

BIOCHEMICAL VARIATIONS IN RESISTANT AND SUSCEPTIBLE BRINJAL VARIETIES INFECTED BY ROOT KNOT NEMATODE

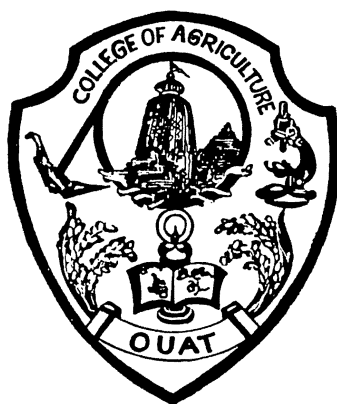
Meloidogyne incognita

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SUBMITTED TO
THE ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY, BHUBANESWAR
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
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**MASTER OF SCIENCE IN AGRICULTURE
(NEMATOTOLOGY)**

BY

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BHUBANESWAR
1992**

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C E R T I F I C A T E

This is to certify that the thesis entitled, "Biochemical variations in resistant and susceptible brinjal varieties infected by root-knot nematode, Meloidogyne incognita" submitted for the degree of Master of Science in Agriculture (Nematology) of the Orissa Univeristy of Agriculture & Technology, Bhubaneswar is a faithful record of Bonafide and original research work carried out by **Sri Suresh Chandra Swain** under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigation have been fully acknowledged.


22.6.93
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C E R T I F I C A T E - I I

This is to certify that the thesis entitled, "Biochemical variations in resistant and susceptible brinjal varieties infected by root-knot nematode, Meloidogyne incognita" submitted by Sri Suresh Chandra Swain to the Orissa University of Agriculture & Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of Master of Science in Agriculture (Nematology) has been approved by the student's advisory Committee after an oral examination on the same in collaboration with an External Examiner.

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ABSTRACT

Investigation on biochemical changes were carried out in two bringal varieties viz. pusa purple long (susceptible) and Ghatikia White (resistant) inoculated with root-knot nematode, Meloidogyne incognita. Among various amino acids detected, five amino acids like L-cystine, L-serine, L-tryptophan, L-leucine and L-isoleucine were found to be common in both the varieties. Higher concentration of various amino acids and amides were detected in each variety upon nematode inoculation except L-tryptophan, by paper chromatographic and TLC scanning technique. The content of chlorogenic acid, total sugar, peroxidase and catalase activities were higher in inoculated samples than their healthy counter parts. However, the catalase activity was reduced in inoculated susceptible sample.

CHAPTER I

INTRODUCTION

I N T R O D U C T I O N

plant parasitic nematodes are recognized in India and other countries as potentially serious constraints to crop productivity. It is because, at present due to intensification of world agriculture, particularly in developing countries to feed the over exploded millions with small holdings, changing pattern of agroecological conditions, and monoculture of major food crops not only increases their population in an increasing rate but also brings on devastation in several fields like cereals, millets, pulses, vegetables, fruit crops and ornamentals. This leads to productive researches in several areas of plant nematode interactions. In this context, an intimate knowledge of physiology and biochemistry of plant as well as nematodes are absolutely essential to understand the complicated areas of host-nematode interaction.

The present production and consumption status of vegetables in our country is very much inadequate, being only about one-fourth to one-third of the requirement. Therefore, it is very much essential to boost up the vegetable production to meet the quality as well as quantity of our daily diet and among vegetables, brinjal is one of the most consumed and major vegetable

grown throughout the country irrespective of rural or urban areas. And it is severely affected by the most notorious root knot nematode, Meloidogyne incognita (Kofoid & White, 1919) Chitwood, 1949, due to its ever growing population and wide spread distribution. Besides, this nematode also plays a vital role in with complexes.

Over the last few decades, the use of chemicals as one of the important components of nematode management received utmost attention of researchers. However, unfavourable cost-benefit ratio, nonavailability of conventional nematotoxicants, growing awareness of world community against pollution and possible health hazards due to application of nematicides made nematologists some what compulsive to look for other alternative to chemical control. Keeping in view the limitations and problems of chemical control in back ground, the use of resistant variety is considered as the most important management strategy over others.

Traditional approaches to the development of nematode-resistant cultivars are time consuming and often limited by intra-specific barriers. Biotechnology and genetic engineering provides potential for efficient development of nematode resistant plants, which may be

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enhanced by elucidation of mechanism limiting nematode development.

Resistance responses of host plant during post infection period have often been regulated by several internal factors. In diseased condition, the sequences of biochemical changes is rather more important than the external symptoms, which are nothing but the manifestation of internal disorders. So it is very much essential to have an adequate knowledge about the biochemistry and physiology of the host as well as the parasite in order to understand the actual mechanism of resistance.

Considering the importance of the subject, the present investigation was under taken to gather some information on 'mechanism of resistance' and 'biochemistry and physiology of pathogenesis', relating to various biochemical parameters like free aminoacids and amides, total sugar content, phenolic compounds, enzyme peroxidase and catalase activities in brinjal roots infected by root knot nematode.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

In recent years, many scientists have shown their deep appreciation and interest on biochemical and physiological basis of resistance relating to plant-nematode interaction. However, some of the important literatures available concerning free aminoacids and amides, phenolic compounds, total sugar content, enzyme peroxidase and catalase activities, their requirements, biosynthesis, metabolism and antimetabolism, which are indispensable to highlight any possible relevance to the present study are given below.

A. FREE AMINO ACIDS

Krusberg (1961) had determined the free amino acid content in Ditylenchus dipsaci and Aphelenchoides ritzemabosi. The extracts of D. dipsaci were found to contain serine, glycine, threonine, alanine, tyrosine, valine, histidine, arginine, leucine and isoleucine and that of A. ritzemabosi contained serine, threonine, glycine, alanine, arginine, valine, lysine and methionine.

Myers (1963) while working on the discharged materials of Ditylenchus dipsaci and Meloidogyne incognita,

observed the presence of 15 to 20 amino acids in aseptic incubates of D. triformis and D. myceliophagus.

Srivastava (1969) reported eight free amino acids in gravid females of Meloidogyne javanica which were alanine, arginine, cystine, lysine, glutamic acid, tyrosine, methionine and phenylalanine.

Saxena (1972) reported the free amino acids of mature Meloidogyne javanica females and of the infected and uninfected roots of jute (Corchorus capsularis L.). He found 14, 13 and 9 amino acids in the nematode, root-knots and the healthy roots of jute respectively by 2-dimensional paper chromatography.

Rebois and Johnson (1973) studied the effect of Rotylenchulus reniformis on the amino acid content of seeds of Glycine max. He reported that the amount of amino acid particularly leucine in the seeds from infected plants is lower as compared to those from uninfected plants.

In 1973, Lewis found that with resistant and susceptible varieties of cotton (Gossypium hirsutum) nematised with M. incognita, in the susceptible cultivar had a greater percentage increase of the free amino acids.

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Midha and Swarup (1974) showed the presence of amino acids and sugar in wheat seed galls caused by Anguina tritici.

Giebel and Stobieka (1974) reported that in the roots of potato infected by Heterodera rostochiensis, the ratio of proline to hydroxyproline increased significantly in susceptible varieties whereas decreased in resistant varieties. Possibly this decreased ratio is the cause of resistance.

Masood and Husain (1975) found out the role of amino acids and proteins in the resistance and susceptibility of tomato varieties to the infection of Meloidogyne javanica. There is increase in the concentration of the amino acids with the increase of infection in resistant and moderately resistant varieties but decrease in highly susceptible ones. It was also observed that the protein content had an inverse relationship with the amino acids.

Lewis and McClure (1975) worked with the susceptible and resistant cultivars of cotton to M. incognita and found that the sum total of free amino acids was greater in resistant cultivar. The concentration

of glycine declined markedly over a 10 days period following inoculation, proline increased to a great extent and tyrosine and phenyl-alanine varied as function of infection, cultivar and time of harvest.

In 1975, Hanounik and Osborne reported about the influence of M. incognita on the content of amino acid and nicotine in tobacco grown under gnotobiotic conditions that the amount of 16 amino acids (including nicotine precursors) and nicotine were significantly greater in infected roots of both resistant and susceptible cultivars.

Wright (1975) reported the presence of significant amount of urea and amino acids along with ammonia as major compounds with the elimination of Panagrellus redivivus. The elimination of amino acids increased while that of ammonia decreased with the increase of stress condition.

Bird and McClure (1976), in their chemical analysis of the hydrolysis products of the egg shells of Meloidogyne javanica, Rotylenchulus reniformis, Pratylenchus minyus and Tylenchulus semipenetrans have revealed a high proline content (up to 25 %), which is apparently a characteristic of nematode egg shells examined so far.

Mohanty and Das (1976) reported L-proline in the nematode infected ragi roots in addition to the eight amino acids present in uninfected root extracts.

In 1980, Ahmed and Naqvi studied the biochemical changes in papaya plant infected with papaya mosaic virus (PMV) and Meloidogyne incognita and found an increase in total free phenols of leaves and roots and free amino acids and soluble proteins of roots. Free amino acids and soluble proteins of leaves decreased compared to control.

Nasr et al. (1980) analysed the effect of root-knot nematodes on the amino acid concentration of almond and peach root stalks and reported that the combined amino acid content of roots and leaves of bitter almond and Nemaguard peach plants were the same in control and infested plants. The free amino acid threonine was absent in M.incognita infested almond leaves and proline was present only in M.incognita infested roots and leaves of almond.

Osman et al. (1981) found that flowering inoculation of Citrullus vulgaris var. Chilian black with M.incognita, Fusarium oxysporum, F.niveum or combination of both, free amino acids increased in root

extracts. When aldicarb or oxamyl was applied to each group, results showed a great variation in the levels of the detected amino acids among different treatments.

Okie et al. (1984) studied the effect of the ring nematode, Criconebella xenoplax on physiology of peach root stocks and reported the reduced free amino acids of shoots and roots as measured by levels of ninhydrin - reactive compounds. They also found that the proportions of specific amino acids were changed and the molar percentage of proline, glycine and alanine increased whereas arginine decreased in roots of both seedlings and herbaceous cuttings in the presence of nematode.

Pradhan and Das (1984) studied the qualitative detection of free amino acids in the homogenate and extractions of a surface sterilised migratory endo-parasitic nematode species, Pratylenchus coffeae by paper chromatographic technique and indicated the presence of the following amino acids :

L-serine, DL-threonine, glycine, L-alanine, L-arginine, L-(1) cysteine and L-proline in the nematode homogenate. Nematode excretions contained L-serine, lysine, L-arginine, L-alanine, L-proline, L-methionine

and L-leucine. Thus, four amino acids were common to both the nematode homogenate and excretion.

In 1985, Freire and Bridge studied the biochemical changes induced by Meloidogyne incognita in the roots and xylem sap of black pepper. They showed by gas liquid chromatography that there is 29% decrease in serine, 20% decrease in glutamic acid and 10% increase in aspartic acid in the 8 month old roots of Piper nigrum seedlings inoculated with 10,000 M.incognita second stage larvae compared to uninoculated plants. However, in the xylem sap of infected plants, alanine, glycine, aspartic acid, glutamic acid increased by 219%, 207%, 80% and 37% respectively. Lysine was detected only in the roots and tyrosine only in xylem of infected plants.

Varma and Yadav (1985) reported about field evaluation of wheat cultivars against ear-cockle infection in VL 421, HW 517 and Narmada-4 with light, moderate and high gall formation respectively and found amino acid constituents of grain flour. In VL 421, out of 16 amino acids detected, alanine, leucine, phenylalanine, serine were negatively correlated but arginine, aspartic acid, glycine, half cystine, histidine, isoleucine, leucine, serine and valine were negatively correlated

while arginine, aspartic acid, glycine, half cystine, histidine, lysine, methionine, phenylalanine, proline, threonine and tyrosine were positively correlated.

Qualitative determination of free amino acids in blackgram roots infected with root-knot nematode, M. incognita, under pot culture conditions, has been demonstrated by Sahu and Mohanty in 1987 by paper chromatographic technique. The alcoholic root extracts of healthy blackgram contained free amino acids such as L-aspartic acid, L-glutamic acid, L-asparagine, L-serine, glycine, L-threonine, L-tryptophan, L-phenylalanine and isoleucine whereas in infected blackgram root extracts, L-glutamic acid was absent but four more amino acids namely, L-cystine, L-lysine, L-proline & L-leucine were present. There was no apparent difference between 15 and 30 days samples with regard to number of amino acids present except for their higher concentration in 30 days samples particularly in diseased tissues.

Quantitative estimation of free amino acids in the above ground parts of clover plants infected by stem nematode Ditylenchus dipsaci was studied by Pokharel in 1989 and concluded that two amino acids tryptophan and cystine were destroyed by hydrolysis and total concentration of free amino acids increased by 22% in infected plants. However, the concentrations of bound

amino acids in both the infected as well as the healthy plant did not differ.

Qualitative and quantitative estimation of free amino acids and amides in greengram roots infected with root-knot nematode, M. incognita under pot culture conditions, has been demonstrated by Mohanty and Pradhan in 1990 by paper chromatographic technique. The alcoholic root extracts of healthy greengram contained nine free amino acids and one amide such as L-cystine, L-arginine, L-histidine, L-lysine, L-serine, L-tyrosine, L-alanine, L-tryptophan, L-isoleucine and L-aspartic acid in both the cultivars of 15 and 30 days old samples. But in infected greengram roots L-alanine was absent but five more amino acids namely, L-glutamine, L-threonine, L-proline, L-valine and L-leucine were present except 30 days old sample of Pusa Baisakhi (susceptible) where L-arginine was absent.

Marlies et al. (1991) determined the changes in the concentrations of 19 amino acids in the syncytia (nurse cell systems) on the development of the cyst nematode Heterodera schachtii in the seedling roots of Brassica rapa var. silverstris f. compestris (stielmus) by using sensitive micromethods. They suggested that glutamine

appeared to play a beneficial role in the "+" variants whereas methionine, phenylalanine, lysine and tryptophan played harmful roles in the "-" variants. Changes in the concentrations of the 14 other amino acids had no influence on nematode development.

Sundararaj and Mehta (1991) reported that the changes in enzymes and free amino acids of sugarcane roots infected by lesion nematode, pratylenchus Zeae, were investigated. It was found that poly phenol oxidase, peroxidase, ascorbic acid oxidase, glucose-6-phosphate dehydrogenase, cellulase, protease and acid phosphatase increased in the P. Zeae inoculated roots as compared to the control. However glutamate dehydrogenase and malate dehydrogenase remained unchanged while ribonuclease decreased in the inoculated roots. In the control plant roots, seven amino acids were identified while the inoculated roots contained nine amino acids.

B. BOUND AMINO ACIDS.

In 1978, Wang and Bergeson identified 6 carbohydrates and 15 amino acids in water in which larvae of Meloidogyne incognita were incubated. The suggestion is made that amino acids and carbohydrates contribute part of the exogenous nutrients for the microflora in the rhizosphere of roots infested with Meloidogyne.

Okopnyi (1980), in his experiment on metabolism of nitrogen and amino acids in the galled tissues of cucumber, melon and tomato observed that 65 days after infestation only the soluble fraction of the protein nitrogen were higher in galls than in control tissue. In comparison with healthy tissue, galls contained more of alanine, leucine, lysine and valine and less of serine, histidine, arginine and proline. Tryptophan was reduced in both gall and surrounding tissue suggesting a metabolic path, tryptophan _____ tryptamine _____ indole. The total amino acid content in tomatoes with different degrees of Meloidogyne resistance was in an increasing series _____ resistant /__ tolerant /__ susceptible. Aspartic acid and glutamic acid levels were significantly lower in resistant than in susceptible varieties and amino acids those play an important role in alkaloid synthesis were found as traces in resistant and in very small amounts in tolerant tomato roots.

In 1983, Singh and others experimented on attractiveness of Meloidogyne incognita larvae to roots of tomato and changes in biochemical content of plants as affected by oil cakes and nematicides determined that plants grown from seeds treated with oil cakes had higher amount of total free phenols and amino acids as compared to untreated plants. Plants from seeds treated with

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nematicide showed no difference in amino acid contents.

Suresh and Bagyaraj (1984) studied arbuscular mycorrhizal fungus Glomus fasciculatum and the root-knot nematode, M. incognita and its effect on growth and chemical composition of tomato found that mycorrhizal inoculation reduced the root-knot infestation. Mycorrhizal plants had increased amino acids like phenylalanine and serine.

C. BIOSYNTHESIS AND AMINO ACID REQUIREMENTS.

Rothstein and Tomlinson (1961) experimentally showed the biosynthesis of amino acids in Caenorhabditis briggsae by adding radio active amino acid precursor to the medium and subsequently determining the labelled amino acids by chromatography and autoradiography. The amino acids are alanine, proline, glycine, cystine, serine, arginine, threonine, tyrosine, valine, leucine, isoleucine, histidine, and lysine.

The same scientist in 1962 found that C. briggsae synthesized not only the non-essential amino acids like aspartic acid, glutamic acid, alanine, glycine, serine and arginine but also the essential amino acids like threonine, valine, leucine, isoleucine, lysine and histidine by feeding them with ^{14}C labelled amino acids.

Balasubramaniam and Myers (1971) determined by labelling and deletion method the amino acid requirements of Aphelenchoides sp. The absolute dietary required amino acids were histidine, tyrosine, methionine, phenylalanine, threonine, lysine, leucine, or isoleucine. The limited required were alanine, proline, serine, glycine and valine. Aspartic acid and glutamic acid were found synthesized by the nematode, hence they are not required to be supplemented in the diet.

The same workers in 1973, demonstrated in axenic culture of Aphelenchoides rutgersi that amino acids like isoleucine, leucine, methionine, threonine, phenylalanine, histidine, tryptophan and lysine are essential for the reproduction of this nematode.

Jackson (1973) reported that in addition to 9 amino acids lysine, tryptophan, histidine, leucine, isoleucine, phenylalanine, threonine, methionine and valine those essential for mammals, Neoaplectana glasseri required arginine essentially and tyrosine marginally.

In 1976, Lu et al. from their experiment proved that C. briggsae essentially required methionine at optimal levels of Vit-B₁₂ and folic acid, methionine could be substituted by homocystein, thereby suggesting the presence of a mechanism that biosynthesize methionine from homocystein as substrate.

Vanfleteran and Avau (1977) reported that treatment of C. briggsae with aminopterin in growth promoting media led to thymine deficiency and sterility. The medium during thymine starvation prevented larval growth at every stage.

In 1978, Husain studied the effect of amino acids on the larval hatching of Heterodera mothi in the laboratory. They reported that the highest larval hatching occurred in L-leucine, closely followed by DL-tryptophan, L-cystine, L-lysinehydrochloride, DL-glycine and DL-tyrosine.

Krishnan and Vaitheeswaran (1984) studied the effect of organic amendment on the quantitative changes of amino acid in the infected host plant of Dolichus lablab. They estimated the total free amino acid contents employing paper chromatography method and reported that there was increase in total free amino acid content in inoculated untreated plant as against control plant and uninoculated plants subjected to varying dosages of oil cake from single to triple showed a gradual increase in total free amino acid content as against control plant. It is suggested that the increased amino acid content might be due to host response in synthesizing new amino acids through metabolic pathways and degradation of old

protein. In infected plant subjected to various dosages of oil cakes, from single to triple, the impact of infection was found to be reduced by nutritional stress as seemed through the decreased amount of free amino acids as against the infected plant not subjected to nutritional stress.

D. AMINO ACID ANTIMETABOLITES

In 1967, Prasad and Webster experimented on the antimetabolic effects of some amino acids on Nacobbus, Aphelenchoides and Heterodera. They found that the number of Aphelenchoides and Nacobbus galls decreased significantly by 91% and 89% respectively by DL-amino butyric acid. DL-alanine also significantly reduced the number of Aphelenchoides and Heterodera females over control. The other reported amino acids having antimetabolitic activity were DL-valine and DL-methionine.

Krishnamurthy Rao and Prasad (1969) studied the soil application effects of 11 DL-form and 8 L-form amino acids on Rotylenchulus reniformis infesting tomato seedlings. It was found that DL-forms of tyrosine, serine, valine, threonine, proline and methionine were found effective in controlling the population of nematodes in root and soil whereas their corresponding L-forms increased the population both in root and soil. The

L-forms of serine, lysine and methionine showed lower population than the control but was more than in DL-forms. Above all, treatments receiving DL-forms of amino acids had significantly lower population of nematodes both in soil and roots in comparison with that of L-forms. Application of total amount of amino acid at one dosage was better effective than its split application in two equal dosages.

In 1974, Krishnaprasad and Setty investigated on root-knot nematode, Meloidogyne incognita in tomato the effect of 3 amino acids, DL-threonine, DL-serine and DL-alanine. The last 2 amino acids significantly affected the development and reproduction of nematode but did not have any adverse effect on the growth and vigour of tomato plant.

Reddy et al. (1975) further studied the action of DL-methionine on M. incognita in tomato. The development of the nematode in tomato roots was reduced when larvae were inoculated one week after application of DL-methionine but not when inoculated 14 days or more later. DL-methionine also reduced tryptophan and methionine content of roots. Methyl mercaptan, an intermediate breakdown product of methionine metabolism was detected in infested roots.

The same scientist in 1975, demonstrated on Meloidogyne incognita in tomato the action of DL-methionine and found the amino acid to reduce root galling, egg mass production and also delay the life cycle of the nematode.

Krishnaprasad et al. (1976) reported the effects of amino acids as soil drenches on Meloidogyne incognita. In pot experiments, soil drenches of DL-alanine, DL-serine and DL-threonine applied at concentrations equal to their molecular weights to tomato plants one week after inoculation with M. incognita significantly lowered the reproduction of the nematodes as compared to the controls. The only effect of the amino acids on growth of the plants was a reduction of shoot weight (but not height) with DL-serine.

Krishnaprasad et al. (1977) investigated chemotherapeutic action of L-cysteine against M. incognita. Solutions of L-cysteine at different concentrations were applied either as drench or foliar spray to tomato plants previously inoculated with M. incognita. The amino acid had no significant effect on plant growth but nematode development was significantly reduced by both drench and spray applications at the higher concentrations.

Setty et al. (1977) studied the effect of Dl-Phenyl-alanine on M.incognita and reported that the foliar spray of this amino acid is more effective than soil drenches for inhibiting the development of this nematode on tomato.

Osman and Viglierchio (1981) investigated on M.incognita development on soyabean treated with selected amino acids by alternate methods. Foliar spray of phenylalanine and methionine reduced gall formation, but valine and cysteine were less effective. All amino acids as soil drenches decreased the number of galls. Seed treatment with phenylalanine, methionine, valine or cysteine affected gall formation erratically. Most treatments significantly reduced rhizobium nodulation except phenylalanine which increased the number of nodules.

The same scientists in the same year reported the foliar spray effects of selected amino acids on sunflower infected with M.incognita. Phenylalanine, valine and Cysteine at different concentrations were sprayed on inoculated plants. Plant shoot height and weight were not affected by any treatment nor were there any sign of growth disorders, phenylalanine at 1000ug/ml was most effective in reducing galling and number of egg masses but was less effective at higher concentrations. Cysteine and valine were less effective.

Mahamood (1983) studied on the changes in amino acid content in different cultivars of tomato and egg plant as a result of infection with Rotylenchulus reniformis and reported that the different amino acid contents of different tomato and egg plant cultivars were higher in those inoculated with R. reniformis than in uninoculated plants. The amino acid contents increased with the increased inoculum level of 100 larvae. Amino acid contents were higher in egg plant than in tomatoes in all cultivars irrespective of infection. Egg plant and tomato cultivars in which the concentrations of the amino acids were low, supported the multiplication of the nematode. The results suggested that the amino acid contents affected the resistance, tolerance or susceptibility of the plant to the nematode.

E. AMIDES

Myers and Krusberg (1965) studies the synthesis of amino acids and amides by Ditylenchus triformis and mixed population of Meloidogyne incognita and M. arenaria larvae incubated in solutions containing glucose ^{14}C or acetate ^{14}C by chromatography and radiography. D. triformis synthesized amides like glutamine, asparagine and amino acids like L-alanine, cysteine, glycine, serine and ornithine whereas Meloidogyne sp. synthesized only amino acids without amides and cysteine and ornithine.

Bumbu et al. (1967) reported that potato infected with Ditylenchus destructor caused a decrease in amides and some free amino acids in the plant tissue while those were increased significantly in cucumber infected with M.incognita, particularly glutamine asparagine, alanine, glutamic acid, serine, histidine and arginine.

Sharma and Tiagi (1984) studied five amino acids and one amide such as DL-alanine, DL-aspartic acid, DL-Leucine, L-leucine, L-tryptophan and L-glutamine as foliar sprays for their efficacy in controlling M.incognita infecting pea plants. DL-amino acids reduced the nematode galling whereas L-amino acids (except L-leucine) had no such effect. DL-aspartic acid was most effective against the nematode and on the other hand L-tryptophan favoured gall production.

F. PHENOLIC COMPOUNDS

Dropkin et al. (1969) reported from an experiment that in absence of exogenous plant growth regulatory substances, approximately 73% of larvae that entered roots of susceptible plants showed growth, non-induced necrosis and nearly all induced gall formation. Further in roots of a resistant variety only 4% of the larvae grew, 88% induced necrosis of host cells and only 29%

induced galls. Exogenously supplied cytokinins shifted the response of resistant plants towards the susceptible reaction. Exogenous kinetin at 0.4 and 0.8 μM allowed 55% and 57% of the nematodes to grow, reduced the incidence of nematodes to 32% and 31% and increased gall formation to 73% and 65%. Three additional cytokinins produced effects similar to kinetin. In the experiment the authors measured initiation of larval growth, induction of cell necrosis and gall formation in the host as criteria of resistance or susceptibility.

On experimenting with phenolic content in roots of 21 species of solanaceae, Giebel (1970) determined the quantities of total phenols, mono-and poly-phenols and the ratio of mono-to poly-phenols. Root extracts from good hosts of Heterodera rostochiensis, were characterised by low ratio of mono-to poly-phenols (0.5 - 3.1) and they strongly inhibited the enzymatic destruction of IAA. Root extracts from non-hosts, showed a high ratio of mono-to poly-phenols (3.4 - 9.3) and they did not inhibit or very slightly inhibited IAA destruction.

Hung and Rhode (1973) reported that chlorogenic acid was the major phenolic compound in healthy tomato roots. Nemared (resistant), contained the highest

concentration of the acid and B-5 (susceptible), the lowest. Histochemical test showed that chlorogenic acid was concentrated in the endodermis.

Knypal et al. (1975) reported increased level of total phenols and chlorogenic acid in the galls and side roots of carrot infested with northern root-knot nematode, Meloidogyne hapla as compared to healthy plants. However, the youngest 2 month old plants of carrot cv. Slendero showed higher IAA oxidase activity in response to M.hapla infestation and side roots contained neither more chlorogenic acid nor more IAA oxidase compounds compared to healthy tissues. Galled side roots of older plants showed higher IAA oxidase inhibitory activity and contained more chlorogenic acid. But carrot cv. Perfekcja, in contrast, had a decreased level of phenolics in the secondary xylem, effect not dependent upon age of plants.

In 1979, Sitaramaiah and Pathak studied the effect of phenolics and an aromatic acid on Meloidogyne javanica infecting tomato and reported that cinnamic acid, catechol and salicyclic acid when applied as soil drench, root treatment, pre-inoculation and post-inoculation foliar spray, imparted partial resistance in susceptible tomato plants. The total phenol content in the roots

of susceptible tomato plants sprayed with the chemicals, was, equal to phenolic of a resistant var. 'Nematex'.

Ganguly and Dasgupta (1982) studied the cellular change and phenolic contents in two varieties of tomato susceptible (var. Pusa Rubby) and resistant (var.SL-120) infected with root-knot nematode, Meloidogyne incognita. Necrotic cells were observed along the entire rate of invading larvae in resistant variety. Resistant response were characterised by the failure of nematode to establish its feeding site. The higher initial increase in phenols in SL-120 synchronized with cell necrosis and hypersensitive reaction. Free phenols and bound phenols increased in Pusa Ruby at any stage after inoculation while in case of resistant variety, initially there was increase till 7 days after inoculation followed by a decrease for two subsequent intervals and levelled off at 28 days after inoculation.

Quantitative estimation of phenolic compounds in the okra roots infected by Meloidogyne incognita was studied by Agarwal et al. (1985). They concluded that the infected roots showed increased level of phenolic compounds.

Out of the 28 phenolic compounds tested, Mahajan et al. in the same year reported that greatest nematicidal

activity against Meloidogyne incognita was exhibited by transcinnamic acid followed by pyrogallol, 2-OH naphthoic acid and ethyl gallate. Transcinnamic acid and 2-OH naphthoic acid also markedly suppressed the egg hatch of M. incognita.

Singh et al. in the same year reported that amending the soil with saw dust, urea, cowdung, leafmold, castor, mustard and neem cakes, reduced the development of Meloidogyne incognita on tomato. Total free phenols, O-dihydroxyphenols and amino acids content increased in infected plants grown in amended soil.

Thakar and Yadav (1986) in their experiment with 3 pigeon pea varieties, with variable reactions to the reniform nematode, viz., 556-1 (resistant), Mukta (moderately susceptible) and 3430-1 (susceptible) for estimation of total phenols, reported presence of high total phenol content in resistant variety (0.86 mg/g fresh roots of total phenols as compared to 0.27 mg/g fresh roots in susceptible var. 3430-1). Possibly the increased phenolic level in 556-1 may be the reason for its resistance to reniform nematode.

Zinoveva (1986) studied the host immunity and observed significant differences in the ammonia and poly-phenol content in infected and intact onion and cucumber plant.

Rezk et al. (1987) determined the phenolic contents of barley and wheat infected by root-knot nematode, Meloidogyne incognita race-1. It is observed that the initial phenolic content in healthy roots of barley cv. Bonus was lower than that of cv. cc 163. The concentration of phenolic content in Bonus roots infected with MI-3 was also increased while decreased in case of MI-1 infection conversely, the phenolic content of CC 163 roots infected with MI-1 was increased while decreased with MI-3 infection. Amounts of total phenol in healthy roots of wheat cv. Stork were less than those of cv. Giza 155. On the other hand, the total phenols were decreased in roots of cv. Giza 155 infected with Meloidogyne javanica while increased in Stork infected roots. However, amounts of phenols in Stork (resistant) infected roots were higher than those of Giza 155 (susceptible) healthy and infected roots.

In the same year, Singh et al. reported that oil cakes of castor, mustard, neem and nematicides, i.e., Furadon 3G and Temik 10G when applied in nursery beds of apricot, lemon, pear, peach and walnut reduced the population of phytonematodes. Neem cake exhibited highest efficacy. Roots of seedlings raised in soil treated with oil cakes had higher amount of total free phenols, O-dihydroxyphenols and amino acids compared to untreated and those treated with nematicides.

Patel and Patel (1990) studied the biochemical changes brought out by the infection of stunt nematode, Tylenchorhynchus vulgaris and reported that transplanted Crop of bidi tobacco cv. Anand 119 and Gujurat tobaccos increased peroxidase activity compared to check. Similar trend was also observed for total phenol content in transplanted crop of both the varieties while it reduced polyphenol oxidase activity in seedling as well as transplanted crop of both the varieties.

Sharma and others (1990) reported higher proline and phenol content in resistant brinjal variety PHR-51 as compared from susceptible pusa purple long infected by root-knot nematode Meloidogyne incognita.

G. SUGAR

Gill and Uppal (1977) estimated the phenolic and sugar contents of Zinnia elegans leaves infested with Aphelenchoides ritzemabosi and found that higher concentrations of total phenols, orthodihydroxy phenols and total sugars and reducing sugars were present in the diseased leaves in comparison to healthy ones.

Farooqi et al. (1980) studied the effect of Meloidogyne incognita in tomato plants and suggested

that both reducing and total sugar was increased in nematode infected roots.

Tayal and Agarwal (1982) studied the effect of Meloidogyne incognita on some biochemical parameters in brinjal plants and indicated low contents of starch, non-reducing sugar, total sugar and high level of reducing sugar, soluble protein and total free amino acids in infected plants.

Ganguly and Dasgupta (1983) investigated some chemical changes in brinjal plants inoculated by Meloidogyne incognita and suggested that nitrogen, phosphorus, reducing and total sugar, crude protein were increased in nematode infected plants.

Agarwal and his associates (1985) while analysing the okra plants infected by root-knot nematode, Meloidogyne incognita reported the decrease level of non-reducing and total sugars with increase level of reducing sugar in infected plant tissue.

Meiakerberhan et al. (1990) studied the effect of Meloidogyne incognita on the concentrations of reducing and non-reducing sugars at the nematode feeding sites of french colombard (susceptible) and thompson seedless (moderately resistant) cultivars of Vitis vinifera.

They reported that nematodes did not affect the concentration of reducing sugar but the concentration of non-reducing sugars increased in french colombard and decreased in thompson seedless.

H. ENZYME PEROXIDASE AND CATALASE

Okopnyi et al. (1977) studied the effect of root knot nematode in resistant and susceptible roots of tomato and reported that higher activities of nucleic acids, enzyme peroxidase, catalase and poly phenol oxidase in the infected lines.

Chang (1979) worked with resistant and susceptible cultivars of tomato to Meloidogyne incognita and found that infected resistant lines had higher peroxidase activity than infected susceptible one and it is suggested that peroxidase was related to resistance reaction.

While working on resistant and susceptible cultivars of tomato, Hasseb & others (1979) reported lower catalase activity from infected plants than those of their respective controls.

Ganguly and Dasgupta (1980) found that with resistant and susceptible cultivars of tomato nematised with Meloidogyne incognita showed higher percentage of

enzyme peroxidase and oxidase activities over their respective controls.

Ahuja and Ahuja (1980) showed the presence of lower peroxidase level and higher poly phenol oxidase in brinjal roots caused by root-knot nematode.

In 1981, Hasseb and others studied the biochemical changes in the roots of Solanum melongena infected by root-knot nematode and reported higher peroxidase and saponin activities in inoculated plants.

Arrigoni et al. (1981) studied the peroxidase and super oxide dimutase activities in pea roots infected by Heterodera goettingiana and reported increased peroxidase activity in resistant plants and there was either no change or decrease in susceptible one.

In 1982, Zacheo and others experimented on peroxidase activity and reported that it increased in Meloidogyne incognita infected tomato plant while that of decreased in susceptible plants.

Khan and Hasseb (1984) reported lower catalase and oxidase activities in root-knot nematode infected okra roots while higher peroxidase activity was observed from inoculated one.

Bajaj et al. (1985) reported higher peroxidase activity in tomato roots infected by Meloidogyne incognita.

Agrawal and others (1985) reported higher peroxidase activity in root-knot nematode inoculated okra roots over their respective controls.

Mohanty et al. (1986) investigated on peroxidase activity in resistant and susceptible cow pea cultivars inoculated with root-knot nematode and reported higher peroxidase activity in infected plants as compared to their respective controls.

While analysing the peroxidase activity on soyabean var clarke - 63, infected by root-knot nematode Simite and Dasgupta (1987) found the elevated levels of peroxidase activity and it is suggested that the increased level of peroxidase activity was due to de novo synthesis of peroxidase iso enzymes.

Zacheo et al. (1988) reported higher peroxidase activity in resistant tomato cultivar VFN 8 as compared from susceptible cultivar Roma VF infected by Meloidogyne incognita.

The same scientists in 1988 reported also peroxidase activity increased in root-knot nematode infected tomato roots at 27°C but not in infected roots grown at 34°C.

In 1988, Shukla and Chakraborty reported that root-knot nematode infected resistant var of tobacco and tomato had higher peroxidase activities than their respective susceptible plants.

While working with two coffee cultivars, Mazzafera and others (1989) reported higher peroxidase activity from both the cultivars infected by Meloidogyne incognita.

Zacheo et al. (1990) while working with resistant and susceptible var of Pisum sativum nematised with Heterodera goettingiana, reported that resistant tissue was associated with increased peroxidase activity.

Mote and others (1990) investigated on sequential development of deaminases in resistant and susceptible tomato var. infested with root-knot nematode and reported the content of chlorogenic acid (CGA) and activities of Phenylalanine lyase (PAL) and tyrosine ammonia lyase (TAL) were higher in inoculated root and shoot samples and the values for resistant variety were considerably higher than for the corresponding samples from the susceptible variety.

While analysing the extracts of Meloidogyne incognita and M. javanica propagated on tobacco plant Ibrahim (1991) reported that only adult females of root-knot nematode showed higher peroxidase activity, where as males, eggs did not show the same under the same conditions of the test.

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

A. COLLECTION OF NEMATODES

The experimental stock cultures of the root-knot nematode, Meloidogyne incognita for this study were originally obtained from a single egg mass progeny, maintained and multiplied on brinjal (Solanum melongena L. cultivar pusa purple long). The brinjal plants were grown in 35 cm pots containing sterilized soil, autoclaved at 15 lbs pressure for 20 minutes. The populations of root-knot nematodes were subcultured periodically at 50-60 days intervals either by transplanting fresh brinjal seedlings in infested soil or by inoculating new transplants with infective second stage larval suspension of root-knot nematode, M. incognita after removal of old plants to ensure a constant supply of sufficient egg masses for infective larvae.

B. EXPERIMENTAL PROCEDURE

Sterilized glasswares, oven dried and double distilled water were used throughout the experiment.

C. PREPARATION OF SOIL, POTS AND PLANT MATERIALS

Earthen pots of 14 cm height x 16 cm dia were surface sterilized with formaldehyde solution (1:100,v/v) and then were filled with soil which had already been autoclaved at 15 lbs pressure for 20 minutes.

Brinjal (Solanum melongena L.) cultivars, pusa purple long and Ghatikia white obtained from vegetable Improvement Project, Orissa University of Agriculture & Technology, Bhubaneswar, were used in this study. Brinjal cultivars pusa purple long and Ghatikia white were used as resistant and susceptible hosts respectively for M. incognita seeds were surface sterilized by immersing these in 0.1% $HgCl_2$ for about 5 minutes followed by repeated washing with sterile water. Surfacesterilized seeds were sown in the pots containing soil, which was also sterilized. Four sets of seedlings, two of each cultivar were maintained under perfect sterilized conditions. The seedling were thinned leaving one seedling per pot. Cultural operations like watering and weeding were attended to as a routine. Procedures for collection, storage and examination of second stage larvae were the same as described previously (Dasgupta and Ganguly, 1975).

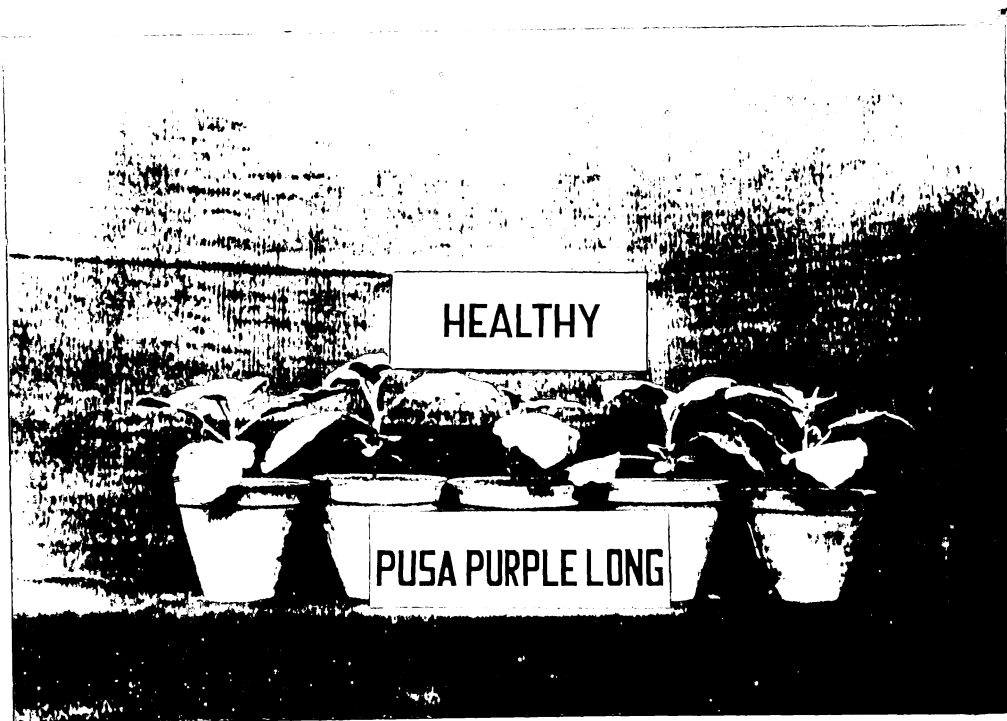


Fig.1. Healthy brinjal plants of variety pusa purple long

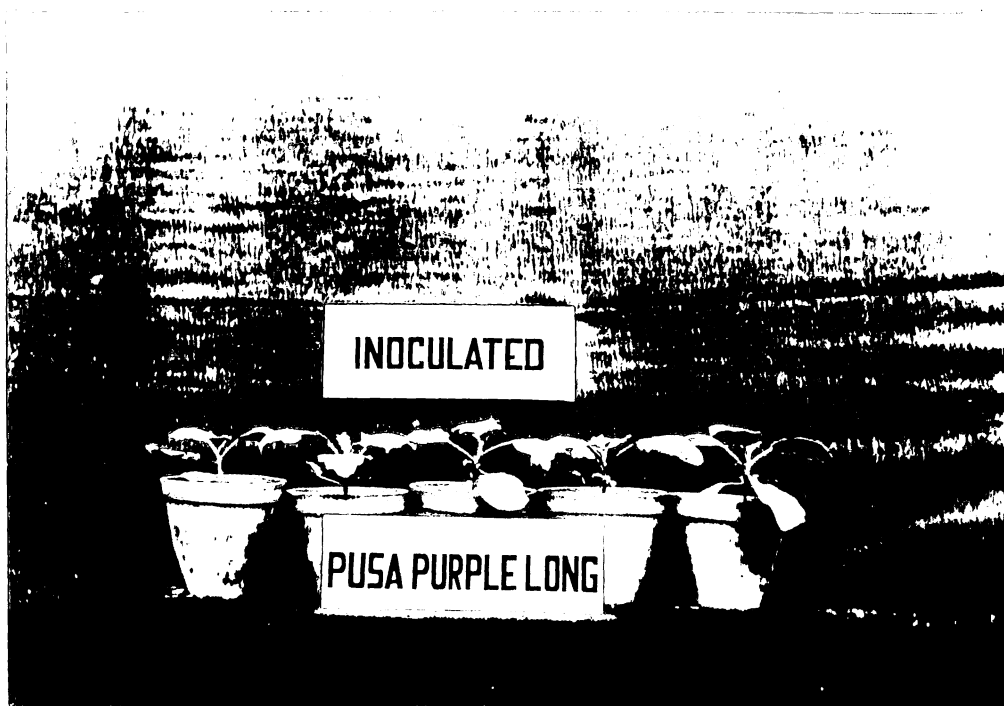


Fig.2. Nematode inoculated brinjal plant of variety pusa purple long



Fig.3. Healthy brinjal plants of variety, Ghatikia white

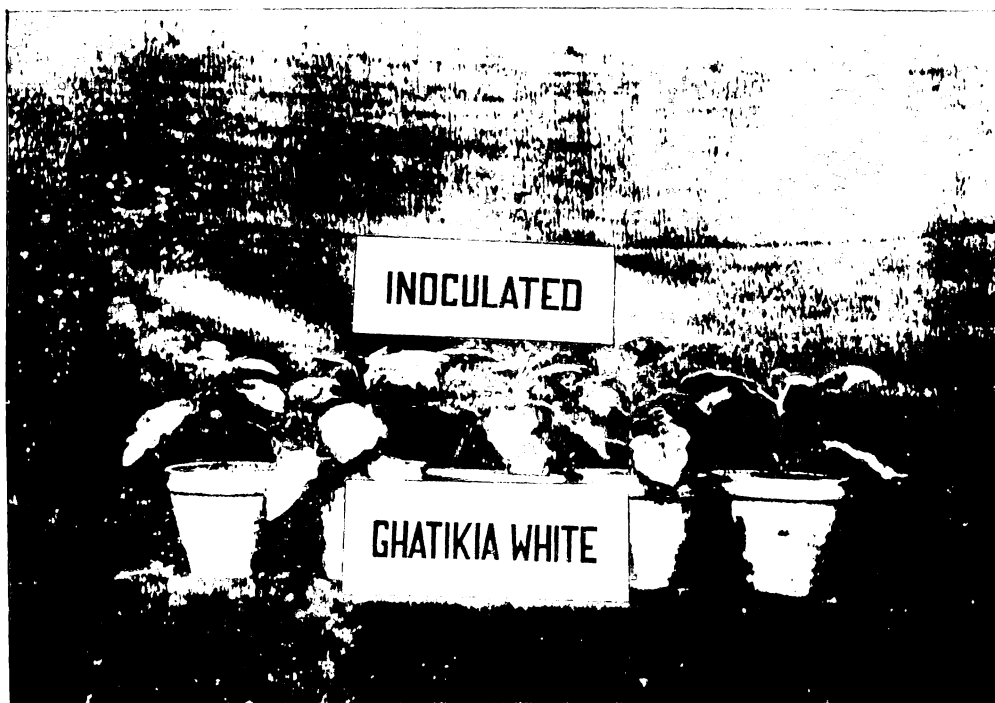


Fig.4. Nematode inoculated brinjal plants of variety, Ghatikia white

D. INOCULATION OF NEMATODES

The nematodes in the second stage, once collected in suspension, were surface sterilized by placing them in 0.5% streptomycin sulphate solution for 12 hours at 4°C. This was followed by further rinsing of nematodes 3 - 4 times with sterile water.

The week old seedlings of each variety were inoculated with axenised suspension of approximately 1,000 infective larvae of M. incognita per seedling. A set of plants of each variety were left uninoculated to serve as control (Fig.1 and Fig.3). The plants were maintained in the departmental net house under controlled environmental conditions of winter, 1992.

E. HARVESTING AND CLEANING OF THE PLANTS FOR ANALYSIS

Plants of various treatments were harvested at 45 days after inoculation. After harvest, the roots were washed free of soil adhering to the roots under running tap water, rinsed in sterile water and surface sterilized in 0.1% mercuric chloride solution. It was followed by thorough and repeated washings by distilled

water. The harvested roots were blotted and kept separately for biochemical analysis.

F. PREPARATION OF TEST SAMPLE FOR ANALYSIS OF
AMINO ACIDS AND PHENOLS

Ten gram of root samples of various treatments were taken, ground separately with 80% ethyl alcohol added at the rate of 20 ml for each sample in a sterile mortar and pestle till the roots were macerated to pulp. These materials after grinding were boiled for 5 to 10 minutes in a hot water bath. After boiling, the alcoholic roots extracts were filtered in a double layered fine cheese cloth so as to remove the floating plant debris. The extracts were centrifused at 5000 rpm for 10 minutes. All the materials were settled at the bottom and top portion became very clean. The supernatant liquid in the test tubes were carefully poured into separate watch glasses, pellets were discarded. The supernatant liquid was allowed to evaporate to complete dryness by keeping them in room temperature and the process was speeded up by means of hot air blower. Then finally a syrupy material was obtained in the watch glass which was slight yellowish in colour. This syrupy material was dissolved in 2 ml of 10% isopropyl alcohol and transferred to

several clean glass vials each representing for a single lot, were stored in the freeze compartment of a refrigerator for subsequent use in paper chromatography.

G. TECHNIQUES FOR PAPER CHROMATOGRAPH

The Whatman No.1 chromatographic paper of size 22.5 cm x 22.5 cm were used for paper chromatography. It was marked and spotted with the test sample which had been prepared earlier and kept in the deep freeze of refrigerator. The spotting of test sample was made with the help of a very fine glass capillary tube of 1 u litre capacity. The size of the spot was tried to be restricted to the smallest possible area on the origin, which was to be facilitated by means of hot air blower at the time of application of sample on the paper. In the same way, spotting of sample was repeated 7 times on the same point after completely drying the previous spot.

For amino acid, two dimensional ascending paper chromatographic technique was used. The two solvents used were n-butanol - acetic acid - water and phenol - water - ammonia in the proportion of 160:40:40; and 180:20:1 respectively. The n - butanol acetic acid -

water was used as the first solvent, whereas Phenol-water-ammonia as the second solvent.

After spotting with the test sample and drying it completely, the paper was folded so as to form the shape of a cylinder, its edges were fastened together carefully avoiding overlapping. The paper cylinder was placed in an upright position in a petridish (15 cm dia) containing 50 ml of n-butanol - acetic acid - water, the first solvent. This petridish has been placed on a glass plate and these were covered with a glass belljar (32.5 cm x 30 cm). The space between its rim and the glass plate was sealed with petroleum jelly, to make the belljar air tight, which served the purpose of a chromatographic chamber ensuring a saturated atmosphere inside the belljar as required for the stationary phase of the paper (Fig.5). The test sample spot was at the lower end of the paper, just above the solvent, but not in direct contact. The belljar chamber was made air tight as before. The paper cylinder was left as such for about 10 hours when the first solvent reached the close proximity (below 2.5 cm) of the upper edge of the paper. After the completion of the flow of first solvent, the paper was carefully taken out of the belljar and dried for about 2 hours under fan to allow evaporation

of n-butanol-acetic acid-water solvent. The chromatogram was again folded in the form of a cylinder in perpendicularly opposite direction of the first solvent. Then the paper cylinder was placed in 50 ml of the second solvent in the other petriplate (15 cm dia) and run in the same manner for about 12 hours as in the first solvent inside the air tight chamber. Towards the end of this period the second solvent reached close to the upper edge (below 2.5 cm) of the paper, again the chromatogram was taken out of the belljar and dried under fan.

For phenolic compounds also, one dimensional ascending paper chromatography technique was used. The solvent used was n-butanol-acetic acid-water in the proportion of 4:1:1 (Block et al., 1958). The chromatogram was prepared in same manner as for amino acid and the paper was dried for 12 hours under fan.

After the chromatogram was completely air dried by hanging with plastic clips inside the laboratory at room temperatures for 12 hours for amino acids and phenols, they were developed. The reagent used for amino acid was 0.25% ninhydrin solution in acetone and for phenols was 2% ferric chloride solution in ethanol.

The chromatograms were developed by dipping them in the reagent taken in the enamel tray. Prepared amount of fresh coloured reagent was poured into the tray and the chromatograms were individually immersed in it. One edge of the paper was first immersed in it and from the other edge the chromatogram was briskly pulled out after allowing it to remain in contact with the solution for a few seconds to give an uniform flow of the colour reagent. Solvents (acetone and ethanol) were allowed to evaporate from the chromatogram again by hanging them at room temperature (30°C). After air drying, the chromatogram was put in an oven maintained at 75°C for 10 minutes when the coloured spots of various amino acids became distinctly visible on the chromatograms. Immediately after detecting the amino spots on the chromatograms, they were outlined with a pencil so as to retain their original location. The central points, the darkest points of the spots were also marked simultaneously for calculation of R_f values. For phenolic compounds, the drying was done in oven like that of amino acids. Similar procedures were followed for detection of central points for calculation of R_f values.

The identification of free amino acids, amides, and phenols was done by preparing standard chromatograms. Eighteen amino acids, two amides and five phenolic compounds

were used for the preparation of standard maps. Amino acids and amides were spotted in 2 chromatograph papers containing ten each and phenols were spotted in separate chromatograph papers. The amino acids were L-cystine, L-threonine, L-serine, L-lysine, L-histidine, L-arginine, L-glycine, L-alanine, L-proline, L-tryptophan, L-tyrosine, L-methionine, L-valine, L-leucine, L-isoleucine, L-phenylalanine, L-aspartic acid and L-glutamic acid and the amides were L-glutamine and L-asparagine. The phenolic compounds were caffeic acid, chlorogenic acid, catechol, pyrocatech and pyrogallol.

The standard solutions of each amino acid and amide were made by dissolving 10 mg of each in 20 ml of 10% isopropyl alcohol solution. The amino acids, namely, L-cysteine, L-phenylalanine, L-tryptophan and L-glutamic acid could not be dissolved very easily. Consequently, a few drops of 100% hydrochloric acid were added to dissolve them. With a view to concentrate the solutions, the volume of each was reduced from 20 ml to 10 ml by evaporating the isopropyl alcohol. To prepare a mixture of the eighteen amino acids and two amides, 0.2 ml from each stock solution was pipetted out and mixed in a glass vial. Each of the eighteen amino acids and two amides was individually spotted on Whatman No.1

chromatographic papers taking 10 in one paper by the procedure described above. Moreover, on the another sheet of paper the mixture of all the eighteen amino acids and two amides were spotted. The former chromatogram (spotted with individually 18 amino acids and two amides) was run in single dimension by n-butanol-acetic acid-water and the latter (spotted with mixture) was run similarly two dimensionally as described earlier with n-butanol-acetic acid-water as first solvent and then with phenol-water-ammonia as second solvent. These were also developed with 0.25% ninhydrin colour reagent in acetone after proper drying of the chromatogram. Acetone was allowed to evaporate from chromatogram by hanging them at room temperature (30°C). After it was air dried, the chromatograms were outlined with a pencil and their central points were marked. These standard maps were used to identify the free amino acids and amides developed on the chromatograms with unknown root extract samples.

The standard solutions of each phenolic compounds were made by dissolving 15 mg of each in 20 ml of distilled water. Spotting of five phenolics was done in a similar manner and was run in single dimension with n-butanol-acetic acid-water. After drying, development was done in 2 % ferric chloride solution in ethanol. That was

hanged in room temperature (30°C) for the solvent to be evaporated and spots were outlined with a pencil to prepare a standard map for identification of the phenolics in unknown samples.

Throughout the chromatographic technique, same proportion and grade of solvent chemicals and colour reagents were used for each treatment with a view to maintain maximum possible uniformity in the experimental procedures.

H. QUANTITATIVE ESTIMATION

Amino acids, amides and phenolics were analysed quantitatively by paper chromatographic technique and subsequently by using TLC scanner (densiometer). After measuring the density of various amino acids, amides and phenolic compounds from developed chromatograms, their concentrations were calculated with the help of standard graph of known concentration. The results were expressed in terms of milligrams of amino acids, amides and phenolic compounds per gram of fresh roots.

I. ESTIMATION OF TOTAL SUGARS

Fresh roots (0.1 g) of various treatments were homogenised separately in 80 per cent ethanol and the volume made up to 10 ml with 80 per cent ethanol. The homogenate was then centrifuged at 2000 g for 20 minutes.

The method followed was that of Dubois et al. (1956). Reagents used were 1. phenol 5 % in distilled water, and 2. concentrated sulphuric acid.

A suitable amount of the supernatant (0.2 to 0.5 ml) was taken in a test tube and to this 1 ml of phenol reagent and 5 ml of concentrated sulphuric acid were added and mixed thoroughly. The absorbance was measured at 490 nm after allowing 20 minutes for colour development. The amount of sugar was calculated from a calibration curve prepared with glucose and expressed as mg sugar (glucose equivalent) g^{-1} fresh weight of roots.

J. DETERMINATION OF ENZYME PEROXIDASE AND CATALASE ACTIVITIES IN ROOTS

One gram of root material was ground with 5 ml cold 0.1 M phosphate buffer (PH-7.0) in a prechilled

Mortar & Pestle. The homogenate was centrifuged at 15,000 rpm at 4°C for 30 minutes & the aliquat was used as the source of the enzymes.

(A) ESTIMATION OF ENZYME PEROXIDASE

Peroxidase activity was assayed following the modified method of Kar & Mishra (1976). Assay mixture for peroxidase contained 2 ml 0.1 M phosphate buffer (PH 7.0), 1 ml 0.01 M pyrogallol, 1 ml 0.005 M H_2O_2 and 1 ml well diluted enzyme extract. The reaction was stopped by adding 1 ml 1.5 N H_2SO_4 after 5 minutes incubation at 25°C & the amount of purpurogallin formed was estimated by measuring the absorbance at 420 nm. The enzyme activity was expressed in absorbancy units.

(B) ESTIMATION OF ENZYME CATALASE

Catalase activity was assayed by the modified method of Barber (1980). 1 ml of enzyme extract was added to 2 ml 0.005 M H_2O_2 & 3 ml phosphate Buffer (PH 7.0). The Reaction was stopped by adding 10 ml 0.7 N H_2SO_4 after 1 min. incubation at 20°C. The residual

: 48 :

H_2O_2 was titrated with 0.01 N KMnO_4 . A blank was prepared by adding the extract to an acidified solution of the reaction mixture of Zerotime. Catalase activity was expressed as mol H_2O_2 used per g fresh wt of root.

CHAPTER IV

EXPERIMENTAL FINDINGS

EXPERIMENTAL FINDINGS

The qualitative and quantitative estimation of free amino acids and amides, phenolic compounds relating to root extracts of two cultivars of brinjal as influenced by Meloidogyne incognita has been done following paper chromatography and TLC scanning techniques.

QUALITATIVE DETERMINATION OF STANDARD AMINO ACIDS AND AMIDES

A. R_f VALUES OF STANDARD AMINO ACIDS AND AMIDES

The eighteen amino acids and two amides of known strength were spotted individually and run in n-butanol-acetic acid-water solvent one dimensionally. After drying and subsequently developing them with ninhydrin solution in acetone the central points of the ninhydrin positive spots were marked and measured from the base line (X-axis). This gave the distance travelled by the substance from the origin. It was divided by the distance the solvent front had travelled and the R_f values were calculated in percentage and presented in Table 1.

B. R_f VALUES OF STANDARD AMINO ACIDS AND AMIDES IN MIXTURE

A mixture of the above 18 amino acids and 2 amides was spotted and separated by two-dimensional chromatography.

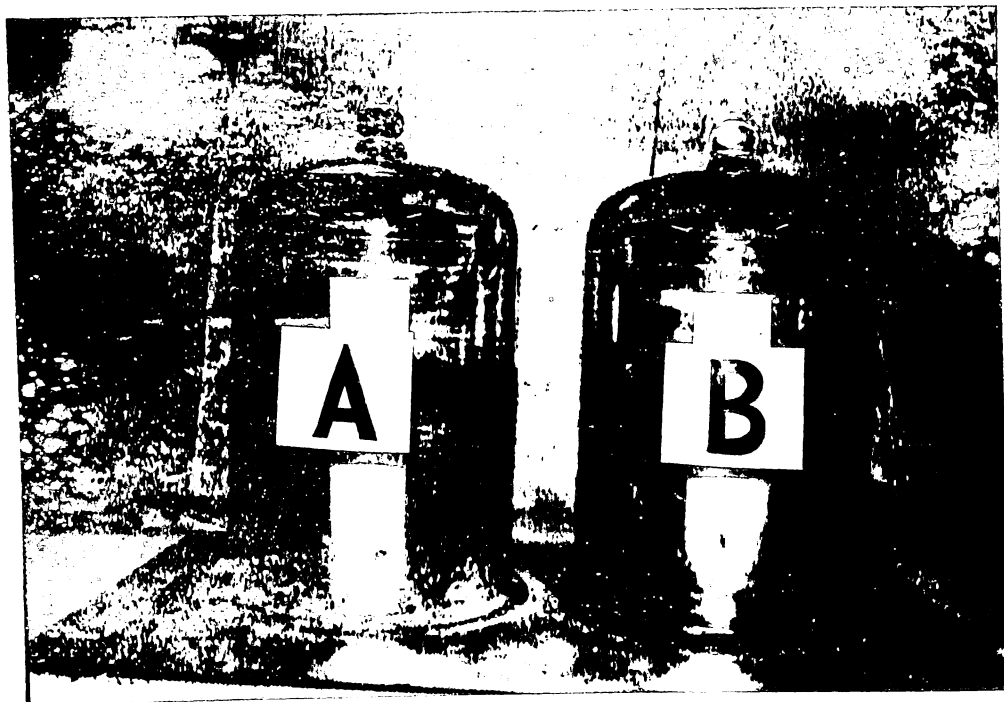


Fig.5. Chromatographic process in progress

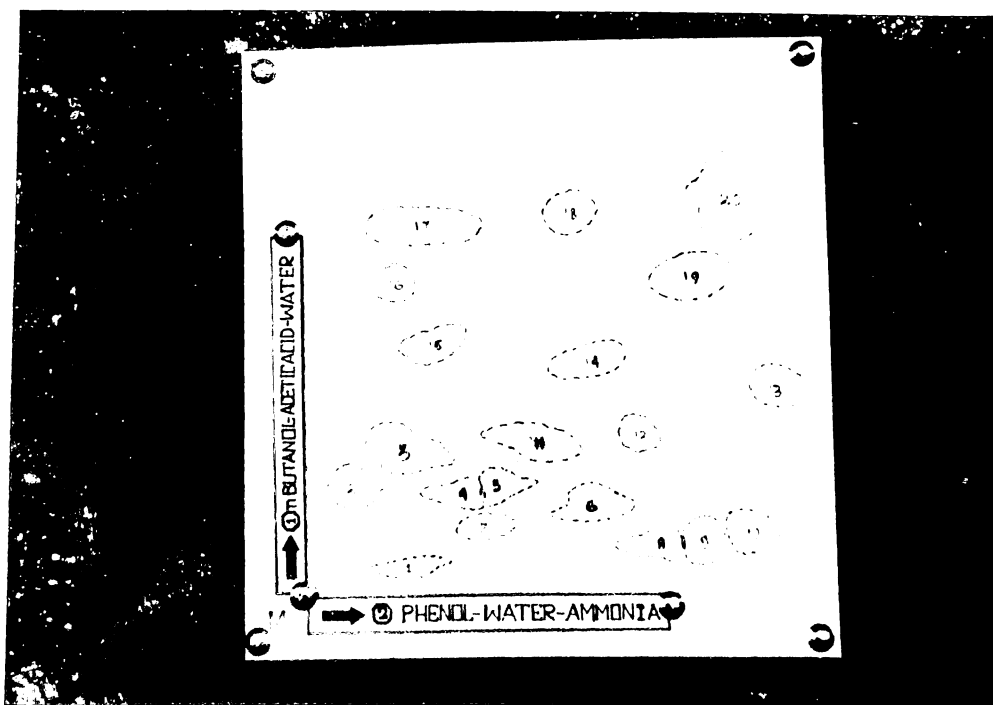


Fig.6. Chromatogram showing relative positions of standard amino acids and amides mixture

The Rf values of individual amino acid and amide spots were calculated against n-butanol-acetic acid-water front.

Subsequently the amino acids and amides were identified by comparing their colour reaction and Rf values with the colour and Rf values of individual amino acids and amides. The Rf values of the separated ninhydrin positive spots from the mixture are tabulated in Table 2 (Fig.6).

Table 1. The Rf values of standard amino acids and amides (1-D, Chromatography)

Sl.No. of Spots	Rf values in percentage			Name of the amino acids and amides
1.	1.4/22.0	=	0.06 x 100 = 6	L-cystine
2.	5.5/22.5	=	0.24 x 100 = 24	L-glutamic acid
3.	6.0/22.0	=	0.27 x 100 = 27	L-threonine
4.	4.5/22.0	=	0.20 x 100 = 20	L-aspartic acid
5.	4.0/22.5	=	0.17 x 100 = 17	L-serine
6.*	3.5/22.5	=	0.15 x 100 = 15	L-glutamine
7.*	2.7/22.0	=	0.12 x 100 = 12	L-asparagine
8.	2.3/22.5	=	0.10 x 100 = 10	L-lysine
9.	2.1/22.5	=	0.09 x 100 = 9	L-histidine
10.	2.9/22.5	=	0.13 x 100 = 13	L-arginine
11.	4.8/22.5	=	0.21 x 100 = 21	L-glycine
12.	7.3/22.0	=	0.33 x 100 = 33	L-alanine
13.	8.0/22.5	=	0.35 x 100 = 35	L-proline
14.	11.3/22.5	=	0.50 x 100 = 50	L-tryptophan
15.	9.3/22.5	=	0.41 x 100 = 41	L-tyrosine
16.	12.5/22.0	=	0.56 x 100 = 56	L-methionine
17.	14.0/22.0	=	0.63 x 100 = 63	L-valine
18.	16.5/22.5	=	0.73 x 100 = 73	L-leucine
19.	13.5/22.5	=	0.60 x 100 = 60	L-phenylalanine
20.	17.3/22.5	=	0.77 x 100 = 77	L-isoleucine

* marks indicate amides.

Table 2. The Rf values of ninhydrin positive spots separated from standard amino acids and amides mixture (2-D, Chromatography) (Fig.6)

Sl.No. of Spots	Rf values in percentage	Name of the amino acids and amides
1.	$1.4/22.5 = 0.06 \times 100 = 6$	L-cystine
2.	$5.3/22.5 = 0.24 \times 100 = 24$	L-glutamic acid
3.	$6.0/22.5 = 0.27 \times 100 = 27$	L-threonine
4.	$4.3/22.5 = 0.19 \times 100 = 19$	L-aspartic acid
5.	$4.1/22.5 = 0.18 \times 100 = 18$	L-serine
6.*	$3.7/22.5 = 0.16 \times 100 = 16$	L-glutamine
7.*	$2.6/22.5 = 0.12 \times 100 = 12$	L-asparagine
8.	$2.3/22.5 = 0.10 \times 100 = 10$	L-lysine
9.	$2.1/22.5 = 0.09 \times 100 = 9$	L-histidine
10.	$2.9/22.5 = 0.13 \times 100 = 13$	L-arginine
11.	$4.8/22.5 = 0.21 \times 100 = 21$	L-glycine
12.	$7.4/22.5 = 0.33 \times 100 = 33$	L-alanine
13.	$8.2/22.5 = 0.36 \times 100 = 36$	L-proline
14.	$11.5/22.5 = 0.51 \times 100 = 51$	L-tryptophan
15.	$9.5/22.5 = 0.42 \times 100 = 42$	L-tyrosine
16.	$12.6/22.5 = 0.56 \times 100 = 56$	L-methionine
17.	$14.4/22.5 = 0.64 \times 100 = 64$	L-valine
18.	$16.3/22.5 = 0.72 \times 100 = 72$	L-leucine
19.	$13.7/22.5 = 0.61 \times 100 = 61$	L-phenylalanine
20.	$17.1/22.5 = 0.76 \times 100 = 76$	L-isoleucine

* marks indicate amides.

C. IDENTIFICATION OF AMINO ACIDS AND AMIDES IN HEALTHY AND INOCULATED BRINJAL ROOT EXTRACT CHROMATOGRAMS :

The Rf values of ninhydrin positive spots on the chromatograms of different lots have been calculated and comparing these RI values with those of mixture

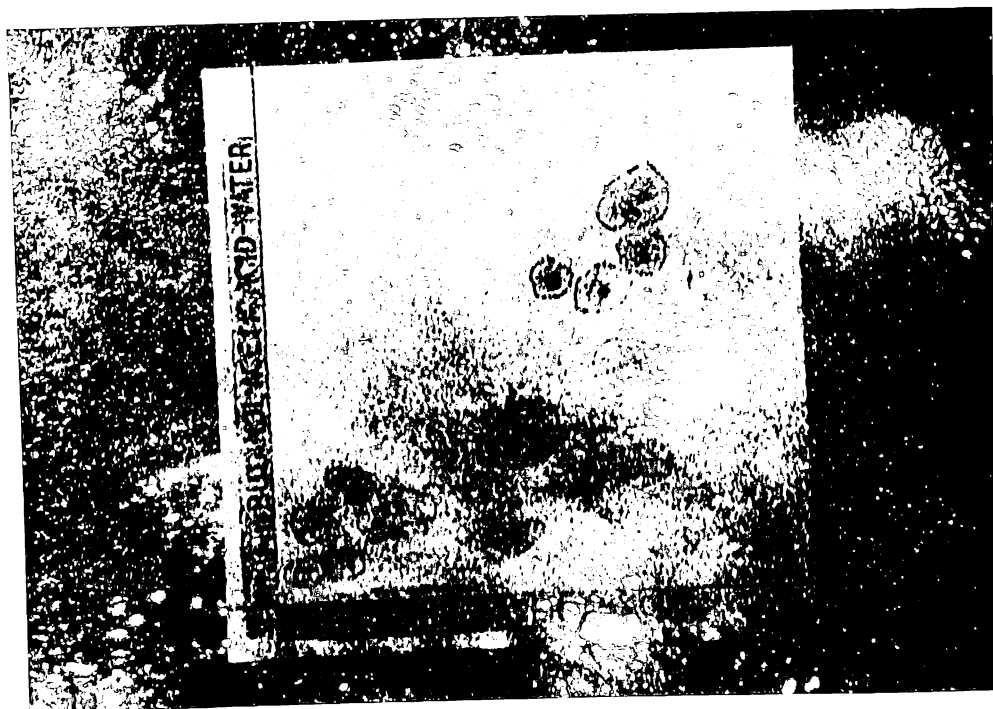


Fig.7. Chromatogram showing relative positions of amino acids and amides in healthy plants of variety, pusa purple long

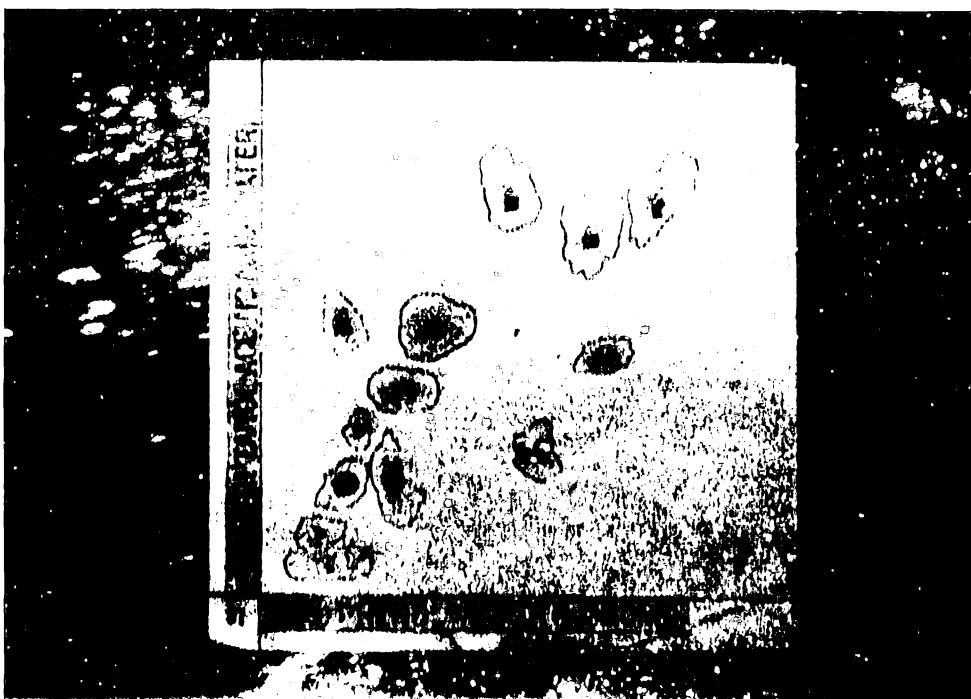


Fig.3. Chromatogram showing relative positions of amino acids and amides in nematode inoculated plants of variety, pusa purple long

chromatogram, the free amino acids and amides were identified and presented in the corresponding tables.

Table 3. The Rf values of ninhydrin positive spots on susceptible healthy cultivar of brinjal, pusa purple long root extract chromatogram (2-D chromatography) (Fig-7).

Sl.No.	Rf values in percentage	Name of amino acids & amides identified
1.	$1.4/22.5 = 0.06 \times 100 = 6$	L-cystine
2.	$2.9/22.5 = 0.13 \times 100 = 13$	L-arginine
3.	$5.3/22.5 = 0.24 \times 100 = 24$	L-glutamic acid
4.	$4.1/22.5 = 0.18 \times 100 = 18$	L-serine
5.	$7.4/22.5 = 0.33 \times 100 = 33$	L-alanine
6.	$2.3/22.5 = 0.10 \times 100 = 10$	L-Lysine
7.	$6/22.5 = 0.27 \times 100 = 27$	L-threonine
8.	$11.5/22.5 = 0.51 \times 100 = 51$	L-tryptophan
9.	$13.7/22.5 = 0.61 \times 100 = 61$	L-phenylalanine
10.	$14.4/22.5 = 0.64 \times 100 = 64$	L-valine
11.	$16.3/22.5 = 0.72 \times 100 = 72$	L-leucine
12.	$17.1/22.5 = 0.76 \times 100 = 76$	L-isoleucine

Table 4. The Rf values of ninhydrin positive spots on nematode inoculated susceptible brinjal cultivar, pusa purple long root extract chromatogram (2-D, chromatography) (Fig-8).

Sl.No. of spots	Rf. values in percentage	Name of the amino acids & amides identified
1.	$1.4/22.5 = 0.06 \times 100 = 6$	L-cystine
2.	$2.6/22.5 = 0.12 \times 100 = 12$	L-asparagine
3.	$5.3/22.5 = 0.24 \times 100 = 24$	L-glutamic acid
4.	$4.1/22.5 = 0.18 \times 100 = 18$	L-serine
5.	$7.7/22.5 = 0.34 \times 100 = 34$	Un-identified
6.	$9.5/22.5 = 0.42 \times 100 = 42$	L-tyrosine
7.	$8.2/22.5 = 0.36 \times 100 = 36$	L-proline
8.	$12.6/22.5 = 0.56 \times 100 = 56$	L-methionine
9.	$11.5/22.5 = 0.51 \times 100 = 51$	L-tryptophan
10.	$16.3/22.5 = 0.72 \times 100 = 72$	L-leucine
11.	$17.1/22.5 = 0.76 \times 100 = 76$	L-isoleucine
12.	$13.1/22.5 = 0.58 \times 100 = 58$	Un-identified
13.	$18.3/22.5 = 0.81 \times 100 = 81$	Un-identified

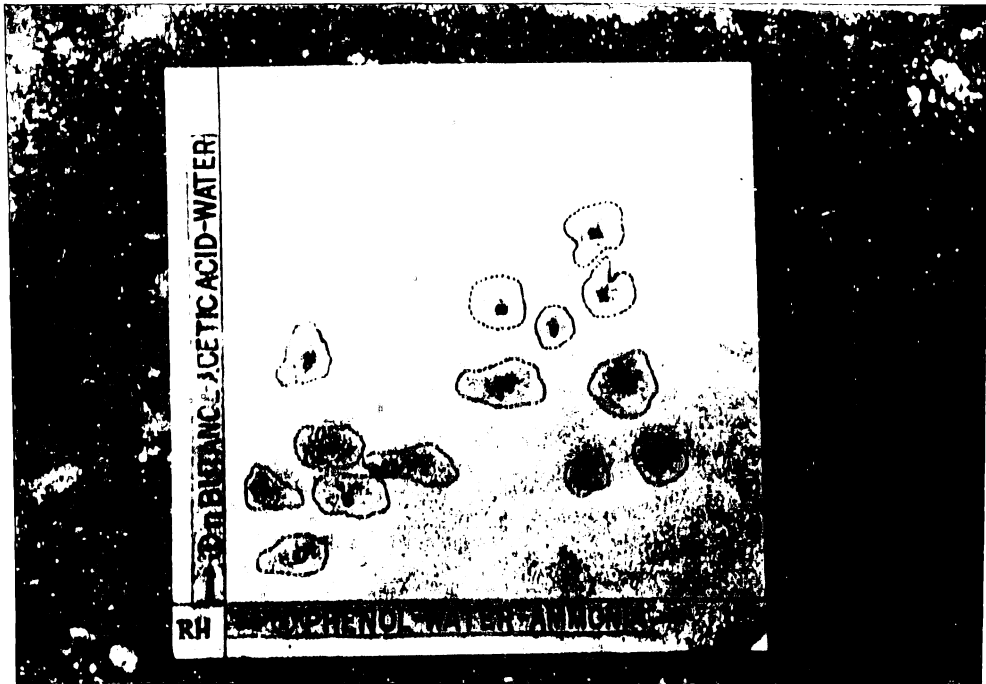


Fig-9. Chromatogram showing relative positions of amino acids and amides in healthy plants of variety, Ghatikia white

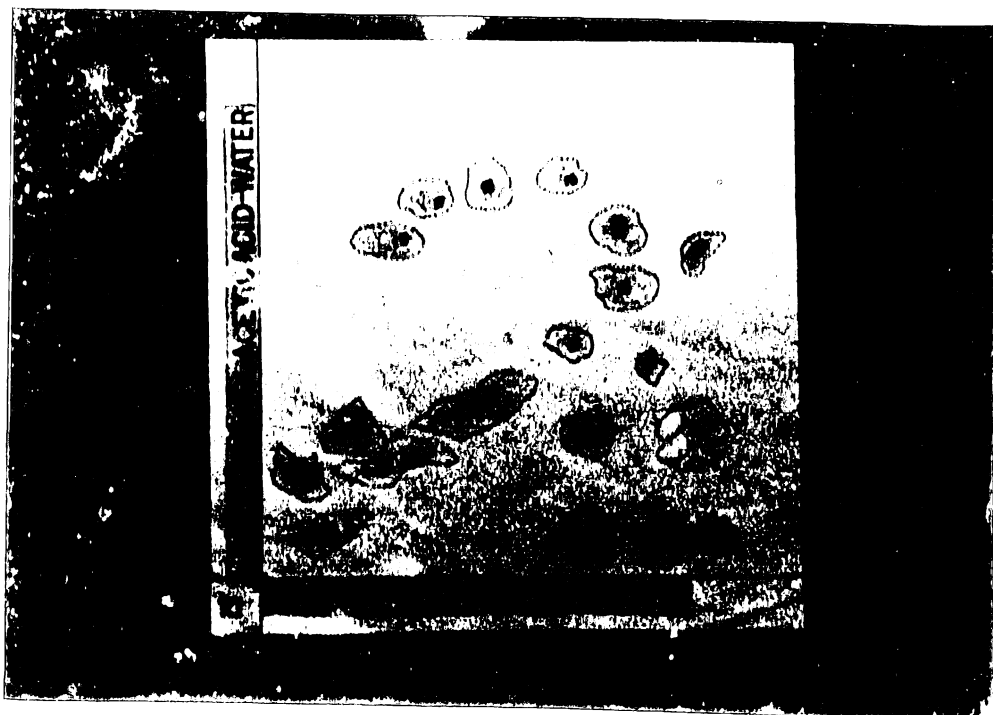


Fig.10. Chromatogram showing relative positions of amino acids and amides in nematode inoculated plants of variety, Ghatikia white

Table 5. The Rf values of ninhydrin positive spots on resistant healthy cultivar of Brinjal, Ghatikia white root extract chromatogram (2-D, Chromatography) (Fig. 9).

Sl.No. of Spots	Rf values in percentage	Name of the amino acids and amides identified.
1.	$1.4/22.5 = 0.06 \times 100 = 6$	L-cystine
2.	$4.8/22.5 = 0.21 \times 100 = 21$	L-glycine
3.	$4.1/22.5 = 0.18 \times 100 = 18$	L-serine
4.	$7.7/22.5 = 0.34 \times 100 = 34$	Un-identified
5.	$7.4/22.5 = 0.33 \times 100 = 33$	L-alanine
6.	$11.5/22.5 = 0.51 \times 100 = 51$	L-tryptophan
7.	$6/22.5 = 0.27 \times 100 = 27$	L-threonine
8.	$9.5/22.5 = 0.42 \times 100 = 42$	L-tyrosine
9.	$13.7/22.5 = 0.61 \times 100 = 61$	L-phenyl alanine
10.	$14.4/22.5 = 0.64 \times 100 = 64$	L-valine
11.	$16.3/22.5 = 0.72 \times 100 = 72$	L-leucine
12.	$17.1/22.5 = 0.76 \times 100 = 76$	L-Isoleucine
13.	$12.6/22.5 = 0.56 \times 100 = 56$	L-Methionine
14.	$6.5/22.5 = 0.29 \times 100 = 29$	Unidentified

Table 6. The Rf values of ninhydrin positive spots on nema - tode inoculated resistant cultivar of Brinjal, Ghatikia white root extract chromatogram (2-D, chromatography) (Fig.10).

Sl.No. of Spots	Rf values in percentage	Amino acids and amides identified
1.	$1.4/22.5 = 0.06 \times 100 = 6$	L-cystine
2.	$4.8/22.5 = 0.21 \times 100 = 21$	L-glycine
3.	$4.1/22.5 = 0.18 \times 100 = 18$	L-serine
4.	$7.7/22.5 = 0.34 \times 100 = 34$	Unidentified
5.	$7.4/22.5 = 0.33 \times 100 = 33$	L-alanine
6.	$11.5/22.5 = 0.51 \times 100 = 51$	L-tryptophan
7.	$6/22.5 = 0.27 \times 100 = 27$	L-threonine
8.	$8.2/22.5 = 0.36 \times 100 = 36$	L-proline
9.	$9.5/22.5 = 0.42 \times 100 = 42$	L-tyrosine
10.	$13.7/22.5 = 0.61 \times 100 = 61$	L-phenyl alanine
11.	$14.4/22.5 = 0.64 \times 100 = 64$	L-valine
12.	$16.3/22.5 = 0.72 \times 100 = 72$	L-leucine
13.	$17.1/22.5 = 0.76 \times 100 = 76$	L-Isoleucine
14.	$2.3/22.5 = 0.10 \times 100 = 10$	L-lysine
15.	$2.1/22.5 = 0.09 \times 100 = 9$	L-histidine
16.	$16.6/22.5 = 0.74 \times 100 = 74$	Unidentified
17.	$18.3/22.5 = 0.81 \times 100 = 81$	Unidentified
18.	$19/22.5 = 0.84 \times 100 = 84$	Unidentified

D. QUANTITATIVE ESTIMATION OF AMINO ACIDS AND AMIDES

The quantity of individual amino acids and amides have been recorded through TLC scanner 11 and the result has been given in Table 7.

Name of amino acids and amides	Pusa purple long			Ghatikia white			
	Healthy (SH)	Inoculated (SI)	% increase (+) or decrease (-)	Healthy (RH)	Inoculated (RI)	% increase (+) or decrease (-)	
L-Cystine	0.041	0.053	29.3%	0.045	0.054	20%	
L-Serine	0.068	0.081	19.1%	0.071	0.083	16.9%	
L-Tryptophan	0.047	0.023	-51%	0.052	0.039	-25%	
L-Leucine	0.084	0.091	8.3%	0.077	0.081	5.1%	
L-Isoleucine	0.067	0.079	17.9%	0.069	0.076	10.1%	
L-glutamic acid	0.078	0.087	11.5%	-	-	-	
L-Alanine	0.071	-	-	0.066	0.075	13.6%	..
L-Threonine	0.053	-	-	0.065	0.076	16.9%	
L-Phenylalanine	0.056	-	-	0.059	0.079	33.8%	54
L-valine	0.038	-	-	0.033	0.038	15.1%	..
L-Arginine	0.027	-	-	-	-	-	
L-Lysine	0.058	-	-	-	0.064	-	
L-Tyrosine	-	0.053	-	0.049	0.061	24.4%	
L-Methionine	-	0.048	-	0.054	-	-	
L-Proline	-	0.087	-	-	0.032	-	
L-Asparagine	-	0.032	-	-	-	-	
L-Histidine	-	-	-	-	0.061	-	
L-Glycine	-	-	-	0.063	0.075	19%	

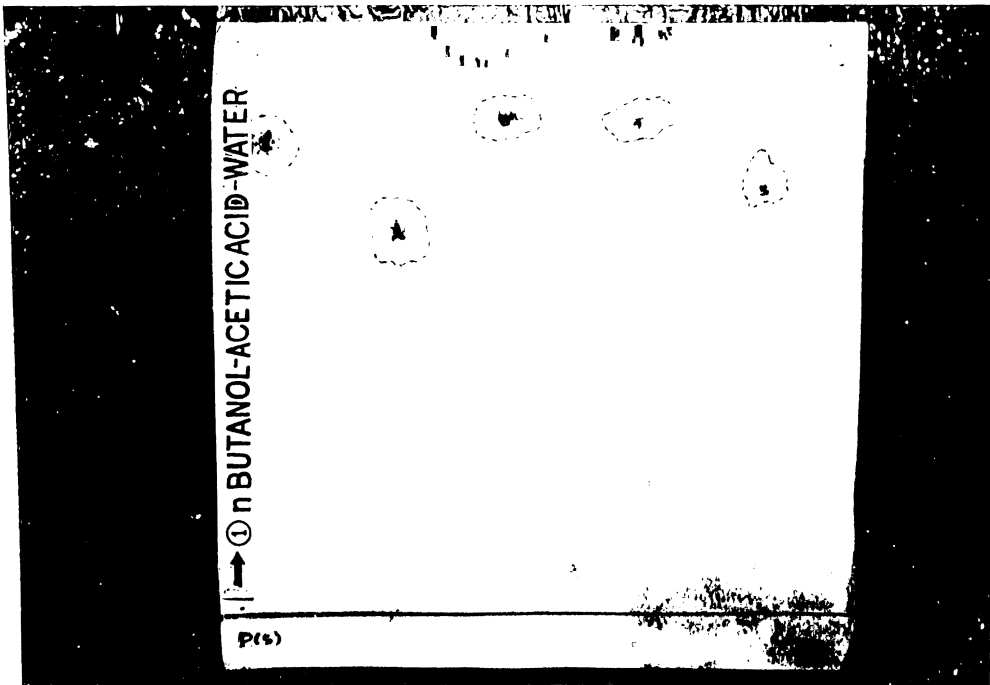


Fig.11. Chromatogram showing relative positions of standard phenolic compounds

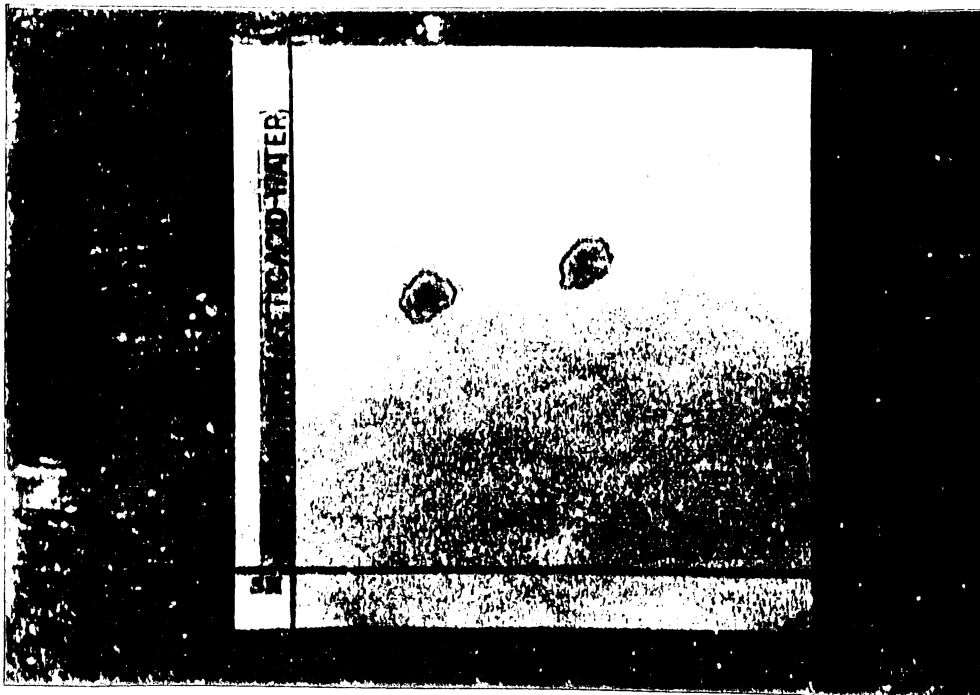


Fig.12. Chromatogram showing relative positions of phenolic compounds in healthy plants of variety, pusa purple long

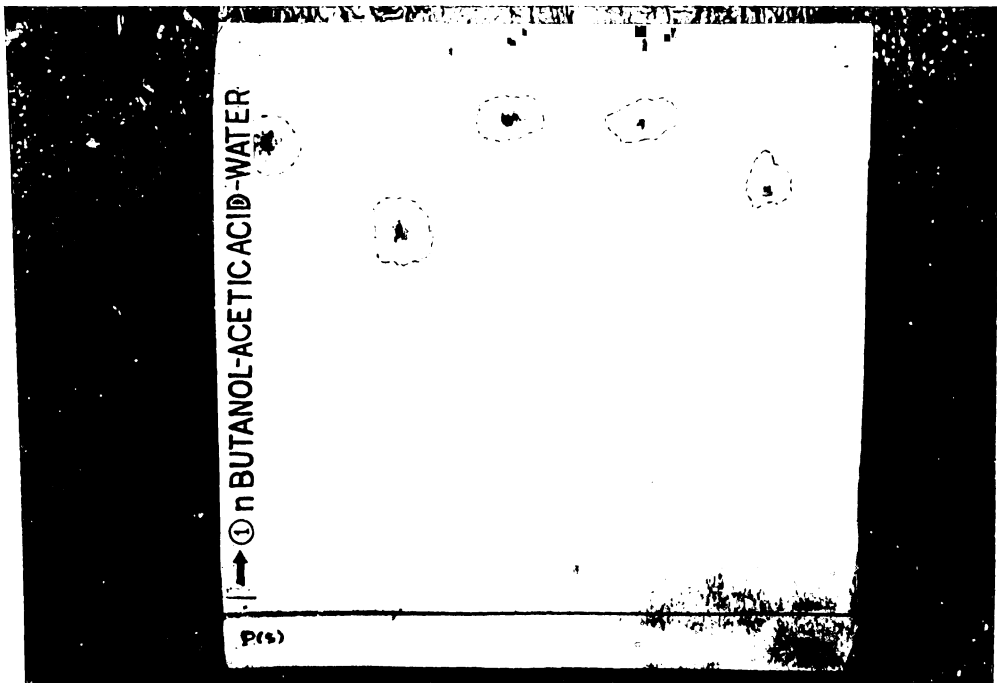


Fig.11. Chromatogram showing relative positions of standard phenolic compounds

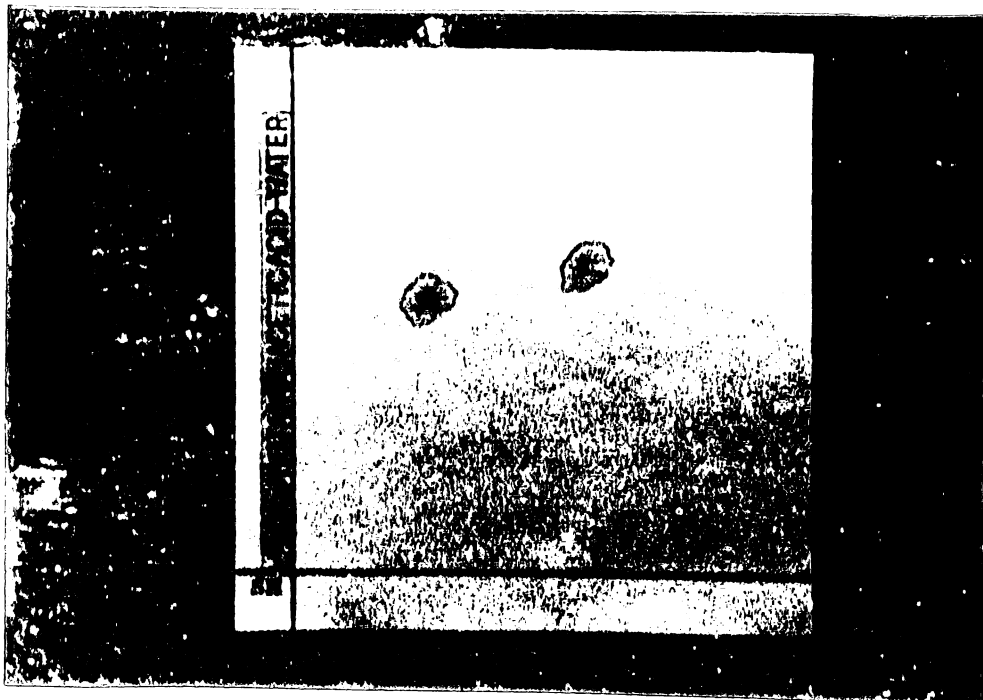


Fig.12. Chromatogram showing relative positions of phenolic compounds in healthy plants of variety, pusa purple long

Results present in Table 3-6 (Fig.7-10) revealed that five amino acids viz. L-cystine, L-serine, L-tryptophan, L-leucine and L-isoleucine were found to be common in healthy and inoculated root samples of both the varieties. Whereas the susceptible healthy samples contained seven other amino acids namely L-glutamic acid, L-alanine, L-threonine, L-phenylalanine, L-valine, L-arginine and L-lysine over above listed common amino acids in Table 3 (Fig.7).

But the nematode inoculated susceptible root samples contained five more amino acids namely L-glutamic acid L-tyrosine, L-methionine, L-proline, L-asparagine (Amide) and three unidentified positive spots over common in Table 4 (Fig.8).

The resistant healthy root samples contained seven more amino acids namely L-alanine, L-threonine, L-phenylalanine, L-valine, L-tyrosine, L-methionine, L-glycine and two unidentified ninhydrin positive spots over the common amino acids tested above in Table 5 (Fig.9).

Whereas nematode inoculated resistant samples contained nine more amino acids namely L-alanine, L-threonine, L-phenyl alanine, L-valine, L-lysine, L-tyrosine, L-proline, L-histidine, L-glycine and four unidentified ninhydrin positive spots over common in Table 6 (Fig.10).

The quantitative changes revealed that there is higher concentration of all amino acids and amides except L-tryptophan in nematode inoculated samples. It was also very much peculiar that L-arginine disappeared from both the varieties with Meloidogyne incognita infection (Table 7).

P H E N O L

QUALITATIVE DETERMINATION OF STANDARD PHENOLIC COMPOUNDS

A R_f VALUES OF STANDARD PHENOLIC COMPOUNDS

The five phenolic compounds of known strength were spotted individually and run in n-butanol-acetic acid-water solvent one-dimensionally. After drying and subsequently developing them with ferric chloride solution in ethanol, the central points of developed spots were marked and measured from the base line (X-axis). This gave the distance travelled by the substance from the origin. It was divided by the distance the solvent front had travelled and the R_f values were calculated in percentage as presented in Table 8 (Fig.11).

Table 8. The R_f values of standard phenolic compounds (1-D, chromatography) (Fig.11)

Sl.No. of Spots	R _f values in percentage	Name of the phenolic compounds
1.	$20.0/22.5 = 0.89 \times 100 = 89$	Caffeic acid
2.	$15.5/22.5 = 0.69 \times 100 = 69$	Chlorogenic acid
3.	$22.0/22.5 = 0.98 \times 100 = 98$	Catechol
4.	$21.5/22.5 = 0.96 \times 100 = 96$	Pyrocatechol
5.	$18.5/22.5 = 0.82 \times 100 = 82$	Pyrogallol



Fig.13. Chromatogram showing relative positions of phenolic compounds in nematode inoculated plants of variety, pusa purple long



Fig.14. Chromatogram showing relative positions of phenolic compounds in healthy plants of variety, Ghatikia white

B IDENTIFICATION OF PHENOLIC COMPOUNDS IN HEALTHY AND INOCULATED BRINJAL EXTRACT CHROMATOGRAMS :

Comparing the Rf values, colour obtained by the colour reagent and configuration of ferric chloride positive spots on sample chromatogram with that of standard chromatogram, the following phenolic compounds were arranged according to the code number of spots identified and presented in the corresponding tables.

Table 9. Rf values of ferric chloride positive spots on susceptible healthy cultivar of Brinjal, root extract chromatogram (1-D, chromatography)(Fig.12).

Sl.No. of Spots	Rf values in percentage	Name of the phenolic compounds identified
1.	$14/22.5 = 0.62 \times 100 = 62$	Unidentified
2.	$15.5/22.5 = 0.69 \times 100 = 69$	Chlorogenic Acid

Table 10. Rf values of ferric chloride positive spots on susceptible inoculated cultivar of Brinjal, root extract chromatogram (1-D, chromatography)(Fig.13).

Sl.No. of Spots	Rf values in percentage	Name of the phenolic compounds identified
1.	$14/22.5 = 0.62 \times 100 = 62$	Unidentified
2.	$15.5/22.5 = 0.69 \times 100 = 69$	Chlorogenic acid

Table 11. Rf values of ferric chloride positive spots on resistant healthy cultivar or Brinjal root extract chromatogram (1-D, chromatography)(Fig.14).

Sl.No. of Spots	Rf values in percentage	Name of the phenolic compounds identified
1.	$14/22.5 = 0.62 \times 100 = 62$	Unidentified
2.	$15.5/22.5 = 0.69 \times 100 = 69$	Chlorogenic acid



Fig.15. Chromatogram showing relative positions of phenolic compounds in nematode inoculated plants of variety, Ghatikia white

Table 12. Rf values of ferric chloride positive spots on resistant inoculated cultivar of Brinjal root extract chromatogram. (1-D, chromatography) (Fig.15).

Sl.No. of Spots	Rf values in percentage	Name of the phenolic compounds identified
1.	$10.5/22.5 = 0.47 \times 100 = 47$	Unidentified
2.	$14/22.5 = 0.62 \times 100 = 62$	Unidentified
3.	$15.5/22.5 = 0.69 \times 100 = 69$	Chlorogenic Acid

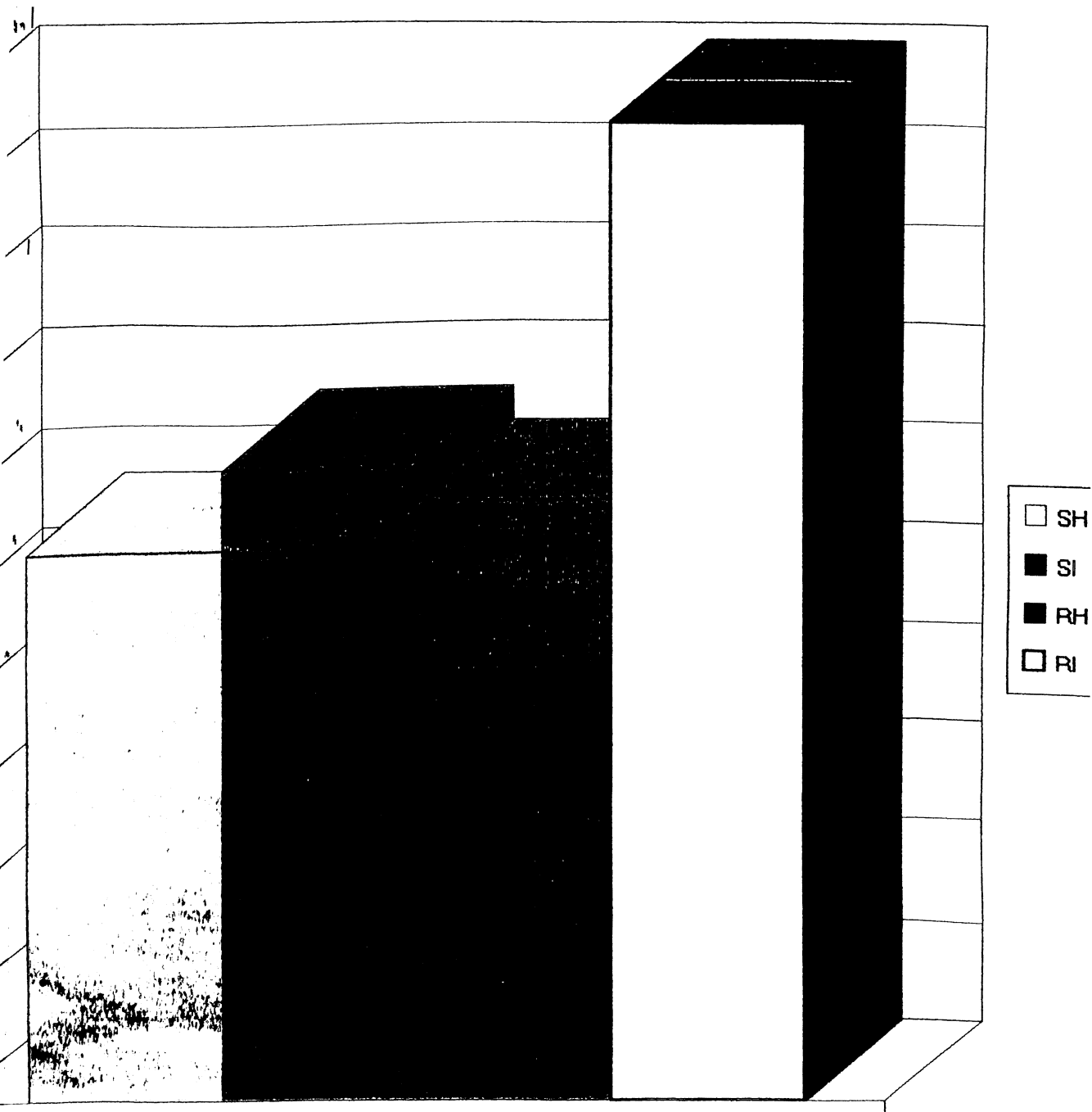
C. CHANGES IN QUANTITY OF PHENOLIC COMPOUNDS (FERRIC CHLORIDE POSITIVE SPOTS) IN BRINJAL ROOT EXTRACTS.

The quantity of individual phenolic compounds have been found out similarly by TLC scanner 11 which is given in the following table 13.

Table 13. Quantity of individual phenolic components of Brinjal roots.

Name of the cultivar	Name of phenolic compound	Phenolic content in mg/gm fresh weight of roots		% increase(+) or % decrease(-)
		Healthy	Inocu- lated	
Pusa purple long	Chlorogenic acid	0.554	0.635	+ 14.62
Ghatikia white	Chlorogenic acid	0.601	0.982	+ 63.39

Fig.16

**EFFECT OF ROOT KNOT NEMATODE M.INCOGNITA ON PHENOLIC
CONTENT IN BRINJAL ROOTS**

The phenolic compound chlorogenic acid (CGA) and one or more unidentified ferric chloride positive spots were found to be commonly present in all the treatments of both the varieties, (Fig.12-15). Quantitative result of phenolic compounds revealed increasing tendency of CGA content in nematode inoculated samples as compared to their healthy counter parts. But the values relating to CGA content for resistant var was considerable higher than that of susceptible var (Table 9-12).

TOTAL SUGAR

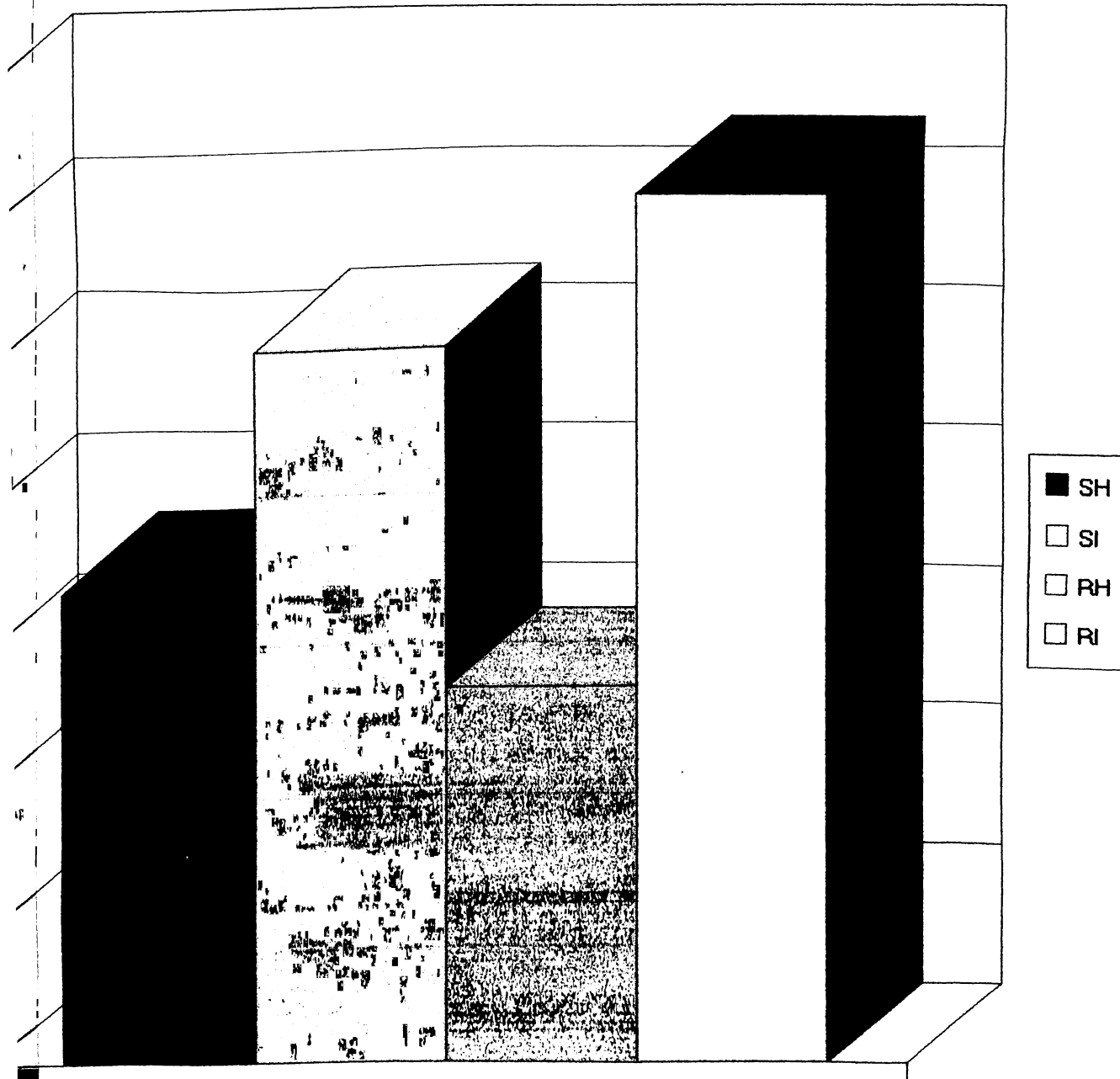
Table 14. Changes in total sugar content in roots of brinjal.

Name of the cultivar	Total sugar content in mg/gm fresh weight of roots		% increase (+) or % decrease (-)
	Healthy	Inoculated	
Pusa purple long	6.848	10.278	+ 50.0
Ghatikia white	5.357	12.441	+132.2

Total sugar content in nematode inoculated root samples of both susceptible and resistant var. were increased by 50 and 132.2 percent, respectively, over their healthy samples. However total sugar content was reduced in healthy resistant samples as compared to its susceptible healthy treatment as shown in Table 14 (Fig.17).

Fig.17

INFLUENCE OF M.INCOGNITA ON TOTAL SUGAR CONTENT IN BRINJAL ROOTS



ENZYME PEROXIDASE

Table 15. Effect of root knot nematode M.incognita on peroxidase activity in Brinjal.

Name of the variety	Enzyme activity in OD units/gm fresh weight of roots		% increase (+) or % decrease (-)
	Healthy	Inoculated	
Pusa purple long	1.05	1.20	+ 14.28
Ghatikia white	1.35	1.85	+ 37.03

The increased peroxidase activity was observed in nematode inoculated samples of both pusa purple long and Ghatikia white. But the higher enzymatic activity was recorded in resistant one, Table 15 (Fig.18).

ENZYME CATALASE

Table 16. Effect of root knot nematode M.incognita on enzyme catalase in Brinjal roots.

Name of the cultivar	Enzyme activity in "mole gm ⁻¹ fresh weight of roots		% increase (+) or % decrease (-)
	Healthy	Inoculated	
Pusa purple long	7.50	6.25	- 16.67
Ghatikia white	8.75	10.00	+ 14.28

From Table 16 (Fig.19), it was observed that catalase activity was lower (16%) incase of nematode inoculated susceptible cultivar. Where as it's activity increased considerably inoculated resistant sample (14.28%) over its control.

Fig.18

**FFECT OF ROOT KNOT NEMATODE, M.INCOGNITA ON ENZYME
PEROXEDASE IN BRINJAL ROOTS**

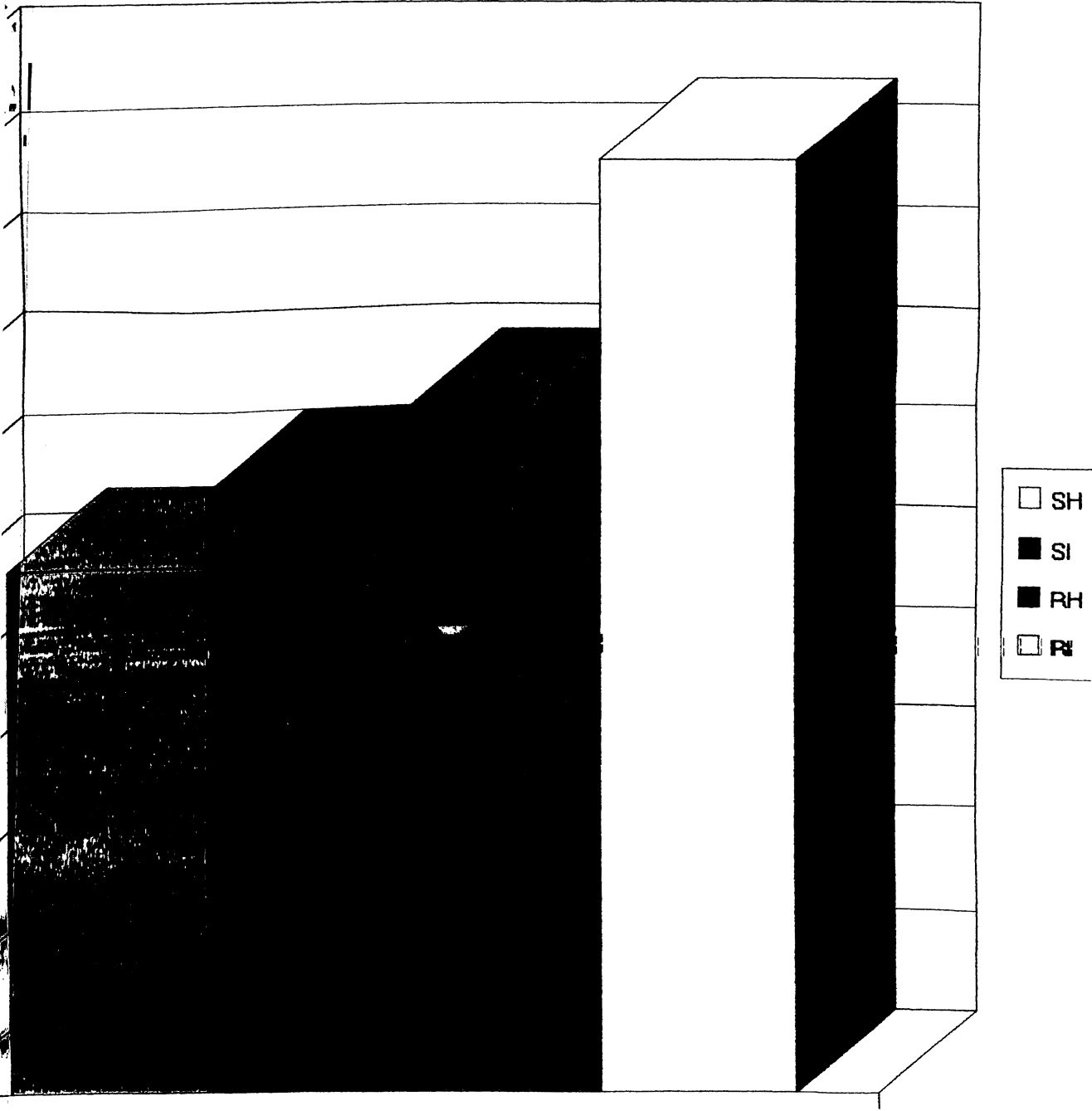
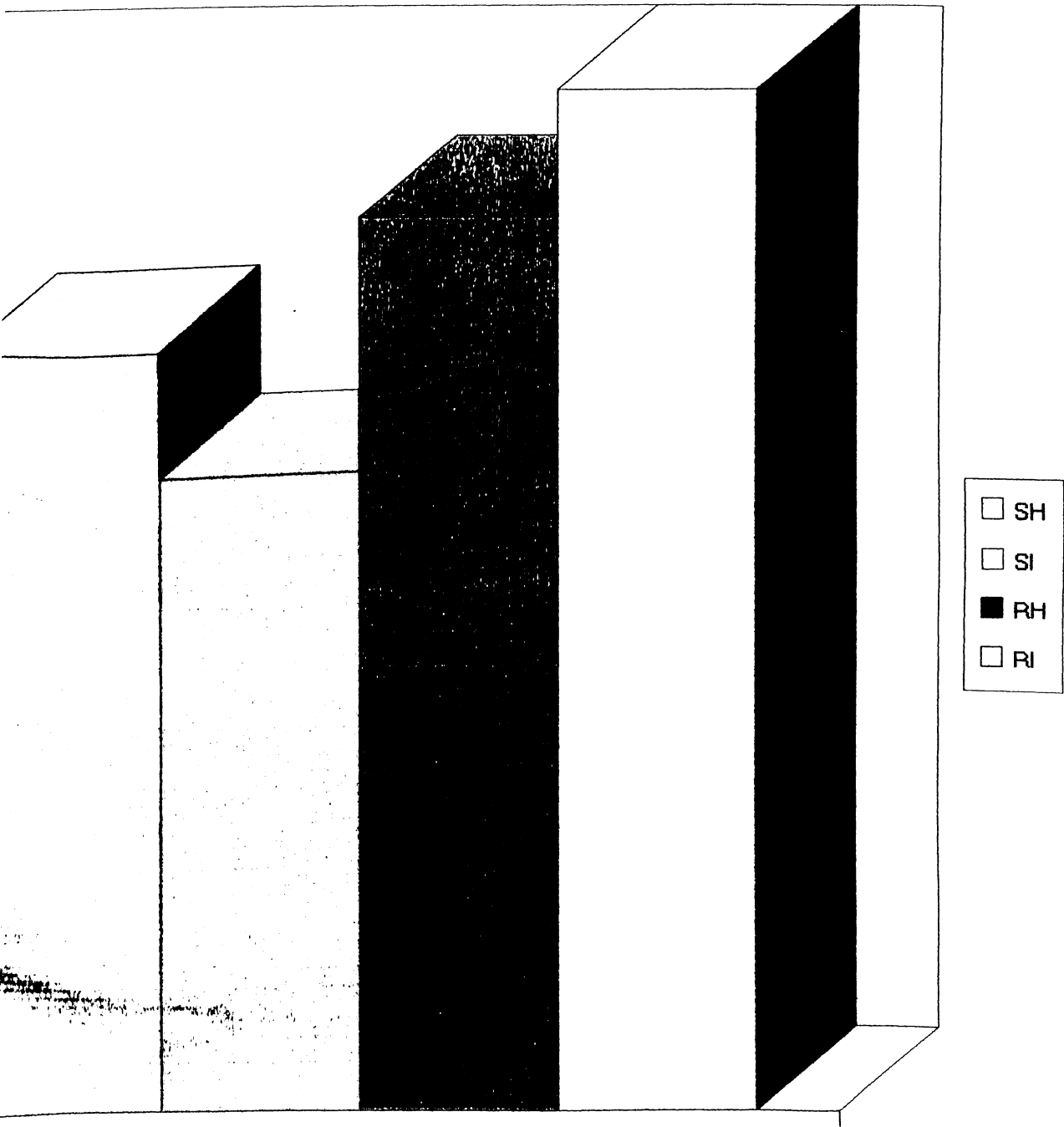


Fig.19

JENCE OF M.INCOGNITA ON ENZYME CATALASE IN BRINJAL
ROOTS



CHAPTER V

DISCUSSION

D I S C U S S I O N

Recent advances in molecular biology and genetic engineering have ushered in a new era equipping scientists with the power to tailor the biological system at will through recombinant DNA technology. This technology could be of immense utility in introducing pesticidal gene into root colonizing organisms including nematodes. In this context, full knowledge of nematode physiology, biochemistry along with its host is absolutely essential. Biochemical changes induced by plant parasitic nematodes relating to various crops have been well documented in a series of publications (Mohanty and Das, 1976; Farooqi, Ganguly and Dasgupta, 1980; Gommers, 1981; Mohanty, Ganguly and Dasgupta, 1988; Mote, Dasgupta and Ganguly, 1990; Sundararaj and Mehta, 1991). A series of biochemical and physiological reactions occur in plants in response to root knot nematode infection. As a result of which the plant is either overcome by the nematode and disease ensues, or the nematode is localised by the plant and disease development is limited. Detailed characterization of these biochemical and physiological processes is essential to advance our understanding of plant nematode interaction. This information would greatly aid plant

breeder and nematologists in breeding for cultivars resistant to root knot nematode. Keeping in view the importance of the subject the present investigation, therefore, undertaken to find the changes in amino acids, amides, phenolic compounds, total sugar, enzyme peroxidase and catalase activities in relation to resistant and susceptible brinjal cultivars inoculated with root-knot nematode, Meloidogyne incognita.

Results present in Table 7 revealed that five amino acids viz. L-cystine, L-serine, L-tryptophan, L-leucine and L-Isoleucine were found to be common in healthy and inoculated root samples of both the cultivars. However, in susceptible healthy, six amino acids detected were absent in infected samples but four new amino acids like L-tyrosine, L-methionine, L-proline and L-asparagine were developed. Similarly in resistant var, six aminoacids were found to be common but L-methionine disappeared and three new amino acids developed.

The results obtained in this investigation indicated increase in amount of the amino acid content during post infection period in both susceptible and resistant brinjal cultivar except L-tryptophan which

are in excellent agreement with the finding of previous researchers (Mohanty and Das, 1976; Farooqi, Ganguly and Dasgupta, 1980; Mohanty and Pradhan, 1990). This increase was presumably due to proteolysis of existing tissue protein or synthesis of new compound through various metabolic path ways by nematode.

In present study, more reduction of L-tryptophan in inoculated root of susceptible cultivar may have role in gall production as a precursor of DAA.

The accumulation of L-proline in inoculated of both the cultivar is considered to be significant. The more accumulation of proline in infected susceptible plant is in accordance with the observation of earlier workers (Lewis and Mc Clure, 1975). The ratio of proline and hydroxy proline play vital role in imparting susceptible and resistant reaction. Epstein, 1972; has observed an unique therapeutic quality of L-proline which is produced in Longidorus infected plants as a defence mechanism against infection. Disappearance of arginine in susceptible inoculated and resistant healthy may be due to conversion of L-arginine to L-proline through ornithine cycle.

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The presence of methionine in susceptible inoculated and resistant healthy samples appears to be very interesting. Methionine is widely involved in transmethylation reaction in the synthesis of methylated compounds like thiamin, methylated phenols, lignin and so on and play vital role in impacting resistance mechanism.

It is also interesting to note that some of the amino acids appeared new and also disappeared. This indicates possible inter conversion of the amino acid to other (Steward and Bid Well, 1962).

The presence of glutamic acid in susceptible variety and glycine in resistant variety appear to be interesting and significant, which may be linked with a distinct co-relationship between the degree of plant resistance and the above said amino acids present in the plant tissue.

The quantitative increase in phenolic compounds like chlorogenic acid in inoculated roots due to deamination of L-phenylalanine and L-tyrosine and there by providing the phenyl propane skeleton necessary for biosynthesis of aromatic compounds (Mote, Dasgupta and Ganguly, 1990).

There is an increase of total sugar in inoculated samples of both the cultivars. Infection by the nematode may alter the metabolism of tissue, so that respiratory substrates move towards the site of infection from elsewhere in the plant. Increased sugar content was also reported in tomato infected by Meloidogyne incognita (Farooqi et al. 1980) and in brinjal (Ganguly and Dasgupta, 1983).

Results presented in this investigation indicated an elevated level of peroxidase and catalase activities during post infection period both in susceptible and resistant brinjal cultivars. The increased level of peroxidase activities were reported by Ganguly and Dasgupta, 1980; Mohanty, Ganguly and Dasgupta, 1986; and Simte and Dasgupta, 1987; This increase in Peroxidase activity may be due to synthesis of new iso-enzymes as a response of nematode infection. Further super oxide radicals and siglet O_2 can be generated from NADH and hydrogen peroxide through the action of catalase and peroxidase respectively superoxide and siglet O_2 are toxic to living organisms and may play role in inhibiting pathogen development.

The present study thus provides some basic information relating to biochemical mechanism of resistance and host pathogen interaction which may be vital to the understanding of the actual mechanism of resistance. There are still many gray areas in understanding host defence mechanism against nematodes which needs special attention.

CHAPTER VI

SUMMARY

S U M M A R Y

Basic studies relating to biochemical mechanism of resistance in brinjal to the root knot nematode, Meloidogyne incognita were made to elucidate the biochemical basis of resistance in host to the nematode. Observations were made in the changes in various biochemical parameters like amino acids and amides, phenolic compounds, total sugar content, enzyme peroxidase and catalase activities in two varieties of brinjal following nematode inoculation. Among various amino acids detected, Five amino acids like L-cystine, L-serine, L-tryptophan, L-leucine and L-isoleucine were found to be common in susceptible (pusa purple long) and resistant (Ghatikia white) varieties. The results of quantitative study and intensity of ninhydrin positive spots on chromatograph indicated higher concentration of various amino acids and amides in both the varieties except L-tryptophan. The content of chlorogenic acid, total sugar and peroxidase activity were higher in inoculated samples than their healthy counter parts. But the values relating to chlorogenic acid and peroxidase activities for resistant variety were considerably higher than that for the corresponding samples of susceptible var. However, the catalase activities were reduced in inoculated susceptible samples.

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