Chapter - 4

RESULT

The most obvious morphological responses to infection by *Meloidogyne* spp. is the characteristics of knotting or galling in host plant roots. Root galls which were induced by the present test organism *M. incognita* on *V. mungo* varied in size and location. Generally medium sized, spherical or oval shaped galls along root axes were present on root tips. Galls occurred either singly or in clusters which could encircle the entire root (Plate I -1).

Histopathology

The effect of *M. incognita* infection on cellular aspects in the host plant *V. mungo* and the infected plant treated with varied concentrations of methanol leaf extract of *P. pinnata* are presented here.

Control Uninfected Root (CUI)

The normal root of *V. mungo* contained both tetrarch and triarch and the diameter was 350 µm (tetrarch). The thin walled epidermis (Rhizodermis) was preserved in the form shrunken layer. The cortex was 150 µm wide and contained 3 or 4 layered thin walled compact cells and the cortical cells were 30 µm wide. The vascular strand was intact with well preserved xylem element. The xylem elements were wide, thin walled and angular in outline measuring about 30-40 µm wide. Phloem was appeared as a thin layer around xylem. The
thick walled protoxylem elements were 10µm in wide. Metaxylem was wide angular, measuring 50 µm in diameter (Plate I-2).

The triarch root had intact, thin walled epidermis. The cortex had highly dilated, thin walled compact cells, alternating with the xylem arms along with small groups of phloem. The metaxylem was 30 µm wide (Plate I-3).

**Infected Untreated Root (IUT)**

Infected root showed cellular alterations in the cortex and stele. After the entry of the nematode due to pressure exerted on the cortical cells, the passage was widened and cavity was formed around the nematode. Nematodes appeared to have migrated intracellularly and their path (through the cortical tissue often) were broken and posterior to the cells occupied by the nematode (Plate I-4).

The infected root contained well developed dense vascular cylinder. The structure of infected root just in the vicinity of infected portion was drastically changed. The xylem elements were reduced in number due to heavy accumulation of giant cells in stele (Plate II-1) and the xylem was cleaved into two masses (Plate II-2). In some of the galls, the nematode and the eggs occupied a portion of root (Plate II-3). The xylem elements were wide, fairly thick walled and angular in outline. The nematode occupied in the central region of the root, so the xylem and phloem vessels were pushed on to one side (Plate II-4). Phloem elements were observed to be in collapsed condition (Plate III-1). The xylem elements were believed to be crushed due to pressure exerted
by the giant cells (Plate III-2) and remnants of digested materials found in the giant cells by the action of digestive enzymes secreted by the nematode. The giant cells induced by *M.incognita* were generally quite large with 110µm in diameter round or oval in shape and the wall was thicker than those of adjacent cells. Giant cells with dense granular protoplasam stained deep blue with toludine blue showed hypertrophic and hyperplasia nuclei of varied size. Some giant cells were empty without any contents inside or they contained just a few remnants of scattered nuclei. The mature giant cell complex of *V.mungo* consisted of a group of 6-8 multinucleated cell. The nucleus scattered throughout cytoplasm are of varied size and tended to be aggregated towards the centre of giant cells (Plate III-3). Giant cells appeared in parenchyma tissue within the secondary xylem, phloem and in the enlarged pericycle. Giant cells in cluster in the vicinity of nematode head and oblique lines in the xylem vessels were observed (Plate III-4).

**Infected Treated Plant I (IT₁)**

The infected plants were treated with 2500 ppm methanol leaf extract of *P.pinnata* showed limited number of giant cells which occupied the vascular cylinder region. Xylem elements were thin walled and reduced in number. Phloem seemed to have increased in quantum (Plate IV-1). Due to the effect of the plant extract in some of the galls dissoultion of giant cells wall and deformed nematode were observed (Plate IV-2 and IV-3).

**Infected Treated Plant II (IT₂)**
Infected plants treated with 3000 ppm methanol leaf extract of *P.pinnata* showed reduced number of giant cells. The cortex was intact and the xylem and phloem elements were more in number (Plate IV-4). The giant cells were reduced in size (Plate V-1) and more number of deformed nematodes were found after this treatment.

**Infected Treated Plant III (IT₃)**

The infected plants treated with 3500 ppm methanol leaf extract of *P.pinnata* showed much reduced size and number of giant cells with deformed nematode in the stele (Plate V-2). The thick walled wide angular xylem elements were intact and lignified. The metaxylem elements were 40 µm wide and retained the normal shape and size. Protoxylem elements were reduced in number and size (Plate V-3) with intact cortex. Greater number of deformed nematodes were found after this treatment.

**Developmental Stages of Nematode in the Host Plant:**

The development of *M.incognita* in infected-untreated host plant and under varied treatment of infected plants are given in Table 1 to 4.

**Development of Different Larval Stages in Infected Untreated Plant (IUT)**

Infected second stage juveniles of root knot nematode penetrated the host roots at the tips and because of this almost all the larvae had their heads pointing towards the plant. Invasion commenced from 2nd day onwards and continued till 24th day. The third larval stage appeared on 6th day and sex
differentiation of the larvae was noted on 10th day onwards. The fourth male and female larval stages were found on 12th and 10th days respectively, whereas matured adult males and females first appeared on 24th day and 26th day respectively. The developmental duration for the second stage larva to establish itself was 5 to 6 days to grow and moult into the third larval stage and 3 to 5 days for moulted into the fourth stage. The fourth stage larva developed into adult in about 12 to 13 days. The life cycle was completed within 24 to 26 days. Reinfection of second stage larva to the host plant was observed from 28th day onwards. Generally more number of second stage larvae penetrated in the 14th day of infection (Table 1).

**Development of Larval Stages in IT₁ Plant**

The penetration of second stage larva commenced from 4th day onwards. The development of third larval stages were observed on 6th day and fourth stage male and female were observed on 12th and 10th days respectively. It was observed that adult male and female appeared on 26th and 28th days respectively. Reinfections of second stage larvae were noted from 30th day onwards. A reduction on the penetration of second stage larvae was noted in the methanol leaf extract treated plants. However, more number of deformed larvae of different stages were noted (Table 2).

**Development of Larval Stages in IT₂ Plant**
Penetration of second stage larva into the host plant root had greatly reduced at this concentration (Table 3). The second stage larvae were observed only on 6\textsuperscript{th} day and continued till 22\textsuperscript{nd} day. The third larval stages were observed on 8\textsuperscript{th} day and fourth male and female larval stages were observed on 14\textsuperscript{th} and 12\textsuperscript{th} day respectively. Mature male and female appeared on 28\textsuperscript{th} and 30\textsuperscript{th} day respectively. More number of deformed third and fourth larval stages of both sex and adult female in addition to normal appearance of all stages were discernible. Reinfection was observed on 32\textsuperscript{nd} day (Plate VI - 1 to 6 and Plate VII - 1 and 2).

**Development of Larval Stages in IT\textsubscript{3} Plant**

The number of second stage larval penetration into the host plant drastically reduced. Initial juvenile penetration was observed on 8\textsuperscript{th} day only and continued till 22\textsuperscript{nd} day. The third larval stages were observed on 10\textsuperscript{th} day and fourth male and female larval stages of both sex were observed respectively from 16\textsuperscript{th} and 14\textsuperscript{th} days onwards. Adult male and female were observed on 30\textsuperscript{th} and 32\textsuperscript{nd} day respectively. More number of deformed larvae of different stages were also observed (Plate VI - 2, 4, 6 and Plate VII - 2). Substantial egg deformation (Plate VII - 3) was discernible. Reinfection was noted from 34\textsuperscript{th} day onwards (Table 4).

**Effect of Nematode Infection on Host Plant Growth**
The effect of nematode infection on wet and dry weights of root and shoot system of *V. mungo* are given in Tables 5 and 7. The statistical analysis of student ‘t’ test reveals that the growth characters of the host plant are significantly affected by the nematode. This significance indicated a reduction in plant weight (Table 6).

Due to nematode infection, the total growth of the host plant was found to be decreased by 21.53% (Table 5) when compared to CUI plant (Plate VIII - 1, 2, 3 and 4). Amidst this reduction, the following features emerged with regard to shoot and root growth. The root and shoot growth of the IUT host plants were observed to be decreased by 31.66 and 14.75% (Table 5) respectively over that of their respective control uninfected plant. The root suffered more loss than the shoot. The root / shoot ratio (R/S) of CUI plant was found to be 0.42 and due to nematode infection, it decreased to 0.30.

**Effect of Methanol Leaf Extract of *P. pinnata* on Growth of Infected Host Plant**

The impact of different concentrations of methanol leaf extracts of *P. pinnata* on mean wet and dry weights of root and shoot systems of *V. mungo* infected by *M. incognita* are presented in Table 5. The statistical analysis of the critical difference at 5 and 1% reveals that the growth characters of the infected plant are significantly (Table 7) influenced by the varied concentrations of methanol leaf extract of *P. pinnata*. This significance indicated the growth increment of both root and shoot systems of infected treated host plant.
However such increment in total plant growth was found to be maximum (119.11 %) in IT₃ plant (Table 5), whereas a minimum percentage increment (35.29 %) was observed in IT₁ host plant (Table 5), when compared to its respective IUT plant (Plate IX - 1, 2, 3 and 4).

Amidst this increment the following features were observed with regard to the shoot and root growth. A maximum percentage increment in wet weight of root (Plate X - 1, 2, 3 and 4) and shoot was discernible in IT₃ plant when compared to respective IUT plant (Root 187.50 %, Shoot 98.07 %) (Table 5).

Infected untreated plants that received varied concentrations of leaf extracts showed increments in root / shoot ratio. However such increment was found to be maximum in IT₃ plant (Table 5).

**Water Content:**

The water content of CUI plant was observed to be 84.61% (Table 8). It was observed to be reduced by 82.84% in IUT plant. The water content was found to be progressively increased in treated plants and the maximum increment of 86.12% was observed in IT₃ host plant compared to IUT (Table 8).
Dynamics of Root-Knot Nematode Population on *M. incognita* on *V. mungo*

The number of galls/plant, egg masses/plant, eggs/egg mass and root and soil population of the nematode/plant in the IUT and infected treated host plants are tabulated (Table 9). The effect of varied concentrations of methanol leaf extract of *P. pinnata* on reproduction and population build up of *M. incognita* on *V. mungo* are given in Table 9 and 10.

The statistical analysis of critical difference at 5 and 1% reveals that varied concentrations of the leaf extract of *P. pinnata* significantly influenced the reproduction and population buildup of *M. incognita* on *V. mungo* (Table 10). All the infection related parameters studied were observed to be suppressed by methanol leaf extract treatment to the infected plants. However a maximum percentage reduction in the above said parameters like galls per plant etc., was noted in IT$_3$ plant which received 3500 ppm concentration of leaf extract. The gall number increased by the nematode infection in IUT was found to be decreased by 87.28% in IT$_3$ plant (Table 9). The number of egg masses / plant also decreased with increased concentration of plant extract treatment and the percentage of decrease was observed to be maximum in IT$_3$ plant (74.07 %) (Table 9) when compared to infected untreated plant. The eggs per egg masses were found to be more in IUT plant where it was found to be decreased by 28.55, 47.37 and 70.53% in IT$_1$, IT$_2$ and IT$_3$ plants respectively (Table 9).
The total number of eggs produced by the root-knot nematodes on the host was greatly suppressed in all treatments. However, such reduction was pronounced in IT₃ plant (92.49 %), when compared to infected untreated plants (Table 10).

The nematode populations in the root and soil were observed to be decreased by 73.79 and 86.64% respectively in IT₃ plant which received 3500 ppm concentration of the plant extract over that of their respective IUT plant (Table 10).

There was a marked decrease in total population of the nematodes and number of mature female per plant noticed in different concentrations of leaf extract treated infected plants. However, IT₃ plant which received 3500 ppm concentrations showed maximum reduction on these parameters studied (Table 10).

The rate of nematode reproduction (RR) was found to be 4.13 in IUT plant (Table 11) whereas in different concentrations of methanol leaf extract treated infected plants, it was observed to be decreased. However such decrease was found to be maximum in IT₃ plant in which flourished plant growth was discernible (Table 11).

The rate of population increase (RPI) of the nematode was 0.52 in IUT plant (Table 12). In different concentrations of extract treated infected plant, it was found to be decreased. However a maximum percentage decrease (97.69%) in RPI was noticed in IT₃ plant (Table 12).
The decreased rate of reproduction (RR) and the rate of population increase (RPI) of the nematode in extract treated infected plant which in turn reflected an increased environmental (plant) resistance factor (ERF). The environmental resistance factor of IUT was 7.94 (Table 13) whereas in extract treated infected plant it was observed to be elevated. However IT3 plant showed more environmental resistance factor (130.0) to nematode infection (Table 13).

The statistical analysis of correlation coefficient (r) indicates that a negative significant relationship was discernible between the plant growth and nematode development under varied concentrations of plant extract (Table 14 and 15).

All the above inter-relationships existing between nematode developmental features and the growth parameters regarding the host tolerance under varied concentrations of plant extract are statistically established and represented in table 16.

**Biochemical Constituents:**

The various biochemical profiles (sugar, protein, lipid, phenol, ascorbic acid and starch), energy content and enzymes in root and shoot tissues of control- uninfected, infected- untreated and infected-treated *V.mungo* are presented in Table 17 and 18.

**Sugar**
The total sugar content was found to be reduced in IUT plant. Amidst this reduction, the following features emerged with regard to root and shoot system. In the plant root system it was found to be reduced by 46.40% whereas in the shoot system it was observed to be reduced by 42.53% (Table 17) when compared to their respective CUI plant. The root system showed more sugar reduction than shoot system. The varied concentrations of treatment showed significant increase in total sugar content in both systems over that of their respective IUT plant. A maximum increment was observed in both root and shoot systems of the host plant which received 3500 ppm concentration of the leaf extract (Table 17).

**Protein**

An elevated level of protein was discernible in the root and shoot systems of IUT plant during nematode infection. The protein content was found to be increased by 27.09% in the infected root system whereas in shoot system it was increased by 49.72% over that of their respective CUI (Table 17). Varied concentrations of treatment from 2500 ppm to 3500 ppm to the infected host plant resulted in more protein increment in both root and shoot systems. However such increment was found to be maximum in root (149.15%) and shoot (110.05%) of IT3 plant and a minimum increment was discernible in the host plant which received 3500 ppm concentrations (Table 17).

**Lipid**
During nematode infection, the lipid content was found to be increased both in the root and shoot systems of IUT plant. In the root system, it was increased by 42.21% whereas in the shoot system it was increased by 28.45% over that of their respective CUI plant (Table 17). Varied concentration of leaf extract treatment to the infected plant resulted in an elevated level of lipid content both in the root and shoot systems. A maximum increment in lipid content was discernible both in root and shoot systems of IT$_3$ plant which received 3500 ppm concentration of the leaf extract when compared to their respective IUT plant (Table 17).

**Phenol**

Due to nematode infection an elevated level of phenol content was observed in both root and shoot systems over that of their respective CUI plants. It was increased by 55.75 and 16.12% (Table 17) in the root and shoot systems of IUT plant respectively. Varied concentrations of extract treatment to the infected plant resulted in phenol increment. However such increment was observed to be maximum in IT$_3$ plant when compared to IUT plant. A maximum percentage increment in total phenol was discernible in the shoot system of IT$_3$ plant 84.30% (Table 17).

**Total Organic Content**

Due to nematode infection all the organic constituents were observed to be elevated in the root and shoot systems of the host plant. It was observed to be increased in root and shoot systems by 31.94 and 14.21% (Table 17)
respectively over that of their respective CUI plant. The total organic constituent was found to be increased in root and shoot systems of varied concentrations of extract treated infected plants. A maximum percentage increment in total organic content was discernible in root and shoot system of the host plant which received 3500 ppm concentrations of leaf extract (Table 17).

**Starch**

The nematode infection resulted in decreased level of starch content in the root and shoot systems of the host plant. It was found to be decreased in root and shoot system by 57.57 and 43.60% (Table 18) respectively over that of their respective CUI plant. A progressive increase in total starch content was evident in the root and shoot systems of the host plant, when treated with increasing concentration of leaf extract. A maximum level of increase in total starch content was recorded in the host plant which received 3500 ppm concentration of the leaf extract (Table 18).

**Ascorbic Acid**

Due to nematode infection a decreased level of ascorbic acid was observed in IUT plant. The total ascorbic acid content in the root and shoot systems of IUT plant was decreased by 20.14 and 25.22% (Table 18) respectively over that of their respective CUI plant. The extract treatment to the infected plant resulted in reduced ascorbic acid level in the root and shoot
systems. A maximum percentage reduction was recorded in the root (46.84%) and shoot (58.20%) systems of IT3 plant (Table 18).

**Respiration**

Due to nematode infection, the respiration was found to be increased by 185.87 and 266.98% (Table 18) in root and shoot systems respectively. The respiration was found to be decreased in root and shoot systems of varied concentrations of extract treated infected host plant. In root and shoot systems of IT3 plant, it was found to be decreased by 57.47 and 66.37% respectively over that of their respective IUT plant (Table 18).

**Energy Content**

During nematode infection, the root and shoot systems recorded less energy content. It was observed to be reduced by 24.54 and 23.33% (Table 18) in the root and shoot systems of the host plant respectively over that of their respective CUI plant. When the infected plants were treated with increased concentrations of leaf extract, the total energy content was observed to be elevated. A maximum percentage increment was discernible in the root and shoot systems of IT3 plant, whereas a minimum percentage increment was noted in IT1 plant which received 2500 ppm concentrations of leaf extract (Table 18).

The statistical analysis of critical difference at 5 and 1% levels reveals that the extract treatment significantly influenced the various parameters
studied (Table 17 and 18). The statistical analysis of student ‘t’ test reveals that the nematode infection significantly influenced the parameters studied (Table 19).

**Enzymatic Studies**

Activities of dehydrogenases of alcohol, formate, glucose, glycerol and succinate, ascorbic acid oxidases and the total endogenous reductases in the root and shoot systems of CUI, IUT and extract treated infected plants are summarized in Tables 20 and 21.

**Total Dehydrogenases (TDH)**

The activities of total dehydrogenases in the root and shoot systems were found to be increased in IUT plant when compared to CUI. In the root, it was found to be increased by 10.42% (Table 20), whereas in shoot it was found to be increased by 2.08% (Table 21) over that of their respective CUI plant. Various concentrations of extract treatment to the infected plant resulted in an elevated activity of dehydrogenases in the root and shoot systems. The root and shoot systems of IT$_3$ plant showed maximum activities of dehydrogenases (Table 20 and 21).

**Total Endogenous Reductase (TER)**

The activities of total endogenous reductases were found to be more in root and shoot systems of IUT plants over that of their respective CUI. It was more in root system (72.72%) (Table 20) when compared to shoot system
(17.35%) (Table 21) in IUT host plants. Addition of varied concentrations of the plant extract to the infected plant resulted in increased activities of total endogenous reductases. Maximum activity was noted in the root and shoot systems of IT$_3$ and lesser activities were observed in IT$_1$ plant. In the root and shoot systems of IT$_3$, it was observed to be elevated by 89.47 and 99.69% respectively over that of their corresponding IT$_1$ plant (Tables 20 and 21).

**β-amylose**

The shoot system of CUI showed more β-amylose enzyme activity than the root system. Infection resulted in an elevated β-amylose activity both in the root and shoot systems. In the root and shoot systems of IUT plants, the activities of β-amylose enzyme were found to be elevated by 27.86 (Table 20) and 15.19% (Table 21) respectively over that of their respective CUI plants. The activities were observed to be decreased in root and shoot systems of varied concentrations of extract treated infected plants. β-amylose activity decreased considerably both in root (8.21) and shoot (19.95) systems of IT$_3$ plant (Tables 20 and 21).

The statistical analysis of critical difference at 5 and 1% reveals that the varied concentrations of plant extract significantly influenced the activities of different enzymes (Table 20 and 21). The statistical analysis of student ‘t’ test reveals that the nematode infection significantly influence the various enzyme activities (Table 22).
**Total Chlorophyll Content**

During nematode infection, the total chlorophyll content in the shoot systems of the host plant was observed to be reduced. It was reduced by 24.20% (Table 23) compared to its respective CUI plant. The same trend was also discernible when individual chlorophyll pigments were taken into consideration. Under different concentrations of extract treatment, the total chlorophyll content and individual chlorophyll pigments were found to be increased and reached the highest level in IT$_3$ plant (Table 23).

**Statistical Relationship between the Nematode Population and the Host Metabolites under Different Concentrations of Leaf Extract**

It is evident from the Tables 24 and 25 that the reproductive rate of nematode was observed to be decreased along with increased total dehydrogenase enzymes activities and total organic contents in the shoot ($r = -0.9870; p < 0.01$) (Table 24) and root tissue ($r = -0.9720; p < 0.01$) (Table 25) respectively. The rate of population increase was associated with increased protein content in the shoot tissue ($r = -0.9669; p < 0.01$) (Table 24) and increased lipid content in root tissues ($r = -0.9759; p < 0.01$) (Table 25).

**GC-MS Analysis**

The chromatogram of *P.pinnata* leaf extract as shown in Fig. 1 and enlarged version of chromatogram are shown in Fig. 1a and 1b. Mass spectrum was used to identify the structure of the compound found (Fig. 2 and 3)
comparing with those in NIST ver. 2.1 (National Institute of Standard Technology) Library. The identified compound, percentage and the retention indices are summarized in Table 26. Sixteen compounds were detected in methanol extracts of *P.pinnata*. The nematicidal compounds of the *P.pinnata* leaf extract were found to be Alkaloid (4-Hydroxy-methylpiperidine (29.33%); 4-Piperidinamine, N, 1-dimethyl (1.22%)), Diterpene (Phytol) (0.54%), fatty acid (n-Hexadecanoic acid or Palmitic acid (1.33%)) and Steroids (Stigmastan-3-ol, 5-chloro-acetate, (3β and 5α) (7.40%) and Stigmasta-5, 22-dien-3-ol, acetate, (3β) (1.28%)).