Chapter - 5

DISCUSSION

*Meloidogyne incognita*, one of the species of root-knot nematode has been proved to cause serious agricultural problems. Interfering with anchorage and absorption, the two primary functions of roots of plants, the root-knot nematode *M. incognita* is of serious concern in the field of agricultural economy. Infection by the root-knot nematode *M. incognita* resulted in gall formation and ultimately resulted in reduced production, because of the nutritional stress created in the host during pathogenesis.

Penetration of the second stage larvae of *M. incognita* into the root and migration through the cortex is the first step in establishing a host-parasite association. Histopathological studies on the host parasite relationship (Dropkin, 1969; Endo, 1971; Bird, 1974; Jones and Dropkin, 1975; Hussey and Williamson, 1997; Gopinatha et al., 2004) clearly indicated that there is a general hypertrophic effect on all cells in the vicinity of the nematode including the vascular bundle. For successful development of larvae into adult, the invading second stage larvae depend on giant cells. These cells which become thick walled, multinucleate and much larger than adjacent normal cells have been proved to act as a source of nutrients for the developing nematode. Dropkin (1955), Paulson and Webster (1970) reviewed the light microscopic investigations of the development and structure of giant cells. The cortical cells surrounding the giant cells become hypertrophied and cause typical regions of
localized swelling, the galls. Giant cells usually develop in the vascular cells (Christie, 1936) and are presumed to form through dissolution of cell walls subsequent to coalescence of the cell contents (Dropkin and Nelson, 1960). Histological study suggested that giant cells resulted from cell expansion.

The development of giant cells seems to be correlated with nematode development. The giant cells are maximal in size at about the time when the female nematode is producing eggs and thus require the most nutrient (Bird, 1961). During the early stages of development, the cell wall regions near the nematode head appeared to stain more and there was always a layer of broken wall material between the nematode and the giant cell. The microscopic observation in the present study suggested that chemical or mechanical wall breakdown contributed to giant cell formation. The adjacent cells were merged by the result of cell wall lysis, as a result of which large multinucleate giant cells were formed. These giant cells appeared as clusters in the vicinity of the nematodes head. Giant cells became multinucleate as a result of Karyokinesis. This was supported by earlier studies (Huang and Maggenti, 1969). The presence of abnormal xylem cell was noticed and this was mainly due to the pressure exerted by giant cells as reported by Nirmala and Mehta (1994). Further the walls of giant cells were thickened and this could be due to secondary growth of plasmalemma as observed by Jones and Northcot (1972) and Yousif (1979).

In the present study showed that the mature giant cell complex of *V. mungo* consisted of a group of 7-9 multinucleate cells. The cytoplasm was
evenly granular and (Plate III-2 and 3) dense in stained section. The cell wall was clear and showed attachment of small granules. These granules might be the remnants of digested materials of cell wall due to action of digestive enzymes secreted by the nematode. Linford (1937) proved that the nematode could feed by penetrating giant cells with its stylet. Further it was proved that the nematode might inject materials into the giant cells through its stylet (Krusberg, 1963). Such materials in addition to initiating giant cells, may soften to facilitate easier feeding by the nematode and hence the granular remnants as observed in the present study is justifiable.

Nearly 6-8 oval shaped nuclei have been found in fully developed giant cells and appeared to be in irregularly agglomerated form and shapeless mass in giant cells. The large nuclei are scattered throughout the cytoplasm but tend to be aggregated toward the centre of the giant cell. Similar observation as in the case of present study was discernible in chick pea infected by *M.incognita* (Vovlas et al., 2005). The large cell nuclei might originate from the initial hypertrophied cells, which showed enlarged nuclei in matured gall. The smaller nuclei probably originated in actively growing cells at the border of enlarging giant cells. The reason of appearance of cluster of nuclei in giant cells is not clear (Vovlas et al., 2005). Some empty giant cells have been observed in the present study. Some giant cells contain a few remnants of scattered nuclei. They probably represented dead giant cells as suggested by Dropkin and Nelson (1960).
In the present study nematodes were found in the vicinity of vascular bundles and bundles contained both giant cells and female nematodes. Abnormal xylem vessels were visible around giant cells (Plate II-4) and this could probably be due to the pathological reaction induced by female nematodes. Further the root xylem vessels strands had the appearance of oblique lines and this could be due to nematode feeding or making injury to the xylem parenchyma. The above findings were in accordance with the findings of earlier worker (Yousif, 1979).

The translocation problem created in the root due to rearrangement of conducting cells as observed in the present study ultimately sets a nutritional hazard in the nematode infected plant (Kannan, 1958; Oteifa and Elgindi, 1962; Dropkin, 1972; Suhail Anver et al., 1999). The damage caused by *M. incognita* in cortical and vascular region of *V. mungo* resulted in stunted plant growth. The stunted plant growth due to nematode infection was also reported by earlier workers at various situation (Ramakrishnan and Rajendran, 1998; Pegard *et al.*, 2005).

Infected plants have been reported to appear more susceptible to wilting than healthier plants. Irrespective of the weight of root tissues, as compared to healthy plant, the absorption of nutrients by galled root is appreciably reduced thereby resulting in reduced top growth, as observed in the present investigation. Therefore taking the suggestion of Raut and Sethi (1980) into consideration, assessing the top weight is a better parameter rather than assessing the root weight to assess the pathological effect. In the present study,
vascular elements are broken so that normal translocation of water and nutrients are mechanically hindered. Similar findings were observed by Taylor and Sasser (1978). Nematodes remove plant nutrients, alter nutrient flow pattern in plant tissue and retard root growth (Hunter, 1958), all of which may contribute to reduced plant yield.

Electrophysiological and action potential studies (Wang et al., 1975) indicated permeability changes occurring in the infected plants. Permeability changes bearing on the absorption on the nutritional status ultimately have an impact on the growth of the infected plant.

The formation of galls may create a drain on the plant’s resources in shoot-tissue, resulting in suppression of shoot growth. It was noted earlier that the amount of plant resources diverted would be directly related to high nematode population densities (Wallace, 1969). Hence the reduced root and shoot of V. mungo as discernible in the present study might be due to the adverse effect of high population densities in the host plant (Plate VIII - 2 and 4). The decrease in both wet and dry weight of root and shoot as against the control was on par with the findings by Raut and Sethi (1980).

In the present study, water content of the host plant was found to be reduced significantly due to nematode infection when compared to control. Nematode feeding and intraroot migration of larvae of the nematode damaged the epidermis, cortex and stelar element as observed in the present studies which resulted in restricted water absorption and transport.
A reduction in the root / shoot ratio due to nematode infection was evident in the IUT plants. Such reduction in root / shoot ratio was due to reduced root growth and increased shoot growth. It was discernible that reduced root growth due to nematode infection indirectly induced water stress in plant tops which thereby reduced root / shoot ratios (O’Bannon and Reynolds., 1965).

Oteifa (1952) observed that a reduction in the plant vigour in nematode infection was attributable to shoot growth and the nematode population increase. Kirkpatrick et al., (1964) also noted low vigour of plants that suffered under high population of *Paratylenchus* spp.

According to Seinhorst (1965) an equilibrium exists between the host and the pathogen during the nematode infection in the host plant. According to him under a certain density of nematode population the growth of the susceptible plant does not suffer appreciably. A tolerant host can support relatively high nematode densities without suffering appreciable damage. This idea led to the concept of n/50 (log number of larvae which reduced the plant growth to 50%) by which it can be accepted that a certain number of nematode population is required to produce an appreciable 50% reduction in the plant group, which necessarily upsets the equilibrium density. In the present study, high densities of nematode population (final population) output in the host plant created a stress on the plant.
With an increase in stress due to increased population output on the host plant, a reduction in the plant vigour could be expected. However, the plant under the population stress exhibited differential shoot and root growth. It is evident that the host plant was able to recoup its vigour. Such a recoupment must necessarily be mediated metabolically and expressed superficially by varied growth of the host plant. This is well evidenced in the present study with regard to growth and metabolic phenomena.

Involvement of plant metabolism during pathogenesis is well documented by Giebel (1982); Tayal and Agarwal (1982) and Sharma et al., (1996). It is now accepted that various metabolites in the syncytial region of galls are involved in the combat mechanisms of the pathogenic mechanism. The role of these metabolites and their possible participation are discussed below.

The reduction in the sugar level in the nematode infection (Kannan and Balaji, 1988; Vaitheeswaran and Mohamed Ibrahim, 2003; Mohamed Ibrahim et al., 2008) is a well known factor with attended starch depletion (Nasar et al., 1980; Mohan and Dhawan, 2000; Vaitheeswaran et al., 2006). The derouting of sugar carbon for the synthesis of phenol (Neish, 1964) is also envisaged. This was experimentally supported by Uritani (1971) who recorded a reduction of glucose in sweet potatoes infected with Ceratocystis fimbrita with a corresponding increase in the phenol levels. The major source of energy for the invading pathogen is the stored carbohydrate which is converted into utilizable form by the action of hydrolytic enzymes secreted by the nematode. Hence, the
sugar content of the infected root and shoot tissues showed a depletion in the present study and similar results were reported by many workers (Haseeb et al., 1996; Vaitheeswaran et al., 2003, 2007; Mohamed Ibrahim et al., 2008). Such a reduction might also be due to consumption by the nematode particularly for egg production, its sustenance and part mobilization in the metabolic pool for synthesis of other metabolites viz., protein, lipid, phenol etc., through shikimic acid pathway (Cowling and Horsfall, 1980) as suggested by Kannan (1967a). It is also possible that the reduction in sugar content could be due to impaired photosynthetic activity. Photosynthetic rate may probably be more directly responsible for sugar synthesis. In the present study, it was discernible that the reduction in chlorophyll pigments content would have resulted in reduced photosynthetic rate which in turn resulted in reduced sugar synthesis.

Metabolic leakage of carbohydrate during post infection period particularly in early gall stage, may be another possible reason of quantitative sugar reduction in diseased plants (Rashid et al., 1985). Metabolic leakage has also been observed by Van Gundy et al., (1977) in root-knot nematode, M. incognita induced galls. The biogenesis of amino acids (Stewart et al., 1966), protein (Broyer, 1959) and ascorbic acid (Burns, 1960) from sugar sources have been reported.

The increased β-amylase activity observed in infected tissue can be attributed to the fact that the amylose, one of the components of cell starch is
hydrolyzed into easily assimilable simple sugars such as glucose resulting in low contents of starch. This suggests that amylase is secreted by the nematode as indicated by Orion and Bronner (1973), who observed very little starch and strong amylase activity within giant cells of the tomato galls. Roy (1979) also localized strong amylase activity. The reduced starch content in the present study might be due to increased β-amylase activity is also substantiated.

In the present study, an increase in the protein content was noticed in the host plants due to nematode infection. Similar observations were made earlier by Devarajan and Rajendran (2002) and Vaitheeswaran et al., (2005). According to Howell and Krusberg (1966) and Simte and Dasgupta (1987) the sources that satisfy the protein requirements are greater translocation of aminoacids into the gall, decreased rates of translocation out of the gall, decreased rates of break down, deposition by nematodes and increased rate of synthesis by root-knot nematodes.

It was remarked earlier that the galled roots shifted their metabolism in the direction of protein synthesis (Dropkin, 1972). Chatterjee and Sukul (1981) suggested that the protein synthesis in galled tissue was indirectly influenced through changes in the levels of plant hormones by root-knot nematodes. It was also indicated that the galled root protein level would be directly proportional to root gall number (Sinha Babu and Sukul, 1984). Ganguly and Das Gupta (1979) reported these changes in the protein synthesis after infection to be one
indicating the defense action of the host plants. Resistance may involve a special type of protein synthesis triggered by the presence of pathogen.

Lipid plays an important role in energy source in the biological organization, since its calorific value is twice more than that of carbohydrate and protein. An increased level of lipid observed in the present study is suggestive of the synthetic process taking place during the course of breakdown of tissues by the pathogen (Owens and Specht, 1966). It may be that the breakdown of tissues by the greater number of nematodes in their root system were salvaged by metabolic mechanism of the host that contribute to the increased level of lipid. It was reported also by Van Gundy et al., (1967) that during aging and starvation plant nematode consumed the carbohydrate and protein more than fats and the latter is used at a fog end of starvation.

From the present study, it was discernible that the phenolic content was more in the infected tissues. Similar results were reported in various plants infected by nematodes (Feldman and Hanks, 1968; Chakrabarti and Mishra, 2002; Mohamed Ibrahim et al., 2008). Higher phenol in roots of plants indicated that they might be imparting tolerance to plant against nematode attack (Singh and Choudhury, 1973). It has been recognized that phenolic compounds play an important role in the defence mechanism against infectious agents. The increased accumulations of phenol in the infected tissues could be ascertained by enzymatic glycosidase activity by the parasite, as well as the plant too. The increase in phenolics in nematode inoculated samples might also be due to liberation of conjugated phenols from the glycosidic compound by
the action of hydrolytic enzymes during feeding as reported by Wilski and Giebel (1966); Badra and Elgindi (1979) and Mohanty et al., (2001). The accumulation of phenol in turn inhibits further advance of the pathogen. There is a distinct correlation between the degree of resistance and the phenolic content present in tissues. Phenol seems to interfere with nematode feeding and physiology which ultimately affect the reproductive potential of the nematode. Several workers have implicated phenols as resistance factors because they become highly reactive upon oxidation resulting in the formation of substances toxic to pathogens or which inactive enzymes produced by plant nematodes (Kosuge, 1969; Epstein, 1972). Most phenol occurs in plant tissues in bound form as glucosides of low physiological and chemical activity, activation requires their decomposition to free phenols. Active phenols released from glucosides by increased β glucosidases activities by nematodes may later get oxidized and involved in resistance reactions (Giebel, 1974) by producing necrotic tissues (Acedo and Rhode, 1971). The derouting of carbon for the synthesis of phenol from sugar source is also responsible for increasing phenolic content (Neish, 1964). It is the small known group of phenolic compounds which are ‘abnormal’ metabolites, not found in the uninfected tissues which are directly concerned in disease resistance (Cruickshank and Perrin, 1964). Mere presence of phenols, which are normal metabolites in a host plant and their accumulation at the sight of infection does not warrant the conclusion that they play a role in the resistance of the host to a given parasite.
Ascorbic acid is present in plants as such and also in its oxidized form, dehydro ascorbic acid. Ascorbic acid is an excellent reducing agent which converts quinones to phenols. Ascorbic acid has been said to play a subtle role in the resistance mechanism of plants against invading organism. The amount of ascorbic acid present in the tissue is known to be correlated with the activities of ascorbic oxidase (Farkas *et al.*, 1965). Low levels of ascorbic acid in the nematode infected plants is perhaps a consequence of the higher ascorbic acid oxidase enzyme activity as observed in the present study. Kannan (1977) and Arrigoni (1979) reported that the ascorbic acid synthesis is stimulated during infection. The ascorbic acid thus synthesized may be utilized for the synthesis of mitochondrial hydroxyproline proteins which control the development of cyanide insensitive respiration (Arrigoni, 1979) which resulted in depletion of ascorbic acid content. Zacheo *et al.*, (1977) have remarked that the resistance was based on the ability of the host to utilize the cyanide insensitive respiration to counteract the effect of the pathogen and subsequent pathogenesis. Low levels of ascorbic acid in general would keep the quinone in the unreduced condition and this in turn would contribute resistance to the disease indirectly by entering cyanide auxiliary pathway. The participation of ascorbic acid is an auxiliary respiratory pathway yielding energy is also known (Mapson, 1958). Arrigoni *et al.*, (1979) have indicated the possible role of ascorbic acid in the defense reaction of the nematode infected plants. Such a role of ascorbic acid require intensive studies with regard to its contribution to the dynamics of resistance as well as its origin in *invivo* during pathogenesis.
Kuc (1966) suggested that changes in respiration, proteins and enzymes seem to be primarily important in disease resistance because they reflect the fundamental changes in the metabolism which provide a physical or chemical environment, that is either inhibitory or conducive for the growth of a micro-organism. It has been emphasized that the hydrolytic enzymes like proteases, pectinases and celullases secreted by nematodes are responsible for the inducement of syncytium (Bird, 1974; Endo, 1975). It was also proved that enzymes like phosphatases, esterases, glycosidases and oxido-reductases were also playing major role in the formation of giant cells (Roy, 1979). Oxidizing enzymes catalyzing electron transfer from the donor to an acceptor other than molecular oxygen are the dehydrogenases. The activities of dehydrogenases and endogenous reductases in the infected root and shoot tissues of *V. mungo* were found to be at an increased level in the present study. The enzyme activities were higher in the root than in the shoot. It is not surprising that the activity of the dehydrogenases, especially of pentose phosphate pathway and of the Kreb’s cycle, increase with the general increment in metabolism of injured plants or of those attacked by pathogens (Farkas *et al.*, 1960). Correlations have been found between the total dehydrogenase activity in the tissues of the diseased plants and between the resistance to plants. Since reductases enzymes are known to function in biological reduction, an increased level of total endogenous reductases observed in the present studies would help in synthesis of lipids and proteins which reflected in an elevated level of these components.
Increases of enzyme activity in infected plants according to Farkas and Lovrekovich (1965) might be a consequence of net synthesis of new enzyme, activation of latent enzyme and increase of co-enzymes or decrease of enzyme inhibitors.

Similar results of increased activities of enzymes were reported in the host plant invaded by *M. incognita* (Kannan, 1967; Vaitheswaran et al., 2003) and by *Globodera rostochiensia* (Giebel et al., 1971). Endo and Veech (1969) demonstrated a number of enzymes in the plants infected by *M. incognita acrita* by histochemical methods. Increased activities of malate, isocitrate, succinate, glucose-4-phosphate etc., occurred in cells adjacent to both anterior and posterior regions of the nematode. The oxido-reductases seem to be connected with susceptible/resistance response of plant tissues by modifying host cell components such as phenolics, auxin and amino acids (Giebel, 1974, 1982). During the present study of nematode pathogenesis, an increase in the activities of endogenous reductases was noted apart from the increase in the activities of dehydrogenases and oxidases as reported earlier by Kannan (1977) and Vaitheeswaran (1990).

The depletion of sugar observed and increments for lipids, protein and phenol indicated the metabolic events that occurred during pathogenesis. Since these events required mediation of enzymes, the enhanced activities of the dehydrogenases (Catalytic enzymes) indicated accelerated catalysis while the increased levels of activities of the total endogenous reductases (synthetic enzymes) indicated enhanced synthesis of lipid, protein and phenol. The
reduced activities of dehydrogenases of glycerol, glucose and alcohol observed in the infected - untreated plants when compared to control might be due to reduced availability of sugar in the infected plant, since these enzymes are involved in carbohydrate metabolism (Kannan, 1977). The variations in the metabolites along with enhanced activities of oxidoreductases enzymes imply hypersensitive reactions during pathogenesis. Gall size, tissue damage etc. associated with various degrees of susceptibility or resistance can be correlated with varied levels of biochemical phenomena mentioned above. The activities of oxidoreductases of the metabolism showed increments by way oxygen consumption during pathogenesis. In response to an infection, the respiratory rate was increased in the host plant. Kannan (1977) reported that the increased activities of dehydrogenases in the galls of the plants infected with *M. incognita acrita* might stimulate respiratory metabolism which is connected with the energy release and such energy release gives resistance to plants against infection. The present results are not in accordance with findings of Bird and Millered (1962) and Owens and Specht (1966) who observed that respiration in galls of *Meloidogyne* infected tomato root is similar to that of uninfected roots of the same age.

Increased respiratory activity observed in the present study might be caused through dearrangement of some metabolic pathway which are related to changes in protein and other metabolism as reported by Fawole and Evans (2008). Results of the present study indicated that the plant that exhibited more respiratory activities may be less tolerant to *M. incognita* infection. Cowling
and Horsfall (1980) suggested that, if the host plant utilizes its food energy sources only for repairing the damaged tissues, the dynamic defenses against the pathogen will be weak. The energy content of the diseased plant will be the consequence of all the metabolic dearrangements that has been discussed above. The energy content of any organism is the sum of the protoplasm and its products. A low energy content would mean that the organism has spent much of its energy in overcoming the internal and external stress and is unable to add anything to its constructive activities. Viewed in this light, the nematode infected plant might have spent much of its energy in trying to forestall, inhibit and overcome the pathogen and its effects.

The present study indicates that the energy requirements of the plant cell is provided by the increase in the biochemical components of the tissue during infection, but the plant utilizes excess energy stored by the way of increased organic components, to combat the effect of infection which might have resulted in decreased energy content. The present findings did not confirm with that of Mohamed Ibrahim et al., (2008), who observed an increase energy content of the infected plant. It is presumed that the host expends the energy to combat the infection, so that its growth is not impeded. Externally the hypersensitive reaction which involve in varied growth of root and shoot systems. Such metabolic alteration creates an impact on the pathogen. The initial host responses are quite significant and later get stabilized so that, total destruction of the host does not occur. The energy released out of catalysis is appropriately utilized by the host for many synthetic activities. Such energy
relations might be contributing to the functional or physiological resistance of the genetically susceptible host, since even in severe infection the susceptible host continues to thrive.

The tolerance level of the susceptible hosts can be increased by controlling the nematode population coupled with root and shoot growth. The reproductive rate of the nematode is influenced by the flourished shoot growth, whereas the increased root growth influences the rate of population increase by the way of providing surface area for feeding. The influence of the root system is checked by the excess shoot growth.

Giebel (1974) has extensively detailed some of the above features involved in contributing towards the resistance of the plants to nematode. It is also known that these changes can be brought about by the environmental stress. Such environmental stress seems to control the pathogen to some extent. Extremes of environment like soil, particle size, oxygen content, pH, moisture, temperature, etc., affect the plant growth and nematode development. So that a well nourished plant is able to overcome the pathogenic impact by virtue of the nutritional status presumably under field conditions and judicious water management, good agronomic practices and fertilizers treatment play in this direction.

Control of root-knot nematode therefore is necessary and several means are adopted for this. Although the application of chemical nematicides has been found as an effective measure for the control of nematodes, due to high toxic
residual effect of chemicals on the environment and particularly on non-target organisms (Akhtar and Malik, 2000; Anastasiadis et al., 2008), there is an urgent need to develop alternative strategies for the control of nematodes.

Modern practices of pathogenic control in susceptible plants of economic importance are directed towards improving the tolerance of the host plant. Such practices are around nutritional amendments and generally spoken of as soil amendments. Such amendments are in practice by a way of usage of organic cakes of neem, castor, groundnut, cotton, chopped leaves and mulching of leaves. Such amendments are meant to boost the growth of the susceptible host and at the same time reduce the pathogenic impact (Youssef and Amin, 1997; Haseeb et al., 1998; Saravanapriya and Sivakumar, 2003; Youssef et al., 2004).

It is presumed that during the slow breakdown of organic additive in the soil, the phenolics, aminoacid, sugars and other organic acids which are released, act as nemostatic factors when nematode control is attempted (Badra et al., 1979). Nematicidal activities of organic amendments in soil can be attributed to chemical mineralization with the ultimate release of ammonia and increased nitrogen and carbon-dioxide levels (Akhtar, 1998). Various compounds including alcohols, aldehydes, fatty acids derivatives, terpenoids, isothiocyanate, thiophenics, alkaloids, glycosides and phenolic compounds exist in plant jointly or independently. Several plant terpenoids, diterpenoids, fatty acids and phenolic compounds are known to have nematicidal properties (Akhtar and Mahmood, 1994; Oka, 2001; Shaukat and Siddiqui, 2001; Siddiqui
These compounds are known as secondary metabolites. All these chemicals have been reported to be highly injurious to many plant parasitic nematode (Alam, 1976). The mode of action of methanol leaf extract of *P. pinnata* used in the present study may be similar to that reported for other organic additives.

The histopathological study of present work indicated that roots of methanol leaf extract of *P. pinnata* treated infected plants had infection site with galls containing one or few syncytia like cells that did not support the development of mature females. The lack of normal syncytia formation and poor nematode development support the concept that the maturation of larvae depends on the formation of syncytia or giant cells or giant transfer cells. Most of the larvae that entered into the root failed to develop into mature females (Table 4). It was already pointed out that during the slow break down of organic additives in the soil, some nemostatic factors are released. The plants might have absorbed any one of the nemostatic factors from the soil and the presence of this factor might have affected the nematode development and prolonged the life cycle of the nematode (Goswami and Swarup, 1972; Zaki and Bhatti, 1990; Wen yan-hua and Feng Zhi-xin, 2003). The present work was reconciled with the findings of Singh and Singh (2001) who observed that the reduction in entry of second stage juveniles, delayed development and decrease in the population of *Heterodera cajani* within host plant grown in amended soil might be also a result of altered resistance of the plant against the nematode. The organic amendments could reduce nematode population by repelling the
juveniles or by adversely affecting the development of those juveniles that have entered into the root system.

Orion et al., (1980) who noticed that higher concentration of ammonia as a nemostatic agent liberated during the decomposition of organic additives inhibited the syncytium formation which is essential for the development of the nematode, since syncytium is the feeding site for the nematode. In most studies syncytia development was also depressed by a growth retardants namely maleic hydrazide (Davide and Triantaphyllou, 1968). In the present study also the possible release of ammonia due to decomposition of organic additive could have well controlled nematodes as reported above. Usually green manure causes reduction of nematodes by increasing the release of ammonia (Rodriguez-Kabana, 1986) and enhancing antagonists to nematode (Sikora, 1992).

The methanol leaf extract of P.pinnata treatment to the infected plant caused substantial egg deformation in M.incognita in addition to killing the eggs, juveniles and females of the nematode. The present findings are in harmony with Yousif and Badra (1978), Badra (1980) and Khan et al., (1986) who reported that the efficacy of organic manure against phytonematodes could be due to stimulation of specific nematode-antagonistic micro-organisms that are capable of parasitizing eggs and other developmental stages of nematodes. Organic manures may accelerate proliferation of the microbial forms which are capable of synthesizing and producing substances toxic to nematodes. These deformed / parasitized eggs never matured or hatched. A similar finding had
been reported by Jatala et al., 1979 in fungus treated nematode infected potato plants. It was suggested that the nemostatic principles in the extract might have caused some alteration in the egg shell make-up or by their inward seepage into the egg caused physiological disorders leading to aborted embryonic development. Aborted giant cells are common in resistant plants.

The application of plant extract resulted in a small size of the final population reflecting the alleviating effect of the extract by reducing the pathogen impact. The final population load in the plant host under the influence of plant extract indicates the host responses. Host pathogen relation is the resultant of two forces viz., plant and pathogen, both of them subjected to soil influences. It can be observed that the success of the pathogen is the result of a low resistance encountered by it in the plant also the soil which is finally exhibited as high or low pathogen impact on the host. It is clear that the final interpretation of the host pathogen relations are based on the interactions of the two forces namely susceptibility rating and environment resistance factor. These factors are applicable in the present study as indicated by the varying final population loads in different concentrations of extract treated infected plants. The influence of the environment resistances over the pathogen, inside the plant and outside the plant (soil) was well observed in the varied population loads in these two environments and the specific influence of the extract was well observed (Table 9). With increased environmental resistance factor in both the environments (Plant and soil) the final population load was depressed. This resulted in altered host pathogen relations in the host plant, which is now
presumed to exhibit improved vigour through plant extract amendment in the soil.

It was evident that plant extract treatment exerted a depressed effect on the nematode by drastically reducing the nematode’s fecundity and galling phenomena (Table 9). The significantly increased shoot and root weights of plants grown in soil with (organic) plant extract additives compared to those in infected untreated soil could be attributed to the reduction in nematode densities and to the fact that this extract serves as organic manure. The present results are also in accordance with the findings of Abbasi et al. (2008) who observed that soil treated with dried powder of Barleria acanthoides improved the height and fresh shoot weight of nematode infected brinjal and okra plant when compared to untreated control. In the present study, the nematode population within the plant decreased following varied concentrations of plant extract treatment. A negative relation was obtained (Fig. 4) \((r = -0.9872)\) with regard to shoot growth, as the shoot weight increased with decreased nematode population density within the plant. The same relationship was also witnessed between shoot growth and rate of reproduction of the nematode (Fig. 5) \((r = -0.9576)\).

Similar observations on better growth of plants in nematode amended soils have been reported by Goswami and Meshram (1991); Haseeb and Butool (1993); Youssef and Amin (1997); Jacob and Haque (1998) and Uma Maheswari and Rajeswari Sundarababu (2001). It was noted that general plant vigour was more correlated with reduced incidence of disease rather than to
any other factor. The present findings were also in agreement with those of Goswami and Vijayalaxmi (1986), Siddiqui et al. (1987), Govindh et al. (1989) and Tiagi et al. (1988). The low galling phenomena and small number of juveniles recovered from plant and soil amended with plant extract suggest that this extract was believed to be toxic to *M. incognita*.

Significant reduction in population of phytonematodes by the use of marigold, tobacco, raw sewage sludge and poultry droppings has been reported earlier (Ahmed *et al.*, 1996). Dropkin (1980) also suggested that breakdown of organic matter in soil releases compounds including acetic, propionic and butyric acids which remain for several weeks in the soil sufficient to induce death of some phytonematodes.

Most of the organic amendments increased the general vigour of the plants and growth of the root system. This may also be one of the important reasons for lesser occurrence of root-knot nematode in the soil. When organic amendments were applied, the establishment of plant was found to be quick and the root growth and number of rootlets increased rapidly. This could have favourably improved the tolerance of plants to nematode invasion and infection.

The effect of amending factors as extrinsic factors applied to host reveal that these factors exert a favourable role on plant growth along with controlling the nematode. A progressive increase in sugar, lipid, protein, phenol and redox enzyme activities was discernible, indicating the dynamics of pathological
metabolism of the infected plants under varied concentrations of plant extract
treatments. Abbasi et al., (2008) also observed increased level of protein in root
knot nematode infected okra and brinjal plant when treated with leaf extract of
*B.acanthoides*.

During metabolic stress due to dietary deficiency or during pathogenesis
with tissue lysis, the various metabolites might have modulated by routing the
carbon reserves through shikimic and other pathways leading to production of
sugar, protein, lipid and phenol. The metabolic modulations and their effect on
growth of the host during pathogenesis have been established in the present
study (Table 7 and 17). During stress, the shoot and root reactions vary and
what was depleted in one region would be compensated through mobilization
from other region. The variations in the different metabolites in the shoot and
root reflected some of these phenomena. The effect of plant extract could be
evidenced in the improved metabolic performance, improved growth and
depressed pathogenic activity in the infected host indicating the ideal nature of
the same towards nematode control.

It is known that smaller molecules of aminoacids, sugars, phenol and
phenolics that formed during the decomposition of plant extract in the soil, are
actively absorbed by plants and metabolized and these products are known to
interact with the nematode’s enzyme (Wallace, 1963; McIntyre, 1980). Under
this situation a possible altered enzyme substrate relations could be sensed and
this situation may not be conducive for the root-knot nematode, *M.incognita*,
since it is an obligate endopathogens, requiring the proper substrate for feeding,
development and egg laying. Under this condition, lesser output of progeny was noted in the present study (Table 9).

The presence of break down products and secondary metabolites in the extract seemed to create a stress on the pathogen by altering the substrate-enzyme feeding relations of the nematode. Under this stress the pathogen could have been impeded from consuming the sugar faster, with the result not only the subsequent progeny output of the nematode was getting reduced, but also a high sugar level was discernible in the plant extract treated infected plant and reached maximum level in higher concentration (3500 ppm) of extract treated infected plant. An inverse significant (Fig. 6) \( r = -0.9622 \) relationship observed between the sugar content and rate of nematode reproduction under varied concentration of plant extract treatment indicated that the rate of reproduction of the nematode was lowered as the sugar levels in the root increased. The relationship between the protein levels in the root and root gall number index (average number of galls / plant) was found to be a negatively significant one (Fig. 7) \( r = -0.9661 ; p < 0.01 \). This correlation clearly indicated that the root protein level in the varied concentrations of extract treated infected plants would be indirectly proportional to root gall number. The relationship between the total number of eggs and protein content in root tissue of varied concentrations of extract treated infected plant was also observed to be negatively significant one in the present study (Fig. 8) \( r = -0.9904; p < 0.01 \). Earlier studies of McClure et al., (1974) and Shepherd (1979) also indicated that the egg count was the most appropriate criterion in
assessing the nematode development and the degree of the host resistance, as ‘empty’ galls without nematodes (McClure et al., 1974) and egg masses with varying number of eggs (Shepherd, 1979) were observed.

In the presence of these higher sugar levels, the sugar carbon might lead to enhanced lipid, protein and phenol synthesis as observed in the extract treated infected plant. As a consequence of this, growth of the infected plant was not reduced but got increased (Plate IX - 2, 3 and 4). Improved growth could probably be resulted in improved vigour and hence the pathogenic impact was presumably lessened.

A perusal of the redox enzyme activities showed a specific increase of dehydrogenases activities in the infected host under plant extract treatment. This indicated greater energy yield through catalysis, due to expected tissue damage by the nematode, as suggested by Kannan and Balaji (1988) who observed that amended nutrient application improved root weights and synthesis of metabolites with reduced galling and fecundity of the pathogen, which signified the improved vigour of the infected host.

However, this seems to be offset by the increased velocities of endogenous reductases involved in synthesis. The synthetic velocities of endogenous reductases are almost two fold greater than the lytic enzymes ie dehydrogenases. The resultant of these two enzyme activities was quite evidenced in increments of sugar, protein, lipid, phenol and other biochemical parameters, when the plant extract was introduced as treatments to infected
plants. The defensive role of sugars, proteins and phenols was well established in all plant pathogens (Goodman et al., 1967).

**GC-MS study**

In the present study, the GC-MS analysis of methanol leaf extract of *P. pinnata* revealed the presence of Alkaloids (4-Piperidinamine, N, 1-dimethyl; 4-Hydroxy-N-methylpiperidine), Diterpene (phytol), Fatty acid (n-Hexadecanoic acid or palmitic acid) and Steroids (Stigmasta-5, 22-dien-3-ol, acetate, (3β); Stigmastan-3-ol, 5-chloro-, acetate, (3β,5α)). These compounds are believed to have nematicidal effects. The phytochemical compound such as alkaloids present in different plants have nematicidal activity (Gommers and Barker, 1988; Chitwood, 1992). The different alkaloids viz., ricinine isolated from *Ricinus communis* (Rich et al., 1989), Peniprequinolene from *Penicillium simplicissimum* (Kusano et al., 2000), Paraherquanide from *penicillium paraherquanide* (Guohong Li et al., 2007) have nematicidal activities against root-knot nematodes. *Azadirachta indica* contains a number of alkaloids and lipid associates in various tissues in various concentrations (Ghosh, 1994a) which have antinematicidal properties. The leaves of papaya possess alkaloid which decreases the percentage of egg hatching in *M. incognita* (Ghosh, 1994; Anuja Bharadwaj and Satyawati sharma, 2007).

The alkaloids might have altered the movement of the juveniles of *M. incognita* to a jerking motion and probably caused paralysis of the juveniles (Plate V-2, VI-2). The present finding are comparable with that of Fassuliotis
and Skucas (1969), who isolated monocrotaline, a pyrrolizidine alkaloid from *Crotalaria spectabilis* which altered the movement of juveniles of *M. incognita* *in vitro* to a jerking motion and significantly reduced infestation of tomato plants. The presence of pyrrolizidine alkaloids such as grantianine and grantaline (Smith and Culvenor, 1984) in *C. grantiana* caused paralysis of juveniles of *M. incognita* and delayed the penetration of the larvae to the susceptible tomato plants.

The secondary metabolite diterpenoids present in some medicinal and aromatic plants possess nematicidal and allelopathy activities (Mathela, 1994; Chitwood, 2002). Diterpenoids compounds from wild sun flower have acted as antifeedants to several insect species and growth inhibit properties (Cooper-Driver and Le Quesne, 1987). From the above findings it could be established that the presence of the metabolite diterpene in the experimental plant extract would also have antinematicidal effect.

The fatty acids, heptanoic-undecylenic are nematicidal in nature (Tarjan and Cheo, 1956; Loos, 1958). Myristic, palmitic and oleic acids were identified as the nematicidal properties in benzene extracts of roots of *Iris japonica* (Iridaceae) (Munakata, 1983) and fatty acids found in most plants are nematicidal in nature (Saleh et al., 1987; Anke and Sterner, 1997; Perez et al., 2003; Park et al., 2005) Dodecanoic acid and myristic acid isolated from *Tagetes erecta* flower also showed antinemical activity (Ray et al., 2000).
Several essential oils of plant origin inhibit acetylcholine esterase activity in insects (Sridhar and Sulochana Chetty, 1989). They also reported that *Pongamia glabra* leaf extract contains fatty acid which is responsible for blocking the pores of the cellular membrane of the alimentary canal of *Euproctis fraterna* and reduction of growth and growth rates. In the present study, the life cycle duration of the nematode was prolonged in extract treated infected plants. Accordingly, Dryer *et al.*, (1979) showed that Pinitol, a fatty acid extract isolated from soya bean leaves reduced the weight gained by the larvae of *Heliothis armigera* and prolonged the life cycle duration. Further the fatty acid might also disrupt the cell membrane of the insect and change its permeability as suggested by Sridhar and Sulochana Chetty (1989). The presence of fatty acid, n - Hexadecanoic acid (palmitic acid) in the extract might have prolonged the lifecycle duration of the nematode and also interfered in the permeability of cell membrane. The nematode control achieved by decomposition of plant residues in soil was found to be due to liberation of fatty acids (Montasser, 1991; El-Naggar *et al.*, 1993). Badra *et al.*, (1979) also reported that the presence of high concentrations of fatty acids in organic amended soil was toxic to *Rotylenchus reniformis*.

The plant steroids such as furostanol glucosides or glycoalkaoids have nematicidal activity on *M.incognita*  (Udalova *et al.*, 2004). The presence of two different steroids in the extract could be considered for nematicidal activity.
The presence of secondary metabolites in the extract might have reduced and delayed the penetration of second stage juveniles to the host plant, *V. mungo*. The protection of the host plant from *M. incognita* infection by methanol leaf extract of *P. pinnata* added to the soil may be either due to various suggestions like loss of mobility due to paralysis of the second stage juveniles, disturbed orientation and host finding (linked to disturbing of chemoreception or injury to sensory organs) thereby disturbing nematode coordination in stimuli gradient or affected response to root attractants or the combinations of all (Green, 1971) have been given for protection of the host plant from *M. incognita* infection by methanol leaf extract of the *P. pinnata* added to the soil.

The pathogenic stress in plants might be compensated by an increased metabolic continuum to combat the stress and in this direction the host plant spends minimal energy for the combat, conserving the rest for repair and growth. The reduced galling phenomena, coupled with the improved weight of the host plant and the enhanced metabolism observed in the present study seemed to indicate improved vigour of the host plant under varied concentrations of extract treatment.

In agricultural soil where humus is lesser, naturally parasites are more abundant and soil amendment with plant extract, oil cake, lay, etc., promote humus formation and decomposition which improve plant growth and tolerance to pathogens.
The readily available nemostatic agents in the extract and other organic amendments are easily absorbed and metabolized by the plant for its sustenance inspite of infection. The nematicides present in the extract had an induced resistance property which complied also with Giebel’s (1982) definitions that induced resistance could be considered when the chemicals used for this purpose do not interfere with host metabolism to such a degree as to be phytotoxic. The present study provided evidence of plant extract performance as an agent to induce resistance when applied to the soil in a greenhouse under controlled environmental condition. Though the extract contains different secondary metabolites a further future study is imminent to ascertain the role of a particular metabolite in controlling the nematode population with an improved tolerance level of any host plant.

It may be stated that from the economic point of view, cost of control is also within the farmer’s reach. Further, pollution hazards of chemical pesticides are not involved in this method, and the hazards of biological pollutions are minimal. This method of controlling nematodes is of paramount importance to countries like India, where large scale use of nematicides is impractical.