INFLUENCE OF SOIL FUNGI ON THE MANAGEMENT OF PLANT-PARASITIC NEMATODES BY ORGANIC AMENDMENTS

DISSertation submitted to the Aligarh Muslim University, Aligarh in partial fulfilment of the requirements for the degree of Master of Philosophy in Botany

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CERTIFICATE

This is to certify that the dissertation entitled "Influence of soil fungi on the management of plant-parasitic nematodes by organic amendments" is a faithful record of the bonafide research work carried out by Miss Aparna Yadav under my guidance and supervision. Her work is up-to-date and original. She is allowed to submit the dissertation to the Aligarh Muslim University, Aligarh for the consideration of the award of the degree of Master of Philosophy in Botany.

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INTRODUCTION & REVIEW OF LITERATURE
1. INTRODUCTION & REVIEW OF LITERATURE

Plant-parasitic nematodes are microscopic multicellular invertebrate animals. They spent entire life or at least a part of it in soil. Plant-parasitic nematodes are potentially serious disease causing agents. As a matter of fact there is not a single plant which is not being parasitized by one or more kinds of nematodes at a given time. The plant damage due to nematodes results in reduced crop yields and lowered quality of the product. There are several reports regarding the crop losses due to nematodes; some workers have attempted to estimate the crop losses in terms of money. Cairns (1955) estimated an annual loss of $500 million resulting from plant-parasitic nematodes. Hutchinson et al. (1961) reported about $250 million loss and Taylor (1967) about $372,335,000 on various crops. An average annual loss of 50,000,000 Kroners resulting from _Heterodera avenae_ in Denmark is reported by Stapel (1953). Southey & Samuel (1954) reported a loss of about 200,000 tones of potato costing about £2 million due to _Globodera rostochiensis_ in U.K. and Wales. Recently, Feldmesser et al. (1971) reported annual losses in the United States due to nematodes to the tune of $1,038,374,300 in 16 field crops, $225,145,900 in 23 fruits and nut crops, $266,939,100 in 24 vegetable crops and $59,817,634 in ornamental crops.

In India, there are few reports regarding the crop losses due to nematodes. Vanberkum & Seshadri (1970) were first to
estimate the crop losses in wheat, barley and coffee in India. They estimated the losses of $10, 8 and 3 million due to **Anguina tritici**, **Heterodera avenae** and **Pratylenchus coffeae** respectively. The assessment of Sasser & Freckman (1987) for crop losses due to nematodes on world basis has given a very grim picture. They have pointed out that losses must be more than $100 billion per annum. Although nematode problems occur in all areas of the world, however, serious problems are more frequent in warm regions with long growing season resulting in more damage due to multiple generations in a season.

The losses caused by nematodes are so enormous that considerable efforts have been made to reduce crop losses by means of different control measures. These methods can be categorised into the following groups: Regulatory, Physical, Chemical, Biological and Cultural.

**REGULATORY METHOD**

Nematodes themselves are incapable of moving long distances. Their spread, therefore, largely depend upon different agencies, such as rain, flood water, wind, etc. These are also transferred from place to place through infested soil adhering to the plants (e.g., **Heterodera**, **Globodera**, etc.), or infected plants (e.g., **Meloidogyne**), seeds and other propagating plant parts (e.g. **Anguina**, **Aphelenchoides**, **Ditylenchus**).

Uncontrolled exchange of infested agricultural products results in the introduction of pests and pathogens to the otherwise
free areas. In the absence of natural checks, these exotic organisms easily establish and sometimes increase to noxious levels. In case of plant-parasitic nematodes, there are several examples where important species have been introduced into new areas by way of transference of nematode-infested materials. For example, *Globodera rostochiensis*, the famous golden cyst nematode of potato, was introduced in Europe during the last century along with potatoes imported from South America for breeding purposes. Since then this nematode has spread to Asia, North Africa, North and Central America. The banana plants which were imported to Australia from Fiji during 1890-1920 were mainly responsible for the dissemination of *Radopholus similis* to that country.

Like other pests, the spread of nematodes is easily checked by regulatory method by way of Quarantine Laws inacted by different Governments. These laws restrict the movement of infested plants, fruits, seeds and other propagating materials and also soil adhering the roots. Plant Quarantine may be defined as an endeavour to prevent or limit the spread or introduction of dangerous diseases and disease causing organisms.

First Plant Quarantine Law was inacted in Roumen, in France in 1660 in order to suppress and prevent the spread of barberry when it was suspected to be responsible for stem rust of wheat. In India, the first such act "The Destructive Insect and Pest Act" was inacted in 1914, Quarantine regulations are meaningful only when the pests and pathogens are not disseminated by natural agencies.
PHYSICAL METHOD

Physical method of nematode control involves different treatments such as solarization, steam sterilization and pasteurization of soil, electrical soil heating, short wave diathermy, peak-heating, "cooking" out of mushroom composts, hot water treatment, washing process, seed cleaning, etc. Soil steaming is common practice in glasshouse-raised crops to remove the nematode infestations of soil. Small quantities of soil may be placed in steam chamber or are autoclaved under pressure. Christie (1959) suggested that all plant-parasitic nematodes are killed at temperatures between 44°C to 48°C. In soil solarization, destruction of nematodes has been done by desiccation or exposure to high temperature during summer ploughing. Another important physical methods of nematode control, i.e., hot-water treatment is recommended for disinfesting seeds and other propagating materials. Hot-water treatment is found useful in controlling citrus nematode (*Tylenchulus semipenetrans*), burrowing nematodes of banana (*Radopholus similis*) and a large number of nematodes attacking ornamental plants. The different types of treatment as mentioned above, require specialized equipments and a high degree of accuracy. These treatments also affect the non-target organisms and also influence the viability of the propagating materials. Use of radiation and electricity for the control of plant-parasitic nematodes may be risky and hazardous for the operator.
For medium and high value crops nematicides are one of the most important and reliable means of controlling a wide variety of nematodes.

Kuhn (1881) was first to use a nematicide. He suggested the use of carbon bisulphide as soil fumigant to control sugar-beet nematode *Heterodera schachtii*. Chloropicrin, another soil fumigant was found to be very effective in controlling root-knot nematodes in field by Matthews (1920), Johnson (1935) tested a number of chemicals against root-knot nematodes and found carbon bisulphide and chloropicrin highly effective. Christie & Cobb (1940) and Taylor & McBeth (1940) used methyl bromide (MBR) against nematodes. Modern era of nematicides started with the discovery of D-D mixture (1,2 dichloropropane + 1,3, dichloropropene) by Carter (1943). This fumigant was found to be highly satisfactory for large scale applications. In 1946, Thorne & Jenson used ethylene di bromide in the control of nematodes. The finding of DBCP (1,2 dibromo-3-chloropropane) or Nemagon gave a new impetus in the management of nematodes because at that time this was the only nematicide which could be used in standing crops. This very discovery was considered at that time to be ultimate means of nematode control. However, the recent observations about its side effects on the workers and its hazardous nature has led to the banning of this and many other nematicides by different Governments. Since then a large number of chemicals, such as phorate/Thimet [O, O, diethyl
S-[(ethyl thio) methyl] phosphorodithioate, fensulfothion/
Dasanit [O, O-diethyl-O[4-methylsulfinyl-phenyl-monothiophosphate]
Vydate/oxamyl {methyl N-N dimethyl-N-[methyl carbamyl] oxy-1-thio oxamimidate}, dimethoate/Rogor {0,0-dimethyl-S-[N-methyl carbomylmethyl] phosphorodithioate}, aldicarb/Temik {2-methyl-2
(methylthio) propionaldehyde-O-(methylcarbamoyl) oxime} 
carbofuran/Furadan {2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate} etc., have been found very effective in controlling the nematodes.

The advantage of using nematicides is to get sure and instant control of nematodes, but in most cases it is observed that use of chemicals for control of plant-parasitic nematodes is a short term solution as nematode populations tend to increase many fold in the next crop if the treatment is not repeated. Besides, the extensive use of nematicides leads to the accumulation of toxic compounds in soil which affects the beneficial soil microflora and microfauna. These chemicals are very costly and sometimes not easily available. These problems have given importance to the researches on non-chemical methods of nematode control.

**BIOLGICAL METHOD**

Sewell (1965) defined biological control as "the induced or natural direct or indirect, limitation of a harmful organism or its effects by another organism or group of organisms". Many predaceous or parasitic organisms such as
fungi, bacteria, protozoans, viruses, nematodes, tardigrades, collembolans, tubellarians, mites, oligochaetus, enchytraeids, etc. have been used in the biological control of plant-parasitic nematodes. Cobb (1920) expressed optimism about introducing exotic nematode antagonists. In his words "There can be no doubt, however, that the enormous losses due to plant-infesting nemaes fully justify the expenditurer of even large sums of money in an effort to apply this remedy more particularly because the remedy, when successful, bids fair to be permanent and self-sustaining." Though biological control appears to be highly promising. Yet it has many constraints in its practical application. It is a great problem to artificially raise and introduce nematode antagonists to the field. However, it is possible to create conditions which increase environmental resistance against nematodes. Alam & Jairajpuri (1990) have shown optimism in the utilization of organic amendments in the manipulation of natural enemies for the control of plant-parasitic nematodes. This aspect has been discussed in detail elsewhere in the dissertation.

CULTURAL METHOD

In spite of development of the chemical method, cultural practices have shown excellent promise for nematode control. Cultural practices involve the principles of avoidance, exclusion as well as eradication of plant diseases. Various cultural practices such as ploughing, flooding, prevention of spread,
crop rotation, use of resistant varieties, selection of healthy propagating materials, addition of organic additives have been found very useful in the management of plant-parasitic nematodes. With rotation of crops, the parasite is deprived of food for a given length of time. With summer fallowing, in addition to starvation the nematode is also affected by soil desiccation and direct heat from the sun. But fallowing causes monetary losses to the grower. Similarly when the land is submerged in water for 1-2 years it results in disappearance of root-knot and other nematodes but the grower is also deprived of farm income during the period of flood treatment. The development of resistant varieties in itself is a very time-consuming and costly affair. Moreover, every resistant variety may not be suitable for all kinds of agro-climatic zones. Continuous use of resistant variety may help evolve new races of nematodes due to selection pressure. Resistant-breaking pathotypes of nematodes have also been reported. Among different cultural practices, manuring has been found highly suitable against a number of nematode diseases. Recent studies have indicated it to be a possible alternative to the chemical control, particularly in the developing countries, where organic wastes are available in plenty. Moreover, it provides safer disposal of these wastes. A detailed review is given below.

**Organic amendments**

Modification in the soil environment is an important mean of nematode control because their populations at any time
are determined by habitat conditions. So, much attention has been given to exploration of effects of various kinds of organic amendments on nematodes, e.g., compost, green crop residues, meals, oil-cakes, cellulose materials, sugars, sewage-sludge, bagasse, etc. The materials when added to soil cause reduction of populations of plant-parasitic nematodes.

Linford and his associated (1936-38) were first to demonstrate the value of decomposition of vegetable matter in soil in the reduction of population of plant-parasitic nematodes (Linford, 1937; Linford et al., 1938). Considerable work has been done on the use of organic amendments in the control of nematodes. The subject has been reviewed by Oostenbrink (1960), Singh & Sitaramaiah (1970), and Muller & Cooch (1982).

Oostenbrink (1954) found reduction in the population of *Pratylenchus* sp. by adding farmyard manure to the soil. Van der Laan (1956) reported reduction in the severity of *Globodera rostochiensis* infection in potato. There is also a report from India, about the use of farm-yard manure in the control of root-knot nematodes. Desai et al. (1969) suggested deep ploughing of field during summer followed by the application of farm-yard manure at the planting sites. It not only decreased the infestation level of root-knot nematode but also increased yields of tomato. Morgan & Collins (1964) observed the reduction in the population of *Pratylenchus penetrans* by amending the composted timothy hay into the soil. Mankau & Minteer (1962) reported reduction in numbers of *Tylenchulus semipenetrans* by amending
the soil with steer and chicken manure. Mankau (1963) made laboratory, greenhouse and field studies on the control of *Tylenchulus semipenetrans* and *Meloidogyne* sp. in soils receiving organic fertilizers. Sewage sludge, a source of organic nitrogen, reduced the populations of *Belonolaimus longicaudatus* and *Hypsoperine graminis* in turf grass and was found to be more effective than ammonium nitrate (Heald & Burton, 1968).

Otiefa et al. (1964) reported that pigeon droppings favoured the development of nematophagus fungi and thus was effective in reducing nematode population. Vega & Gatica (1970) used horse dung manure in fruit tree nursery for the control of phytonematode. Upadhyay et al. (1975) observed reduction in nematode population by adding poultry manure in the soil. Yousif & Badra (1981) observed the significant reduction in population of root-knot nematodes with sheep dung and rice straw, which were found to be more effective in controlling the root-knot nematode in comparison to pigeon droppings, horse dung and poultry manure.

Green manuring is conventional practice to improve organic matter and nutritional status of the soil for succeeding crop. Green plants are grown and ploughed or are directly incorporated to the soil. The green organic matter, during its decomposition brings about important changes in physical and chemical condition of soil besides influencing the microflora and microfauna. Linford & Yap (1939) reported that incorporation of 50 to 200 tons/acre chopped pineapple leaves to the soil infested with
root-knot nematodes reduced the disease incidence significantly. Duddington & Duthoit (1960) and Duddington et al. (1961) noticed high reduction in cereal root-eelworm (*Heterodera avenae*) when chopped cabbage leaves were applied to the infested soil. Hams & Wilkin (1961) observed that green manuring had given better crops in soil infested with *Heterodera* sp. Hutchinson et al. (1960) reported low populations of *Hoplolaimus tylenchi-formis* and *Pratylenchus penetrans* in soil where pieces of pumpkin were left to rot as compared with rest of the soil. In root-knot infested soil, Patel & Desai (1964) tested five green manuring crops. They found *Melilotus alba* var. *annua* and *Sorghum vulgare* effective in reducing root-knot development. Singh (1965) obtained 50% reduction in root gall intensity by addition of chopped leaves of karanj (*Pongamia glabra*). Mankau (1968) used alfalfa green manure for controlling root-knot nematodes. Singh & Sitaramaiah (1967) found that 5-10% (wt/wt) of leaves of *Azadirachta indica*, *Melia azedarach*, *Cassia fistula*, *C. occidentalis*, *Crotalaria juncea* and *Sesbania aculeata* applied to infested soil (in pot cultures) suppressed the incidence of root-knot of tomato and okra. They also worked out the effective dose of *C. occidentales* which should be given to the field for getting maximum control. They found it to be 8000 kg/acre.

Application of leaves and different parts of neem (*Azadirachta indica*) were found to be effective in reducing the plant-parasitic nematodes on different hosts (Singh & Sitaramaiah,
1967; Lall & Hameed, 1969; Vijaylakshmi et al., 1979; Ram & Gupta, 1980, 1982; Gokte & Swarup, 1988).

There are also reports regarding the use of wild plants as green manures. Alam (1986) reported some wild plants, e.g., Solanum xanthocarpum, Calotropis procera, Crotonbonplandianum Argemone maxicana and Datura metel to be highly effective in reducing the incidence of Meloidogyne incognita and Tylenchorhynchus brassicae in eggplant significantly. In a later study Alam (1987) found that chopped leaves of other weeds when applied to naturally infested soil, effectively suppressed populations of plant-parasitic nematodes and improved the growth of tomato. Alam (1986, 1987) claimed that this type of management of phytonematodes was economical, easy and pollution-free. Siddiqui & Alam (1989) got satisfactory control of Meloidogyne incognita and Rotylenchulus reniformis on tomato and eggplant with soil application of chopped plant parts of a noxious weed, water hyacinth (Cichhornia crassipes). Water extracts of water hyacinth also showed nematicidal and nemato-static properties. Soil amendment with plant wastes of marigold (Siddiqui & Alam 1987, 1988a) and latex bearing plants (Siddiqui et al., 1987) have also shown promise in the control of plant-parasitic nematodes.

Haseeb & Alam (1984), Haseeb et al. (1984), Tiyagi et al. (1988) and Akhtar & Alam (1989) have used chopped plant parts of a large number of cultivated as well as wild plants against plant-parasitic nematodes.
Use of mature dry crop residues has also been found beneficial against the plant-parasitic nematodes. A mixture of legume hay and pine straw suppressed the development of root-knot and lesion nematodes on tobacco (Caines, 1945). Johnson (1959, 1962, 1963) Johnson et al., (1967) reported that dry crop residues of lespedeza, alfalfa, oat and flax suppressed the number of galls in plants. It was also observed that greatest control of nematodes was possible when residues were left for decomposition for 30 days. Mountain & Elliott (1962) suggested the control of *Pratylenchus penetrans* by ploughing rye straw into infested soil during summer. They also found that manuring of soil with rye straw was as effective as fumigation with dichloropropane-dichloropropene mixture. Mankau & Minteer (1962) applied cotton waste, lucerne pellets, lucerne hay, alfalfa hay, alfalfa pellets, sugar-beet pulp to the soil for the control of *Tylenchulus semipenetrans*. Tomerlin & Smart (1969) found that reduction in the population of *Belonolaimus longicaudatus* and other plant-parasitic nematodes by applying rice straw at 9.0 or 17.9 t/ha.

Since cellulose is the major constituent of crop residues, so, workers have also tried other sources of cellulose such as wood shavings, waste paper, etc. Miller & Edginton (1962) demonstrated that chopped paper or white pine sawdust reduced *Pratylenchus penetrans* significantly. Miller & Wihrlieh (1966) reported control of *Heterodera tabacum* with 1% amendment of paper and white pine sawdust. Singh & Sitaramaiah (1967, 1971) and
Singh et al. (1967) amended saw-dust of Shorea robusta (salwood) in the soil in order to reduce root-knot of okra and tomato. Miller & Edgington (1962) and Miller et al. (1968) reported that cellulose amendment checked the larval emergence and eggplant root invasion by larvae of Heterodera tabacum. Mankau (1963) observed that addition of cellulose in infested soil lead to reduction in numbers of Tylenchulus semipenetrans and increase in numbers of microbivorous and fungivorus nematode species in soil. Srivastava et al. (1971) reported that the development of galls caused by Meloidogyne on tomato and eggplant was significantly reduced by the addition of saw-dust to the infested soil. Sikora et al. (1973) found that addition of sugarcane bagasse at 4000 kg/ha to field before planting tomatoes, caused 22% reduction in galling, while 100-150 days after planting, the reduction was 90% in the population of Meloidogyne sp. Roy (1979) used the decaffeinated tea and water hyacinth for the control of root-knot nematode.

Use of oil-cakes and meals as soil amendment have been found effective against a variety of plant-parasitic nematodes. Oil-cakes of castor, groundnut, cotten seed, mustard, mahua, rapeseed, neem and karanj have been found nematicidal in action. Oilcakes have been found more effective in moist soils than in dry soils. Lear (1959) observed the reduction in root-knot nematode (Meloidogyne javanica) and Heterodera schachtii populations as well as in root-galling in tomato when soil was
amended with castor pomace. Mankau (1963) reported 100% reduction in populations of *Tylenchulus semipenetrans* and *Meloidogyne sp* by amending the soil with castor pomace. Singh & Sitaramaiah (1966, 1969a) tried several oilcakes such as neem/margosa (*Azadirachta indica*), castor (*Ricinus communis*), linseed (*Linum usitatissimum*), mustard (*Brassica juncea*), groundnut (*Arachis hypogaea*) and mahua (*Madhuca indica*). They found that all the above oilcakes were capable of reducing galls/plant when applied in the infested field, 3 weeks before planting okra and tomatoes. Similar, results were also observed by Alam *et al.* (1977c), Goswami & Midha (1987), Prakash Rao & Bajaj (1984). Oilcakes were also found effective in controlling nematode associated with cereals, legumes and other crops. (Prasad *et al.*, 1972; Mathur & Prasad, 1974; Mishra & Prasad, 1974; Alam & Khan, 1974; Ismail *et al.*, 1976; Sharma *et al.*, 1981; Vijaylakshmi & Prasad, 1982; Alam & Ashraf, 1986). Mathur & Prasad (1973) obtained efficient control of rice root-nematode, *Hirschmanniella oryzae* by amending the soil with several oilcakes. Alam *et al.* (1977c) also provided evidence that efficacy of oilcakes persisted even in the next crop after a lapse of six months.

Oilcakes have also been reported to be highly effective in controlling phytonematodes in nurseries of perennial plants, viz., papaya, pomegranate, mango, black-berry, lemon, bougainvillia and rose (Khan *et al.*, 1974; Alam *et al.*, 1977a). Alam (1976) noticed significant reduction of plant-parasitic nematodes with
oilcakes in nurseries of vegetables like tomato, eggplant and chilli.

Alam (1976) made a comparative study about the efficacy of oilcakes in two different seasons - summer and winter, and also in two different soil types, one with high organic content and pH at 8.4 and another with low organic content and pH at 7.7. In all these conditions oilcakes were found highly efficacious against a variety of nematodes attacking different crops.

Several vegetable and animal oils have been found to reduce populations of soil-borne plant-parasitic nematodes. Ellenby (1945a, 1945b, 1946, 1951) reported that 0.1 cwt mustard oil/acre decreased the emergence of larval from cysts but increased the yield of potato by 100%. Walker et al. (1967) used the vegetable oils such as corn oil, cotton seed oil, groundnut oil and soybean oil for reducing the population of Pratylenchus penetrans. They found corn oil most effective against the nematode. Renninger et al. (1953) sprayed 1.5% water soluble hydrogenated fish oil on the surface of the soil to reduce the populations of Radopholus similis in the infected roots of citrus.

Chitin amendment to the soil, significantly reduced root-knot nematode, Meloidogyne and other plant-parasitic nematodes on different hosts (Mankau, 1963; Mankau & Das, 1969, 1974; Saka, 1978; Mian et al., 1982; Godoy et al., 1983; Rodriguez-kabana et al., 1983, 1984).
Miller et al. (1973) demonstrated that addition of chitin along with hemicellulosic wastes to soil suppressed Pratylenchus penetrans and Tylenchorhynchus dubius. Culbreath et al. (1986) observed that application of chitin alone and in combination with hemicellulosic waste and oil cakes not only declined the population of plant-parasitic nematodes but also reduced the phytotoxic effects of chitin.

Walker et al. (1967) have reported the reduction in populations of Pratylenchus penetrans in soil amended with corn and soybean meal. Tomerlin & Smart (1969) have also found similar results for alfalfa and cotton seed meal. Alam et al. (1977b) demonstrated that use of bone meal as phosphatic fertiliser effectively suppressed the population build up of Hoplolaimus indicus, Helicotylenchus indicus, Rotylenchulus reniformis, Tylenchorhynchus brassicae, Tylenchus filiformis and Meloidogyne incognita in twelve different crops. Similar results were also obtained by amending the soil with horn meal (Alam, 1976).

The presence of high concentration of sugar in soil and solution kill the nematodes, due to osmotic destruction of body. As little as 1,000 ppm of dextrose had been found nematicidal by Feder (1960). Feder et al. (1961, 1962) demonstrated that addition of 1,000 ppm of monosodium-lauryl sulphate detergent to dextrose enhanced the osmotic effect of dextrose. Roman (1963) observed that 0.5 to 10% sugar amendment to soil suppressed the
root-knot nematodes. He also reported phyto-toxicity beyond 10% dose of the treatment.

Several workers have attempted to workout the mode of action of organic amendments against plant-parasitic nematodes. The different theories put forward by them from time to time are given below:

1. Organic amendments cause changes in the physical and chemical properties of soil which are inimical to nematodes (Ahmad et al., 1972).

2. As a result of the application of organic amendments, plant nutrients are released which accelerate rapid root development and overall plant growth and thus helping the plants to escape nematodes (Van der Laan, 1956; Alam et al., 1977, 1980; Sitaramaiah & Singh, 1978a).

3. Nematicidal/nematostatic substances, present in the amendments, are released after dissolution in water, or, in other words the organic matter itself is toxic to nematodes (Khan et al., 1974; Alam et al., 1978, 1979).

4. Predacious and parasitic activity of soil microorganism is enhanced by incorporating the organic matter to the soil (for references, see the review on the topic).

5. Toxicants are produced/released during microbial decomposition of organic amendments (Khan et al., 1974; Alam et al., 1982; Sitaramaiah & Singh, 1978b).
6. Metabolites of microbes, which are activated by organic amendments, are toxic to plant-parasitic nematodes. (Rodriguez-Kabana et al., 1965; Walker et al., 1965; James, 1966; Mankau, 1969; Alam et al., 1973; Khan et al., 1981).

The last three modes of action of organic amendments as listed above, are closely related to the topic of research. A brief review of the subject is as follows:

It has been observed that addition of decomposable organic matter stimulate the development of saprozoic nematodes and naturally occurring populations of nematophagous fungi (Linford, 1937; Linford & Yap, 1938; Linford et al. 1938) used chopped pineapple (Ananas comosus) leaves as soil amendment and obtained significant control of root-knot nematode, Meloidogyne sp. attacking cowpea (Vigna unguiculata). They suggested that organic amendments supported microbial and animal species inimical to root-knot nematode. Duddington et al. (1956) applied bran and a fungus, Dactylaria thomasia in order to control Heterodera schachtii on beet. Duddington et al. (1961) found a significant reduction in the numbers of Heterodera avenae in roots when a green manure (chopped cabbage leaves) and Dactylaria thomasia were incorporated into the microplots. Reduction was found to be mainly due to stimulation of the predacious fungus rather than the organic amendments. Mankau (1962) observed the effects of several organic amendments,
e.g., alfalfa green manure, rotted wood shavings, Oat hay, dung, steer manure and chicken manure on the nematode fauna of agricultural soil and found that dung and green manure promoted greatest activity of predacious fungi which reduced the population of nematodes significantly. Cooke (1968) found that when chopped cabbage leaf tissues were added to a medium loam, there was a rise in the population of free-living nematodes and in the activity of nematode-trapping fungi. Cooke (1968) further stated that for successful predaceous activity it was necessary to have organic matter in a particular phase of decomposition with readily available carbon sources not being exhausted, as mycelial growth and trap formation are energy requiring processes that precede predation.

Singh & Sitaramaiah (1969b) isolated Curvularia pallescens from the females of Meloidogyne javanica obtained from root galls of tomato plants grown in soil treated with oilcakes of margosa, peanut and castor. Kirmani et al. (1978) found that development of root-knot on eggplant was reduced significantly in soil infested with saprophytic fungi, e.g., Aspergillus versicolor, Penicillium corylophylum and Alternaria tenuis isolated from decomposing oil-cakes. The inhibitory effect of these fungi was enhanced when soil was also amended with oil-cakes.

Telplyakova et al. (1982) effectively controlled Meloidogyne incognita by adding organic humus, prepared from
pig manure and a fungus *Arthrobotrys oligospora* into the soil and observed better growth of plants.

Veldkamp (1955) found that actinomycetes were predominant microorganisms in chitin degradation and chitinase formation. Saka (1978) added waste mycelium, sewage sludge and crab chitin as soil amendment to control the plant-parasitic nematodes. Godoy et al. (1983) observed that chitin amendment at 0.4% and above reduced root-galling in *Cucurbita pepo* as fungal populations were stimulated by chitin amendments at 1% or above. Culbreath et al. (1986) amended the soil with chitin and rice colonized with *Paecilomyces lilacinus* to control root-knot nematode, *Meloidogyne arenaria* in tomato.

Davide & Zorilla (1985) applied *Paecilomyces lilacinus* either as soil drench or mixed with substrates such as rice hulls and rice bran (50:50) or chopped water-lily and later incorporated it into soil and found significant reduction in the numbers of root-knot nematode.

Jatala (1980) made a comparative study about the effect of application of fungi, organic matter and nematicides for the control of *Meloidogyne incognita* on potatoes and reported that plants grown in plots inoculated with the fungus *Paecilomyces* spp. had significantly lower root-galling than those grown in plots applied with the organic matter and the nematicide.

Singh & Sitaramaiah (1973) found an increase in the number of free-living and predatory nematodes when two inches thick
pine needle mulch was applied on the infested soil; it also resulted in the reduction of numbers of total plant-parasitic nematodes.

Schaeffenberg (1951) gave an explanation the control of sugarbeet cyst nematode by the addition of dung and green manure to the infested soil. According to him the addition of dung and green manure provided better conditions for the development of enchytraeids. Singh & Sitaramaiah (1975) noticed the activity of an unidentified tardigrade to be higher in oil-cake amended than in nonamended soils.

Murphy & Doncastor (1957) reported an increase in population of collembola when organic matter was added to the soil whereas populations of plant-parasitic nematodes declined.

**PLAN OF WORK**

The review of literature as presented above clearly indicates that considerable work has been carried out on the control of plant-parasitic nematodes with organic amendments. However, few attempts have been made to investigate the influence of soil fungi, that are stimulated during the decomposition of organic matter, in such a control of nematodes. Moreover, these studies have been mostly confined to other countries. Therefore, it is considered worthwhile to systematically study this problem. The following shall be the plan of work.
1. To study the effect of different organic amendments such as leaves and oil-cakes of neem/margosa (*Azadirachta indica*) and castor (*Ricinus communis*), on the population of soil fungi and nematodes and plant growth/yield of certain vegetables (okra and chilli) and grain legumes (chickpea and cowpea) in field.

2. To study the effect of selected saprophytic fungi on the root-knot development caused by *Meloidogyne incognita* and plant growth of okra and chilli, in the presence and absence of organic amendments in pots.

3. To study the effect of selected nematophagous fungi on the root-knot development caused by *Meloidogyne incognita* and plant growth of okra and chilli, in the presence and absence of organic amendments in pots.

4. To study the effect of selected saprophytic fungi on the root-knot development caused by *Meloidogyne incognita*, root-nodulation and plant growth of chickpea and cowpea in the presence and absence of organic amendments in pots.

5. To study the effect of selected nematophagous fungi on the root-knot development caused by *Meloidogyne incognita*, root-nodulation and plant growth of chickpea and cowpea in the presence and absence of organic amendments in pots.

6. To study the effect of selected saprophytic fungi on the population of *Rotylenchulus reniformis* and plant growth of okra and chilli, in the presence and absence of organic amendments in pots.

7. To study the effect of selected nematophagous fungi on the population of *Rotylenchulus reniformis* and plant growth of okra and chilli, in the presence and absence of organic amendments in pots.

8. To study the effect of selected saprophytic fungi on the population of *Rotylenchulus reniformis*, root-nodulation and plant growth of chickpea and cowpea in the presence and absence of organic amendments in pots.

9. To study the effect of selected nematophagous population of *Rotylenchulus reniformis*, root-nodulation and plant growth of chickpea and cowpea in the presence and absence of organic amendments in pots.
10. To study the effect of culture filtrates of test fungi on the mortality and larval hatching of *Meloidogyne incognita* *in vitro*.

11. To study the effect of culture filtrates of test fungi on the mortality of *Rotylenchulus reniformis* *in vitro*.

12. To study the colonization/association of nematophagous fungi with different stages of the test nematodes in the presence and absence of organic amendments.
2. MATERIALS & METHODS

2.1 Selection of test materials

Organic amendments, viz., chopped leaves and oil-cakes of neem/margosa (*Azadirachta indica* A. Juss.) and castor (*Ricinus communis* L.) will be used in different experiments. Few saprophytic/nematophagous fungi, which will appear as dominant species after treating the soil with organic amendments, will be selected for the studies. The root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood and the reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira, which are most important parasitic nematodes in Aligarh soils, will be used as test nematodes. Two vegetables, e.g., okra (*Abelmoschus esculentus* Moench.) and chilli (*Capsicum annuum* L.) and two grain legumes, e.g., chickpea (*Cicer arietinum* L.) and cowpea (*Vigna sinensis* Savi,) will serve as test plants.

2.2 Maintenance of nematode culture

The nematode cultures shall be maintained or tomato in concrete microplots. Details are as follows:

In case of root-knot nematode, a single eggmass will be collected from the infected roots of tomato with the help of forcep and placed on a small coarse seive (1 mm pore size) fitted with moist tissue paper and placed in petridish (10 cm diameter) containing distilled water. Second stage juveniles, which will be hatched out, will be collected along
with water from petri dish after every 24 hrs. Fresh water will be added to the petri dish each time after withdrawing the nematode suspension. This process will be repeated up to 5-7 days. These second-stage juveniles will serve as the primary inoculum of the root-knot nematode. Tomato plants growing in 15 cm clay pots containing 1 kg autoclaved soil- manure mixture will be inoculated with the nematode larvae. Two months after inoculation these plants will be carefully uprooted and the roots will be washed gently with water. Eggmasses of the nematode will be removed from roots of these plants and the larvae will be obtained in a similar manner as described above and will be used for further multiplying the inoculum on tomato plants growing in concrete microplots. This will serve as the source of inoculum of the nematode for different experiments. The species of the nematode will be ascertained by close examination of the perineal pattern of the females.

For obtaining the reniform nematode, soil from around the roots of tomato will be collected from different places and it will be brought to the laboratory. The soil will be processed using Cobb's sieving and decanting technique along with modified Baermann funnel technique (Southey, 1986). From the nematode suspension, specimens of *Rotylenchulus reniformis* will be picked with the help of nematode picking device of Khan et al. (1972). These nematode specimens will serve as primary inoculum for
multiplying the nematode on tomato plants in concrete microplots. Soil from these microplots will be processed in the same manner described above for obtaining the inoculum of the reniform nematode which will be used in different experiments.

Before nematode inoculation in different experiments, separate water suspensions of the nematodes will be gently stirred for making homogenous distribution of nematodes and than 5 ml suspension will be transferred to the counting dish (Southey, 1986) and nematodes in each sample will be counted under stereoscopic microscope. An average of five counts will be made in each case to determine the density of nematodes per unit volume of the suspension.

2.3 Maintenance of Fungal culture

Different organic amendments as listed above will be separately added to thoroughly prepared field plots (10 sq meter) at the rate of 110 kg/ha. Untreated plots as well as those treated with inorganic fertilizers (urea @ 110 kg N/ha, super phosphates @ 55 kg P/ha, murate of potash @ 55 kg K/ha) will serve as control. There will be five replicates for each treatment. Besides, there will be a buffer zone of 0.5 meter width in between different beds. Immediately, after treating the soil, the beds will be watered for ensuring proper decomposition of the organic additives. After a
waiting period of one week, 3-week old seedlings of tomato will be transplanted in different beds. Soil population of nematodes and fungi will be determined, before application of organic amendment as well as at regular intervals of one month upto three months.

Nematodes will be isolated from 200 g soil samples of different beds as per procedure given in 2.2. In case of fungi, soil plate method will be used for determining the population of different fungi (Warcup, 1950, 1955).

Few saprophytic/nematophagous fungi which will be dominant species after treating the soil with different organic amendments, will be selected for further studies. These fungi will be separately isolated and their cultures will be maintained in culture tubes containing potato–dextrose–agar (P.D.A.) which will be prepared from the following constituents:

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato</td>
<td>250.00 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>17.00 g</td>
</tr>
<tr>
<td>Agar</td>
<td>20.00 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00 ml</td>
</tr>
</tbody>
</table>

First, the peeled pieces of potato will be boiled in distilled water and extract obtained, to which other constituents will be added. This medium in liquid condition (when still hot) will be transferred to culture tubes which
will be plugged with cotton plugs. These tubes will then be autoclaved. After cooling of P.D.A. test fungi will be transferred to those culture tubes in an aseptic chamber by using all the precautions prescribed to such an operation.

Inoculum of the test fungi will be raised on Richard's liquid medium (Riker & Riker, 1936) having the following composition:

- Potassium nitrate = 10.00 g
- Potassium dihydrogen phosphate = 5.00 g
- Magnesium sulphate = 2.50 g
- Ferric chloride = 0.02 g
- Sucrose = 50.00 g
- Distilled water = 1000.00 ml

One hundred ml of the above medium will be transferred to 250 ml Erlenmeyer flasks which will be plugged with cotton plugs covered with butter paper. Then these flasks will be autoclaved. Small bits of test fungi will be transferred to these conical flasks in an aseptic chamber taking all the precautions prescribed for such an operation. The fungi will be incubated for 15 days in an incubator running at 28±2°C temperature.

After the incubation period the mycelial mats will be removed, washed in distilled water to remove the traces of the medium and then it will be gently pressed between sterile
blootting paper to remove the excess amount of water. Inoculum will be prepared by mixing 10g fungal mycelium in 100 ml of distilled water and blending it for few seconds in a waring blender (Stemerding, 1964). In this way each 10 ml of this homogenate will contain 1g of fungus. This will serve as inocula of different fungi for use in different experiments.

2.4 Maintenance of test plants

Sandy loam soil, which is commonly found in Aligarh will be collected from a field and will be passed through a coarse sieve (1 mm pore size) to remove stone particles, debris, etc. To the soil, compost will be added at the ratio of 3:1. Fifteen cm claypots containing 1 kg soil-manure mixture will be autoclaved. Seeds of chilli will be sown in these pots to raise the seedlings for use in different experiments, whereas in case of other plants, e.g., okra, cowpea and chickpea, seeds will be directly sown for different experiments.

2.5 Field Experiment

To study the effect of different organic amendments on the population of soil fungi and nematodes and plant growth/yield of test crops, the experiments will be done as per procedure described in 2.3. Recording of final data will be determined as in 2.7.
2.6 Pot Experiment

Fifteen cm claypots containing 1kg field soil will be autoclaved and then treated with different organic amendments @ 1g N/pot. Untreated as well as those treated with inorganic fertilizers (urea, @ 1g N/pot, super phosphate 0.5g P/pot, murate of potash 0.5g K/pot) will serve as control. There will be five replicates for each treatment. The pots will be kept on glasshouse/green house bench in a random manner. The pots will be watered immediately after treating the soil for ensuring proper decomposition of the organic additives. Seedlings of chilli will be transplanted singly to each pot after a waiting period of one week. In case of other plants seeds will be sown directly. At the age of 3-week, the plants will be inoculated with 1g of fungal mycelium and/or 5000 specimens of the test nematodes. The experiments will be terminated two months after inoculation. The final data will be determined as per 2.7.

2.7 Recording of data

The experiments will be terminated two months after inoculation. Plants will be uprooted and the roots will be thoroughly and gently washed with running water. The recording of data will be done as under:

2.7.1 Water absorption capability of roots

The water absorption capability of roots will be determined following the method described by Alam et al. (1974)
Erlenmeyer flasks (250 ml capacity) will be filled with weighed amount of water. The plants will be kept singly in these flasks with their roots dipped in water. Flasks without plants will serve as control. After 24 hrs the remaining quantity of water will be weighed. Amount of water lost from the control flasks will be taken as water lost by surface evaporation and will be deducted from the amount of water lost from other flasks and thus the actual amount of water absorbed by the roots will be determined.

2.7.2 Plant growth

The length (in cm) and fresh/dry weights (in g) of shoot and root will be taken separately. Before taking the fresh weight excess amount of water will be removed by putting the roots and shoots between blotting sheets.

2.7.3 Nematode population

Nematodes from soil will be isolated by processing the pot soil as per procedure described in 2.2. The population of nematodes will be determined by counting nematode specimens as in 2.2. Chopped root pieces of nematode-infected plants will be blended with some water in Waring blender for few seconds. Thus root population of the nematodes will be obtained in a suspension which will be counted in the same manner. Total sum of soil and root populations of nematodes will give the final population of the nematodes. Reproduction
factor (R) of the nematodes will be calculated by the formula of Oostenbrink (1966) as follows:

\[ R = \frac{P_f}{P_i} \]

(where, \( P_f \) represents the final population and \( P_i \) represents the initial population of the nematodes).

2.7.4 Root-knot index

The degree of root infection caused by the root-knot nematode will be assessed according to the rating scale of Taylor & Sasser (Sasser et al., 1984):

<table>
<thead>
<tr>
<th>Gall index (GI)</th>
<th>Number of galls</th>
</tr>
</thead>
<tbody>
<tr>
<td>or</td>
<td>Eggmasses</td>
</tr>
<tr>
<td>Eggmass index (EI)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1 - 2</td>
</tr>
<tr>
<td>2</td>
<td>3 - 10</td>
</tr>
<tr>
<td>3</td>
<td>11 - 30</td>
</tr>
<tr>
<td>4</td>
<td>31 - 100</td>
</tr>
<tr>
<td>5</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

2.7.5 Association/colonization of fungi

Different stages of nematodes including egg will be stained with 0.01% cotton blue and mounted in lectophenol on a slide. Association/colonization of nematophagous fungi,
if any, will be confirmed by studying under compound microscope. Suitable photographs will also be obtained. Fungi from infected nematodes will also be isolated by transferring them to P.D.A. This will help in confirming the identity of the fungi.

2.7.6 **Statistical analysis**

Statistical analysis of the data for critical difference (C.D.) at $P = 0.05$ and $P = 0.01$ will be done as per procedure described by Pansey & Sukhatme (1978).

2.8 **Hatching of the root-knot nematode in culture filterates**

Five freshly picked average sized eggmasses of the root-knot nematode will be transferred separately to 5 cm diameter petriplate containing 5 ml of different dilutions of culture filterates of test fungi. Number of second stage juveniles which will be hatched out will be counted with the help of counting dish after five days.

2.9 **Mortality of nematodes in culture filterates**

About hundred specimens of second stage juveniles of the root-knot nematode or different vermiform stages of the reniform nematode will be separately transferred to 5 cm diameter petriplates containing different dilutions of culture filterates of the test fungi, as per procedure described by Alam (1986). Mortality of the nematodes will be determined after 12, 24 and 48 hours of exposure.
3. REFERENCES


Cairns, E.J. (1955). Nematodes tiny, but might research underway points to development at better and cheaper control. Highlights of Agr. Res., 2:


