STUDIES ON
THE INTERACTION OF NEMATODES
AND FUNGI

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INTRODUCTION AND REVIEW OF LITERATURE

Plant - parasitic nematodes constitute one of the most important non-insect pests which limit the agricultural production and at times the losses are tremendous (Hutchinson et al., 1961; Taylor, 1967; Van Berkum et al., 1970).

The plant nematodes, by interacting with other components of soil biota, specially the soil microflora, affect the disease situations forming the so called "disease complexes". Thus the pathogenicity of plant-nematodes under monopathogenic conditions may not be very important in the etiology of plant diseases than interactions between nematode and soil microflora. The subject has been critically reviewed by Pitcher (1965), Powell (1963, 1971).

The association between plant-parasitic microflora and plant-parasitic nematodes is directly related to the mode of parasitism of plant nematodes being less developed in ectoparasitic nematodes than endo-parasitic nematodes. In the former it appears to be more of "additive" and in the latter "synergistic". The subject matter has thus been reviewed nematode wise.

MIGRATORY ECTOPARASITIC NEMATODES:

_Hoplolaimus_

_Hoplolaimus uniformis_ has been found associated with _Fusarium oxysporum f. pisi_ in early yellowing of peas (Labruyere, et al., 1959). Inoculation of nematode alone caused a slight
grey discolouration of portions of the root and cracks in the cortex within which nematode occurred. Inoculation with the fungus alone produced a superficial discolouration of the cortex when the inoculum was placed in direct contact with the root and no visible damage when the inoculum was placed at a short distance from the root. Inoculation with both the organisms resulted in extensive dark brown discolouration along the roots and complete decay at the point of inoculation. In the absence of nematode, the fungus colonized only outer layers of the cortex, whereas in the presence of nematode, the fungus penetrated into the vascular bundle.

Brodie and Cooper (1964) reported that cotton seedlings grown in soil infested with *H. tylenchiformis* became predisposed to the attack of *Rhizoctonia solani*.

Whunt and Weaver (1972) observed the strongly combined effect of nematode and *Fusarium oxysporum* on the growth of peach seedlings. They further reported that seedlings grown in soil infested with *Heterodera* and *Fusarium* were smaller as compared to those grown in soil infested with either of the pathogens alone. Further, using *H. galeatus*, *Tylenchorhynchus claytoni* and *Criconemoides xenoplax* in combination with *Fusarium oxysporum*, the combination of *H. galeatus* and *F. oxysporum* gave the highest reduction in the growth.
The role of *H. indicus* on the severity of seedling blight of rice has been observed by Ramana et al., (1974) wherein *H. indicus* increased the severity of seedling blight of rice when present together with Helminthosporium oryzae.

*H. galeatus* alone has no effect on the development of wilt of cotton caused by *Fusarium oxysporum f. vasinfectum* but when present together with other nematodes such as Belonolaimus longicaudatus and Meloidogyne incognita, considerably increased the wilt (Yang Henry et al., 1976).

**Helicotylenchus:**

There is only one report about the association of *Helicotylenchus* wilt diseases. Stover (1966) reported that *Rhizoctonia solani* was more commonly found in shallow lesions on Banana roots caused by *Helicotylenchus* spp.

**Tylenchorhynchus:**

*Tylenchorhynchus claytoni* caused a slight to moderate increase in the incidence of blackshank of tobacco on the resistant variety Dixie Bright 101, in association with *Phytophthora parasitica var. nicotianae* (Graham, 1958). Holdeman (1956) observed that *T. claytoni* increased the incidence of wilt caused by *Fusarium oxysporum f. nicotianae* in a susceptible variety of tobacco. Similar synergistic effect of nematode-fungus has been observed by Haglund & King (1961) in
the root-rot of peas between Aphanomyces euteiches and I. martini. Brodie and Cooper (1964) pointed out that _T. claytoni_ increased the incidence of root-rot caused by Rhizoctonia solani in cotton but did not lengthen the period of susceptibility. Hendrix et al., (1965) observed that Tylenchorhynchus app., in addition to other nematodes, was responsible for increasing the peach decline. Khan et al., (1971) observed the interaction of Rhizoctonia solani and _I. brassicae_ in pre-emergence damping off of cauliflower seedlings. _I. brassicae_ alone did not affect the percentage of emergence of cauliflower seedling, however, the nematode considerably increased the pre-emergence damping off cause by Rhizoctonia solani.

_Paratylenchus:_

The pin nematode _Paratylenchus hamatus_ has been associated with a species of Rhizoctonia in root-rot of celery (Lownsbery and Lownsbery, 1952) and with _Rhizoctonia, Pythium_, and _Fusarium_ in root-rot of mint (Horner and Jensen, 1954) but these have not been studied critically.

_Belonolaimus:_

Holdeman and Graham (1952) reported that _Belonolaimus gracilis_ greatly facilitated the development of _Fusarium_ wilt in susceptible and resistant varieties of cotton. Minton and Minton (1966) have reported that _B. longicaudatus_ did not affect cotton seedling emergence, however _Fusarium_ wilt developed as plants become older if they were simultaneously exposed to nematode and the fungus.
SEDENTARY ECTO-PARASITIC NEMATODES:

Tylenchulus:

Feldmesser, et al., (1962) observed that rough lemon feeder roots and crowns infected with Tylenchulus semi-penetrans for a long time had the highest incidence of infection with Fusarium solani and F. oxysporum. Roots from the areas newly invaded or not yet invaded by the nematode had much lower percentages of Fusarium infection. There was no apparent relation between this nematode and infection by Pythium, Phytophthora or Thielaviopsis. O'Bannon et al., (1967) observed that lemon root decay by Fusarium solani increased considerably when I. semi-penetrans was present but this was not true when Fusarium oxysporum was present with the nematode.

MIGRATORY ENDO-PARASITIC NEMATODES:

Ditylenchus:

A very interesting relation between Ditylenchus dipsaci and Botrytis allii in collar rot of onion has been reported by Myuge (1959). When water into which the nematode had excreted enzyme was injected into onion plants in the presence of Botrytis allii, 100% plants became infected with collar rot in contrast to disease incidence of 30 percent when pure water was used under similar circumstances.

Radopholus:

Radopholus similis is commonly associated with citrus decline but species of Fusarium may be associated with the
Fusarium, together with Sclerotium and Thielaviopsis, appeared to be important in the "decay phase" of the disease (Du Charme and Hanks 1957). The incidence of infection of citrus crown pieces by Fusarium was increased four-fold in the presence of R. similis (Feder and Feldmesser 1961). There may be somewhat complex interactions between these two organisms in citrus because varieties that were tolerant to the nematode appeared also to be tolerant to the fungus (Feder and Ford, 1961).

Although R. similis was not a prerequisite to Panama wilt in the Gros Michel variety of banana caused by Fusarium oxysporum f. cubense, the disease expression was aggravated and the period between inoculation and appearance of symptoms was considerably shortened when the nematode was also present (Loos, 1959).

The root rot disease of banana varieties, Dwarf Cavendish and Williams in Australia appeared to be entirely due to R. similis and the fungi, Fusarium oxysporum and Rhizoctonia solani acted only as secondary invaders (Blake, 1961; Stover, 1966).

Pratylenchus:

Root lesion nematodes have been associated with Fusarium wilt of cotton (Smith, 1940; Holdeman, 1954), but the relation has never been examined critically. Rhizoctonia solani and Pratylenchus neglectus were very closely associated
in root-rot of winter wheat (Benedict and Mountain, 1956). The combined effect of the fungus and the nematode in reducing the growth of wheat was approximately twice that produced by either of the pathogens alone. The incidence of fungal infection of the roots may be correlated with nematode population but the effect of the two parasites appeared to be additive rather than synergistic.

The classical studies on the interrelation of P. penetrans and Cylindrocarpon radicicola (Hastings and Bosher, 1938) showed that the fungus alone reduced the growth of a number of plants including potato, carrot, red clover and violet by 6 to 11 per cent where as the fungus and the nematode reduced the growth by 50 to 75 per cent. More recently, Slootweg (1956) reported that Cylindrocarpon radicicola alone produced no lesions on the root of narcissus, whereas the fungus and P. penetrans produced extensive lesions.

True synergism between Verticillium dahliae and P. penetrans has been demonstrated in the etiology of egg-plant wilt (Mc Keen and Mountain, 1960) and to a lesser extent in tomato wilt (Mountain and Mc Keen, 1962). The incidence of wilt was strikingly increased by the nematode if the level of the fungus in the soil was low. Symptoms of wilt in the egg-plant appeared 20 days earlier and the incidence of wilt at the end of 8 weeks was 65 per cent higher in plants growing in soil containing both the
organisms than in soil containing only the fungus. The exact mechanism of interaction has not been known but the nematode causes extensive cortical necrosis within 24 hours on the roots of egg-plant and the fungus may utilize the necrotic pathway to the vascular tissues. The root population of the nematode was usually much higher when the fungus was also present and there were indications that complex interrelations existed between the host plant, the fungus and the nematode (Mountain and Mc Keen, 1962).

The presence of P. penetrans resulted in earlier and more severe symptoms of Verticillium wilt in a susceptible variety of strawberry. However, varieties of strawberry resistant to wilt were not affected by Verticillium albo-atrum when the nematode was also present, although the latter reproduced readily and caused many root lesions (Abu-Gharbieh, et al., 1962).

In the etiology of charcoal rot of sorghum, the nematode P. hexincisus and the fungus, Macrophomina phaseoli caused a significantly higher rating under dry soil conditions than did the fungus alone (Norton, 1958).

The presence of P. penetrans resulted in earlier and more severe symptoms of Verticillium wilt in a susceptible variety of strawberry resistant to wilt were not affected by Verticillium albo-atrum when the nematode was also present, although the latter reproduced readily and caused many root lesions (Abu-Gharbieh, et al., 1962).
Peppermint is subjected to *P. minyus* - *Verticillium* wilt interaction. The optimum temperature for wilt development was influenced by *P. minyus*. It shifted from 24°C when fungus was present alone to 27°C when both organisms were present (Bergeson, 1963). Faulkner and Bolander (1969) reported that optimum temperature for nematode reproduction was also changed in complex situations. These authors have also reported (1970) that nematodes positively influenced the incubation period as well as the incidence and severity of the wilt.

Dwinell and Sinclair (1967) have concluded that *P. penetrans* might interact with *Verticillium dahliae* on elm and maple. Nematode increased invasiveness by *V. dahliae* in the first year elm seedlings treated with *Pratylenchus*. This effect was nullified by the addition of potassium.

Root lesion nematode was also involved in certain root-rotting complexes. Mountain (1954); Benedict and Mountain (1956) reported that *P. minyus* interacted with *Rhizoctonia solani* resulting of wheat growth from root-rot. Edmunds and Mai (1966) have shown that *P. penetrans* combined with *Trichoderma viride* caused more reduction in root and shoot growth in alfalfa and celery than either of the organisms alone. The fresh weight of corn was reduced more when *P. scribneri* and *Fusarium moniliforme* were present together than when either of the organisms was present alone (Palmer et al., 1967).
Wilt of peas on var. 'Rondo' caused by *Fusarium oxysporum* f. *pisi* race 2 was enhanced by *P. penetrans* (Sinhorst and Kuniyasu, 1971).

Michell and Powell (1972) have reported the influence of *P. brachyurus* on the incidence of *Fusarium* wilt in cotton. A great percentage of plants wilted when the nematode and the fungus were applied simultaneously than when nematode was added two weeks prior to the fungus or when fungus alone was used. Colonization of roots was most extensive in plants simultaneously treated with nematode fungus combination.

Burpee and Bloom (1974) have observed the interaction of *Verticillium albo-atrum* and *P. penetrans* on potatoes. Initial symptom development in the fungus and the nematode plus fungus treatments appeared one week and two weeks respectively prior to the development of natural senescence. The nematode reduced the inoculation period for the development of wilt.

Maximum population of *P. penetrans* occurred at about 12 per cent soil moisture, 20°C temperature 4.5 pH. Damage to the potato crop was minimal due to low nematode population densities (Gould, 1974). A glasshouse experiment suggested an additive pathogenic effect of *P. penetrans* and *Verticillium albo-atrum* on *Solanum tuberosum* (Gould, 1974).
Coomans (1977) has studied the interaction of P. penetrans and Verticillium app. on flax. According to him P. penetrans and Verticillium app. acted synergistically under certain circumstances, but a minimum population of both nematode and the fungus was required. When the initial population was higher either the fungus or the nematode induced minimum growth and maximum wilt and thus masked the effect of the other pathogen.

There was positive correlation between the presence of P. thornei on potato and the severity of Verticillium dahliae wilt where there was fairly high population. Even relatively Verticillium tolerant varieties sustained losses of 30-40 per cent in presence of P. thornei (Krikun and D’orlon, 1977). Muller (1977) observed the interaction between different species of Pratylenchus and Verticillium albo-strum. Out of five species used P. penetrans and P. vulnus enhanced the wilt.

SEDENTARY ENDO-PARASITIC NEMATODES:

Heterodera:

Ross (1965) reported that Heterodera glycines was more effective in predisposing soyabean to Fusarium wilt than was Meloidogyne incognita. Soyabean, however, were somewhat more tolerant to root-knot nematodes than cyst nematodes and this could explain the difference in the complex. In sugarbeet Jorgenson (1970) noted that nematicides controlled the disease caused by H. schachtii and Fusarium oxysporum. The fungus
apparently inhibited nematode invasion and development resulting in the decrease in the nematode population.

Cyst nematodes are also capable of interacting with certain fungi to promote root decay. Polychronopoulos et al., (1969) reported that roots and seedlings of sugarbeet were greatly damaged by a complex of _H. schachtii_ and _Rhizoctonia solani_. Wounds and root proliferations induced by nematodes, appeared to facilitate subsequent penetration and colonization by the fungus. Seedling symptoms developed rapidly after infection by both the pathogens. These authors concluded that giant cells resulting from _H. schachtii_ as well as adjacent areas provided a highly suitable substrate for fungal growth.

Dunn (1968) reported interesting interaction involving _H. rostochiensis_ with _Rhizoctonia solani_ and _Colletotrichum atramentarium_ on tomato. James (1968) failed to observe any influence of _H. rostochiensis_ on disease caused by _C. atramentarium_. Ray (1968) studied sites in tomato roots jointly infected with _H. rostochiensis_ and grey sterile fungus. When both the pathogens were present, giant cells were either not formed or deformed. Nematode larvae failed to develop into adults. Plants inoculated with nematodes only produced typical giant cells.

The adverse effect of _Pythium ultimum_ and _P. aphanidermatum_ was far greater when the nematode infestation occurred along with fungal infection. The effect with the former was synergistic
while with latter additive (Whitney, 1971). Whitney (1974) observed the synergistic effect of *P. aphanidermatum* with *Heterodera suachi* on sugarbeet. The increased damping off of sugarbeet appeared to be associated with the increased growth of the fungus around the infected centres because the number of centres remained constant whether or not the nematode was present. The susceptibility of the fungus was not lengthened by nematode infection. The effect of *Pythium aphanidermatum* and the nematode in combination was additive for damping off and root-rot of sugarbeet (Whitney, 1974).

Adeniji *et al.*, (1975) observed interrelationship between *H. glycines* and *Phytophthora megasperma* var. *Sojae* in soyabean. According to the authors soyabean seedlings (2, 5 and 10 days old) of 3 cultivars varying in susceptibility to race 1 of *Phytophthora megasperma* var. *Sojae* were inoculated with each of the organisms alone and in combination. Seedling disease was more severe in susceptible cultivars when both the organisms were present than fungus alone. *P. megasperma* infection significantly reduced the population of *H. glycines* on roots but did not break resistance.

The development of *Verticillium* wilt was more severe in tomatoes growing in soil to which 100 cysts of *H. tabacum* had been added than in soil without the nematodes, whereas *Fusarium* wilt development was less severe (Miller, 1975).
Melloidogyne:

There are large number of evidences wherein interaction between this genus and the soil microorganisms occurred. The most common of these appears to be with genus Fusarium especially with varieties of plants that are resistant to the fungus.

The association of root-knot nematode with cotton wilt has been observed in the fields for the last several years. Atkinson (1892) was among the first to notice this association when he observed that infection by root-knot nematode increased the severity of Fusarium wilt in cotton. Since then the interaction between these two pathogens has received much attention on a variety of host plants. As early as 1928 Rosen postulated that the action of root-knot nematodes on roots was largely that of producing localised hyperplastic overgrowths consisting mostly of soft parenchyma and a reduced amount of cork and wood; this type of tissue offered excellent opportunity for growth of cotton wilt fungus. Martin, et al., (1956) reported that the incidence of wilt in both susceptible and resistant varieties of cotton by Fusarium oxysporum f. vasinfectum was significantly increased in the presence of M. incognita and M. incognita acrita.

Critical work has been carried out on the interaction between root-knot nematode and Fusarium wilt of tomato. Jenkins and Coursen (1957) found that F. oxysporum f. lycopersici alone or with root wounding, did not cause wilt in tomato variety chesapeake. But when M. hapla was also present 60% of
the plants wilted; and when *M. incognita acrita* was present 100 per cent of the plants wilted. Similar studies by Cohen and Minz (1960) showed conclusively that root-knot nematode interacted with *Fusarium* increasing the incidence in tomato wilt. Interaction between *Meloidogyne* and *Fusarium* have also been demonstrated on alfalfa (McGuire, et al., 1958), black beans (Thomason, 1958), Cowpea (Thomason, et al., 1959), minosa (Gill, 1958) and carnations (Schindler, et al., 1961). The fact that certain species of *Meloidogyne* are frequently more effective than others in causing interaction, shows it's not simple wounding by nematodes responsible for disease complex but something else.

The root-knot - *Fusarium* wilt interaction in tobacco has been more interesting. Melendez and Powell (1967) reported that giant cells and adjacent xylem elements in both *Fusarium* susceptible and resistant plants were extensively colonized. Giant cells, however, were sensitive to fungus invasion and became devoid of protoplasm soon after invasion. Fungal hyphae present in such cells gradually became debilitated. Occasionally the female nematodes and gelationous matrix of eggmasses were invaded. Porter and Powell (1967) observed that in some hosts, the nematodes apparently were capable of maximum predisposition only after 3-4 weeks. This time interval would be sufficient to permit the formation of galls along with accompanying morphological and physiological changes. The appearance of *Fusarium* wilt symptoms was more closely related to the time of
nematode inoculation. This fact does not hold true of other crops (Johnson and Littrell, 1969). Goode and Mc Guire (1967) pointed out that root-knot nematode infection enabled certain races of *F. oxysporum f. lycopersici* to attack tomato varieties ordinarily resistant to those races and suggested that fungus mutated in the host in the presence of the nematode.

The interrelation between *M. incognita* and *Phytophthora parasitica var. nicotianae* in black shank of tobacco is also very interesting. Sasser, et al., (1955) observed that the resistant variety Dixie Bright 101 was much more susceptible to the fungus in the presence of the nematode. The fungus had a distinct affinity for hyperplastic and hypertrophied areas of galled root tissue. In such regions, the mycelium was more extensive and vigorous than in the non-galled areas. The hyphae progressed rapidly and directly into the hyperplastic tissues and syncytia. After invasion, a compatible relation was formed between the fungus and the host cells undergoing hyperplasia. Powell and Nusbaum (1960) concluded that nematode provided a highly suitable substrate for the development of the fungus.

Interactions between *Meloidogyne* and *Rhizoctonia* have been reported on cotton (Reynolds and Hanson, 1957), soybeans (Taylor and Wyllie, 1959) and peas (Sayed, 1961). *M. hapla* has a much greater effect than *M. javanica* on incidence of damping-off of soybeans (Taylor and Wyllie, 1959).
Kushner and Crittenden (1967) observed that decay in alfalfa roots by either Fusarium roseum or F. oxysporum f. batatas increased when M. incognita acrita was present with the fungus. Root-knot nematode also interacted with other rhizosphere fungi. The frequency of occurrence of Aspergillus flavus from peanut shells increased manyfold if M. arenerea was present (Minton and Jackson, 1967).

Very often the status of normally unimportant fungal pathogens has been elevated to major significance by nematode infection. Powell and Batten (1967) and Melendez and Powell (1969) reported that both Rhizoctonia solani and Pythium spp. caused damping off of tobacco seedlings, but usually was insignificant. The Rhizoctonia also caused root and stalk disease known as "Soreshin", but the occurrence of this condition in the fields was sporadic. However, tobacco plants previously inoculated with M. incognita by 3-4 weeks were infected with either of these fungi, the disease intensity was aggravated. On the other hand, little damage resulted if the fungus and the nematode were applied simultaneously.

Powell (1971) observed some effect with fungi which, although pathogenic on other crops, have not been reported on tobacco. Species of Curvalaria, Botrytis, Aspergillus and Penicillium invaded and caused decay of tobacco roots if roots were predisposed by prior root-knot nematode infection. Without the predisposition, these fungi appeared to be incapable of
establishing a parasitic relationship of tobacco. Similarly Melendez and Powell (1969) reported that Trichoderma sp. although poor pathogenic, became pathogenic and induced decay of tobacco roots if roots were infected with M. incognita and these losses due to the disease syndrome were comparable to that caused by the interaction of Pythium sp. and M. incognita.

Several cases have been reported where the susceptibility period of the plants has been increased in the presence of plant parasitic nematodes (Brodie and Cooper, 1964). Cotton plants were susceptible for longer duration to Pythium debaryanum only when either M. incognita or M. hapla was present. Minton and Minton (1963) observed that on cotton roots jointly infected with M. incognita acrita and Fusarium oxysporum f. vasinfectum, there was abundant fungal growth in nematode induced giant cells as well as the xylem. The fungus also grew well on decaying cortical and epidermal tissue but poorly in apparently healthy tissues. M. incognita predisposed cotton seedlings to Altemaria tenuis, Fusarium oxysporum f. vasinfectum, Rhizoctonia solani and Glomerella gossypii (Hefner, 1967).

Overman and Jones (1970) reported that in tomatoes both the stunt nematode, Tylenchorhynchus capitis and root-knot nematode M. incognita increased the incidence of Verticillium wilt.
Bergeson, et al., (1970) reported that populations of actinomycetes was low in the rhizosphere soil of cotton roots, infected with *M. incognita*, whereas population of *Fusarium* was more in the rhizosphere of galled roots. According to Powell, *et al.*, (1971) the cotton roots showed symptoms of necrosis when inoculation with *M. incognita* in combination with some of the non-pathogenic fungi like *Oryzaloria*, *Botrytis*, *Aspergillus*, *Penicillium* and *Trichoderma*. Necrosis was more especially severe in treatments in which nematodes preceded the fungi by several weeks. None of the fungi induced the disease unless *M. incognita* was present. Agrawal and Goswami (1973) have reported greatest reduction in seedlings, root and shoot weight and highest mortality rate was observed in plants inoculated with nematodes followed 3 weeks latter by the fungus. More severe symptoms and an elevated mortality rate were observed in plants inoculated with two pathogens simultaneously, as compared with when the nematode followed the fungus by 3 weeks of when plants were inoculated with either pathogens alone.

Similarly simultaneous inoculation with root-knot nematode and any of the fungi such as *Rhizocotonia solani*, *Pythium* sp or *Colletotrichum atramentarium* resulted in more damage to the egg plants (Azam, 1975).
Sumner and Johnson (1973) have observed that more plants wilted in soil when *M. incognita* was present even in the varieties of watermelon resistant to wilt. The severity of wilt symptom was proportional to the initial populations of the root-knot larvae.

Effect of the fungus and nematode interaction on nematode reproduction and host root development was studied by Conroy and Green Jr. (1974). In the presence of *Trichodorus Christei* the incidence of *Verticillium* wilt on tomato increased at all the inoculum densities of the fungus. Such relation was observed with *M. incognita*.

Palmer and Mac Donald (1974) have observed the interaction of *Fusarium* spp. and nematodes on maize. Average dry weight of seedlings inoculated with both *M. incognita* and *Fusarium moniliforme* was less than those of seedlings inoculated with either of the organisms alone. Further *F. moniliforme* alone decreased root and shoot weight of maize seedlings more than when fungus was combined with either *Pratylenchus scribneri* or *P. penetrans*. Golden and Van Gundy (1975) while working with okra and tomato reported that okra and tomato roots infected with *M. incognita* in the field and in the greenhouse were highly susceptible to infection by *Rhizoctonia solani*. Root decay by fungus occurred 4-5 weeks after nematode infection. Gracia and Mitchell (1975) reported that in peanut, pod rot was more severe when pods were exposed to soil containing
combination of Pythium myriotylum and Fusarium solani or M. arenaria than when pods were exposed to P. myriotylum alone.

Webster (1975) observed that formation of the giant cells in response to M. javanica in tomato made the plants susceptible to wilt caused by Fusarium oxysporum f. lycopersici.

Ferraz and Lear (1976) reported Criconemoides curvatum, Paratylenchus dianthus and M. hapla showed a synergistic interaction with Fusarium oxysporum f. dianthi in causing carnation wilt. The plants wilted earlier and more severe symptoms were observed with the nematode-fungus combination than with the fungus alone.

Ndubizm (1977) reported that although M. hapla, Pratylenchus penetrans resulted in a greater growth reduction in cherry seedlings but presence of these nematodes with Verticillium dahliae caused more severe growth reduction than either nematode or the fungus alone.

Fields of gram in and around Agra have been found heavily infected with gram wilt caused by Fusarium oxysporum f. ciceri. At many places the damage to the crop has been tremendous and the growth of the plants has been scanty and in patches. The soil type and other agronomic practices did not appear to be very much different from the remaining part of the field and also with the adjacent relatively good crop.
A close examination of such roots revealed the presence of root-knot galls in addition to the nodules and Fusarium lesions. This situation has arisen as a result of the presence of the two pathogens together forming "disease complex" syndrome in gram. Hence, with this aim in view the following aspects will be studied.

1. Effect of Fusarium oxysporum f. ciceri on the emergence of seedlings of different varieties of gram.

2. Effect of Meloidogyne incognita on the emergence of seedlings of different varieties of gram.

3. Effect of inoculating the seeds with F. oxysporum f. ciceri and M. incognita simultaneously and 5, 10, 15 days prior to one another on emergence of the seedlings of different varieties of gram.

4. Effect of inoculating gram varieties resistant and susceptible to gram wilt, different inoculation densities of root-knot nematode M. incognita, and the fungus F. oxysporum f. ciceri, simultaneously and 5, 10 and 15 days prior to each other on the development of wilt symptoms.

5. Histopathology of roots infected with F. oxysporum f. ciceri and M. incognita alone, and when infected with two pathogens together.
6. Effect of different doses of various oil-cakes, nematicides, fungicides and herbicides on the development of gram wilt with both under monopathogenic and bi-pathogenic conditions.

7. Screening of different varieties of gram against *M. incognita* and *F. oxysporum f. ciceri* under monopathogenic and bi-pathogenic conditions.
MATERIALS AND METHODS

2.1 Raising of Seedlings:

Seedlings of different varieties of gram, *Cicer arietinum* will be raised in steam sterilized soil-sand mixture (3:1) and will be inoculated with the fungus *F. oxysporum f. ciceri* and *Meloidogyne incognita* both singly and together.

2.2 Maintenance of cultures of the nematode and the fungus:

A single egg-mass of *M. incognita* obtained from infected roots of tomato/gram will be surface sterilized with 1:500 solution of Chlorax for 5 minutes and washed thrice in sterilized distilled water. The egg-mass will then be transferred to sterilized distilled water for larval hatching. The seeds of gram will be inoculated with larvae hatched from egg-mass. The culture of nematodes thus raised will further be multiplied on gram/tomato for subsequent studies.

Culture of *F. oxysporum f. ciceri* originally isolated from infected seedlings of gram will be maintained on P.D.A. The inoculum of the fungus for inoculation purposes will be raised in 250 ml Erlen meyer flasks containing Czapek's Dex Agar medium (Distilled water-1000 ml; Agar-20 g; Sucrose-30 g; NaNO₃-2 g; MgSO₄-500 mg; KCl-500 mg; KH₂PO₄-1 g; FeSO₄-Traces).
Three weeks old mycelium mat will be macerated in a blender and plants will be inoculated by pouring 100 ml of suspension containing 10 gms of mycelium around the roots of the plants.

2.3 Inoculation of plants with fungus and nematode:

For studying the effect of the two organisms on emergence of seedlings, sterilized soil contained in sterilized pots will be infested with the fungus by mixing 10 gms of mycelium with 100 gms of soil or 1000 larvae of *M. incognita* per 250 gms of soil. Sterilized soil will also be infested with the two organisms together in the following manner:

1. Nematode and fungus simultaneously.
2. Nematode 5, 10, 15 days prior to infestation with fungus.
3. Fungus 5, 10, 15 days prior to infestation with nematode.

Known number of seeds of different varieties of gram will be surface sterilized with 1:1000 mercuric chloride solution and will be sown in the soil infested with the fungus and nematodes separately and together in the manner given above. After 5, 10, 15 and 30 days of sowing the number of seedlings emerged will be counted and post-emergence losses would be determined.

In order to study the effect of *M. incognita* on the development of wilt symptoms the seedlings raised in the manner
given above, will be inoculated with 100 ml of suspension containing either 10 gms of fungus mycelium or 1000 larvae of root-knot nematode. In mixed inoculation a 200 ml suspension would be prepared by mixing the two inocula. The suspension will be poured around the seedlings by making 4 holes at equidistance. Uninoculated plants would serve as the control.

2.4 Effect of oil cakes, fungicides, nematicides and herbicides on the development of disease syndrome.

Oil cakes of neem, castor, ground nut and mustard at the rate of 1 gm of oil cake per 100 gms of soil, and certain systemic nematicides (Tenic and Dasanit) and fungicides (Bevisitin, Calyxine, Aureofungin) at the required doses will be incorporated in the soil both before and after infestation with the two organisms separately and together (2.3). The seeds will be sown in both infested and uninfested soil treated with oil cakes and nematicides and fungicides. Herbicides (2,4-D; MCPA; 2,4,5-T) will be sprayed on the seedlings after their emergence.

2.5 Histopathological studies:

Mixing and staining of materials:

Killing and fixing of infected roots will be done after 1, 2, 4, 6 and 8 weeks of inoculation. The root pieces will be fixed in F.A. 4:1, heated upto 95°C. After cooling the material will be transferred in Bouin's fluid (Picric acid saturated aqueous solution 75 ml, formalin 25 ml and glacial acetic acid 25 ml). To ensure the full and rapid penetration of the fixative,
reduced pressure in vacuum desiccators will be used to get rid of air in diseased tissues. The root pieces will be left in 70 per cent ethanol until used for sectioning.

The material will be dehydrated by washing it in ascending ethanol series and ultimately will be stored in absolute alcohol. The dehydrated material will be brought to solution of methyl benzoate and will be kept there for about 10-16 minutes or until they sink to the bottom. This would make the material transparent. The material will be stored in a 2 per cent celloidin solution for about 3-6 days. The material will then be transferred to benzene with three changes of 3 minutes each. Thus the material will be ready for embedding in paraffin.

To ensure the complete penetration of wax, the material will be passed twice to warm paraffin. The embedding dish after being cleaned with xylene, will be covered with glycerine and will be half filled with warm melted paraffin of melting point 56°C. The material would be placed in the dish and the dish would then be filled with melted paraffin up to the brim. The blocks will be cooled and stored for sectioning.

Sections will be cut at about 15 μ - 20 μ thickness. The paraffin ribbons containing sections will be mounted on a slide by using albumin-glycerine, as adhesive.

The slide so prepared will be kept in an incubator running at 60°C for an hour. It will then be passed through
xylene series in order to ensure complete removal of paraffin. The slides will be passed through descending series of alcohol and will be brought to 50 per cent alcohol and will be stained with Corson's staining fluid for 5 minutes. After staining, the slides would again be passed through ascending series of ethanol. The section will then be mounted in Canada Balsam.

Finally the slides will be examined under the microscope. The details of the histological changes brought about by the presence of nematode alone, nematode-fungus together and fungus alone, as compared to uninoculated control will be studied. Necessary diagrams would be drawn, and microphotographs would also be taken.

2.6 Recording of observations:

After 60 days of inoculation plants will be uprooted and would thoroughly be washed in running water. The length, fresh and dry weight of root and shoot will be determined. Roots will be examined carefully and root-knot index will be determined as follows:

0  - No galling
1  - Few galls without eggmasses
2. - Few galls with eggmasses
3. - Moderate galling with eggmasses
4. - Heavy galling with eggmasses
5. - Severe galling with eggmasses

The nodulation of the roots will also be rated by weighing nodules per gm of roots and will be expressed in terms of nod-root ratio.
Root-knot infected roots will be macerated in a Waring blender and number of larvae present per gm of root will be determined. Nematodes will also be isolated from the soil by Cobb's sieving and decantation technique. The number of plants exhibiting typical wilt symptoms will be counted and percentage of wilted plants will be determined. While making observation the severity of wilt symptoms will also be taken into consideration. Throughout the studies proper controls and proper replicates would be maintained. All the data will be subjected to statistical analysis.
BIBLIOGRAPHY

   Relationship of meadow nematodes to Verticillium

   Interrelationship of Heterodera glycines and
   Phytophthora megasperma var. Sojae in soyabean - Soybean.
   Phytopathology 65: 122-725.

   between a fungus Macrophomina phaseoli and root-knot
   nematode, Meloidogyne incognita in soybean - Glycine
   max. Proc. Indian National Science Acad. 39:701-704


   root-knot nematode, Meloidogyne incognita alone
   and in combination with Rhizoctonia Solani, Pythium sp.
   and Colletotrichum atramentarium. Ph.D. Thesis
   Aligarh Muslim University, Aligarh.

   of root-rot of winter wheat in South Western Ontario.

   alone and in combination with Verticillium albo-
   atrum on growth of peppermint. Phytopathology 53:1164-66

   of Meloidogyne incognita on the rhizosphere microflora
   and Fusarium wilt of tomato. Phytopathology 60:1245-49


