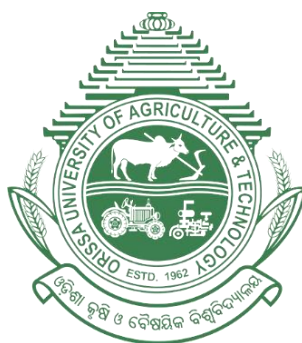


**Physiological and biochemical variation analysis
of resistant and susceptible varieties of gerbera
as influenced by root-knot nematode,
Meloidogyne incognita.**

A
***Thesis submitted to the
Orissa University of Agriculture and Technology
in partial fulfilment of the requirement for the degree of
Master of Science in Agriculture
(Nematology)***

By

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2018**



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CERTIFICATE - I

This is to certify that the thesis entitled “**Physiological and biochemical variation analysis of resistant and susceptible varieties of gerbera as influenced by root-knot nematode, *Meloidogyne incognita***” submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE(NEMATOLOGY)** of the Orissa University of Agriculture and Technology, Bhubaneswar is a faithful record of bonafide research work carried out by **SANJUKTA BAA** 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.

**CHAIRMAN
ADVISORY COMMITTEE**



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CERTIFICATE-II

This is to certify that the thesis entitled “**Physiological and biochemical variation analysis of resistant and susceptible varieties of gerbera as influenced by root-knot nematode, *Meloidogyne incognita***” submitted by **SANJUKTA BAA** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirement for the degree of **MASTER OF SCIENCE IN AGRICULTURE (NEMATOLOGY)** has been approved by the students’ advisory committee and the external examiner.

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ACKNOWLEDGEMENT

“Gratitude can turn common days into thanksgiving, turn routine jobs into joy and change ordinary opportunities into blessings”.

First and foremost, keeping aside arrogance of enthusiastic human nature, 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, Assoc. Professor, 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. S. Sahoo Head, Department of Nematology and Dr. (Mrs). Gayatri Biswal, Associate Professor 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. G. R. Rout, Professor and Head, 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, Mr. R. K. Panda, Assoc. Professor, Dr.(Mrs) R. Bhola, Asst. Professor, Department of Plant Physiology, Dr. S. K. Panda, Professor & Head, Dr. L. K. Rath, Professor, Department of Entomology, OUAT, Bhubaneswar for providing necessary laboratory facility during the biochemical analysis of my plant materials.

I also express my heartfelt gratitude to Dr. N. K. Sahoo, Ex-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 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 Binay, Krupali, Narmada, Anita, Nangki, Anu, Anwessa, Basant, Sujit, Subhasis, Rosy, Lopa, Sudepta, Surbhi, Kullu bhai and also to my seniors Manaswini didi, Ritu didi, Diksha didi, Poornata didi, Shilpi didi, Mukesh bhai 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 this OUAT campus, Bhubaneswar.

Bhubaneswar

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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
r.p.m	revolutions per minute
S.E.(m)	Standard error mean
CD	Critical difference

ABSTRACT

Nematode infection tends to interact with various biological processes of the plant which are marked with changes occurring in physiology of the plant like stunting, wilting, yield loss and nutritional imbalances. So, this has made it critical to study about the life history of root knot nematode and its post infection development in the host plant. Seven genotypes of gerbera were collected and screened in green house condition for testing their resistance and biochemical reactions to root-knot nematode, *Meloidogyne incognita* in pot culture experiment. Out of the seven varieties one was found resistant, five were moderately resistant and one was susceptible to *M. incognita* according to the number of galls and gall indices. Only one variety showed resistant reaction with an average of 8.66 no. of galls per plant while five varieties showed moderate resistance with number of galls ranging from 22.82 to 29.10 and the other one was susceptible with an average of 67.66 number of galls/plant. There was noteworthy reduction in plant growth parameters with marked decrease in chlorophyll "a", "b" and total chlorophyll content. The chlorophyll "a", "b" and total chlorophyll decreased to the tune of 27.36 to 27.50%, 14.70 to 38.43% and 27.38 to 32.10% respectively. The host nutritional content was altered which affected the physiology of the host. The nitrogen content was decreased in the shoot system of varieties from 14.15 to 33.33% and same was decreased by 27.31% in root system of resistant Mammut variety than susceptible one *i.e.* Seth. The phosphorus content was decreased in the shoot system of infected varieties but was found to have increased in the roots and it was recorded highest as 55.00% in infected Seth variety. There was a significant increase in potassium content of both susceptible and resistant infected plants and it was more pronounced in the shoots of resistant varieties (11.20 to 52.94%). Total Phenolic and Proline content increased significantly in infected plants as compared to their healthy counterparts. Maximum percentage increase in total phenol was observed in shoots of Mammut with 61.18% and minimum was 60.15% in Pink elegance. Similarly the Proline content increased from 60.49 to 61.23% in shoots and 56.90 to 61.07% in roots of the infected plants. As a result of nematode infection the amount of micronutrient was also found to be altered. Due to inability to take up nutrients and translocation to other parts of the plant the concentration of micronutrient in roots is seen to be more than shoots. Iron content was recorded highest in roots 36.84% of variety Seth and lowest as 10.81% in Mammut. Magnesium was recorded as 83.33% in Seth and 28.57% in Mammut in the infected plants. Calcium content decreased by 13.04 to 30% in the infected varieties. Similarly sulphur was recorded to be increasing by 22.22 to 66.66 % and manganese content was recorded to be decreasing by 10.00 to 40.00 % respectively in the infected varieties.

CHAPTER-1

INTRODUCTION

INTRODUCTION

India has a long tradition of floriculture. The floral industry today has grown in large proportions and offers a wide scope for growth and profits. Commercial floriculture in our country has assumed importance only in the recent times. The use of cut flowers raised the status of living and serve as a symbol of happiness. As per the statistics of 1999, the area under cut flowers in our country is 64,768 ha. Five hundred hectares climatically controlled greenhouses are available for growing quality flowers for export purpose. Karnataka is regarded as a progressive state for horticulture development with more than 20,000 ha area under cultivation of various types of flowers accounting for 75% of India's total cut flower production.

Among the major cut flowers growing in India, gerbera is one most popular commercial cut flower which not only meets the requirements of domestic market but is also being exported outside. It is the fifth most used cut flower in the world. "*Gerbera jamesonii*" popularly known as "Transvaal daisy" belongs to Compositae family and is native to South Africa and Asiatic regions. The success of gerbera as commercial cut flower is primarily due to wide range in colour and shape of flower. Major gerbera cut flower production states in India are West Bengal, Maharashtra, Andhra Pradesh, Orissa, Karnataka, Gujarat, Uttar Pradesh, Uttaranchal, Arunachal Pradesh. In the year 2015-2016, Assam was the leading producer of gerbera with 12.42 000 tonnes. (Source: National Horticulture Board).

Under cultivation, the crop is affected by several biotic stresses among which the most significant damage is caused by root knot nematode i.e. *Meloidogyne incognita*. Infection by this nematode results in heavy loss to the crop both in quality and quantity. Infection by this nematode can be easily recognized by the formation of prominent root galls at the infection site. *Meloidogyne* spp. is among the most prevalent economical crop pests worldwide (Sasser, 1989; Stirling *et al.*, 1992; Oka *et al.*, 2000) and they interfere with anchorage and absorption of water and nutrients. They are the most destructive and devastating important polyphagous crop pests. Manju *et al.* (2014) estimated a yield loss of 31.1% in gerbera due *Meloidogyne incognita* infection.

Plant parasitic nematodes like root-knot nematode are capable of altering the host metabolism by bringing about the physiological and biochemical modifications in the host plants (Ganguly *et al.* 1991; Zinove'eva *et al.*, 2004). In nematode infected plants there occurs a considerable meddling in the metabolism of protein, nitrogen and carbohydrate (Pathak *et al.*, 1983). Phenolic compounds play an important role in the defense mechanism of plants and consequently there is accumulation of phenolic compounds in the cells damaged due to nematode feeding. (Acedo and Rohde, 1971; Valette *et al.*, 1998). The increase in phenol compounds aids in the formation of hypersensitive reaction towards nematode infection (Mazzafera *et al.*, 1989). Phenolic compounds helps in larval mortality by rendering host roots less attractive to nematode (Sitaramaiah and Singh, 1978; Nicholson and Hammerschmidt, 1992). The increase in phenolic concentration coupled with increase in the activity of polyphenol oxidase in the diseased tissue (Ahuja and Ahuja, 1980; Bajaj *et al.*, 1983, 1985 and David 2002). The invasion of plant parasitic nematodes caused an augmentation in sugar content and it was also observed that the increased sugar content was helpful for the survival of nematode (Owensh and Specht, 1966; Dropkin, 1969). They have also reported the increase in sugar content in the infected leaves which might have occurred due to the degradation of starch or inhibition of starch synthesis from sugar. Farooqi *et al.* (1980) confirmed the increase in total as well as reducing sugar in root-knot infected tomato plants. The content of total carbohydrates, total protein, total soluble sugars and the minerals Ca, Fe, Mg, N, P, K and Zn; in host is decreased as a result of nematode invasion, however total phenol and tannins are increased. Hofmann *et al.* (2007) and 2008 and Nayak and Mohanty (2010) agreed with the increase in sugar and protein levels which occur due to high metabolic activity in decreased tissues.

In diseased condition, the sequence of biochemical changes is rather more important than the external symptoms which are nothing but the manifestation of internal disorders. A series of biochemical and physiological reactions that occur in host plants in response to root-knot nematode infection results in disease occurrence or limitation of disease development due to localization of nematodes by plants. Detailed characterization of this biochemical and physiological processes is essential to advance our understanding of plant nematode interaction and also help us find out a suitable management strategy. Considering the importance of this object, an investigation entitled "Physiological and biochemical variation analysis of resistant and susceptible varieties

of gerbera as influenced by root-knot nematode, *Meloidogyne incognita*” was undertaken to study the host -pathogen reaction and biochemical alterations in the host plant to screen out resistant varieties against the nematodes.

To better understand the mechanism of resistance, the physiology and biochemistry of pathogenesis the following analysis were done:

1. Screening of gerbera cultivars or germplasms/ cultivars
2. Evaluation of resistant and susceptible gerbera varieties
3. Estimation of chlorophyll content (a, b, total) of leaves (spectrocolourimeter method)
4. Estimation of nitrogen content (micro-kjeldahl method)
5. Estimation of crude protein content (Lowry method)
6. Estimation of phosphorus content (spectrophotometer method)
7. Estimation of potassium content (flame photometer method)
8. Estimation of total sugar content (spectrocolourimeter method)
9. Estimation of starch content (spectrocolourimeter method)
10. Estimation of Phenolic substances(spectrocolourimeter method)
11. Estimation of Proline substances(spectrocolourimeter method)
12. Estimation of micronutrients (SS method)
 - I. Iron (Fe)
 - II. Magnesium (Mg)
 - III. Calcium (Ca)
 - IV. Sulphur (S)
 - V. Manganese (Mn)



CHAPTER-2

REVIEW OF LITERATURES

REVIEW OF LITERATURE

The feeding action of plant parasitic nematodes brings about metabolic changes in the cells and tissues of the host plant. The visible symptoms are of the root-knot nematode infection is production of galls which are produced as a result of root-knot nematode invasion. The physiological and biochemical changes brought about in the host have been studied earlier by many workers which are a basis for classifying resistant and susceptible varieties. Some of them are enumerated below.

2.1 Nematode association

Dasgupta *et al.* (1981) studied about the system of biochemical resistance in tomato to the root-knot nematode, *M. incognita* to understand biochemical source of resistance in hosts to the nematode. Biosynthesis of certain benzoid compounds during post-infection period of hosts due to enzymatic directive of phenyl propanoid pathway and the quantity of these compounds varied considerably in the resistant-reacting and susceptible tomato varieties. These studies indicated that the nematode mediated disease resistance in tomato var. SL-120 was due to fabrication of antibiotic molecules which were induced due to nematode attack.

Roberts (1995) revealed that root knot nematodes are the most important cause of crop losses taking place due to plant parasitic nematodes. A few species like *Meloidogyne arenaria*, *M. chitwoodi*, *M. hapla*, *M. incognita*, *M. javanica* are said have very extensive host range that edge non host rotation options. Numerous host resistance genes in different crops have been recognized and could be used in cropping sequences to deal with root- knot nematodes. To maximize effectiveness and safeguard durability their combined approaches need to be employed, which consist of pyramiding genes in a crop and in a cropping system, which are measured with resistance gene specificity and assortment to nematode species and population.

Ehwaeti *et al.* (1999) experimented with the four populations of *Meloidogyne incognita* and tested them on 57 species of plants from temperate and tropical regions. It was found that juveniles of one or more populations attacked, and stimulated galls on almost all plants. Except the population from West Indies that induced most galls on *Nicotiana* spp. And it was observed that there was no obvious association between the

genesis of populations and their capacity to stimulate galls on plants from particular regions. Even though, it was seen that most of the plants were hosts to all four populations nevertheless many produced only a few galls/egg masses with some of the populations. There was a significant relationship ($P < 0.001$) between differences in galling between populations, and the interaction with plant species. It was seen that poor hosts often contained fewer juveniles and the rate of juvenile development was time and again decreased as compared to good hosts.

Ploeg *et al.* (2001) studied about the association between increasing *Meloidogyne incognita* inoculum density and the growth of melon plants in pots and portrayed it well by the Seinhorst model. It was found that the yield loss resulted principally from fewer fruits being harvested per plant, relatively than from the fruits being smaller in size. Redirection of plant nutrients towards supporting the nematodes developing in the root systems and hampering fruit set might be the cause of decline in yield. The outcome indicated that avoiding immediate access of the nematodes to very young seedlings may prevent relentless yield losses, but might also upshot in high populations during harvest.

de Almeida Engler *et al.* (2005) revealed that among plant pathogens, sedentary endoparasitic nematodes are one of the most destructive pests in global agriculture. These obligate parasites are known to act together with their hosts in a quite exclusive and fascinating way. The redifferentiation of root cells into specialized feeding cells is essential for their growth and reproduction and the redifferentiations are induced by these nematodes and thus these nematodes have developed the ability to make the most of plant genes and use the host functions for their own necessities. Diverse approaches towards plant genetic engineering with resistance to parasitic nematodes have been carried out. The main focus of this was the introduction of resistance genes. And an alternative tactic to achieve resistance is to make use of the vulnerability of plant diseases. Better understanding of the plant reaction during the attuned interface would help in the detection of targets to engineer resistance to parasitic nematodes in crop species.

Gheysen *et al.* (2011) revealed that the sedentary plant-parasitic nematodes set up a long term relationships with their hosts. The root vascular cells have been known to get distorted into large multinucleate feeding cells from which the nematodes feed for more than one month. Transcriptome analyses put forwarded that feeding cells are unlike from other plant cell. The advancement of feeding cells, however, remains poorly

understood, regardless of the new evidence that appears to confirm previously proposed models, such as the significance of auxin. From the investigation of nematode effector proteins that are known to work together with plant proteins, it has become clear that nematodes control many aspects of plant development, auxin transport and plant cell differentiation pathways. These studies also revealed about the roles of effectors in the inhibition of plant stress and defense reactions to establish feeding cells.

Baldacci-Cresp *et al.* (2015) found that root-knot nematodes are obligatory plant parasitic nematodes that set up and preserve an intimate relationship with their host plants. These nematodes have been found to induce the redifferentiation of root cells into multinucleate and hypertrophied giant cells (GCs) during a compatible interaction. The elite sources of nutrients for these nematodes are these metabolically active feeding cells. Analysis for modifications of water status, ionic content and buildup of metabolites in the development of galls, mature galls induced by *Meloidogyne incognita* and in uninfected roots of *Medicago truncatula* plants were done. It was seen that water potential and osmotic pressure were considerably changed in mature galls as compared to developing galls and control roots. Ionic content was extensively changed in galls in contrast to the roots.

Manju *et al.* (2016) assessed the yield loss caused by root knot nematode in gerbera (*Gerbera jamesonii* Hook) as 30.21 to 36.90 percent, correspondingly in two different experiments run all together with different varieties viz., Alabanda Red and Palm Beach Yellow. It was seen that there was considerable decrease in shoot length, root length and shoot weight due to root knot nematode infestation. And also it was found that the nematode depreciates the quality parameters like stalk length and flower diameter of gerbera.

2.2 Pathogenecity

Pankaj *et al.* (1990) experimented on the pathogenecity of *Meloidogyne incognita* on bittergourd and round melon by inoculating different levels of second-stage juvenile (J2) of the *Meloidogyne incognita* to the plants. The plant growth characters showed a considerable reduction owing to nematode infection. But the final nematode population increased with increase in the inoculum levels.

Govindaiah *et al.* (1991) studied the consequence of different inoculum levels of *Meloidogyne incognita*, 0, 10, 100, 1000 and 10,000 second stage larvae/plant in case of mulberry and the degree of avoidable leaf yield loss were anticipated in pot culture as well as field situation. *M. incognita* was found to be extremely pathogenic to mulberry. At 10,000 larvae/plant it caused considerable decline in plant growth, leaf yield and moisture per cent as well as shoot and root weight. The avoidable leaf yield loss in field situation was found to be 11.8 percent.

Barool *et al.* (1998) examined the influence of different initial inoculum densities of *Meloidogyne incognita* on growth of henbane (*Hyoscyamus muticus*) in a glasshouse. The entire alkaloid concentration, physiological and biochemical changes, root-knot development, and nematode multiplication were investigated 60 days after inoculation. Preliminary inoculum densities and various growth variables illustrated a negative correlation. It was observed that there was a great decline in growth of the plants inoculated with the highest bulk of nematodes (15,000 juvenile/stage 2 organisms/5 kg soil). The plants inoculated with the lowest level of nematode population tested (50 juvenile/stage 2 organisms/5 kg soil) showed the highest reproduction factor.

Manju *et al.* (2015) studied about the pathogenicity of the root knot nematode, *Meloidogyne incognita* on gerbera and saw a noteworthy reduction in plant growth compared to uninoculated control plants at an initial inoculum level of 1000 nematodes. This was considered as the damaging threshold level. It was observed that the rate of reproduction of *M. incognita* was highest at the lowest initial inoculum level and vice versa.

Duggal *et al.* (2016) studied about the life cycle and pathogenicity of root knot nematode on gerbera in polyhouse as well as under net house conditions. It was found that nematodes completed their life cycle in 45 days under net house conditions. It was seen that development was more rapid in polyhouse and life cycle was completed within 40 days. Besides this, pathogenicity of root-knot nematode was studied at the different inoculum levels of 0, 10, 100, 1000 and 10000 J₂ of *Meloidogyne incognita*/cm³ soil. Noteworthy reduction in plant growth factors at all the initial inoculum level of 100 J₂/kg soil was found. And the pathogenic level of root-knot nematode in Gerbera was documented at an inoculum level of 1,000 J₂ and above, under both the conditions. The plant growth parameters like shoot length (26.9 cm), fresh shoot weight (12.8g), dry

shoot weight (3.3g), fresh root weight (22.8g), dry root weight (6.5g), number of galls per root system (14), no. of egg masses per root system (8) and final nematodes population (110 J₂) was confirmed elevated under polyhouse condition.

2.3 Screening and evaluation of gerbera varieties against root-knot nematode, *Meloidogyne incognita*

Mukhtar *et al.* (2014) revealed that the root-knot nematode, *Meloidogyne incognita*, is one of the major limiting factors affecting plant growth and yield. It has caused a \$100 billion estimated yield loss per year worldwide. Synthetic pesticides, though immediately effective but are generally prohibitively pricey, not readily accessible, and might cause threats to both man and livestock, and impose harm to the environment. Use of resistant cultivars is a replacement to nematicides as these are economical and eco-friendly. The study consisted of assessment of twelve okra (*Abelmoschus esculentus* L) cultivars for their resistance against *M. incognita* under field conditions. Ten-days old okra plants of test cultivars were inoculated with 3000 freshly hatched second stage juveniles of *M. incognita* and it was seen that the nematode caused drop in various growth parameters of all the cultivars to varying levels over their respective controls. None of the cultivars was found to be totally resistant. The cultivar Sharmeeli was noted to be highly susceptible with more than 100 galls on the roots and it also showed maximum reductions in growth among the cultivars evaluated. The varieties Anmol and Sindha were found to be susceptible with 71–100 galls. Sabz Pari, Super Star, PMS-55 and PMS Beauty were found to be moderately susceptible with 31–70 galls and relatively less decrease in growth was observed. The cultivars Sanam, Dikshah, Arka Anamika, Ikra-1 and Ikra-2 with 11–30 galls were rated as moderately resistant, these showed a lesser amount of damage by the nematode as compared to susceptible cultivars and their planting would offer a useful tool for controlling root-knot nematodes.

Manju *et al.* (2015) reported that root knot nematode (*Meloidogyne incognita* (Kofoid and White) Chitwood) is the major pest of gerbera which causes a yield loss upto 31.1%. 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. An experiment was carried out to evaluate the resistant level of twenty five gerbera varieties. Two gerbera varieties Atletico orange and

Bombero were found to be resistant. Two varieties such as Orinaco yellow and Opium red exhibited moderately resistant, thirteen varieties viz., Ruble purple, Jinx yellow, Poubledate orange, Mammut cream, Bell water white, Aqua pink, Aveance pink, Bismack pink, Mariatta pink, Alamo red, Estoria red, Aragon red and Lexington red were found to be susceptible and the rest remaining varieties namely Palm beach yellow, Cunzone cream, Esmara pink, Bellwater pink, Aqua melone pink, Avenida red, Kaiser red and Alabanda red were found to be highly susceptible for *M. incognita*.

NA Tamilselvi *et al.* (2016) conducted a pot culture experiments under *in vitro* to understand the biochemical transformations taking place during nematode attack and thus 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*) included the 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 bare least values and these were found to be moderately resistant. The authentication to these resistant responses was done by assessment biochemical components like total phenol and ortho dihydroxy phenol (OD phenol). This study revealed that there was brisk increase total phenol and OD phenols activities and were detected in resistant rootstocks namely *C. colocynthis*, *C. metuliferus* and *C. moschata* and these began to amplify at 24 hours after inoculation and these were seen to have reached utmost at 96 hours after inoculation and subsequently declined. These outcomes suggested that the aforementioned resistant rootstocks were probable foundation of managing root knot nematode annoyance in bitter gourd by grafting.

Pandey *et al.* (2016) investigated on the biochemical alterations taking place in black gram varieties inoculated with root-knot nematode, *Meloidogyne incognita*. Various parameters like total chlorophyll, totalsugar contents, protein, and proline content were taken into observation during post infection period. The observations regarding the transformations in these biochemical parameters were documented. 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 and noted down. It was found that

the percentage of total chlorophyll contents decreased and this was observed in inoculated samples than the healthy counterparts. On the other hand, it was observed that in the diseased tissues the amount of total protein, proline and total sugar contents was increased.

2.3 Effect of the nematode infection on chlorophyll content.

Melakeberhan *et al.* (1985) experimented on one week-old *Phaseolus vulgaris* plants which were inoculated with 0, 2000, 4000 & 8000 *Meloidogyne incognita* juveniles/plant and were grown under controlled conditions of 21°C, 14 h day at 400 μ E.m-2.s-1, 16°C, and 10 h night cycle. Upto 28 days subsequent to inoculation, morphological and physiological distinctiveness of the bean plants were measured using destructive and nondestructive assays at intervals. Due to nematodes there was noteworthy reduction of total carbon, hydrogen, and nitrogen content, and plant dry weight from 8 day, and flowers, pods, seeds and leaf area after 15 day. Photosynthetic rate, based on leaf area and/or chlorophyll and nitrogen content decreased within one week and chlorophyll content within two weeks. Chlorophyll content and photosynthetic rate for all treatments decreased with time. The relation between the physiological and chronological age of the nematode and host in relation to the reduced production of infected plants was discussed.

Mohanty *et al.* (1997) accounted about the intrusion of *Meloidogyne incognita* with the performance of nodules. Besides being pathogenic to green gram *M. incognita* also influenced the normal root biochemistry of the plant. The nematode infected plants showed decline in the chlorophyll content in the leaves. There was reduction in leghaemoglobin and nitrogenase activities in nematode infected nodules.

Joshi *et al.* (2012) found that an inoculum level of 1000 J₂/500 g soil was found damaging threshold level on cv. RCH-1 of chilli. It was 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 J₂/500 g of soil. It was observed that there was less uptake of N, P and K in the roots due to parasitization by nematodes. It was found that there was considerable decline in N, P, K uptake in the plant and total chlorophyll content in the

leaves with inoculum of 1000 J₂/500 g soil and above. Accordingly, higher accumulation of the nutrients was seen in the rhizospheric soil.

Thakur (2014) observed that *Meloidogyne incognita* altered mentha plants physiology, disrupted vascular tissue, causing reduced amount of water and mineral supply to the foliage and this hampered the photosynthetic process. He found that *Meloidogyne incognita* reduced the chlorophyll 'a' and chlorophyll 'b' and total chlorophyll content and assimilation rate of mentha plants. It was observed that there was significant reduction of chlorophyll 'b' and total chlorophyll contents at 10 nematodes and above where as chlorophyll 'a' reduced at 100 nematodes and above.

Meena *et al.* (2016) conducted two pot culture experiments in two different seasons on carnation var. Redking to study about the interactive consequence of *Fusarium oxysporum* and *Meloidogyne incognita* on the growth, yield and amends in physiological parameters of the plant. Highest diminution of growth and yield factors was seen in the plants that received sequential inoculation of the nematode followed by fungus. It was observed that there was significant reduction in the nematode population in the sequential inoculation of the fungus followed by nematodes and combined inoculation of the two pathogens. Decline in chlorophyll content and boost in protein and sugar content was apparent in the plants due to the interaction of nematode and fungus.

2.4 Effects of nematode infection on macronutrient and micronutrient contents

Oteifa (1952) conducted experiments on lima bean plants which were infected by nematodes and supplied with potassium. A drop in plant development was observed due to the infection by *Meloidogyne incognita* and this was confirmed by notable changes observed in total fresh and total dry weights. Infected plants had lesser total amounts of nitrogen, phosphorus, calcium, magnesium and potassium as judged against the control plants and this was determined by chemical analysis. The nematodes impinged on the rate of absorption of these elements.

Melakeberhan *et al.* (1985) experimented on one-week-old *Phaseolus vulgaris* cv. Topnotch Golden Wax plants which were inoculated with 0, 1000, 5000 or 10 000 freshly hatched *Meloidogyne incognita* larvae per plant, and were maintained under controlled conditions of 21 °C, 14 h day at 400 μE m⁻²s⁻¹; 16 °C and 10 h night cycle. Leaf area, dry weight, number of flowers, the total carbon, hydrogen, nitrogen, calcium,

copper, iron, manganese, potassium and zinc content of shoots and roots, leaf chlorophyll content, and dark respiration and photosynthetic rates were measured at 3 weeks after inoculation. Respiration rate, percentage shoot nitrogen content, and calcium, copper and iron content per unit shoot weight: root weight ratio basis, increased considerably with increasing inoculum level. Other measured parameters showed a significant decline. Calcium, copper and iron in the shoot and potassium in the root augmented per unit weight as a consequence of nematode infection. Taken as a whole the total nutrient elements content per plant was notably decreased due to nematode infection. Differences in the physiology and nutrient content of *P.vulgaris* plants and distorted growth and loss of yield of nematode-infected plants was discussed.

Melakeberhan *et al.* 1987) conducted experiments on different stages of beans with different inoculum densities of *Meloidogyne incognita* under controlled condition. They found that potassium and iron showed an increasing trend in roots whereas calcium decreased in roots. It was seen that generally the concentration of K and Mn increased with the duration of infection and Ca and Fe decreased, with duration of infection in all treatments. In general, the concentration of all the nutritional elements decreased with the increase in treatment with nematodes.

Jacob *et al.* (1996) assessed the shoot and root samples of resistant tomato (Mangla) and susceptible tomato (Pusa ruby) varieties after inoculations with *Meloidogyne incognita* and the concentrations of bivalent cations along with other minerals was recorded. The percentage boost in the concentrations of calcium and magnesium, sodium, manganese and iron over check in the roots of susceptible and resistant plant was 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. In 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*.

Verma *et al.* (2006) experimented to see the effect of suggested, half and one and a half times of recommended doses of nutrients N, P, Zn individually on *Meloidogyne incognita* infecting American cotton susceptible var. H-777 and also on uptake on N, P, K, and total sugar and phenol contents. The treatments included non-fertilized and uninoculated checks and a treatment comprising of combination of N, P and Zn at recommended dose. The outcome that were recorded 90 days after inoculation disclosed

that amongst the individual fertilizers, appreciably higher plant growth was observed in nitrogen @ 150 kg/ha followed by 100 kg N and 75 P/ha as compared to inoculated check. The fecundity as well as final soil population of *M. incognita* was found to be significantly lowest at 75 kg P/ha followed by 50 kg P and 150 kg N/ha as compared to inoculated checks. It was seen that by increasing the application level of each nutrient, there was corresponding increase in the concentration and uptake of N, P, K and total sugar and total phenol contents. Considerably highest uptake was documented in combined application of nutrients followed by 150 kg N and 75 kg P/ha. It was seen that nematode inoculation reduced the concentration and uptake of these components drastically in comparison to uninoculated check.

El-sherif *et al.* (2013) studied on the impact of three levels of *Meloidogyne incognita* eggs, that is, 1000, 2000 and 3000 eggs/plant on plant growth factors of sugarbeet cv. Nejma along with its development and reproduction under greenhouse situation at $17 \pm 5^{\circ}\text{C}$. End results specified a reduction in the yield of sugarbeet and the growth parameters were also very much affected and the trivial outcome was seen more on the roots than the shoots. Furthermore, it was seen that when the inoculation level increased from 1000 up to 3000 eggs of *M. incognita* /plant, the percentage decline of plant growth parameters also increased and the highest rates were recorded to be 48.97 and 24.18% by 3000 eggs/ plant for shoot dry weight and whole plant fresh weights whereas, their lowest values of 30.93 and 4.05% resulted by inoculation level of 1000 eggs/ plant for the same plant growth criteria, respectively. It was found that *M. incognita* infection to sugar beet plants cv. Nejma at the three levels of egg inoculation reduced N, P, K and total chlorophyll content on leaves of sugarbeet with values of 9.9% (N), 18.5% (P), 8.8% (K), 1.58% (chlorophyll) and 20.0% (N), 27.4% (P), 18.4% (K) and 5.9% (chlorophyll) by 1000 and 3000 eggs/ plant, respectively as compared to the uninoculated plant.

2.5 Effect of nematode infection on crude protein content, total sugar, starch content, phenol and proline substances

Wang *et al.* (1974) conducted test using two monoxenic culture techniques of growing plants *i.e.* using filter paper and silica sand cultures. It was found that sugar in root exudates from tomato plants, infected with *Meloidogyne incognita* amplified from 133 to 836% over controls. The amino acids abridged by 52 to 56%. Galled root exudates

showed large increase in sugar level due to polysaccharide. Up to 6 weeks after inoculation the concentration and the absolute amount of total sugars in the infected plant xylem sap were greater than the healthy plant xylem sap whereas amino acids were moderately lower than in controls throughout the test period. The concentration of sugar increased as the nematode inoculum increased at 2, 4 and 6 wk after inoculation. The amino acids in all samples from the infected plant moderately decreased with an increase of nematode inoculum. The changes in total sugars and amino acids, of infected plant xylem sap and root exudates would incline the tomato plants to *Fusarium* wilt.

Basu *et al.* (1983) reported the changes brought about in the total protein, carbohydrate and lipid substance in the roots of *Hibiscus esculentus* caused due to the infection of root-knot nematodes at 15, 30, 45 and 60 days after inoculation. Emergence of galls on roots within 15 days after inoculation was marked and the number of galls increased very rapidly as the plants grew. The treated plants showed less number of galls. It was seen that there was a peak rise in root-knot nematode populace in 45 days which declined thereafter. The total protein, carbohydrate and lipid in roots were seen to have increased in amount with the development of test plants, both inoculated and uninoculated. The uninoculated plants had lesser amount of protein but higher amount of carbohydrate and lipid in roots than the inoculated ones. Taking into contemplation the root-carbohydrate and root-lipid, the intensity of infection caused by root-knot nematode was evaluated, and these were taken as supplementary biochemical parameters which got abridged due to feeding by nematodes.

Rezk *et al.* (1987) observed that phenolic content was elevated in nematode infected roots of resistant barley and wheat cultivars than those of susceptible cultivars and the control. In the resistant cultivars the concentration of the phenolics gradually increased with time subsequent to nematode inoculation.

Sharma *et al.* (1996) accounted about the biochemical changes brought about in *Abelmoschus esculentus* roots as a result of root-knot infection. The roots were uprooted after 30, 60 and 90 days of *Meloidogyne incognita* inoculation. Quantitative investigation for different metabolites in both highly susceptible (Pusa Sawani) and less susceptible (Punjab-7) cultivars was undertaken and the results of the investigation illustrated that the amount of reducing sugars, proteins, total free amino acids, proline, phenols, ascorbic acid, enzymes, nitrogen and sodium excepting total sugars, non reducing sugars,

phosphorus, and potassium increased in diseased roots of both the okra cultivars over their healthy counter parts.

Haseeb *et al.* (1999) studied in a glass house about the influence of varying primary inoculum densities (Pi) of *Meloidogyne incognita* on growth, physiological and biochemical amends and oil content of *Ocimum sanctum* cv. Shyama. An inversely proportional association was observed between Pi and fresh and dry weights, total chlorophyll, sugar, phenol and oil content of *O. sanctum*. The amount of root galling was directly proportional to the increase in Pi. Plants inoculated with highest Pi (2.1 J₂/g of soil) showed peak reduction in all the growth parameters. Plants inoculated with lowest Pi (0.1 J₂/g of soil) showed the highest reproduction factor (Rf = 20.37).

Nagesh *et al.* (2004) conducted experiments in glasshouse to study about biochemical authentication for variations observed in the roots for resistance to nematode infection, colonized by mycorrhiza, and vulnerability of the fresh flush of roots of the same plant that escaped mycorrhizal colonization. Tomato roots were analyzed for their biochemical outlines with respect to total proteins, total phenols, indole acetic acid, activities of polyphenol oxidase, phenylalanine ammonia lyase and indole acetic acid oxidase. One set of roots of the same plant was given *Glomus fasciculatum* and *G. fasciculatum* plus juveniles of *Meloidogyne incognita* individually; and half the roots of second set of plants received *G. fasciculatum* while the other half of roots did not obtain any treatment. The roots that received juveniles of root-knot nematode recorded least amount of activities of PAL and IAA oxidase and maximum amount of PPO activity. Since the roots of same plant that received mycorrhiza and that did not receive mycorrhiza; and the plant that received nematode only and mycorrhiza plus nematode recorded differential biochemical contents of proteins, total phenols and IAA, and differential behavior of enzymes under study, it was apparent that the biochemical defense retort to mycorrhizal colonization against root-knot nematodes was localized and not systemic. This explained for the response of plant that differed in root galling due to nematode infection in presence of mycorrhizal colonization. The new or fresh roots which missed mycorrhizal colonization, got infected by nematodes and developed root galls.

Swain *et al.* (2004) investigated on chronological development of phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), phenol and lignin-like polymers,

which were undertaken in differential host plants (cotton cv. Deltapine-16 and tobacco cv. NC-95) along with susceptible hosts (cotton cv. H-777 and tobacco cv. FCV-Special) after being inoculated with different host races of *Meloidogyne incognita*. It was observed that all the races induced a faster and early accumulation of these defense parameters upon inoculation to host differentials than their healthy counterparts, whereas race-inoculated susceptible tobacco cv. FCV Special and cotton cv. H-777 showed a steady and deferred buildup in their defense responses. But the per cent increase over their uninoculated controls, was of less degree than that observed in host disparities at different intervals of time.

Sundararaju *et al.* (2008) conducted a pot culture experiment to understand the plant growth, physiological and biochemical transformations occurring due to the infection of root-knot nematode, *Meloidogyne incognita* on banana cv. Robusta (AAA). It was found that accretion of sugar was higher in uninoculated plants (0.60g/100g) as compared to the plants inoculated with 10,000 nematodes (0.30/100g) which recorded the lowest concentration of sugar.

Vaitheeswaran *et al.* (2009) studied the impact of pathogenesis of root knot nematode at tissue level which was revealed with low sugar, high protein, phenol and lipid levels under normal nitrogen stress situation. It was found that there was decline in sugar content in excess nitrogen treated infected plant which might be due to stirred protein synthesis for which large amount of sugar might be considered necessary. The decline in sugar content during nitrogen shortage might be due to reduced activities of the nematode. Nitrogen in excess amount resulted in the buildup of protein. It was observed that nitrogen in surplus encouraged the synthesis of proteins which defied the parasitic effect of the nematodes and protein was found with an increased rate. It was seen that nitrogen shortage resulted in diminished protein content owing to low turnover of the amino acid. A general diminution in the actions of most of the enzymes was observed in the infected plant under normal nitrogen stress situation. This characteristic was observed to be more at infection site and this showed the stress undergone by the root during infection by nematodes.

Nayak *et al.* (2010) investigated about biochemical changes brought about in brinjal cv. Pusa Purple Long inoculated with root-knot nematode, *Meloidogyne incognita*. Various factors like amino acids and amides, total sugar contents, nitrogen,

crude protein, total chlorophyll and organic acid contents during post infection periods were taken into account and the biochemical variations related to these parameters were recorded. It was observed that among the various amino acids detected, 08 amino acids were found to be common in both healthy and inoculated plants. Organic acids and total chlorophyll contents showed diminished percentage in inoculated samples than healthy counterparts. However, it was observed that the diseased tissues showed an increase in quantity of crude protein, nitrogen and total sugar contents.

Chaudhary *et al.* (2013) investigated about the alterations in biochemical and antioxidative parameters brought about subsequent to nematode inoculation in four tomatoes varieties, two resistant i.e. Hisar Lal, PNR-7 and two susceptible i.e. Punjab Varkha Bahar-1 and Punjab Varkha Bahar-2. The four varieties talked about above were infected with root knot nematode (*M. incognita*) at the nursery stage. The egg masses of *M. Incognita* were taken from the infected brinjal plants. The sampling of roots and leaves were done after 7 days and 20 days of infection. Tomato fruits from uninoculated and inoculated plants were taken and evaluated for their nutritional value parameters. It was found that the phenolic compounds showed an increase in the resistant inoculated varieties, whereas susceptible varieties showed a gradual decrease in phenolic compounds following inoculation.

Cabello *et al.* (2014) studied about that the sedentary endoparasitic nematodes of plants which provoke highly specific feeding cells in the root central cylinder. It was seen that the obligate parasites remove all required nutrients from these definite feeding cells. The feeding cells have been depicted as sink tissues in the plant's circulation system and are supplied with phloem-derived solutes such as sugars. The roles of the sucrose-cleaving enzymes sucrose synthases (SUS) and invertases (INV) in the development of *Heterodera schachtii* were studied. Gene expression analyses indicated that both enzymes were controlled transcriptionally. Nematode development was improved on multiple INV and SUS mutants. Syncytia of these mutants were differentiated by alteration in enzyme activity and modification in the sugar pool sizes. Furthermore, the analyses also revealed that the sugar levels were systemically influenced and enzyme activities in the shoots of the tested mutants were also influenced. This suggested about the changes in the source-sink correlation. The development of the root-knot nematode *Meloidogyne javanica* was studied in different INV and SUS mutants and wild-type

Arabidopsis plants. Similar results on the development of both sedentary endoparasitic nematode species (root-knot and cyst nematode) were observed and this suggested a more universal role of sucrose-degrading enzymes during plant-nematode interfaces.

Gautam *et al.* (2014) found that bitter melon (*Momordica charantia*) being an economically important vegetable crop was facing considerable yield loss due to infection by root-knot nematode (*Meloidogyne incognita*). A pot culture experiment was conducted to understand the biochemical changes taking place during the early response to nematode attack in bitter melon. Nematodes were isolated and inoculated (500J₂/pot) into the rhizosphere of seedlings. A rapid increase in the protein concentration in the first week and total sugar level in the fourth week of inoculation in infected roots was observed. The study indicated a higher rate of protein synthesis during infection by nematodes for initiation of early primary resistance mechanism by the plants to counteract nematode attack. Increase in sugar levels in the 4th week coincided with gall formation in the root tissue by the nematode and it indicated greater inflow of sugars into the attacked and diseased tissue for providing nutrition to the nematodes for their growth and survival.

Pandey *et al.* (2017) reported that all living organisms required sugar for their metabolic energy. It was established that there was considerable boost in the amount of total sugars in roots of nematode inoculated plants save for the same was found to be lessened in shoots of nematode infected plants.

Banuelos *et al.* (2012) reported about the activation of different kind of defense mechanism in plants as a result of nematode infection. They reported that there was a reduced concentration of proline in the inoculated plants as compared to the non inoculated plants which showed a higher concentration.



CHAPTER-3

MATERIALS & METHODS

MATERIALS AND METHODS

3.1 Collection of nematodes

Stock culture

The experimental stock cultures of the root-knot nematode, *Meloidogyne incognita* for this study was originally obtained from a single egg mass progeny, maintained and multiplied on susceptible tomato variety, Pusa Ruby. The seeds of tomato plants were sown in 15 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 J₂ in sterile water suspension to the root zone of two 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. J₂-that hatched out were collected daily in beakers and were 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 surface sterilized with formaldehyde solution (1.0 %) and filled with autoclaved soil (15 lbs/20min). These pots were arranged on greenhouse benches in complete randomised design, with four replications. As and when required agricultural operations were done. For all chemical analysis purposes sterilized oven dried glassware and double distilled water were used throughout the experiment.



Fig. 1 Seven gerbera varieties/ cultivars (1) Seth, (2) Mammut, (3) Goldstrick, (4)Snowball, (5) Pink elegance, (6) Real, (7) Rosario.

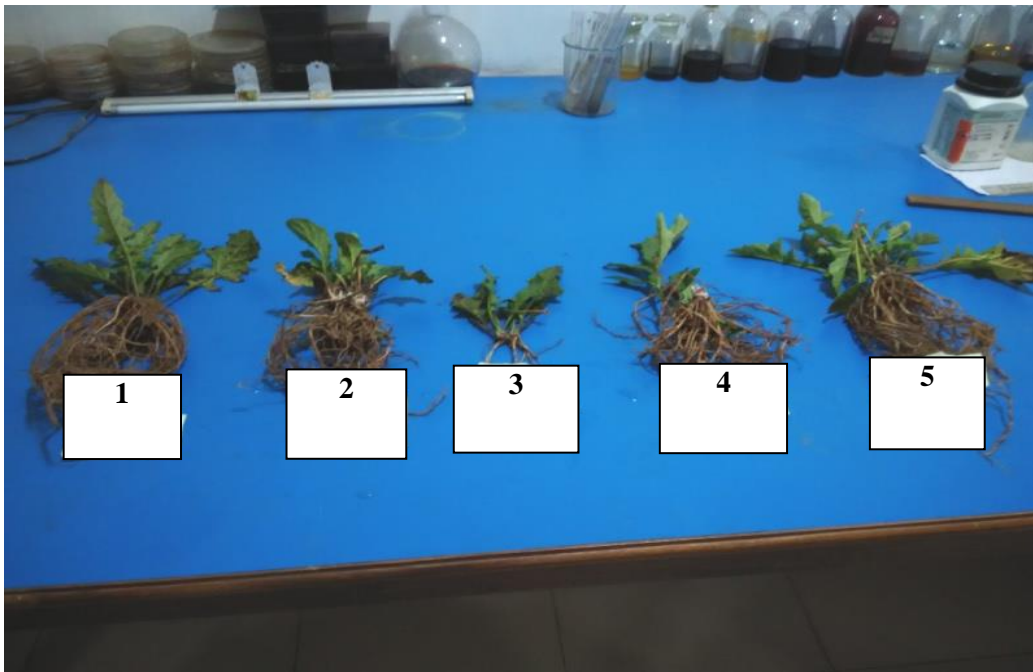


Fig. 2 Five gerbera varieties/ cultivars taken for biochemical analysis (1)Mammut, (2) Pink elegance, (3) Seth, (4) Goldstrick, (5) Real.



Fig. 3 Gallings in roots of gerbera varieties



Fig. 4 Laboratory analysis of total Phenolic substances

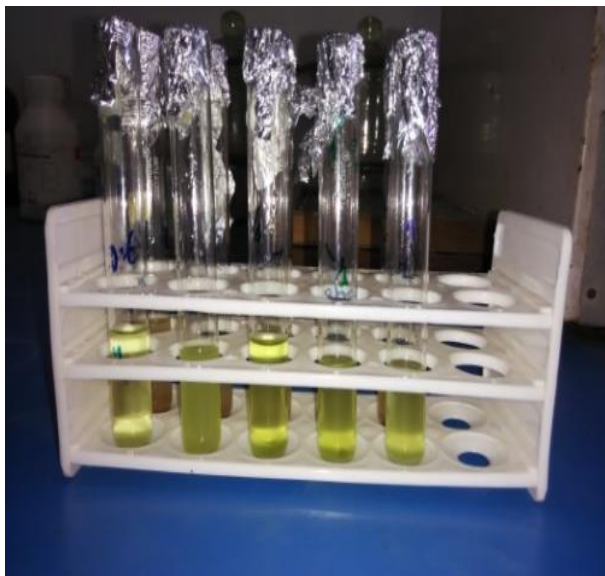


Fig. 5. Laboratory analysis of total proline substance



3.2.1 Collection of gerbera germplasms/cultivars

Culms of 7 gerbera germplasms/cultivars each of 3 to 5 g, were collected from **local market and Biotechnology cum Tissue Culture Laboratory, OUAT, Bhubaneswar**. The culms were surface sterilized by treating them with 0.1 % HgCl₂ for 5 minutes, washed thoroughly with sterile water and air dried. These culms were sown in the pots to conduct the experiments.

Planting of culms and inoculation of nematodes

The soil to be filled in the pots were pulverised, mixed with N, P, K fertilizers @ 200:100:100 per hectare on soil weight basis and filled in the pots @ 1 kg/pot. The surface sterilized culms were planted @ 2 to 3 culms per pot. Pots were arranged in CRD with four replications. Moisture was maintained regularly after sprouting with light irrigation. At 15 days after planting the plants were thinned keeping one seedling per pot at the centre. Four holes were made by small glass tube (2 cm long, 0.5 cm bore) inserting into the soil near the root zone of each of the seedlings. Two weeks after sprouting axenised nematodes were counted under a stereoscopic microscope and released into the holes near the root zones @ 1000 J₂± 20 per seedling in 10 ml sterile water. For chemical analysis another two sets of pots were maintained, one for the uninoculated control (Healthy) and the other infected with the nematodes. Each set was arranged on separate platform in the green house in order to avoid cross infections.

3.3 Screening of gerbera germplasms/ cultivars against root-knot nematode

Root-knot nematodes are polyphagous in nature. Hence, 7 genotypes (varieties) of gerbera were procured for testing and screening against test nematode for their resistance/susceptibility. Pots containing sterilised soil were arranged on greenhouse benches in complete randomized design with four replications.

After 2 weeks of planting J₂ of root-knot were released into holes near the root zone of each plant. Watering was done just to drench the soil avoiding over flooding. Forty five days after planting the pots were washed under tap water. 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 were 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, five gram was stained with lacto-phenol acid fuchsine. The egg masses present in it were counted through stereoscopic microscope. Gerbera varieties or germplasms 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	0	Highly resistant (HR)
2.0	1-10	Resistant (R)
3.0	11-30	Moderately resistant (MR)
4.0	31-100	Susceptible (S)
5.0	More than 100	Highly susceptible (HS)

3.3.1 Evaluation of gerbera varieties against root-knot nematode

Seven gerbera varieties were planted 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 planting of culms and observations were made on number of galls/plant, plant height (cm), root length (cm), fresh shoot weight (g), fresh root weight (g), dry shoot weight (g), dry root weight (g), number of egg masses/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 Mammut (Resistant), Pink elegance, Gold strick, Real (Moderately resistant), and Seth (susceptible) were planted in earthen pots arranged in the greenhouse benches as described earlier maintaining four replications in Completely Randomised Design. These plants were washed 45 days after planting or 30 days after inoculation of nematodes and the following chemical compositions were estimated.

- i. Chlorophyll content (mg/g fresh leaves)
- ii. Nitrogen content and crude protein content (%)
- iii. Phosphorus content (%)

- iv. Potassium content (%)
- v. Total sugar content (mg/g fresh roots)
- vi. Starch content (mg/g fresh roots)
- vii. Total phenol content (mg/g on fresh weight basis)
- viii. Total proline content (mg/g on fresh weight basis)
- ix. Iron content (mg/100g on dry root weight)
- x. Magnesium content (mg/100g on dry root weight)
- xi. Calcium content (mg/100g on dry root weight)
- xii. Sulphur content (mg/100g on dry root weight)
- xiii. Manganese content (mg/100g on dry root weight)

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

One hundred mg leaf portion of each treatment were cut from the composite leaves were immersed in 10 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 shoot

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

Per cent N_2 in sample

$$= \frac{(\text{Sample titer} - \text{blank titer}) \times N_2 \text{ of } H_2SO_4 \times 14 \times 100 \times 2.5}{\text{Sample weight (g)} \times 1000}$$

3.4.3 Crude protein

Percentage of protein present in shoots and roots were 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

Digestion of samples

Powdered plant samples (0.2 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 overnight. 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) HNO₃Then mix the reagents (a) and (b) in a 250 ml volumetric flask
The resulting solution is called molybdate – vanadate solution.
2. 2(N) HNO₃: Dilute the 60 ml concentrated HNO₃ to 480 ml with distilled water.
3. The standard phosphorus solution (25 ppm): Dissolve 55 mg monobasic potassium phosphate (KH₂PO₄) 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) HNO₃ 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

One ml digested sample extract of shoot and root 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

The total sugar content of root was determined by adopting the following procedure.

Extraction of root sugar

One hundred mg of ground root 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 with distilled water. Five ml of this extract was taken in a 25 ml volumetric flask. Simultaneously standards of 0 ml, 1 ml and 1.5 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 % H₂SO₄) 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

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 phenolic substances

Exactly 0.1 g each of 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 Na_2CO_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).

3.4.9 Estimation of total proline substance

Exactly 100 mgs each of shoot and root was macerated with 5 ml of sulfo-salicylic acid.

The residue was centrifuged 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 of fresh sample.

3.4.10 Estimation of micro-nutrients influenced by root knot nematode in gerbera plant samples

Mineral acids like of diacid (HNO_3 - HClO_4) digestion (Jackson, 1973) by SS method through model GCB Awanta

Method

Powdered samples (0.2g) were taken in a 100 ml conical flask. To each flask 10 ml of concentrated HNO_3 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_3 : HClO_4 (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 micro-nutrients like Fe, Ca, Mg, S and Mn by adopting the procedure of Jackson (1973).

Micro-nutrients (Fe, Ca, Mg, S and Mn) Estimation

Digested sample was introduced to AAS for Fe, Ca, Mg, S and Mn analysis after standardizing the AAS with respective standards.

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



CHAPTER-4

RESULT & DISCUSSION

RESULTS

Root knot nematodes are polyphagous in nature and obligate parasites of thousands of plant species, 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, black shank disease 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 nematodes. In this context, an intimate knowledge of physiology and biochemistry of plants as well as nematodes are very essential to understand the complicated areas of host-nematode interactions. Hence, 7 gerbera varieties/germplasms were collected from different sources for testing their resistance to root-knot nematode, *Meloidogyne incognita* under pot culture condition in greenhouse conditions.

4.1 Screening of gerbera germplasms/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 presence egg masses, root-knot indices following 1-5 scale (Taylor and Sasser, 1978).

Scale

- 1 = No egg mass/galls/plant= Highly resistant (HR)
- 2 = 1-10 egg masses/galls/plant = Resistant (R)
- 3 = 11-30 egg masses/galls/plant = Moderately resistant (MR)
- 4 = 31-100 egg masses/galls/plant = Susceptible (S)
- 5 = > 100 egg masses/galls/plant = Highly susceptible (HS)

After thirty days of inoculation, the plants were uprooted and root system was gently washed under running tap water. Number of galls and egg masses per root system of the tested variety were counted and rated according to 1-5 scale of gall index.

Out of the seven varieties one was found resistant, five were moderately resistant, one was susceptible to the test nematode, *M incognita* under greenhouse condition (Table 1).

4.2 Evaluation of gerbera varieties against root-knot nematode, *Meloidogyne incognita*

After 30 days of inoculation, the plants were uprooted smoothly and the root systems were washed carefully under running tap water to remove the soil particles adhering to them. Growth parameters like shoot length (cm), root length (cm), shoot weight (g), root weight (g), no. of egg mass/10g root, final nematode population (number) and gall index (1-5 scale) were studied (Table 2).

Table 1. Screening of gerbera germplasms/cultivars against root-knot nematode, *M. incognita*

(Mean of four replications)

Sl.no.	Varieties	No. of galls	Reaction	Final population *
1	Mammut	8.66	Resistant	1190 (3.075)
2	Pink elegance	23.34	Moderately resistant	1350 (3.130)
3	Gold strick	29.10	Moderately resistant	1270 (3.130)
4	Real	25.48	Moderately resistant	1340 (3.127)
5	Rosario	22.82	Moderately resistant	1285 (3.108)
6	Snowball	28.06	Moderately resistant	1365 (3.135)
7	Seth	67.66	Susceptible	1450 (3.164)
	SE(m)±	0.026		0.004
	CD(0.05)	0.077		0.012

*Figures in parenthesis are log transformed values

Table 2. Evaluation of gerbera cultivars against root-knot nematode, *M. incognita*. (Mean of four replications)

Sl.no	Varieties	Fresh shoot wt.(g)	Fresh root wt.(g)	Shoot length (cm)	Root length (cm)	No. of galls	Reaction	Root knot index (1-5 scale)	Dry shoot wt.(g)	Dry root wt.(g)	Final population *
1	Mammut	19.90	18.90	19.30	29.30	8.66	Resistant (R)	2	3.20	3.90	1190 (3.075)
2	Pink elegance	13.10	15.80	26.90	22.70	23.34	Moderately resistant (MR)	3	3.10	3.70	1350 (3.130)
3	Gold strick	15.20	10.50	19.20	19.90	29.10	Moderately resistant (MR)	3	2.80	3.30	1270 (3.130)
4	Real	10.20	10.80	25.80	16.30	25.48	Moderately resistant (MR)	3	2.70	2.70	1340 (3.127)
5	Rosario	10.60	9.80	17.70	14.20	22.82	Moderately resistant (MR)	3	1.60	2.20	1285 (3.108)
6	Snowball	7.50	8.40	21.40	14.60	28.09	Moderately resistant (MR)	3	2.20	1.80	1365 (3.135)
7	Seth	7.40	7.50	16.10	10.80	67.66	Susceptible (S)	4	1.40	1.60	1450 (3.164)
	SE(m)±	0.216	0.243	0.150	0.159	0.026			0.187	0.168	0.004
	CD(0.05)	0.667	0.751	0.463	0.492	0.077			0.578	0.519	0.012

*Figures in parenthesis are log transformed values

4.2.1 Effect of nematode infection varietal response

Out of seven varieties, one had shown resistant reaction with 8.66 numbers of galls per plants while five entries 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.

4.3 Biochemical composition studies of the varieties upon nematode infection

Different types of chemicals are present in the plant system which influence the metabolism of the pests, feeding upon them. The chemicals may consists 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. During the course of feeding some chemicals may be depleted or some other new may be synthesized, that are detrimental to the pest. In order to know the chemical and genetic basis of resistance, five varieties were chosen for biochemical studies. These varieties were, Pink elegance, Gold strick, Real are moderately resistant, Mammut being resistant and Seth is susceptible check included for detailed study. These varieties were grown with utmost care, both in inoculated and control conditions. One set of each uninoculated (healthy) and inoculated (infected) plants of the varieties were studied to test the effect of root-knot nematode infection on different biochemical parameters.

4.3.1 Effect of the nematode infection on chlorophyll content.

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

In the experiment it was observed that the chlorophyll 'a' contents in the infected plants decreased by 0.261, 0.278, 0.317, 0.292 and 0.583 mg/g and the percent decrease was 27.50, 27.41, 27.45, 27.36 and 27.48 in the varieties Mammut , Pink elegance, Gold strick, Real and Seth, respectively over the healthy (Table 3).

Table 3 Estimation of chlorophyll contents (a, b, total) (mg/g) due to root-knot nematode infection in gerbera varieties/ cultivars.

Sl. No.	Varieties	Chlorophyll" a" content (mg/g)				Chlorophyll" b" content (mg/g)				Total chlorophyll content (mg/g)			
		Infected leaf	Healthy leaf	Mean	Increase)/ decrease over healthy (%)	Infected leaf	Healthy leaf	Mean	Increase /decrease over healthy (%)	Infected leaf	Healthy leaf	Mean	Increase / decrease over healthy (%)
1	Mammut (R)	0.261	0.360	0.311	-27.50	0.086	0.118	0.102	-27.12	0.378	0.521	0.450	-27.44
2	Pink elegance (MR)	0.278	0.383	0.331	-27.41	0.197	0.320	0.259	-38.43	0.578	0.796	0.687	-27.38
3	Goldstrick (MR)	0.317	0.437	0.377	-27.45	0.232	0.272	0.252	-14.70	0.478	0.704	0.591	-32.10
4	Real (MR)	0.292	0.402	0.347	-27.36	0.312	0.431	0.372	-27.61	0.489	0.674	0.582	-27.44
5	Seth (S)	0.583	0.804	0.694	-27.48	0.316	0.436	0.376	-27.52	0.629	0.868	0.749	-27.53
	SE(m)±	0.014	0.002			0.002	0.003			0.003	0.005		
	CD(P=0.05)	0.032	0.006			0.006	0.008			0.009	0.016		

Data revealed that the chlorophyll 'b' contents in the infected plants decreased by 0.086, 0.197, 0.232, 0.312 and 0.316 mg/g and the percent decrease was by 27.12, 38.43, 14.70, 27.61 and 27.52 in varieties Mammut, Pink elegance, Gold strick, Real and Seth, respectively over their healthy counter parts. (Table 3).

The chlorophyll total contents in the infected plants was decreased by 0.378, 0.578, 0.478, 0.489 and 0.629 mg/g and percent the decrease was by 27.44, 27.38, 32.10, 27.44 and 27.53 in varieties Mammut, Pink elegance, Gold strick, Real and Seth, respectively over their healthy counter parts (Table 3).

4.3.2 Effects of nematode infection on nitrogen contents

The total Nitrogen content was decreased in the shoot system of infected varieties of gerbera, Mammut, Pink elegance, Gold strick, Real and Seth by 29.06, 21.73, 19.68, 14.15 and 33.33 percent respectively over healthy one on dry weight basis. Whereas in the roots Nitrogen content was increased by 27.31, 27.27, 27.21, 27.27 and 34.48 percent in all the above mentioned varieties serially.

The total Nitrogen content was decreased in the shoot system of varieties from 29.06 to 33.33% and the same was decreased by 27.31% in the root system of resistant variety as compared to susceptible one (34.48%) (Table 4).

The reduction in Nitrogen content (%) in shoots was significantly lower in the resistant varieties as compared to susceptible variety.

4.3.3 Effect of nematode infection on crude protein content

Due to infection of root-knot nematode, the percentage of shoot crude protein content was decreased by 33.33% in variety Seth, 14.19% in Real, 19.68% in Gold strick, 21.70% in Pink elegance than Mammut (29.11%) whereas crude protein content in root was low in Seth (34.25%) than that of Real (27.23%), Gold strick (27.27%), Pink elegance (27.25%) and Mammut (27.34%) (Table 5). Similar trend was seen in both Nitrogen content and crude protein content of the infected shoot where the crude protein of both the infected shoot decreased and infected root increased.

Table 4 Estimation of Nitrogen Contents (%) in the resistant/susceptible gerbera varieties influenced by root knot, *Melodogyne incognita*.

Sl. No	Varieties	Nitrogen (%) on dry weight basis							
		Shoot				Root			
		Infected(I)	Healthy(H)	Mean	Increase / decrease over healthy (%)	Infected(I)	Healthy(H)	Mean	Increase / decrease over healthy (%)
1	Mammut (R)	1.22	1.72	1.47	-29.06	1.65	2.27	1.96	-27.31
2	Pink elegance (MR)	1.08	1.38	1.23	-21.73	1.52	2.09	1.81	-27.27
3	Goldstrick (MR)	1.02	1.27	1.15	-19.68	1.23	1.69	1.46	-27.21
4	Real(MR)	0.91	1.06	0.99	-14.15	1.12	1.54	1.33	-27.27
5	Seth (S)	0.10	0.15	0.13	-33.33	0.19	0.29	0.24	-34.48
	SE(m)±	0.018	0.011			0.032	0.031		
	CD(P=0.05)	0.055	0.033			0.098	0.095		

Table 5 Estimation of Crude Protein (%) Contents in the resistant/susceptible gerbera varieties influenced by root knot nematode, *M. inconita*.

Sl.no	Varieties	Crude protein content (%) on dry weight basis							
		Shoot				Root			
		Infected(I)	Healthy(H)	Mean	Increase or decrease over healthy (%)	Infected(I)	Healthy(H)	Mean	Increase or decrease over healthy (%)
1	Mammut	7.62	10.75	9.19	-29.11	10.31	14.19	12.25	-27.34
2	Pink elegance(MR)	6.75	8.62	7.69	-21.70	9.50	13.06	11.28	-27.25
3	Goldstrick(MR)	6.37	7.93	7.15	-19.68	7.68	10.56	9.12	-27.27
4	Real(MR)	5.69	6.63	6.15	-14.19	7.00	9.62	8.31	-27.23
5	Seth	0.62	0.93	0.78	-33.33	1.19	1.81	1.49	-34.25
	SE(m)±	0.013	0.043			0.078	0.213		
	CD(P=0.05)	0.040	0.132			0.240	0.655		

4.3.4 Effect of nematode infection on Phosphorus content

The phosphorus content was decreased in the shoot system of infected varieties i.e. Mammut, Pink elegance, Gold strick, Real and Seth by 58.33, 51.42, 53.57, 76.92 and 57.14 percent and the same trend was observed in the infected roots by 49.02, 45.45, 54.76, 43.75 and 55.00 percent in gerbera varieties in order as mentioned above over healthy plant on dry weight basis. Whereas phosphorus content was recorded highest as 55.00% in infected roots of variety Seth followed by 54.76% in Gold strick, 49.02% in Mammut, 45.45% in Pink elegance and 43.75% in Real (Table 5). The above results of the present investigation showed that the infected plants had decreased percentage of phosphorus content in shoots but with increased percentage in roots of susceptible and resistant varieties (Table 6).

4.3.5 Effect of nematode infection on potassium content

The root-knot nematode inoculated plants measured an increase in K_2O content to the tune of 11.20, 13.46, 29.33, 33.33 and 52.94 percent in shoots and 18.11, 22.52, 14.70, 41.26 and 61.90 percent in roots of varieties Mammut, Pink elegance, Gold strick, Real and Seth respectively. There was significant increase of potassium content of both susceptible and resistant infected plants which was more pronounced in shoots of resistant varieties as compared to the susceptible variety (Table 7).

4.3.6 Effects of nematode infection on total sugar content

The amount of sugar present in the roots of the inoculated plants were recorded as 0.38, 0.36, 0.34, 0.32 and 0.28 mg/g and percentage increase was by 31.03, 80.00, 21.42, 10.34 and 12.00 percent in varieties Mammut, Pink elegance, Gold strick, Real and Seth, respectively on fresh weight basis (Table 8). This amount was also increased in all the cases of inoculated roots over healthy in the above mentioned varieties accordingly.

Table 6 Estimation of phosphorus contents (%) in the resistant/susceptible gerbera varieties influenced by root knot nematode, *M.incognita*.

Sl.no	Varieties	Phosphorus (%) on dry weight basis							
		Shoot				Root			
		Infected(I)	Healthy(H)	Mean	Increase or decrease over healthy (%)	Infected(I)	Healthy(H)	Mean	Increase or decrease over healthy (%)
1	Mammut (R)	0.20	0.48	0.34	-58.33	0.26	0.51	0.39	-49.02
2	Pink elegance (MR)	0.17	0.35	0.26	-51.42	0.24	0.44	0.34	-45.45
3	Goldstrick (MR)	0.13	0.28	0.21	-53.57	0.19	0.42	0.31	-54.76
4	Real(MR)	0.03	0.13	0.08	-76.92	0.18	0.32	0.25	-43.75
5	Seth (S)	0.09	0.21	0.15	-57.14	0.18	0.40	0.29	-55.00
	SE(m)±	0.031	0.012			0.011	0.018		
	CD(P=0.05)	0.095	0.036			0.033	0.055		

Table 7 Estimation of potassium contents (%) in the resistant/susceptible gerbera varieties influenced by root knot nematode, *M.incognita*.

Sl.no	Varieties	Potassium % on dry weight basis							
		Shoot				Root			
		Infected(I)	Healthy(H)	Mean	Increase or decrease over healthy (%)	Infected(I)	Healthy(H)	Mean	Increase or decrease over healthy (%)
1	Mammut (R)	1.29	1.16	1.23	+11.20	1.63	1.38	1.51	+18.11
2	Pink elegance (MR)	1.18	1.04	1.11	+13.46	1.36	1.11	1.24	+22.52
3	Goldstrick (MR)	0.97	0.75	0.86	+29.33	1.17	1.02	1.10	+14.70
4	Real(MR)	0.72	0.54	0.63	+33.33	0.89	0.63	0.76	+41.26
5	Seth (S)	0.26	0.17	0.22	+52.94	0.34	0.21	0.28	+61.90
	SE(m)±	0.011	0.015			0.010	0.011		
	CD(P=0.05)	0.033	0.046			0.030	0.033		

Table 8 Estimation of total sugar content (mg/g) in the resistant/susceptible gerbera varieties influenced by root knot nematode, *M.incognita*.

Sl. No.	Varieties	Total Sugar content (mg/g) on fresh weight basis			
		Infected root	Healthy root	Mean	Increase/decrease over healthy (%)
1	Mammut (R)	0.38	0.29	0.34	+31.03
2	Pink elegance(MR)	0.36	0.20	0.28	+80.00
3	Gold strick(MR)	0.34	0.28	0.31	+21.42
4	Real (MR)	0.32	0.29	0.31	+10.34
5	Seth (S)	0.28	0.25	0.27	+12.00
	SE(m)±	0.013	0.015		
	CD(0.05)	0.041	0.046		

4.3.7 Effects of nematode infection on total starch content

The amounts of starch present in the roots of the inoculated plants were recorded as 0.65, 0.56, 0.42, 0.68 and 0.51 mg/g and the percentage of decrease was by 5.88, 10.71, 15.38, 27.45 and 28.57 in varieties Real, Pink elegance Mammut, Seth and Gold strick, respectively on fresh weight basis (Table 9). The amount of total starch was more in both shoots and roots of susceptible varieties than resistants.

Table 9. Estimation of changes of starch content (mg/g) in the roots resistant/susceptible gerbera varieties influenced by root knot nematode, *M. incognita*.

Sl. No.	Varieties	Total Starch content (mg/g) on fresh weight basis of roots			
		Infected	Healthy	Mean	Increase/decrease over healthy (%)
1	Mammut (R)	0.65	0.75	0.70	-15.38
2	Pink elegance (MR)	0.56	0.62	0.59	-10.71
3	Gold strick (MR)	0.42	0.54	0.48	-28.57
4	Real (MR)	0.68	0.72	0.70	-5.88
5	Seth (S)	0.51	0.65	0.58	-27.45
	SE(m)±	0.008	0.013		
	CD(0.05)	0.024	0.042		

4.3.8 Effect of nematode infection on contents of phenolic substances

The phenolic content of inoculated shoots were 0.35, 0.21, 0.22, 0.24 and 0.20 mg/g in Mammut, Pink elegance, Gold strick, Real and Seth, respectively and this was increased by 61.18, 60.15, 60.56, 60.52 and 60.62 percent in shoots and 60.91, 61.19, 60.43, 60.93 and 60.62 percent in roots, respectively in the above mentioned varieties (Table 10). Phenolic content was observed to be more in the present observation.

4.3.9 Effect of nematode infection on contents of proline substances

The proline content of infected shoots of gerbera cultivars were 0.78, 0.35, 0.27, 0.26 and 0.16mg/g in Mammut, Pink elegance, Gold strick, Real and Seth respectively. Moreover, due to infection of root knot nematode the proline contents of these varieties increased by 61.23, 61.19, 60.84, 60.49 and 61.00 percent in shoots and 61.07, 61.02, 56.90 60.94 and 60.34 percent in roots respectively in the above said varieties (Table 11). The amount of proline content was more in both shoots and roots of resistant cultivars than susceptible gerbera cultivars. .

Table 10. Estimation of total phenolic content (mg /g) in the resistant/susceptible gerbera varieties influenced by root knot nematode *M. incognita*,

Sl. No	Varieties	Phenol content (mg /g) on fresh weight basis							
		Shoot (leaf)				Root			
		Infected	Healthy	Mean	Increase/ decrease over healthy (%)	Infected	Healthy	Mean	Increase/ decrease over healthy (%)
1	Mammut (R)	0.35	0.21	0.28	+61.18	0.42	0.26	0.34	+60.91
2	Pink elegance (MR)	0.21	0.13	0.17	+60.15	0.32	0.20	0.26	+61.19
3	Goldstrick(MR)	0.22	0.14	0.18	+60.56	0.29	0.18	0.23	+60.43
4	Real(MR)	0.24	0.15	0.19	+60.52	0.30	0.19	0.25	+60.93
5	Seth(S)	0.20	0.12	0.16	+60.62	0.25	0.16	0.20	+60.62
	SE(m)±	0.001	0.003			0.002	1.165		
	CD(P=0.05)	0.005	0.010			0.008	3.589		

Table 11 Estimation of changes of total proline content (mg/g) in the resistant/susceptible gerbera varieties influenced by root knot nematode, *M. incognita*

Sl. No.	Varieties	Proline content (mg/g) on fresh weight basis							
		Shoot (leaf)				Root			
		Infected	Healthy	Mean	Increase/ decrease over healthy (%)	Infected	Healthy	Mean	Increase/ decrease over healthy (%)
1	Mammut (R)	0.78	0.48	0.63	+61.23	0.80	0.50	0.65	+61.07
2	Pink elegance (MR)	0.35	0.21	0.28	+61.19	0.53	0.33	0.43	+61.02
3	Goldstrick (MR)	0.27	0.17	0.22	+60.84	0.55	0.35	0.45	+56.90
4	Real(MR)	0.26	0.16	0.21	+60.49	0.47	0.29	0.38	+60.94
5	Seth (S)	0.16	0.10	0.13	+61.00	0.18	0.11	0.15	+60.34
	SE(m)±	1.560	1.162			0.002	0.007		
	CD(P=0.05)	4.804	3.578			0.005	0.021		

4.3.10. a. Effects of nematode infection on iron content

Table 12a depicted that iron content was decreased in the root system of infected varieties of gerbera Mammut, Pink elegance, Gold strick, Real and Seth by 0.33, 0.17, 0.11, 0.16 and 0.12 mg/100g, respectively. Whereas iron content was recorded highest in a decreasing order as 36.84% in infected roots of variety, Seth followed by 20.00% in Real, 19.04% in Pink elegance, 15.38% in Gold strick and 10.81% in Mammut over the healthy counter parts on dry weight basis. The results of the present investigation suggested that there was decrease in percentage of iron content in the roots of infected susceptible and resistant varieties. More precisely an increase in trend of iron content from susceptible cultivar to resistant one was observed.

4.3.10. b. Effects of nematode infection on Magnesium content.

As depicted in the Table 12b the magnesium content was decreased in root system of infected varieties of Mammut, Pink elegance, Gold strick, Real and Seth by 1.00, 0.50, 0.10, 0.10 and 0.10 mg/100g respectively whereas magnesium was recorded highest in decreasing order of 83.33% in infected roots of Seth followed by 66.66% in Real, 75% in Gold strick, 54.54% in Pink elegance and 28.57% in Mammut over the healthy counter parts on the dry weight basis. The results of present investigation revealed that the infected plants had decreased percentage of magnesium in the roots of susceptible and resistant varieties.

4.3.10. c. Effects of nematode infection on calcium content.

It was revealed from the Table 12 c that calcium content was decreased in root system of infected varieties of Mammut, Pink elegance, Gold strick, Real and Seth by 2.00, 2.40, 1.40, 2.00 and 1.40mg/100g respectively .Moreover calcium content percentage decreased by 13.04% in Mammut, 17.24% in Pink elegance, 21.42% in Gold strick 23.07% in Real and 30% in Seth over healthy counter parts. The results of present investigations revealed that the infected plants had decreased percentage of calcium content in the roots of susceptible and resistant varieties.

Table 12a Estimation of iron contents in the resistant/susceptible gerbera varieties influenced by root knot nematode *M. incognita*,

Sl. No	Varieties	Iron content (mg / 100 g) dry weight basis			
		Root			
		Infected(I)	Healthy(H)	Mean	Increase/ decrease over healthy (%)
1	Mammut (R)	0.33	0.45	0.39	-10.81
2	Pink elegance(MR)	0.17	0.21	0.19	-19.04
3	Goldstrick(MR)	0.11	0.13	0.12	-15.38
4	Real(MR)	0.16	0.20	0.18	-20.00
5	Seth (S)	0.12	0.19	0.16	-36.84
	SE(m)±	0.011	0.013		
	CD(P=0.05)	0.033	0.040		

Table 12b Estimation of magnesium contents in the resistant/susceptible gerbera varieties influenced by root knot nematode, *M. incognita*

Sl. No.	Varieties	Magnesium content (mg/100g) dry weight basis			
		Root			
		Infected (I)	Healthy (H)	Mean	Increase/ decrease over healthy (%)
1	Mammut (R)	1.00	1.40	1.20	-28.57
2	Pink elegance(MR)	0.50	1.10	0.80	-54.54
3	Goldstrick(MR)	0.10	0.40	0.25	-75.00
4	Real(MR)	0.10	0.30	0.20	-66.66
5	Seth (S)	0.10	0.60	0.35	-83.33
	SE(m)±	0.090	0.120		
	CD(P=0.05)	0.280	0.370		

Table 12c Estimation of calcium contents in the resistant/susceptible gerbera varieties influenced by root knot nematode, *M. incognita*

Sl. No.	Varieties	Calcium content(mg / 100g) dry weight basis			
		Root			
		Infected (I)	Healthy (H)	Mean value	Increase/ decrease over healthy (%)
1	Mammut (R)	2.00	2.30	2.15	-13.04
2	Pink elegance(MR)	2.40	2.90	2.65	-17.24
3	Goldstrick(MR)	1.40	1.70	1.55	-21.42
4	Real(MR)	2.00	2.60	2.30	-23.07
5	Seth (S)	1.40	2.00	1.70	-30.00
	SE(m)±	0.14	0.11		
	CD(P=0.05)	0.44	0.34		

4.3.10d Effects of nematode infection on sulphur content.

It was revealed from the Table 12 d that sulphur content was decreased in root system of infected varieties of Mammut, Pink elegance, Gold strick, Real and Seth by 0.09, 0.07, 0.06, 0.05 and 0.11 mg/100g respectively whereas sulphur content percentage increased by 50.00% in Mammut, 40.00% in Pink elegance, 50.00% in Gold strick 66.66% in Real and 22.22% in Seth over healthy counter parts. The results of present investigations revealed that the infected plants had increased percentage of sulphur content in the roots of infected susceptible and resistant varieties.

4.3.10e Effects of nematode infection on manganese content.

It was revealed from the Table 12 e that manganese content was decreased in root system of infected varieties of Mammut, Pink elegance, Gold strick, Real and Seth by 0.02, 0.06, 0.05, 0.09 and 0.08 mg/100g respectively whereas magnesium content percentage increased by 33.33% in Mammut, 25.00% in Pink elegance, 40.00% in Gold strick, 10.00% in Real and 33.33% in Seth over healthy counter parts. The results of present investigations revealed that the infected plants had decreased percentage of manganese content in the roots of infected susceptible and resistant varieties.

Table 12d Estimation of sulphur contents in the resistant/susceptible gerbera varieties influenced by root knot nematode, *M. incognita*

Sl. No.	Varieties	Sulphur content(mg/100g) on dry weight basis			
		Root			
		Infected(I)	Healthy (H)	Mean	% Increase(+) or decrease(-) over control
1	Mammut	0.09	0.06	0.08	+50.00
2	Pink elegance	0.07	0.05	0.06	+40.00
3	Goldstrick	0.06	0.04	0.05	+50.00
4	Real	0.05	0.03	0.04	+66.66
5	Seth	0.11	0.09	0.10	+22.22
	SE(m)±	0.013	0.010		
	CD(P=0.05)	0.040	0.030		

Table 12e Estimation of manganese contents in the resistant/susceptible gerbera varieties influenced by root knot nematode, *M. incognita*

Sl. No	Varieties	Manganese content (mg/100g) on dry weight basis			
		Root			
		Infected(I)	Healthy(H)	Mean	% increase(+) or decrease(-) over control
1	Mammut	0.02	0.03	0.03	-33.33
2	Pink elegance	0.06	0.08	0.07	-25.00
3	Goldstrick	0.05	0.07	0.06	-40.00
4	Real	0.09	0.10	0.10	-10.00
5	Seth	0.08	0.12	0.10	-33.33
	SE(m)±	0.016	0.013		
	CD(P=0.05)	0.049	0.040		



DISCUSSION

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. Manju *et al.* (2015) reported that root knot nematode (*Meloidogyne incognita* (Kofoid and White) Chitwood) is the major pest of gerbera which causes a yield loss upto 31.1%. 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 gerbera 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 gerbera varieties

For screening purpose seven gerbera germplasms were obtained from the local market and Biotechnology cum Tissue Culture Laboratory, OUAT, Bhubaneswar and were tested in green house condition. Out of the seven gerbera varieties, one was found to be resistant, five were moderately resistant and one was susceptible to the nematode. (Table1).

To substantiate, about the resistance occurring in the plants, biochemical analysis of three moderately resistant, one resistant and one susceptible variety i.e. Pink elegance, Gold strick, Real, Mammut and Seth was done.

5.2 Effects of nematode infection on plant growth parameters

Shoot length and root length (cm)

As a result of the root-knot nematode invasion in the host plant (gerbera) cultivars, the maximum average shoot length was 26.90cm in Pink elegance and root length was 29.30cm in Mammut. The decrease in shoot length and root length was more distinct i.e. 16.10 cm and 10.80 cm respectively in Seth. Improper and altered uptake and transportation of minerals, nutrients and water might be the possible reason of the decline due to nematode invasion.

Shoot weight and root weight (g)

The decrease in fresh shoot weight (19.90g) and dry shoot weight (1.60g) of the gerbera varieties, Mammut and Seth was significantly different from other varieties showing resistant and susceptible reaction to the test nematode. Inadequate supply of nutrients to plant infected by the nematode may be attributed as the possible reason of decline in shoot weight and root weight. However the nematode infection has been espriated to some extent in the resistant variety.

5.3 Chemical analysis of plant samples

5.3.1 Effect of the nematode infection on chlorophyll contents

During the course of investigation the degradation in chlorophyll content of a, b and total of gerbera varieties was observed. (Table3). It was evident from the table that maximum reduction of chlorophyll “a” occurred in variety Mammut (27.50%) and it was minimum in Real (27.36%). The reductions are non significant at 5%CD value when comparing among the infected varieties.

Likewise in chlorophyll “b” maximum reduction was in Pink elegance (38.43%) and minimum was in Gold strick (14.70%) while comparing among the infected varieties the reduction was found to be insignificant at 5% CD value.

The total chlorophyll content was decreased maximum by 32.10% in Gold strick over healthy counterpart and minimum reduction was observed in Pink elegance *i.e.* 27.38% and the reduction of total chlorophyll were at par among the varieties of gerbera (Table 3).

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

As a result of the infection by *M. incognita*, the total Nitrogen content declined in shoot system gerbera varieties to the extent of 33.33 percent in susceptible variety, Seth to 14.15 percent in moderately resistant variety, Real over their healthy counter parts (Table 4.). 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 N₂-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 gerbera 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 10.81 -36.84% (Table 10.a), magnesium by 28.57- 83.33% (Table 10.b), Calcium by 13.04-30.00% (Table 10.c), manganese by 10.00- 40.00% (Table 10.e) except in sulphur it increased by 22.22-66.66% (Table 10.d) in root knot infected plants gerbera varieties over their healthy counterparts.



CHAPTER-5

SUMMARY & CONCLUSION

SUMMARY AND CONCLUSION

Root knot nematode infection generally, is characterized by yellowing, stunting and wilting of the above ground plant parts along with loss in yield. But the diagnostic symptoms occurring as a result of root-knot nematode, *Meloidogyne incognita* is in the below ground plant parts i.e. the formation of root galls or knots. Nematode infection is associated with various alterations in the biochemistry and physiology of the host plant. The alterations in the biochemical reactions in the host plant may be attributed towards imparting of resistance against the root-knot attack. Resistant varieties are an alternative way to control these nematodes apart from the chemical nematicides which are way too costly as well as pose a threat to human health and environment. So, the safest way of controlling the nematodes is by using resistant varieties along with other cultural practices to control nematodes as compared to the conventional breeding methods which are time consuming and tedious. So this has made it quintessential to understand the basis of resistance and resistance breeding. Resistance breeding using Biotechnology and Genetic Engineering paves a way in developing resistant varieties easily.

Various parameters were taken into consideration for this study. As a result of nematode infection shoot growth parameters like shoot length, fresh shoot weight and dry shoot weight of different gerbera varieties were significantly reduced. In the gerbera varieties maximum average shoot length was recorded as 26.90 cm in Pink elegance and minimum average shoot length was recorded as 16.10cm in Seth. A comparative analysis of shoot length of infected and healthy varieties of gerbera showed that there was significant reduction in all varieties i.e. Mammut, Pink elegance, Gold strick, Real, Rosario, Snowball, and Seth. Similarly the root growth parameters like root length, fresh root weight and dry root weight of the different varieties of gerbera undertaken for study were found to be significant. Among all the varieties of gerbera, Seth (10.80cm) showed significant decrease in root length. Nematode population, root gall index and varietal responses basing on number of galls showed a significant relationship while maximum was seen in variety Seth with 67.66 average numbers of galls per root and minimum was seen in variety Mammut with 8.66 numbers of galls per root.

It was evident that due to host pathogen interaction various metabolic changes are established in the host even if the interaction may be a compatible or non-compatible one between the host and pathogen. These changes are attributed to the defense mechanism triggered as a result of the nematode attack. So this study was carried out to analyze the changes occurring in biochemical components of infected and healthy host plants. Due to the extensive relationship between host and root-knot nematode specialized nurse cells are developed which aid in the growth and development of the nematode. To better understand the biochemical alterations out of the seven varieties of gerbera, one resistant variety Mammut, three moderately resistant varieties Pink elegance, Goldstrick, Real and one susceptible variety Seth were selected for investigation. The chlorophyll “a” content in healthy gerbera varieties ranged from 0.360 to 0.804 mg/g and 0.261 to 0.583 mg/g in infected gerbera varieties as per the data from Table 3. Similarly it was observed that chlorophyll “b” content varied from 0.118 to 0.436 in healthy gerbera varieties and 0.086 to 0.316 in infected gerbera varieties as evident from Table 3. The total chlorophyll content ranged from 0.521 to 0.868 mg/g in healthy gerbera varieties and 0.37 to 0.62 mg/g in infected gerbera varieties. Maximum decrease in chlorophyll “a”, chlorophyll “b” and total chlorophyll was seen as 27.50% in Mammut, 38.43% in Pink elegance and 32.10% in Goldstrick respectively.

As per the data presented in Table 4, the range of nitrogen content in shoots was observed to be 0.15 to 1.72 % of healthy gerbera varieties and 0.10 to 1.22% in inoculated gerbera plants. Lower percentage of nitrogen was seen in the shoots of infected plants as compared to their healthy counterparts with highest decrease of 33.33% in the variety Seth. From the Table 5, it was found that crude protein in shoots ranged from 0.62 to 7.62% in infected plants and 0.93 to 10.75% in their healthy counterparts. Phosphorus content was highest as 0.48 % in shoots of healthy Mammut variety and the same was reduced to 0.20% in its infected plant as a result of nematode infection. The data in Table 6, suggested that the phosphorus content reduced in the shoots and roots in the range of 51.42 to 76.92% and 43.75 to 55.00% respectively. Likewise the potassium content increased in the range of 11.20 to 52.94% and 18.11 to 61.90% in the shoots and roots respectively.

All the nematode inoculated gerbera plants showed increase in the sugar content as compared to their healthy counterparts in the range of 10.31 to 80.00%. The total

sugar content ranged from 0.28 to 0.38 mg/g in infected gerbera plants and 0.25 to 0.29 mg/g in healthy gerbera plants as per the data of Table 8. Similarly, the total starch content decreased in the infected gerbera plants as compared to their healthy counterparts in the range of 5.88 to 28.57%. The amount of total starch content was 0.42 to 0.68 mg/g in infected gerbera plants and 0.54 to 0.75 mg /g in healthy gerbera plants.

During the course of investigation it was observed that the protein content of roots increased as compared to the shoots. Excessive hypertrophy and hyperplasia leads to increase in protein content is subsequently due to enzymatic degradation of plant proteins and reduced photosynthesis as a consequence of nematode infection. The data in Table 10. depicted that total phenol content was observed as maximum 61.18% in shoots of variety Mammut and 61.19% in roots of variety Pink elegance. The shoots and roots infected plants showed an increase in phenol content as compared to the healthy plants. This occurs because of interference of nematodes with phenol metabolism leading to decline in phenol content. Similarly from Table 11. it is evident that Proline content was found to be maximum as 61.23% in shoots and 61.07% in roots of variety Mammut.

Owing to nematode infection there is profound alteration in nutrient concentrations in the infected plants. Variation in various nutritional elements was seen as a result of nematode infection in the subsequent course of the study. The elements like Fe, Ca, Mg, S and Mn were taken into consideration for the study. Iron decreased by 10.81 -36.84% (Table 12a.), magnesium by 28.57- 83.33% (Table 12b.), calcium by 13.04-30.00% (Table 12c.) and manganese by 10.00-40.00% (Table 12.e) except sulphur which increased by 22.22-66.66% (Table 12.d), in root knot infected plants gerbera varieties over their healthy counterparts.

The above data indicated that nutritional changes occurred in the host plant due to the nematode infection and probably this was one of the first effects of nematode infection. These may lead to chlorosis and premature defoliation and these effects increase in duration of infection. All the infected genotypes showed reduction in chlorophyll. This decreases the photosynthetic rates of the host. The changes in both concentration and total contents of different elements in host plants to establish a relationship between plant nutrient status and physiological processes like photosynthesis which appears as a reason of yield losses. The total content of various elements decreased over time. Nitrogen uptake decreased and total sugar content in the roots increased. The

change in potassium concentration seemed to be important because of its effect on photosynthesis by altering carbon dioxide uptake or by altering osmotic potential. Lower K concentration in shoots of nematode infected plants attributed to the increase in root galling, giant cell size and metabolic activity. Improper translocation of various elements occur due to deformation of vascular tissue. Considering the role of Fe, Mg and Mn in the synthesis of chlorophyll, the concentration of these nutrient elements have a profound effect on host physiology. Sulphur along with calcium and magnesium are required for normal and healthy growth of the plant. So any variation in their contents impede the host physiology

Thus it can be wrapped up that the plants which endure root-knot nematode infection, subsequently develop physiological and biochemical transformations which may be understood as a probable strategy of the plant to shun or tolerate the adverse effects caused by the nematode infection.



CHAPTER



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