# EFFECT OF ROOT-KNOT NEMATODE MELOIDOGYNE INCOGNITA ON ROOT BIOCHEMISTRY AND FUNCTIONING OF NODULES IN BLACKGRAM

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**( NEMATOLOGY )**

*BY*

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

This is to certify that the thesis entitled "Effect of root-knot nematode, Meloidogyne incognita on root biochemistry and functioning of nodules in blackgram" submitted for the degree of Master of Science in Agriculture (Nematology) of the Orissa University of Agriculture & Technology, Bhubaneswar is a faithful record of Bonafide and original research work carried out by Sri Biswajayee Mishra 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.

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## CERTIFICATE-II

This is to Certify that the thesis entitled "Effect of root-knot nematode, Meloidogyne incognita on root biochemistry and functioning of nodules in Blackgram" submitted by Sri Biswajayee Mishra 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 students advisory committee after an oral examination on the same in collaboration with an external examiner.

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Bhubaneswar

Date : 22nd July 1994.

Biswajayee Mishra

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# ABSTRACT

Comparative biochemical and physiological changes due toinfection of rootknot nematode Meloidogyne incognita was investigated in healthy, rhizobium inoculated, nematode inoculated and both rhizobium and nematode inoculated blackgram cv. T-9. Among various aminoacids detected six amino acids namely LL-tryptophan, L-isoleucine, L-threonine, L-serine, L-aspargine and L-aspartic acid were found to be common in all four treatments Higher concentrations of various amino acids and amides were detected in all the treatments upon nematode inoculation except L-tryptophan and L-aspargine by paper chromatographic and TLC scanning technique. Leghaemoglobin content and nitrogenase activity were decreased where as total sugar content was increased in both rhizobium and nematode inoculated nodules than rhizobium inoculated nodules. Higher total sugar content was detected in nematode inoculated root samples follwed by both rhizobium and nematode inoculated, rhizobium inoculated and healthy root samples. Chlorophyll contents were higher in rhizobium inoculated root sample followed by both rhizobium and nematode inoculated, healthy and nematode inoculated root samples.

# CHAPTER I

# INTRODUCTION

# INTRODUCTION

Pulses, the protein enriched (24%) crops, known as poorman's meat, are grown all over the world and rank second in area of cultivation after the cereals in India. Among the pulses, blackgram, Vigna mungo (L.) Hepper, a native crop of India, is grown in 6.6% of total acerage (2.4 million hectares) under pulses, occupying third position both in acerage and preference. But in Orissa blackgram (urad) occupies second position in terms of acerage (0.346 million hectares) and production (0.183 million tonnes/annum).

Being a leguminous crop, possessing root nodules, this fixes atmospheric nitrogen through symbiotic process and subsequently increases the fertility status of soil. The knowledge of bilogical nitrogen fixation at genetic, biochemical and physiological level has much expanded during recent years. Among the various obstacles that limit maximum nitrogen fixation, plant parasitic nematodes may induce stress condition at various stages of metabolic pathway leading to symbiotic nitrogen fixation in various legume crops.

The root-knot nematode, Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949 is considered as an important and prevelant pest of blackgram causing serious economic loss. The frequent occurance of this nematode in

blackgram fields have been reported from Orissa (Sarangi and Das, 1980) and its pathogenic relationship has been established (Mishra and Gaur, 1981) M.incognita not only attacks the roots but also interferes with the establishment and development of rhizobial nodules (Hussaini and Seshadri, 1975; Sharma and Sethi, 1976; Chahal and Chahal , 1989; Mohanty, 1992) which advessly affects the general vigour, growth and yield of the crop.

Comparative physiological and biochemical studies between healthy and infected nodules have advanced our understanding of legume host and rhizobial gene interaction in the symbiotic association.

Keeping in view, the importance of the subject, an attempt has been made to investigate whether and to what extent the root-knot nematode, M.incognita would interfere with various biochemical parameters like free amino acids, amides, total sugar content, chlorophyll content, leghaemoglobin content and nitrogenase activity relating to nodule formation and symbiotic nitrogen fixation in black gram variety 'T-9'.

CHAPTER II

# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

In any scientific investigation a comprehensive review of literature is essential, not only to provide a basis for development of theoritical frame work and an insght into the methods and procedures but also a basis for interpretation of findings. In this chapter an attempt has been made to present a brief review supporting the biochemical and physiological basis of biological nitrogen fixation particularly with relation to plant parasitic nematodes under the following headings :

A. Free amino acids

B. Bound amino acids

C. Biosynthesis and amino acid requirements

D. Amino acid antimetabolites

E. Amides

F. Leghaemoglobin and nitrogenase

G. Sugar

H. Chlorophyll

## A. FREE AMINO ACIDS

Krushberg (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, glycime, 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.

While analysing the contents of Heterodera rostochiensis cysts during maturation, Smith and Ellenby (1967) found the free amino acids like threonine, serine, proline, glycine, alanine, valine, methionine, aspartic acid, glutamic acid, isoleucine and leucine. The quantity of total amino acids decreased with advancing maturation.

In 1967, Asit and Riggs reported serine, glycine, alanine and two unidentified nihydrin positive spots from the incubation solution of Heterodera glycines larvae. They also identified serine, glycine, alanine, threonine and glutamine and six otherspots tentatively identified as lycine or ornithine, aspargine, leucine or isoleucine, tyrosine, methionine or valine, phenylalanine, as the free amino acids present in the homogenate of the same larvae.

Srivasttava (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 precentage of increase in the free amino acids.

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 decreased in highly susceptible ones. Protein content had an inverse relationship to 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 phenylalanine varied as functions 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 ( upto 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.

Singh et al. (1978) estimated total protein, free amino acids and proline in healthy and Meloidogyne incognita infected roots of Solanum melongena. They reported that quantities of total protein, free amino acids and proline in infested roots exceeded those in healthy roots; the protein by 21-45 %, amino acids by 75-233% and proline by 23-220% .

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 androots and free amino acids and soluble proteins of roots. Free amino acids and soluble proteins of leaves decreased compared to controls.

Nasr et al. (1980) analysed the effect of root-knot nematodes on the amino acid concentration of almond and

peach root stocks 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 from 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 following inoculation of Citrullus vulgaris var. Chilian black with M. incognita, Fusarium oxysporum F. niverum 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.

Mahmood (1983) reported that amino contents of different tomato and eggplant cultivars was higher in those inoculated with Rotylenchulus reniformis than in uninoculated controls. He also found that amino acid content increased with increased inoculum level, and the eggplant and tomato cultivars in which the concentration of amino acid was low, supported the multiplication of nematode appreciably while those cultivars with a higher concentrations of amino acids did not support multiplication of nematode. The results suggested that amino acids affected the resistance/tolerance/susceptibility of the plant to nematode.

Okie et al. (1984) studied the effect of the ring nematode, Criconemella 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 propertions 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 excretions of a surface sterilised migratory endoparasitic nematode species, Pratylenchus coffeae by paper chromatographic technique and indiacted 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.

Verma 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, aspattic acid, glycine, half cystine, histidine, isoleucine, lysine, methionine, proline, threonine, tyrosine and valine were positively correlated. In HW 517, alanine, isoleucine, leucine, serine and valine were negatively correlated while arginine, aspartic acid, glycine, half cystine, histidine, lysine, methionine, phenylalanine, proline, threonine and tyrosine were posittively correlated.

In 1987, Sarna and Trivedi reported that Meloidogyne incognita infection at seedling stage increased the proline, protein and free aminoacids content in Cicer arietinum plants.

Zaki and Bhatti (1987) studied the amino acid content in Heterodera cajani infected pigeon pea. He found that all amino acids showed reduced levels in infected plants except for aspartic acid which increased both in shoots and roots and alanine, glycine and serine which increased in the root only.

Qualitative determination of free amino acids in blackgram roots infected with root-knot nematode, M. incognita, under pot culture conditions, has been demostrated 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-aspargine, L-serine, glycine, L-threonine, L-tryptophan, L-phenylalanine and isoleucine where as in infected blackgram root extracts, L-glutamic acid was absent but four more amino acids namely L-cystine, L-lysine, L-proline and 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 palnts. 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, had 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-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. silvestris f. campestris (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 role in

the "-" variants. Changes in the concentrations of the 14 other amino acids had no influence on nematode development.

## 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 infested with Meloidogyne.

Okopnyi (1980) in his experiment of 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 level were significatly 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 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 and 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 acitive 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, tyrosine, valine, leucine, isoleucine, lysine and histidine by feeding them with $^{14}C$ lablled amino acids.

Balasubramanium and Myers (1971) determined by labelling and deletion method, the amino acid requirements of Aphelenchoides sp. The absolute dietary required amino acidswere 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 namatode, hence they are not required to be supplemented in the diet.

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

Jackson (1973) reported that in addition to 9 amino acids lysine, tryptophan, histidine, leucine, isoleucine, phenylalanine, threoine, 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-$B_{12}$ and folic acid ; methionine could be substituted by homocystein, thereby suggesting the presence of a mechanism that biosynthesize methionine from homocystein as substrate.

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 occured in L-leucine, closely followed by DL-tryptophan, L-cystine, L-lysinehydrochloride, DL-glyine 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 inpact 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, and 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 acitvity 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 mehionine 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, treatment 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-phenylalanine 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 of 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 cystine affected gall

formation erratically. Most treatments significantly reduced rhizobium nodulation except phyenylalanine 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 1000 ug/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 .

## E. AMIDES

Myers and Krusberg (1965) studied 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, aspargine 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, aspargine, alanine, glutamic acid, serine, histidine and arginine.

Sharma and Tiyagi (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. LEGHAEMOGLOBIN AND NITROGENASE

Sharma and Sethi (1975) studied the effect of Meloidogyne incognita and Heterodera cajani on leghaemoglobin content of cowpea nodules and reported that the nematode decreased the leghaemoglobin content of the cowpea root nodules with M. incognita causing more reduction than H. cajani.

The works of Chahal and Chahal (1987) in Meloidogyne incognita on the nodulation of mung bean ( Vigna radiata ) cv. G-65 revealed that functioning of nodules was adversely affected by nematode infection which could be attributed to

reduction in bacteroids, leghaemoglobin contents of nodules and reduced supply of photosynthate to the nodules.

Verdejo et al. ( 1988) studied the influence of Meloidogyne incognita on nodulation and growth of pea and black bean. They reported that effective nodules had more nitrogenase activity/g on plants with M. incognita than on those without nematodes. They also found that M. incognita increased the leghaemoglobin content of nodules of pisum and decreased it on phaseoli.

Chahal et al. (1988) studied the effect of different inoculum levels of Meloidogyne incognita and Rhizobium strain R-5 on nitrogen fixation in Vigna mungo. They found that functioning of nodules was disturbed by the nematodes due to reduction in leghaemoglobin and bacterial contents.

Chahal and Chahal (1988) reported that different population levels of Meloidogyne incognita on chick pea (Cicer arietinum) showed adverse effect on the functioning of nodules as reflected by reduced nitrogenase activity which might be due to a reduction in leghaemoglobin and bacteriod contents of nodules.

Sharma and Tiyagi (1988) estimated the leghaemoglobin content of pea nodules at 30, 60 and 90 days after inoculation with Meloidogyne incognita and found that maximum reduction occured in 30-days-old nodules.

Caroppo and Pelagatti (1988) stated that Meloidogyne inocgnita infection on soyabenas inoculated with Bradyrhizobium japonicum did not induce significant alterations in number and weight of root nodules or on nitrogenase activity.

In 1989, Chahal and Chahal investigated the adverse effect of Meloidogyne incognita on the functioning of nodules produced by Rhizobium spp. on mung bean. They concluded that the functioning of the nodules to fix dinitrogen was adversely affected due to nematode infection as reflected by reduced leghaemoglobin, bacteroid contents and nitrogenase activity of nodules.

Lynd and Ansman (1989) reported that in siratro (Macroptilium atropurpureum) root nodule infected with several Meloidogyne spp. , nitrogenase activity significantly increased compared with nematode free palnts.

Mohanty (1992) investigated that the functioning of nodules of green gram was adversely affected by Meloidogyne incognita infection which could be attributed to reduction in leghaemoglobin content and nitrogenase activity of nodules.

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, arthodihydroxy phenols and total sugars and reducing sugars were present in the diseased leaves in comparison to healthy ones.

Singh et al. (1978) besides other factors, estimated the reducing sugar and starch content in healthy and Meloidogyne incognita infected roots of brinjal 30, 60 and 90 days after inoculation with nematodes and found that reducing sugars were decreased by 30 to 55% in infected roots. Starch content in infested root was less at 30 and 60 days but 12.5% more at 90 days.

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 efect of Meloidogyne incognita on some biochemical parameters in brinjal plants and indicated low contents of starch, nonreducing sugar, total sugar and high level of reducting plants.

Ganguly and Dasgupta (1983) inivestigated 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 rootknot nematode, Meloidogyne incognita reported the decrease level of nonreducing and total sugars with increase level of reducing sugar in infected plant tissue.

Melakeberhan 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 nonreducing sugars increased in french colombard and decreased in thompson seedless.

Das (1991) studied the effect of Meloidogyne incognita on total sugar content of the roots as well as nodules of french bean. He reported that the infection by the nematode interfered with nitrogen fixation and increased the total sugar content in both roots and nodules of french bean.

## H. CHLOROPHYLL

Singh et al. (1977) studied the effect of Meloidogyne incognita in Phaseolus aureus grown with or without nitrogen

fixing bacterium Rhizobium phaseoli. They reported that after 45 days growth the total chlorophyll content showed significant interaction between Rhizobium and nematode inoculation and at harvest there was no significant interaction.

Melakeberhan et al. (1985) reported that total chlorophyll and chlorophyll-a content in Phaseolus vulgaris plants infected with different levels of Meloidogyne incognita, decreased significantly with increased levels of infestation when harvested 3 weeks after inoculation.

In 1985, Vaishnav et al. observed in Arachis hypogaea plants inoculated with Meloidogyne arenaria that chlorophyll content reduced singnificantly after 60 days compared with the controls.

Chahal and Chahal (1987) studied effect of different levels of Meloidogyne incognita on Vigna radiata cv. G-65 and found that nematode caused reduction in the chlorophyll content of leaves as well as adversely affected the functioning of nodules.

Swain and Prasad (1986) estimated the chlorophyll content of different varieties of rice infected with Meloidogyne graminicola. They found that chlorophyll a and b

fractions decreased in the susceptible variety and increased in the resistant variety.

In 1988, Nagesh and Dhawan studied the effect of different inoculum levels of Heterodera avenae on wheat and observed that negative correlation existed between inoculum levels of nematode and chlorophyll content of infected plants.

Al-Sabie et al. (1989) observed the influence of Meloidogyne javanica on the quantity of photosynthetic pigments in 2 tomato varieties (susceptible and resistant to Meloidogyne) at 2 periods after inoculation with 0, 10, 100, 1000 and 10,000 $J_2$/pot. The results indicated that 10and 100 $J_2$ /pot increased the quantity on photosynthetic pigments in susceptible variety 35 days after inoculation, while after 70 days the pigments were higher only in plants inoculated at 10 $J_2$/ pot. The results also showed that the quantity of photosynthetic pigments increased in the resistant variety with all levels of inoculum but the rate of increase after 35 days from the time of inoculation was greater than that after 70 days.

Tiyagi and Alam (1990) compared the effect of Meloidogyne incognita, Rotylenchulus reniformis and Tylenchorhynchus brassicae on chick peas at different inoculum, levels. They found that there was a significant

reduction in chlorophyll content of plants even at low inoculum level in all cases. They also observed that M. incognita caused the most damage.

Haseeb et al. (1990) determined chlorophyll content in Hyoscyamus niger at 90 days after inoculation with Meloidogyne incognita and found that with increase in inoculum level, there was a corresponding decrease in photosynthetic rate and total chlorophyll content.

In 1990, Pathak et al. observed an increase in leaf chlorophyll 'a' and 'b' at 45 and 90 days in wheat cv. HD-1533 infected with Anguina tritici. They also found that the chlorophyll 'a' fraction of the leaf showed a decrease at initial stage (20 days) and later on the accelerated growth of infected plants was correlated with increased percentage of chlorophyll.

# CHAPTER III

# MATERIALS AND METHODS

# MATERIALS & 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 tomato (Lycopersicon esculentum cultivar Pusa Ruby) The tomato 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 tomato 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 sypply 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 12 cm height x 12 cm dia were surface sterilized with formaldehyde solution (1:1000, V/V) and then

were filled with soil which had already been autoclaved at 15 lbs pressure for 20 minutes.

Blackgram Vigna Mungo (L.) Hepper cultivar T-9 obtained from State Seeds Corporation, Bhubaneswar, Orissa, was used in this study. Blackgram cultivar T-9 is a susceptible host for Meloidogyne incognita. One hundred gram of seeds were surface sterilized by immersing these in 0.1% $HgCl_2$ solution for about 5 minutes followed by repeated washing with distilled water.

## D. INOCULATION OF RHIZOBIUM CULTURE (Rhizobium phaseoli)

The culture, used for this work, was collected from the Regional Biofertilizer Development Centre, Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India, Sahid Nagar, Bhubaneswar. One gram of rhizobium culture was suspended in 2 ml of distilled water in a beaker and mixed the contents thoroughly by shaking for 2 minutes to obtain a thick black paste. Fifty grams of surface sterilized blackgram seeds were kept on a clean paper. The rhizobium culture paste were poured slowly on the seeds and mixed then by the hand till an uniform coating was obtained on each and every seed. The seeds were then spread for drying under shade.

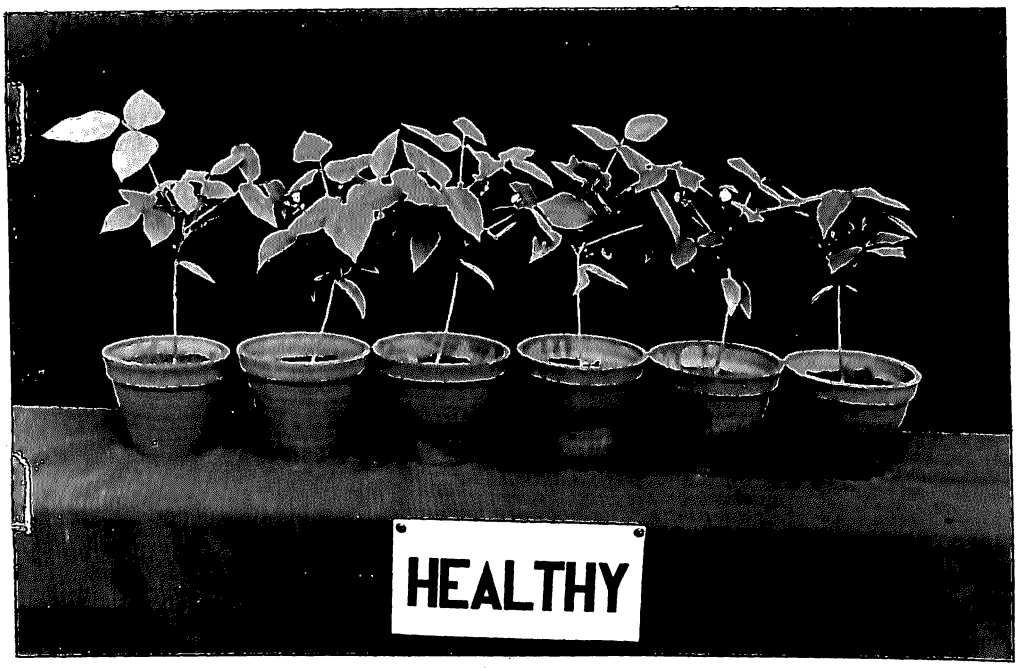


Fig.1: Healthy blackgram experimental plants.

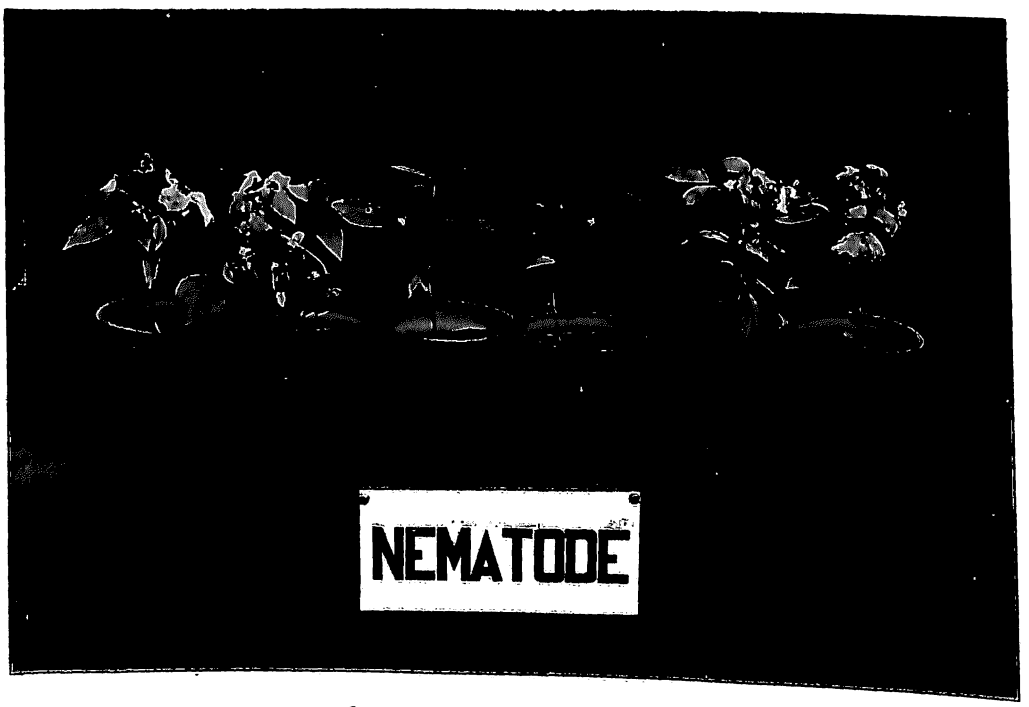


Fig.2: Nematode - inoculated blackgram experimental plants.

## E. RAISING OF PLANTS

Rhizobium treated as well as untreated seeds were sown separately in different pots containing soil which was also sterilized. The pots were thinned leaving one seedling per pot. Cultural operations like watering and weeding were attended to as a routine. Procedure for collection, storage an examination of second stage larvae were the same as described previously (Desgupta and Ganguly, 1975).

## F. 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.

Once week old stedlings of both rhizobium treated and untreated seeds were inoculated with axenised suspension of approximately 1,000 infective larvae of Meloidogyne incognita per seedling. Two sets of plants were left uninoculated out of which one set relates to competely healthy (Fig. 1) and another set relates to rhizobium treatment (Fig. 3) . The plant were maintained in the departmental net house under controlled environmental conditions.

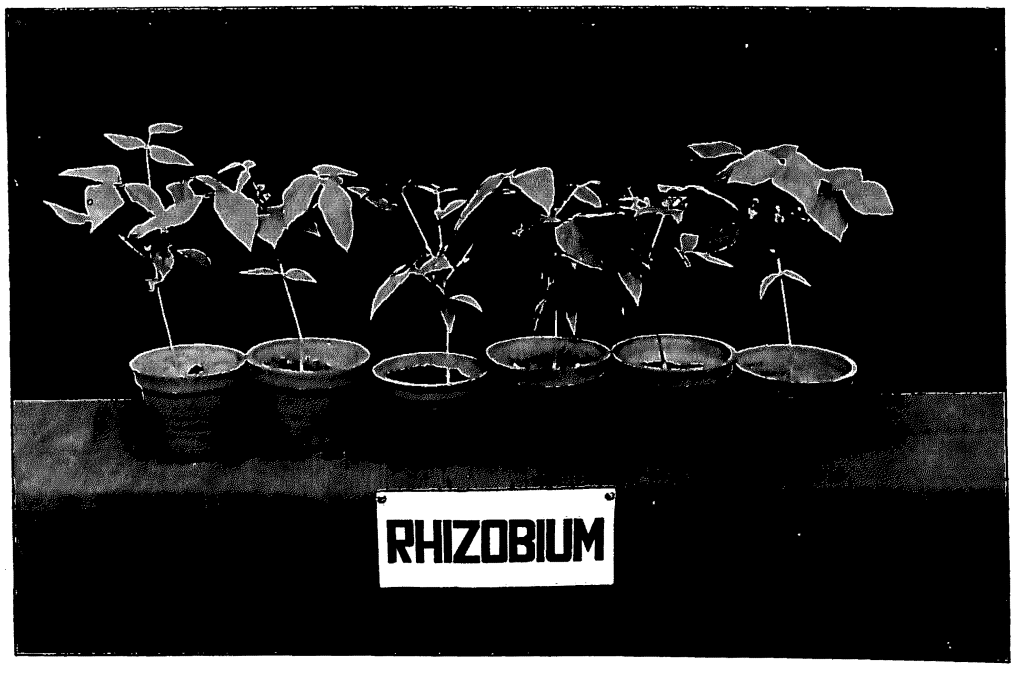


Fig.3: Rhizobium - inoculated blackgram experimental plants.


Fig.4: Rhizobium and nematode inoculated blackgram experimental plants.

## C. HARVESTING AND CLEANING OF THE PLANTS FOR ANALYSIS

Plants of various treatments were harvested at 30 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 ditilled water. The harvested roots were blotted and kept separately for biochemical analysis. The same method was also adopted for nodules.

## H. PREPARATION OF TEST SAMPLE FOR ANALYSIS OF AMINO ACIDS AND AMIDES.

Five grams of roots of various treatments were taken, ground separtely with 80 % ethyl alcohal added at the rate of 20 ml for each sample in a sterile mortar and pestle till the roots were macerated to pulp. These meterials after grinding were boiled for 5 to 10 minutes in a hot water bath. After boiling, the alcohalic root 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 surupy material was dissolved in 1 ml of 10% isopyopul 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.

## I. TECHNIQUES FOR PAPER CHROMATOGRAPHY

The Whatman NO.1 chromatographic paper of size 22.5 cm x 22.5cm were used for paper chromatography. It was marked and spotted with the test sample which has 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$\mu$ capacity. The size of the spot was tried to be restricted to the smllest 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 the sample was repeated 5 times on the same point after completely drying the previous spot.

For amino acids and amides two dimensional ascending paper chromatographic tenchnique 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 repectively. 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 (15cm 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 (32.5cm x30cm). The space between its rim and the glass plate was sealed with pertoleum 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 12 hours under fan to allow evaporation of n-butanol-acetic acid-water solvent. The chromatogram was again folded in the

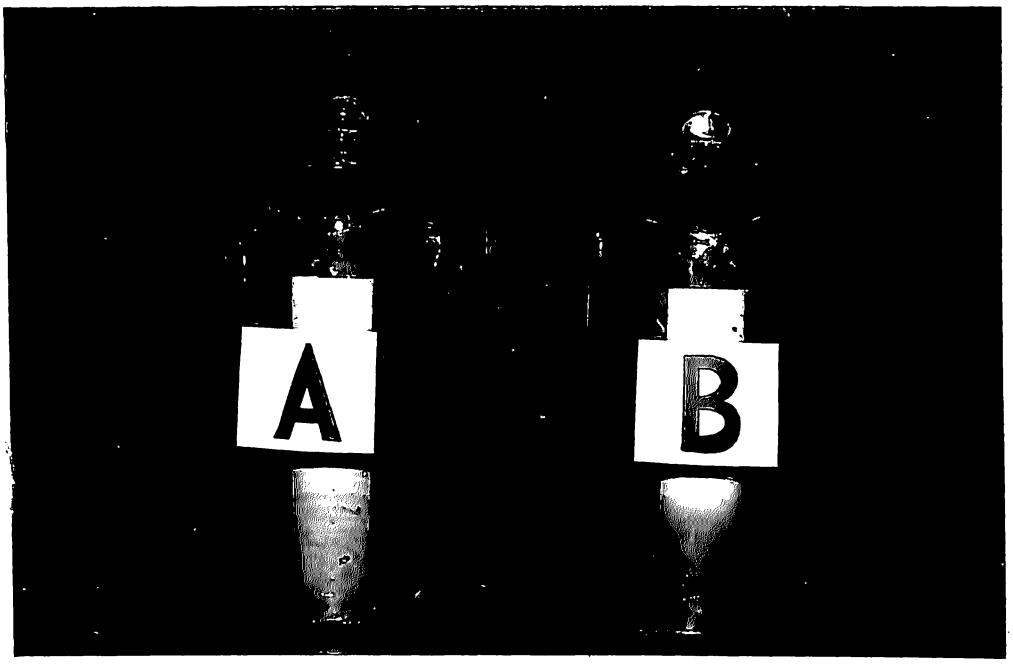


Fig.5: Chromatographic process in progress.

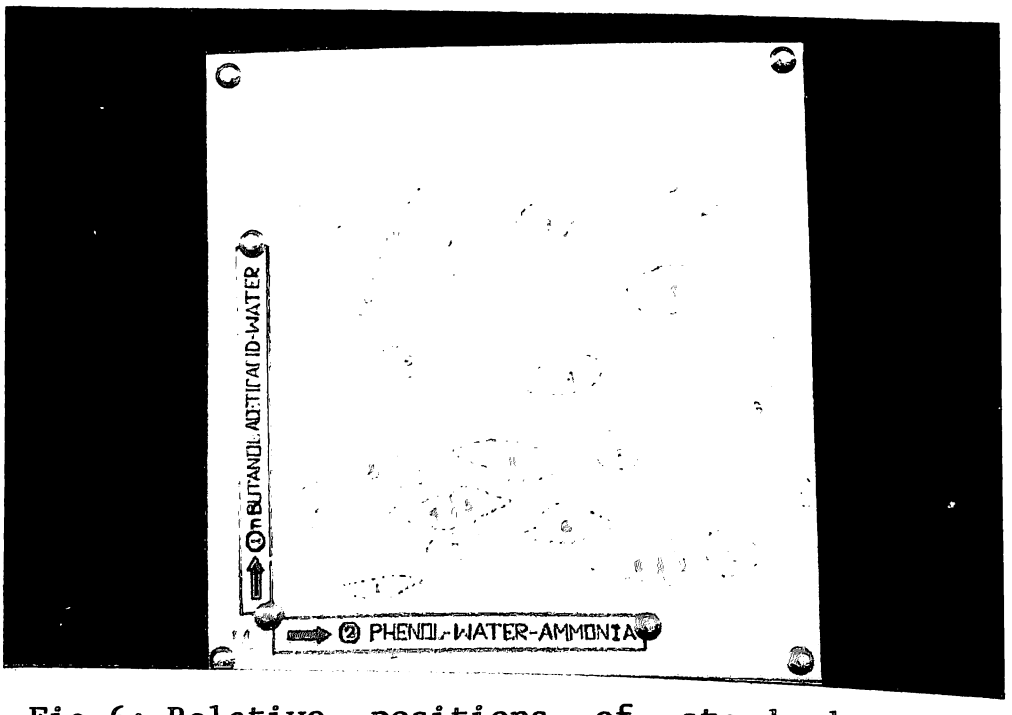


Fig.6: Relative positions of standard amino acids and amides.

distinctly visible on the chromatogram. Immediately after detecting the amino acid 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 Rf values.

The identification of free amino acids and amides were done by preparing standard chromatograms. Eighteen amino acids and two amides were used for the preparation of standard maps. Amino acids and amides were spotted in 2 chromatograph papers containing ten each. The amino acids were L-cystine, L-threonine, L-serine, L-lysine, L-histidine, L-arginine, L-glycine, 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-aspargine.

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-cystine, 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 dissovle them. With a view to concentrate the solutions, the volume of each was reduced from 20 ml to 10ml by evaporating the isopropyl alcohol. To prepare a mixture of the eighteen amino acids and

two amides, 0.2ml from each stock solution was pipetted out and mixed in a glassvial . 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. More over, 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 dimention 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 cromatogram. 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 indentify the free amino acids and amides developed on the chromatograms with unknown root extract 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.

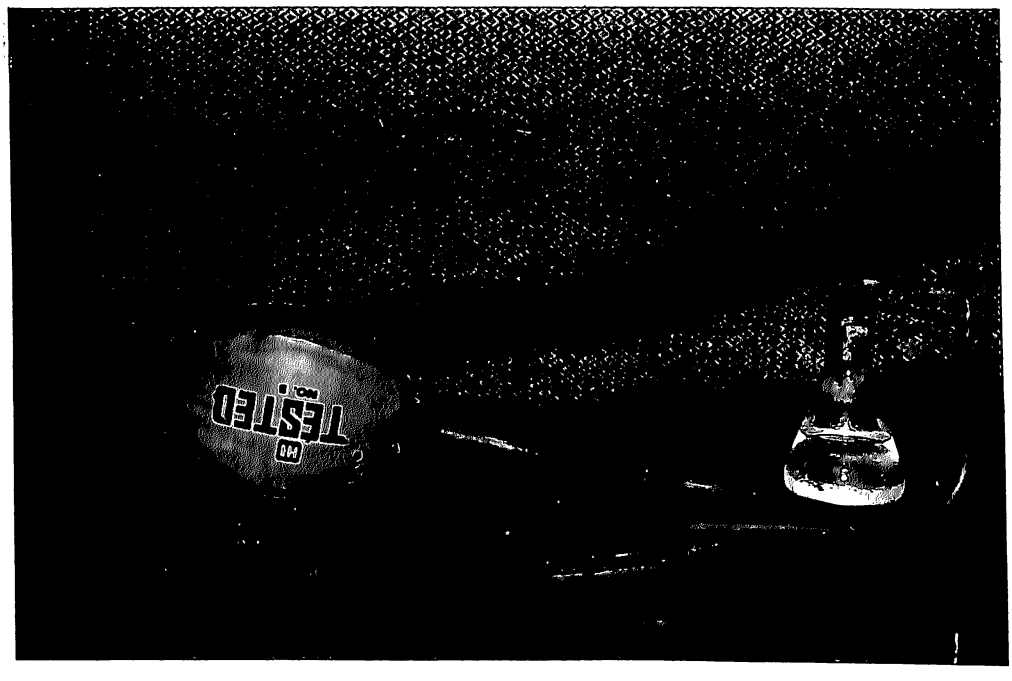


Fig.7: Acetylene generation process in progress.

## J. QUANTITATIVE ESTIMATION

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

## K. DETERMINATION OF NITROGENASE ACTIVITY OF NODULES

Fresh nodules weighing 0.2g were taken for nitrogenase assay. Nitrogenase assay of intact nodules were determined using acetylene reduction technique of Hardy et al. (1968). The method is based on competitive inhibition of $N_2$ fixation by acetylene ($C_2H_2$) in a reaction analogous to the reduction of $N_2$ to $NH_3$. Ethylene ($C_2H_4$)produced were maeasured by gas chromatography.

The assays were carried out in 65 ml capacity gas tight vials fitted with rubber septum stoppers. The gas phase (air) of $10cm^3$ were removed from the vials and replaced with and equal amount of $C_2H_2$ produced by reacting calcium carbide with water in a gas generator. The vials were then incubated for half an hour at room temperature. After incubation, gas samples were drawn with a disposable air tight syringe and

injected into gas chromatograph. $C_2H_2$ produced was worked out by comparing the G.C unit of the sample with that of standard. The results were expressed as micro mole $C_2H_2$ reduced $g^{-1}$ fresh weight of nodule $h^{-1}$ .

## L. DETERMINATION OF LEGHAEMOGLOBIN CONTENT OF NODULES

Leghaemoglobin content was determined following the method of Wilson and Reisenauer (1963).

Fresh nodule tissue weighing 0.5g was crushed and ground in a 10 ml round bottom centrifuge tube in 3ml of Drabkin's solution prepared by dissolving 52mg of potassium cyanide, 198mg of potassium ferricyanide and 1.0g of sodium bicarbonate per litre of distilled water. The homogenate was then contrifuged for 15 minurtes at 500 x g and the supernatant was transferred to a 10ml volumetric flask. The pelleted nodule tissue was extracted twice more with 3ml of Drabkin's solution and the supernatants obtained were combined. The combined extracts were then made to 10ml with Drabkin's solution. The mixture was crntrifuged once again at 20000 x g for 30 minutes. The absorbance of the clear supernatant was read at 540 nm. Drabkin's solution served as blank. The amount of leghaemoglobin present was calculated from a calibration curve prepared with known concentration of cyanmethaemoglobin andthe resultswere expressedasmg $g^{-1}$ fresh weight of noudle.

## M. ESTIMATION OF TOTAL SUGARS.

Fresh roots (1g) and nodules (0.5g) of various treatments were homogenised separately in 80% ethanol and the volume made up to 10ml with 80% 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 supernatant (0.2ml) was taken in a test tube and to this 1ml of phenol reagent and 5ml of concentrated sulphuric acid were added and mixed thoroughly. The absorbance was measured at 490mm after allowing 20 minutes for colour development. The amount of sugar was calculated from a calibration curve prepared with glucose and expreseed as mg sugar (glucose equivalent) $g^{-1}$fresh weight of roots or nodules.

## N. ESTIMATION OF CHLOROPHYLL

Chlorophyll - a, chlorophyll-b and total chlorophyll of leaves were determined by using acetone extraction method given by Arnon (1949).

Fresh leaf weight 250mg from each treatment was macerated thoroughly in a glass mortar and pestle with a

small quantitiy of 80 % acetone to get a fine pulp. The pulp was filtered to a 25ml volumetric flask through whatman No.1 filter paper. The residue was washed twice and the final volume was made upto 25ml with 80% acetone. Then the absorbance of the extract was recorded at 645 nm and 663nm in a spectrophotometer. The contents of chlorophyll a, chlorophyll-b and total chlorophyll were calculated using the formulae and expressed in mg $g^{-1}$fresh weight of leaf.

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# CHAPTER IV

# EXPERIMENTAL FINDINGS

# EXPERIMENTAL FINDINGS

The qualitative analysis of free amino acids and amides present in the root extracts of black gram plants, as influenced by Meloidogyne incognita has been done by following chromatographic technique, and the ninhydrin positive spots on the chro-matogram were identified mainly on the basis of their relative positions on the chromatogram, the colour reactions with spray reagents and the Rf values as compared to those of the known aminoacids and amides in standard chromatograms.

Rf value.

When the solvent front is not allowed to travel off the paper, the Rf value is defined as:

$$Rf = \frac{\text{distance travelled by the substance from origin}}{\text{distance travelled by the solvent front from origin}}$$

The Rf value obtained in this way is a fraction, so it has been conveniently expressed in percentage. Furthermore, the Rf value is an arbitary value, hence for identification of coloured spots of test sample, the basic configuration and colour of the spots on standard chromatograms were also considered.

## A. Rf VALUES OF STANDARD AMINOACIDS 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 devided by the distance, the solvent front had travelled and the Rf values were calculated in percentage as presented in Table 1.

## B. Rf VALUES OF STANDARD AMINOACIDS AND AMIDES IN MIXTURE

A mixture of the above 18 amino acids and 2 amides was spotted and separated by two dimensional chromatography. The Rf values of individual aminoacid and amide spots were calculated against n-butanal-acetic acid-water front. Subsequently the amino acids and amides were identified by comparing their colour reaction and the Rf values with those of individual amino acids and amides and the observation was recorded in Table 2 and Fig. 6.

## C. IDENTIFICATION OF AMINOACIDS AND AMIDES IN VARIOUS TREATMENTS OF BLACKGRAM ROOT EXTRACT CHROMATOGRAMS

The Rf values of ninhydrin positive spots on the chromatograms of different test samples (Fig.8, Fig.9, Fig.10, and Fig. 11) have been calculated and the free amino acids and amides which were identified by comparing these Rf values, colour offered by the reagent and the configuration

Table 1 The Rf of values of standard aminoacids and amides (1-D, Chromatography)

| le No. ts | Name of the aminoacids and amides | Rf values in percentage of |
|---|---|---|
| | L-Cystine | 1.4/22.0=0.06x100=6 |
| | L-glutamic acid | 5.5/22.5=0.24x100=24 |
| | L-threonine | 6.0/22.0=0.27x100=27 |
| | L-aspartic acid | 4.5/22.0=0.20x100=20 |
| | L-serine | 4.0/22.5=0.17x100=17 |
| | L-glutamine | 3.5/22.5=0.16x100=16 |
| | L-aspargine | 2.7/22.0=0.12x100=12 |
| | L-lysine | 2.3/22.5=1.10x100=10 |
| | L-histidine | 2.1/22.5=0.09x100= 9 |
| | L-agrinine | 2.9/22.5=0.13x100=13 |
| | L-glycine | 4.8/22.5=0.21x100=21 |
| | L-alanine | 7.3/22.0=0.33x100=33 |
| | L-proline | 8.0/22.5=0.36x100=36 |
| | L-tryptophan | 11.3/22.5=0.5 x100=50 |
| | L-tyrosine | 9.3/22.5=0.41x100=41 |
| | L-methionine | 12.5/22.0=0.57x100=57 |
| | L-valine | 14.0/22.0=0.64x100=64 |
| | L-leucine | 16.5/22.5=0.73x100=73 |
| | L-phenylalnine | 13.5/22.5=0.60x100=60 |
| | L-isoleucine | 17.3/22.5=0.77x100=77 |

* Marks indicate amides

able 2. The Rf values of ninhydrin positive spots separate from a mixture of standard aminoacids and amides (2-D chromatography)Fig.- 6

| ode No. of spots | Rf values in Percentage | Name of the amino acids/amides |
|---|---|---|
| 1. | 1.4/22.5=0.06x100=6 | L-cystine |
| 2. | 5.3/22.5=0.24x100=24 | L-glutamic acid |
| 3. | 6.0/22.5=0.27x100=27 | L-threonine |
| 4. | 4.3/22.5=0.19x100=19 | L-aspartic acid |
| 5. | 4.1/22.5=0.18x100=18 | L-serine |
| 6. | 3.7/22.5=0.16x100=16 | L-glutamine |
| 7. | 2.6/22.5=0.12x100=12 | L-aspargine |
| 8. | 2.3/22.5=0.10x100=10 | L-lysine |
| 9. | 2.1/22.5=0.09x100= 9 | L-histidine |
| 10. | 2.9/22.5=0.13x100=13 | L-arginine |
| 11. | 4.8/22.5=0.21x100=21 | L-glycine |
| 12. | 7.4/22.5=0.33x100=33 | L-alanine |
| 13. | 8.2/22.5=o.36x100=36 | L-proline |
| 14. | 11.5/22.5=0.51x100=51 | L-tryptophan |
| 15. | 9.5/22.5=0.42x100=42 | L-tyrosine |
| 16. | 12.6/22.5=0.56x100=56 | L-methionine |
| 17. | 14.4/22.5=0.64x100=64 | L-valine |
| 18. | 16.3/22.5=0.72x100=72 | L-leucine |
| 19. | 13.7/22.5=0.61x100=61 | L-phenylalanine |
| 20. | 17.1/22.5=0.76x100=76 | L-isoleucine |

* Marks indicate amides.

Table 2. The Rf values of ninhydrin positive spots separate from a mixture of standard aminoacids and amides (2-D chromatography)Fig.- 6

| Code No. of spots | Rf values in Percentage | Name of the amino acids/amides |
|---|---|---|
| 1. | 1.4/22.5=0.06x100=6 | L-cystine |
| 2. | 5.3/22.5=0.24x100=24 | L-glutamic acid |
| 3. | 6.0/22.5=0.27x100=27 | L-threonine |
| 4. | 4.3/22.5=0.19x100=19 | L-aspartic acid |
| 5. | 4.1/22.5=0.18x100=18 | L-serine |
| *6. | 3.7/22.5=0.16x100=16 | L-glutamine |
| *7. | 2.6/22.5=0.12x100=12 | L-aspargine |
| 8. | 2.3/22.5=0.10x100=10 | L-lysine |
| 9. | 2.1/22.5=0.09x100= 9 | L-histidine |
| 10. | 2.9/22.5=0.13x100=13 | L-arginine |
| 11. | 4.8/22.5=0.21x100=21 | L-glycine |
| 12. | 7.4/22.5=0.33x100=33 | L-alanine |
| 13. | 8.2/22.5=o.36x100=36 | L-proline |
| 14. | 11.5/22.5=0.51x100=51 | L-tryptophan |
| 15. | 9.5/22.5=0.42x100=42 | L-tyrosine |
| 16. | 12.6/22.5=0.56x100=56 | L-methionine |
| 17. | 14.4/22.5=0.64x100=64 | L-valine |
| 18. | 16.3/22.5=0.72x100=72 | L-leucine |
| 19. | 13.7/22.5=0.61x100=61 | L-phenylalanine |
| 20. | 17.1/22.5=0.76x100=76 | L-isoleucine |

* Marks indicate amides.

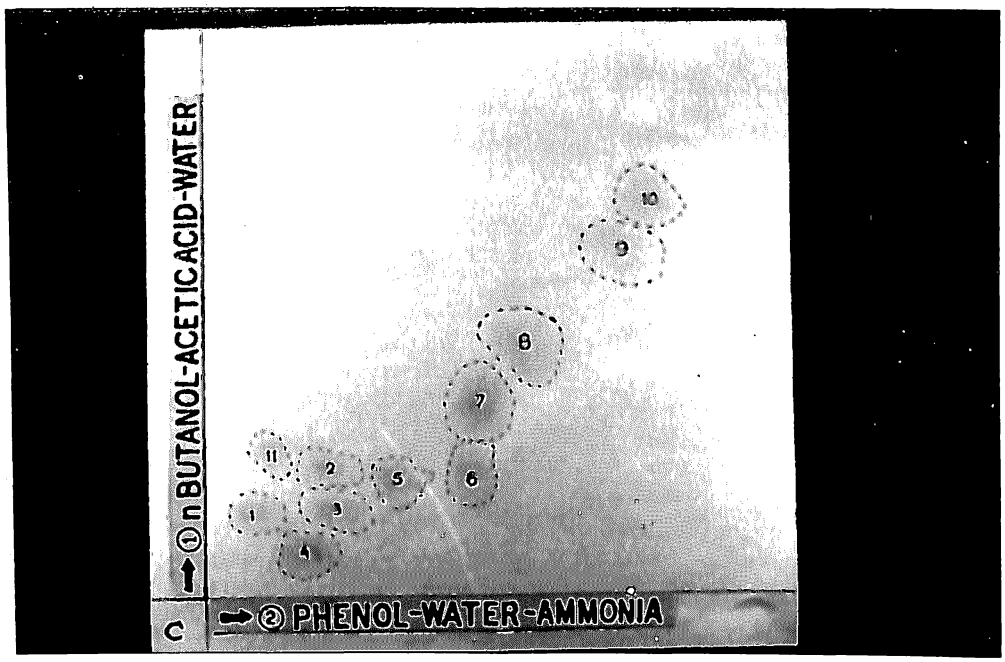



Fig.8: Relative positions of aminoacids and amide in healthy root extracts.

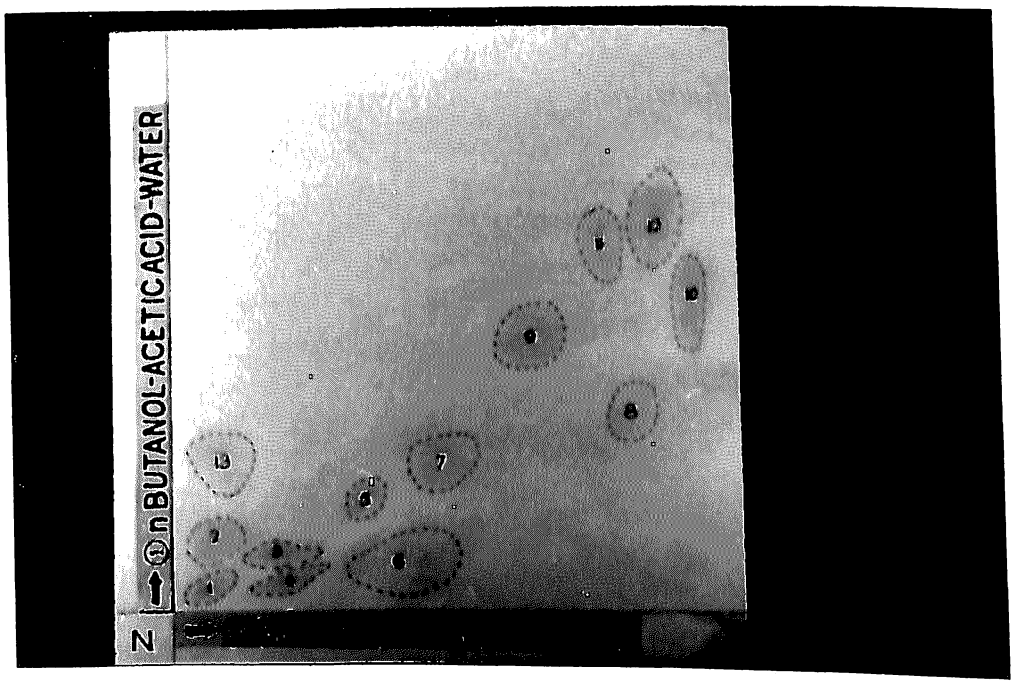



Fig.9: Relative positions of aminoacids and amides in nematode inoculated root extracts.

Table 3. The Rf values of minhydrin positive spots on the chromatogram of healthy blackgram root extact and the aminoacids/amides identified (2-D chromatography) (Fig.8)

| Code No. of spot | Rf values in percentage | Name of the amino acid/ amide |
|---|---|---|
| 1. | 4.3/22.5=0.19x100=19 | L-aspartic acid |
| 2. | 5.3/22.5=0.24x100=24 | L-glutamic acid |
| 3. | 4.1/22.5=0.18x100=18 | L-serine |
| 4. | 2.6/22.5=0.12x100=12 | L-aspargine * |
| 5. | 4.8/22.5=0.21x100=21 | L-glycine |
| 6. | 6.0/22.5=0.27x100=27 | L-threonine |
| 7. | 7.4/22.5=0.33x100=33 | L-alanine |
| 8. | 11.5/22.5=0.51x100=51 | L-tryptophan |
| 9. | 14.4/22.5=0.64x100=64 | L-valine |
| 10. | 17.1/22.5=0.76x100=76 | L-isoleucine |
| 11. | 6.9/22.5=0.30x100=30 | Unidentified. |

* Mark indicates amide

Table 4. The Rf values of ninhydrin positive spots on the chromatogram of nematode inoculated blackgram root extract and the aminoacid/amides identified (2-D chromatography) (Fig.9.)

| ode No. of spot | Rf value in percentage | Name of the amino acid/ amide |
|---|---|---|
| 1. | 1.4/22.5=0.06x100= 6 | L-cystine |
| 2. | 4.3/22.5=0.19x100=19 | L-aspartic acid |
| 3. | 2.6/22.5=0.12x100=12 | L-aspargine * |
| 4. | 2.3/22.5=0.10x100=10 | L-lysine |
| 5. | 4.1/22.5=0.18x100=18 | L-serine |
| 6. | 3.7/22.5=0.16x100=16 | L-glutamine * |
| 7. | 6.0/22.5=0.27x100=27 | L-threonine |
| 8. | 8.2/22.5=0.36x100=36 | L-proline |
| 9. | 11.5/22.5=0.51x100=51 | L-tryptophan |
| 0. | 13.7/22.5=0.61x100=61 | L-phenylalanine |
| 1. | 16.3/22.5=0.72x100=72 | L-leucine |
| 2. | 17.1/22.5=0.76x100=76 | L-isoleucine |
| 3. | 6.9/22.5=0.30x100=30 | Unidentified. |

* Marks indicate amides.

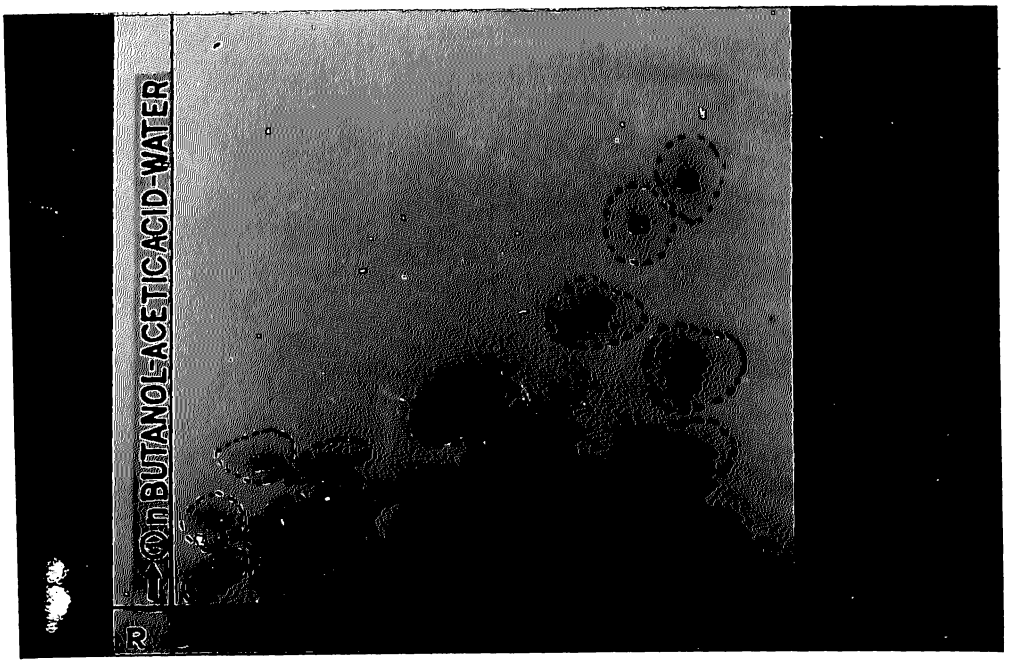



Fig.10: Relative positions of aminoacids and amides in rhizobium inoculated root extracts.

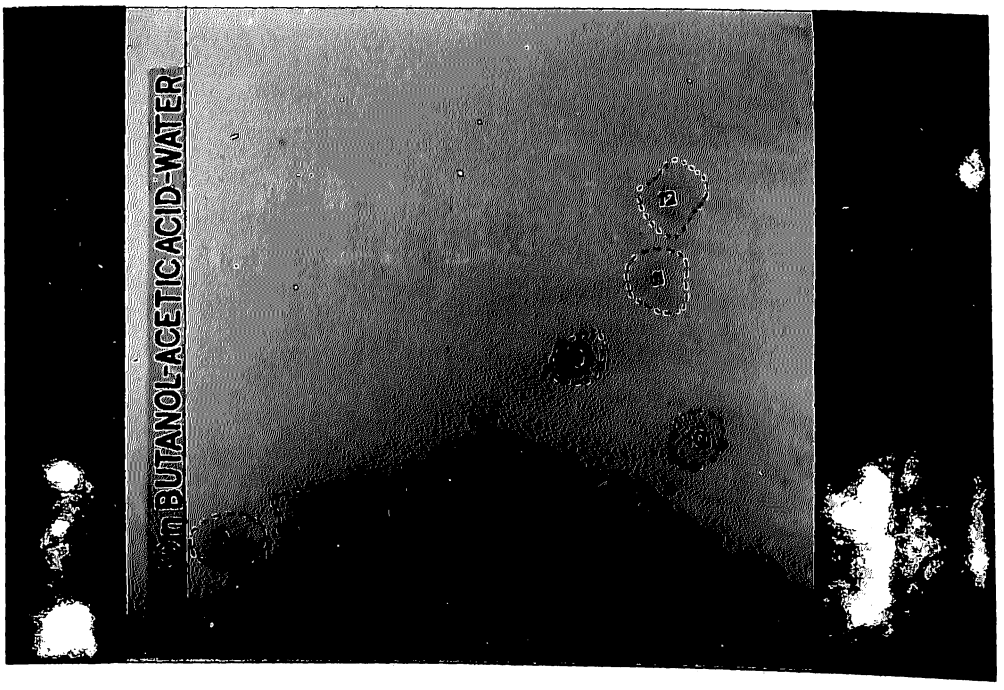



Fig.11: Relative positions of aminoacids and amides in both rhizobium and nematode inoculated root extracts.

able 5. The Rf values of ninhydrin positive spots on the chromatogram of rhizobium-inoculated blackgram root extract and the aminoacids/amides identified. (2-D chromatography) (Fig.10)

| ode No. of pot | Rf value in percentage | Name of the amino acid /amide |
|---|---|---|
| 1. | 1.4/22.5=0.06x100= 6 | L-cystine |
| 2. | 2.3/22.5=0.10x100=10 | L-lysine |
| 3. | 4.3/22.5=0.19x100=19 | L-aspartic acid |
| 4. | 2.6/22.5=0.12x100=12 | L-aspargine * |
| 5. | 4.1/22.5=0.18x100=18 | L-serine |
| 6. | 5.3/22.5=0.24x100=24 | L-glutamic acid |
| 7. | 3.7/22.5=0.16x100=16 | L-gyutamine * |
| 8. | 6.0/22.5=0.27x100=27 | L-threonine |
| 9. | 7.4/22.5=0.33x100=33 | L-alanine |
| 0. | 11.5/22.5=0.51x100=51 | L-tryptophan |
| 1. | 2.9/22.5=0.13x100=13 | L-arginine |
| 2. | 9.5/22.5=0.42x100=42 | L-tyrosine |
| 3. | 16.3/22.5=0.72x100=72 | L-leucine |
| 4. | 17.1/22.5=0.76x100=76 | L-isoleucine |
| 5. | 5.6/22.5=0.25x100=25 | Unidentified. |
| 6. | 6.9/22.5=0.30x100=30 | Unidentified. |

* Marks indicate amides.

ble 5. The Rf values of ninhydrin positive spots on the chromatogram of rhizobium-inoculated blackgram root extract and the aminoacids/amides identified. (2-D chromatography) (Fig.10)

| ide No. of ot | Rf value in percentage | Name of the amino acid /amide |
|---|---|---|
| . | 1.4/22.5=0.06x100= 6 | L-cystine |
| . | 2.3/22.5=0.10x100=10 | L-lysine |
| . | 4.3/22.5=0.19x100=19 | L-aspartic acid |
| . | 2.6/22.5=0.12x100=12 | L-aspargine * |
| . | 4.1/22.5=0.18x100=18 | L-serine |
| . | 5.3/22.5=0.24x100=24 | L-glutamic acid |
| . | 3.7/22.5=0.16x100=16 | L-gyutamine * |
| . | 6.0/22.5=0.27x100=27 | L-threonine |
| . | 7.4/22.5=0.33x100=33 | L-alanine |
| . | 11.5/22.5=0.51x100=51 | L-tryptophan |
| . | 2.9/22.5=0.13x100=13 | L-arginine |
| . | 9.5/22.5=0.42x100=42 | L-tyrosine |
| . | 16.3/22.5=0.72x100=72 | L-leucine |
| . | 17.1/22.5=0.76x100=76 | L-isoleucine |
| . | 5.6/22.5=0.25x100=25 | Unidentified. |
| . | 6.9/22.5=0.30x100=30 | Unidentified. |

* Marks indicate amides.

able 5. The Rf values of ninhydrin positive spots on the chromatogram of rhizobium-inoculated blackgram root extract and the aminoacids/amides identified. (2-D chromatography) (Fig.10)

| ode No. of pot | Rf value in percentage | Name of the amino acid /amide |
|---|---|---|
| 1. | 1.4/22.5=0.06x100= 6 | L-cystine |
| 2. | 2.3/22.5=0.10x100=10 | L-lysine |
| 3. | 4.3/22.5=0.19x100=19 | L-aspartic acid |
| 4. | 2.6/22.5=0.12x100=12 | L-aspargine * |
| 5. | 4.1/22.5=0.18x100=18 | L-serine |
| 6. | 5.3/22.5=0.24x100=24 | L-glutamic acid |
| 7. | 3.7/22.5=0.16x100=16 | L-gyutamine * |
| 8. | 6.0/22.5=0.27x100=27 | L-threonine |
| 9. | 7.4/22.5=0.33x100=33 | L-alanine |
| 10. | 11.5/22.5=0.51x100=51 | L-tryptophan |
| 11. | 2.9/22.5=0.13x100=13 | L-arginine |
| 12. | 9.5/22.5=0.42x100=42 | L-tyrosine |
| 13. | 16.3/22.5=0.72x100=72 | L-leucine |
| 14. | 17.1/22.5=0.76x100=76 | L-isoleucine |
| 15. | 5.6/22.5=0.25x100=25 | Unidentified. |
| 16. | 6.9/22.5=0.30x100=30 | Unidentified. |

* Marks indicate amides.

ɔle-6 The Rf values of ninhydrin positive spots on the chromatogram both rhizobium and nematode inoculated blackgram extract and the amino acids/amides identified.(2-D chromatography) (FIG.11)

| de no of ot | Rf values in percentage | Name of the amino acid/amide |
|---|---|---|
| | 1.4/22.5=0.06x100=6 | L-cystine |
| | 2.3/22.5=0.10x100=10 | L-lysine |
| | 4.3/22.5=0.19x100=19 | L-aspartic acid |
| | 2.6/22.5=0.12x100=12 | L-aspargine * |
| | 5.3/22.5=0.24x100=24 | L-glutamic acid |
| | 3.7/22.5=0.16x100=16 | L-glutamine * |
| | 4.1/22.5=0.18x100=18 | L-serine |
| | 6.0/22.5=0.27x100=27 | L-threonine |
| | 11.5/22.5=0.51x100=51 | L-tryptophan |
| | 8.2/22.5=0.36x100=36 | L-proline |
| | 16.3/22.5=0.72x100=72 | L-leucine |
| | 17.1/22.5=0.76x100=76 | L-isoleucine |

Table-7 Quantity of individual amino acids and amides in the blackgram roots of different treatments and their percentage increase/decrease over the healthy.

| Name of the amino acids and amides | Amino acid and amide content in mg/g fr.wt.of roots | | | | | | |
|---|---|---|---|---|---|---|---|
| | Healthy | Rhizobium inoculated | % increase (+)or decrease (-) | Rhizobium & nematode inoculated | % increase (+)or decrease (-) | Nematode inoculated | % increase (+)or decrease (-) |
| 1.L-cystine | - | 0.021 | - | 0.023 | - | 0.026 | |
| 2.L-glutamic | 0.024 | 0.035 | +45.83 | 0.031 | +29.16 | - | - |
| 3.L-glycine | 0.027 | - | - | - | - | - | - |
| 4.L-alanine | 0.051 | 0.052 | + 1.96 | - | - | - | - |
| 5.L-tryptophan | 0.027 | 0.030 | +11.11 | 0.023 | -14.81 | 0.016 | -40.74 |
| 6.L-valine | 0.004 | - | - | - | - | - | - |
| 7.L-phenyl-alanine | - | - | - | - | - | 0.038 | - |
| 8.L-leucine | - | 0.011 | - | 0.012 | - | 0.014 | - |
| 9.L-isoleucine | 0.021 | 0.023 | +9.52 | 0.027 | +28.57 | 0.033 | +57.14 |
| 10.L-threonine | 0.011 | 0.012 | +9.09 | 0.014 | +27.27 | 0.016 | +45.45 |
| 11.L-serine | 0.009 | 0.010 | +11.11 | 0.011 | +22.22 | 0.013 | +44.44 |
| 12.L-arginine | - | 0.019 | - | - | - | - | - |
| *13.L-aspargine | 0.036 | 0.038 | + 5.55 | 0.032 | -11.11 | 0.027 | -25.0 |
| *14.L-glutamine | - | 0.029 | - | 0.023 | - | 0.020 | - |
| 15.L-tyrosine | - | 0.059 | - | - | - | - | - |
| 16.L-proline | - | - | - | 0.011 | - | 0.018 | - |
| 17.L-lysine | - | 0.017 | - | 0.021 | - | 0.028 | - |
| 18.L-aspartic acid | 0.019 | 0.021 | +15.78 | 0.025 | +31.57 | 0.029 | +52.63 |

* Marks indicates amides

of ninhydrin positive spots on sample chromatograms with that of mixture chromatogram were represented in the corresponding tables (Table 3, Table 4, Table 5, and Table 6).

D. CHANGES IN QUANTITY OF AMINO ACIDS AND AMIDES (NINHYDRIN POSITIVE SPOTS) IN BLACKGRAM ROOT EXTRACT

The quantity of individual amino acids and amides have been found out by TLC scanner II which as been shown in table 7.

E. CHANGE IN NITROGENASE ACTIVITY IN BLACKGRAM NODULES OF DIFFERNT TREATMENTS

Table 8

| Treatments | Nitrogerase activity in micromole $C_2H_2$ reduced per gram fresh weight of nodules per hour | % increase (+) or decrease (-) over rhizobium inoculated |
|---|---|---|
| Rhizobium | 2.67 | |
| Rhizobium + Nematode | 1.79 | -32.9 |

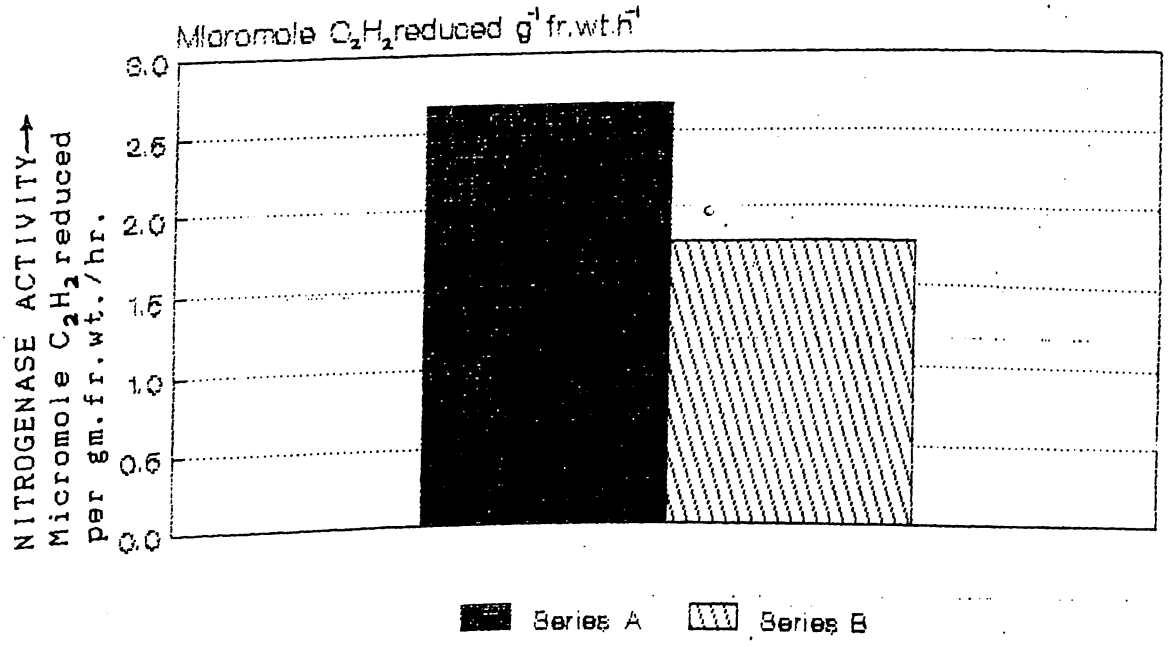
F. VARIATION IN LEGHAEMOGLOBIN CONTENT OF BLACKGRAM NODULES WHEN INFECTED BY M. INCOGNITA

Table 9.

| Treatments | Leghaemoglobin content in mg per gram fresh weight of nodules | % increase (+) or decrease (-) over rhizobium inoculated |
|---|---|---|
| Rhizobium | 1.288 | |
| Rhizobium + Nematode | 0.628 | -51.24 |

Fig. 12

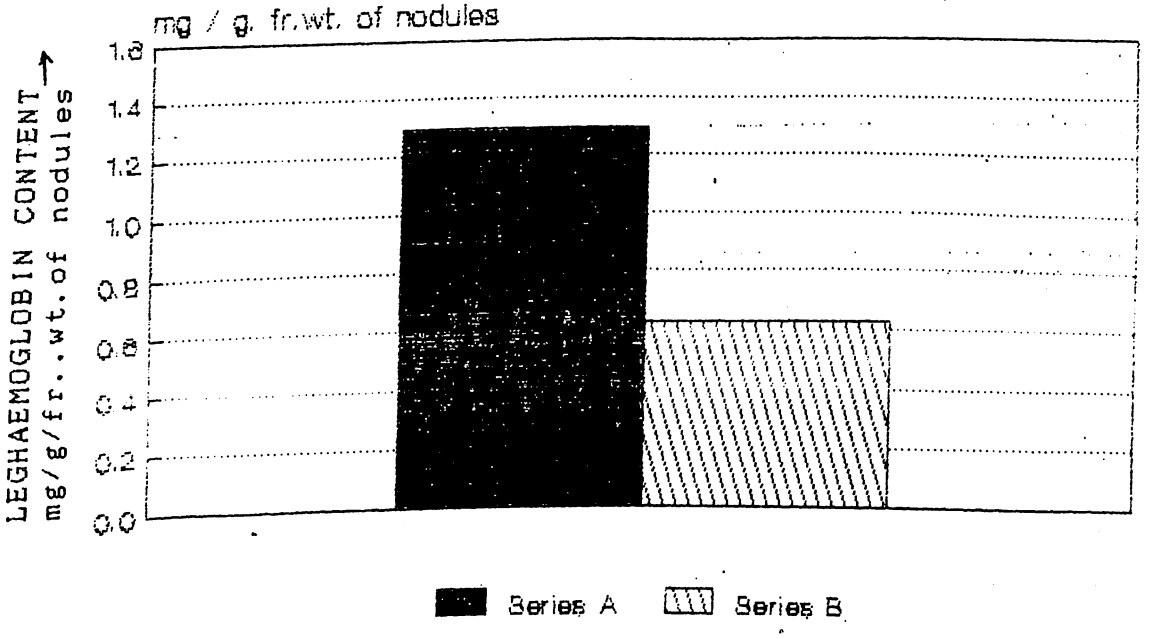
# NITROGENASE ACTIVITY OF BLACK GRAM NODULES




SERIES A - RHIZOBIUM
SERIES B - RHIZOBIUM + NEMATODE

Fig. 13

# LEGHAEMOGLOBIN CONTENT OF BLACK GRAM NODULES




SERIES A - RHIZOBIUM
SERIES B - RHIZOBIUM + NEMATODE

G. EFFECT OF *M. INCOGNITA* AND *R. PHASEOLI* ON TOTAL SUGAR CONTENT OF BLACKGRAM ROOTS

Table-10

| Treatments | Total sugar content in mg per gm fresh weight of roots | % increase (+) or decrease (-) over healthy |
|---|---|---|
| Healthy | 13.32 | |
| Nematode | 17.98 | +34.98 |
| Rhizobium | 14.17 | + 6.38 |
| Rhizobium + Nematode | 15.10 | +13.36 |

H. EFFECT OF *M. INCOGNITA* AND *R. PHASEOLI* ON TOTAL SUGAR CONTENT OF BLACKGRAM NODULES

Table-11

| Treatments | Total sugar content in mg per gm fresh weight of nodules | % increase (+) or decrease (-) over rhizobium inoculated |
|---|---|---|
| Rhizobium | 20.85 | - |
| Rhizobium + Nematode | 27.54 | +32.08 |

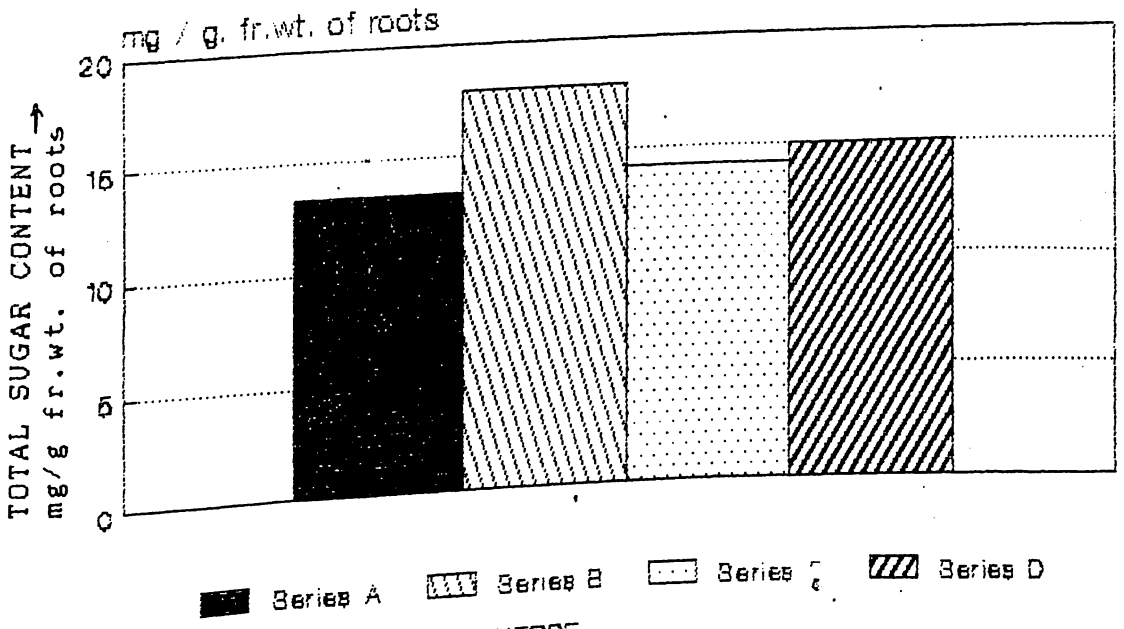
I. VARIATION IN CHLOROPHYLL CONTENT OF BLACKGRAM LEAVES OF DIFFERENT TREATMENTS

Table-12

| Treatments | Chlorophyll content in mg per gm fresh weight of leaves | | | % increase (+) or decrease (-) over healthy | | |
|---|---|---|---|---|---|---|
| | Chl. a | Chl. b | Total chl. | Chl. a | Chl. b | Total chl. |
| Healthy | 0.933 | 0.588 | 1.689 | - | - | - |
| Nematode | 0.790 | 0.494 | 1.466 | -15.32 | -15.98 | -13.2 |
| Rhizobium | 1.533 | 0.986 | 2.605 | +64.3 | +67.68 | +54.23 |
| Rhizobium + Nematode | 1.340 | 0.856 | 2.358 | +43.62 | +45.57 | +39.60 |

Fig. 14

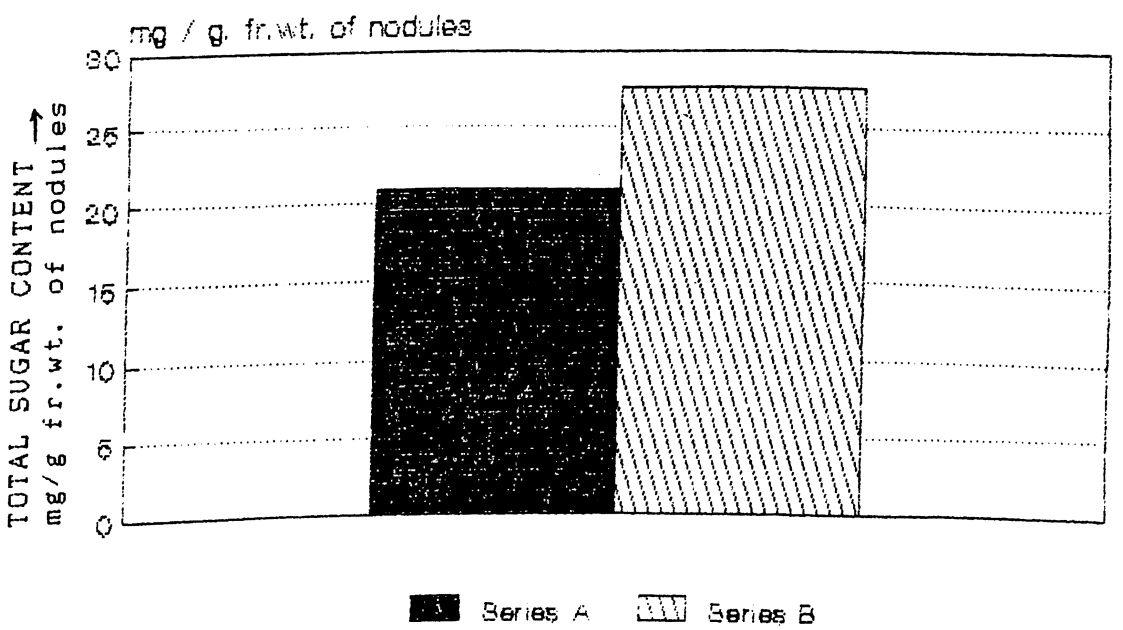
# TOTAL SUGAR CONTENT OF BLACK GRAM ROOTS




SERIES A - HEALTHY, SERIES B- NEMATODE
SERIES C - RHIZOBIUM
SERIES D - RHIZOBIUM + NEMATODE

Fig. 15

# TOTAL SUGAR CONTENT
## OF BLACK GRAM NODULES

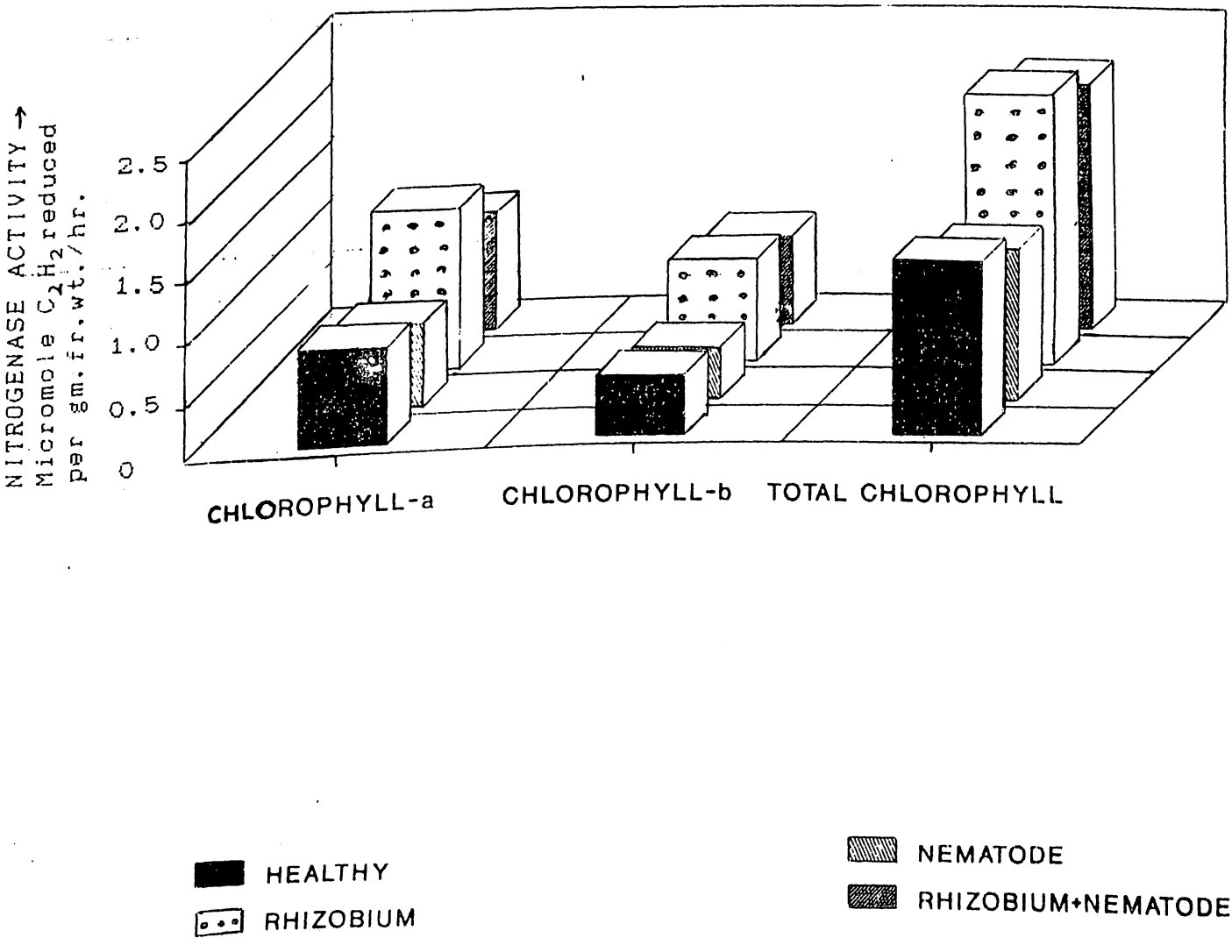



SERIES A - RHIZOBIUM
SERIES B - RHIZOBIUM + NEMATODE

# CHLOROPHYLL CONTENT
## OF BLACK GRAM LEAVES

(mg/g fresh weight of leaves)

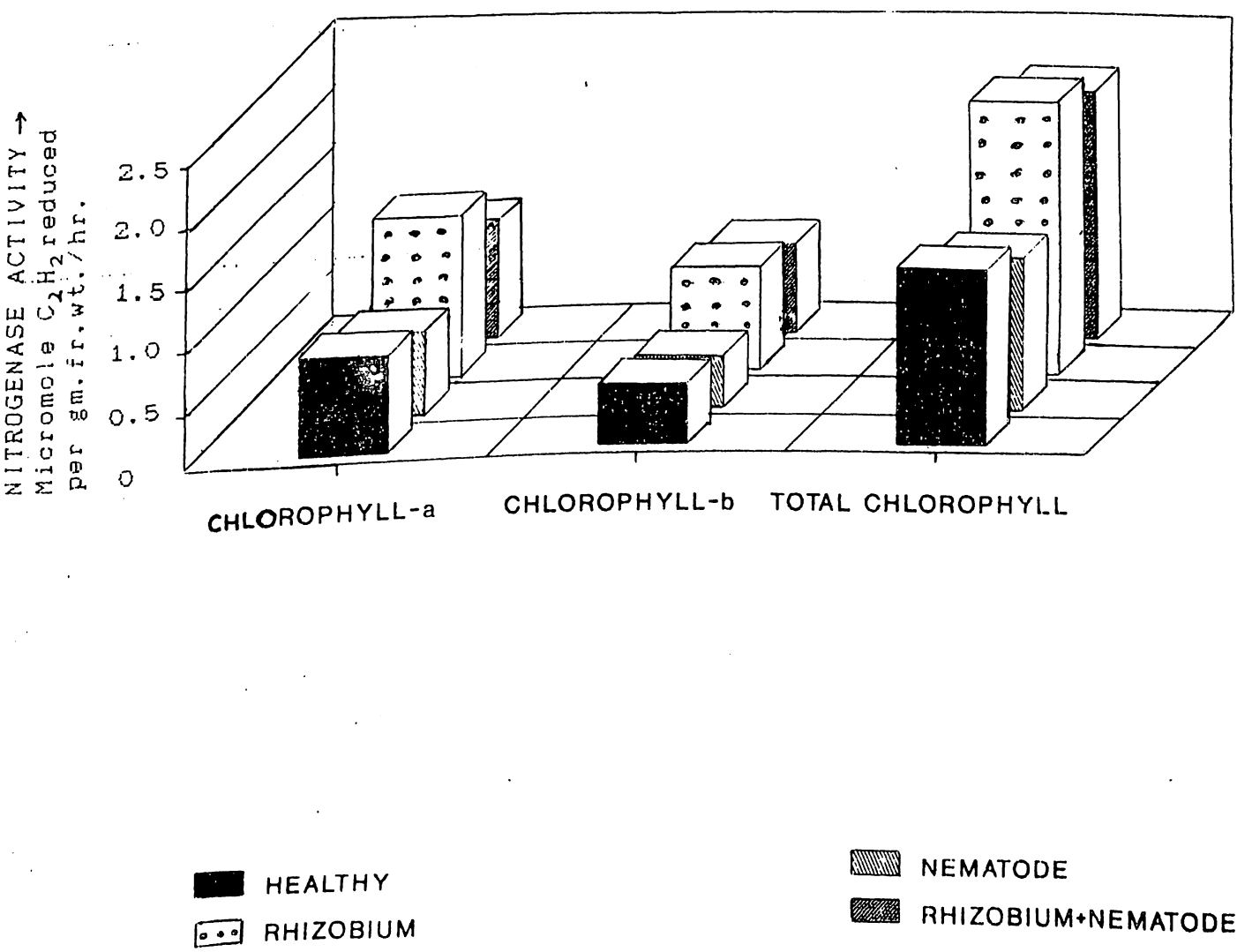
Fig. 16



# CHLOROPHYLL CONTENT
## OF BLACK GRAM LEAVES

(mg/g fresh weight of leaves)

Fig. 16



Six amino acids namely, L-threonine, L-aspartic acid,L-serine, L-aspargine, L-tryptophan and L-isoleucine were found to be common in the root extracts of healthy, nematode inoculated, rhizobium inoculated and both rhizobium and nematode inoculated blackgram crop where as the healthy blackgram root extact contained four other amino-acids namely, L-glutamic acid, L-alanine and L-valine.( Fig.8,Table 3).

The neamtode-inoculated blackgram root extracts contained six more amino acids namely, L-cystine, L-glutamine, L-lysine, L-proline, L-leucine, L-phenylalanine along with one unidentified ninhydrin positive spot over common aminoacids listed above (Fig. 9 , Table 4).

The rhizobium-inoculated blackgram root extracts contained eight more aminoacids namely L-Cystine, L-glutamic acid, L-glutamine L-lysine, L-arginine, L-alanine, L-tyrosine, L-leucine along with two unidentifed ninhydrin positive spots over common aminoacids listed above. (Fig. 10 Table 5 ).

Rhizobium and nematode inoculated root extracts contained six more aminoacids namely, L-cystine, L-glutamic acid, L-glutamine,L-lysine, L-proline and L-leucine over common amino acids listed above (Fig.11 Table 6)

The quantitative changes of amino acids (Table 7 ) revealed that there is higher concentrations of aminoacids except L-tryptophan in nematode inoculated followed by both rhizobium and nematode inoculated, and rhizobium inoculated

root extracts as compared to their healthy counterparts indicated by intensity of ninhydrin positive spots. L-aspargine content in nematode inoculated root extracts decreased over healthy.

The nitrogenase activity decreased in rhizobium and nematode inoculated root nodules as compared to rhizobium inoculated root nodules (Table 8 ) (Fig.12)

The leghaemoglobin content decreased in rhizobium and nematode inoculated root nodules as compared to rhizobium inoculated root nodules (Table 9) (Fig.13)

The total sugar content increased in rhizobium and nematode inoculated root nodules as compared to rhizobium inoculated root nodules (Table11)(Fig.15)

The total sugar content is highest in nematode inoculated root extracts followed by both rhizobium and nematode inoculated, rhizobium incoulated and healthy blackgram root extracts. The total sugar content in nematode inoculated, rhizoibum inoculated and both rhizobium and nematode inocluated roots increased by 34.98, and 6.38 and 13.36 percent respectively over healthy roots (Table10,Fig14)

Chlorophyll-a chlorophyll-b and total chlorophyll content decreased in nematode-inoculated, where as increased in rhizobium inoculated, and both rhizobium and nematode inoculated leaf samples as compared to their healthy counterparts (Table 12) (Fig.16)

# CHAPTER V

# DISCUSSION

# DISCUSSION

In biological nitrogen fixation, establishment of legúme rhizobium symbiosis is a complex process involving physiological and biochemical properties of both the bacterium and the host plant, the development of symbiosis requires a coordinated expression of number of plant and bacterial genes interractions and biochemical phenomenon. In recent years, due to the development of modern physical methods like GLC, ELISA, HPLC,NMR spectroscopy etc, rapid advances have been made towards the understanding of key biochemical process relating to symbiotic nitrogen fixation. Thus, nudulation involves many components in delicately balance state. A genetic defect in either partner, rhizobia or legume host, would arrest one or more of these nodule developmental sequences. Similarly biotic stresses on either partner would also disturb the whole mechanism leading to nodule formation. In this context, plant parasitic nematodes particularly root-knot nematode, Meloidogyne incognita, is considered as potentially serious constraints to grain legume production. It is now realised that root-knot nematode during feeding process along with root, it also disturbs the metabolic process of nodular tissues (Sharma & Sethi, 1975; Das, 1991)

Considering the importance of the subject, the present investigaiton was undertaken to gain

more information on various biochemical parameters like aminoacids amides, total sugar leghaemoglobin, nitragenase activity and chlorophyll content following the infection of Rhizobium phaseoli and/or Meloidogyne incoginta.

The result presented in Table,3,4,5,6 and 7 revealed the presence of six aminoacids viz. L-trypotophan, L-isoleucine, L-threonine, L-serine, L-aspargine and L-aspartic acid to be common in root extracts of various treatments. The result of quantitative study through TLC scanner along with the intensity of ninhydrin positive spots showed higher concentrations of aminoacids excpet L-tryptophan and L-aspargine in nematode incoulated root extracts as compared to their healthy counterparts.Amount of most of the aminoacids increase in inoculated samples are in excellent agreement with the findings of previous researchers (Mohanty and Pradhan, 1990; Sundaraja and Mehta, 1991; Swain and Prasad, 1991). The quantitative increase in various aminoacids may be due to proteolisis of existing tissue protein or synthesis of new aminoacids.Further,the reduction of free tryptophan as infection progressed, might be concerned in the galling activites as it was observed that the gall size increased along with an increase in the post infection period. Higher L-aspargine content in rhizobium inoculated samples appears to be interesting and significant.L-aspargine is considered as major nitrogen

transport compound present in the xylem sap of several legumes. It has C:N ratio which favours low energy cost in nitrogen transport. The synthesis of aspargine from aspartate takes place by glutamine dependant aspargine synthetase and paly important role in symbiotic nitrogen fixation.But in rhizobium-nematode inoculated samples and only nematode inoculated samples the reduction in aspargine content clearly indicates, root-knot nematode play key role in disturbing the metabolic sequence of nodule formation.

The accumulation of prolene, being more pronounced in inocu-latedsamples, is in accordance with the observtions of earlier workers (Lewis & Mc Clure, 1975; Mohanty & Pradhan, 1990; Mohanty, 1992) The absence of L-arginine in rhizobium-nematode inoculated sample may be due to the conversion of L-arginine to L-prolene through ornithine cycle.

It is interesting to note that L-glycine, and L-Valine identified in helathy roots were not detected in inoculated samples,at the same time, L-alanine identified in healthy and rihzobium inoculated samples was missing in nematode inoculated samples,such phenomenon suggests interconversion of one aminoacid to another (Steward and Bidwell, 1962).

The presence of L-tyrosine in only rhizobium inoculated sample and L-phenylalamine in rhizobium and nematode inoculated sample signifies positive role of these aminoacids

in symbiotic nitrogen fixation and host-parasitic interaction.

Total sugar content was increased in nematode inoculated samples. Infection by the nematodes alter the metabolism of the tissues so that respiratory substrates move towards the site of infection from else where in the plants. Increased sugar content was also reported in Zinnia elegans infected by Aphelenchoides ritzemabosi (Gill & Uppal, 1977); in tomato infected by M. incognita (Farooqi et al, 1980) ; in greengram infected by M. incognita (Mohanty, 1992). Increase sugar level in the infected tissues may be considered as a resultant of a complex of plant-nematode interaction which includes the hydrolysis of bound sugar and the utilisation of simple sugar by the pathogen, the reaction of host tissues in the replacement of sequestered carbohydrate.

One of the interesting observation in the present investigation was the reduction of leghaemoglobin content in nematode inoculated nodules. Similar type of results have previously been noted in cowpea (Sharma and Sethi, 1975) and greengram (Mohanty, 1992)when infected by Meloidogyne incognita. Any adverse effect either on the host or on the bacterium would reduce the leghaemoglobin content as the same compound is a complex product of host-bacterium interaction. The intensity of nitrogen fixation is positively correlated

to the amount of leghaemoglobin content in nodules.Acetylene reduction by bacteroid suspension is also dependant upon leghaemoglobin concentration. Hence reduction in the leghaemoglobin content ultimately affects the key process leading to nitrogen fixation.

Nitrogenase activity was reduced in nematode infected nodules which agrees with the findings of earlier workers on various other leguminous crops (Chahal and Chahal, 1987;Mohanty,1992) Nitrogenase is oxygen sensitive and for proper functioning of enzyme, leghaemoglobin is essential which regulates oxygen diffusion through the nodules and into the bacteriod to support oxidative phosphorylation without inactivating nitrogenase. As leghaemaglobin is functionaly directly related, reduction in leghaemoglobin content might be the possible reason for low actvity of the enzyme nitrogenase.

Chlorophyll contents in the leaves were decreased in only nematode inoculated sample where as increased in rhizobium and both rhizobium and nematode inoculated samples. But the percent age of increase is less in both rhizobium and nematode inoculat ed samples which clearly indicates that the disturbances caused by root-knot nemotode in roots have significant impact on the chlorophyll content of the leaves which ultimately led to the reduced nutrient production and supply to the nodules.As a result of which the functioning

of nodules might be drastically hampered.

From the results of present investigation it may be concluded that root knot nemoatode, Meloidogyne incognita, has the capacity to alter the cellular structure of the nodules and possibly the host metabolism to suit its own environment which seems detrimental to Rhizobium sp. The function of nodules was adversely affected by the nematode infection which could be due to alterations of various biochemical parameters like leghaemoglobin content, nitrogenase activity etc.

# CHAPTER VI

# SUMMARY

## SUMMARY

Potculture experiment was conducted to gather information regarding the effect of root -knot nematode, Meloidogyne incognita on root biochemistry and functioning of nodules in blackgram Vigna mungo (L.) Hepper)

Findings of present investigation indicated that out of 18 aminoacids detected, 6 aminoacids namely L-tryptophan, L-isolencine, L-threonine, L-serine, L-aspargine, L-aspartic acid were found to be common to all treatments. Quantitative estimation of aminoacids revealed higher concentration of various aminoacids (except L- tryptophan and L-aspargine) in nematode, rhizobium and both rhizobium & nematode inoculated roots than their healthy counter parts. Total sugar contents in roots increased in all the treatments over healthy and in nodules, it increased in both rhizobium and nematode inoculated samples over rhizobium inoculated ones. The chlorophyll content of leaves increased in all plants (expcept nematode inoculated) over healthy.Proper functioning of nodules was adversely affected by nematode infection which could be due to reduction in leghaemoglobin content and nitrogenase activity of nodules.

It was evident from the results that Meloidogyne incognita inteferes with various physiological and biochemical parameters relating to proper functioning of nodules.

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