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PHYTONEMATODES GROWTH EFFECT STUDIES ON LEAFY VEGETABLE CROPS

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Abstract

Nematodes feed from plants in a variety of ways, but all use a specialized spear called a stylet. In this work, we have identified several nematodes in the soil of leafy vegetable crops like Spinach and Mentha. The nematodes *Radopholus and Criconemella* were specific in *Spinach soil* and *Rotylenchulus* was specific in Mentha. Soil analysis of these vegetable crops was studied and the pH was found to be 7.5 in spinach grown soil and 7.8 in mentha grown soil. The temperature was found to be 25^0C for spinach soil, and 25^0C for Mentha soil. Among the soils selected, Spinach showed high diversity of microorganisms followed by Mentha. Micrococcus roseus, Bacillus cereus, Cellulomonas terrae, Pseudomonas fluorescens, Azospirillum brasilense, Rhizopus microspores, Aspergillus niger, Curvularia clavata, Fusarium oxysporum, Penicillium chrysogenum and AM Spore were found in all the different soils of leafy vegetables. *Hoplolaimus* was found to be in low number and *Aorolaimus* was found to be in high number in leafy vegetables. The nematode population density was increased from first day to 30^{th} day in all the leafy vegetable crops. When compared to control (without leafy vegetables), the soil which contain with leafy vegetables have high nematode number.

KEYWORDS: Phyto Nematodes, Spinach, Mentha Caloosia and Micrococcus roseus.

INTRODUCTION

Phytonematodes are an extremely important limiting factor in vegetable production, therefore crop protection is an integral part of food production and must be considered within the context of modern agriculture and sustainable development. Effective crop protection is essential both to combat the threat of widespread diseases and to provide the effective pest management programmes. The majority of the plant species, which account for the major

world's food supply, is susceptible to attack from phytonematodes which are capable of causing sustainable economic losses in the quantity and quality of the crops.[1,2] The crop losses caused by phytonematodes in economic terms estimated about $ 157 billion annually to the world agriculture.[3] Yield losses due to root-knot nematodes (Meloidogyne spp.) range from 35.0 to 39.7%.[4,5] In India the losses of agriculture by phytonematodes estimated at about Rs. 210 crore annually.[6] Phytonematodes are severely destroying the roots and other parts of various crops. Roots damaged by the phytonematodes are not efficient in the utilization of available moisture and nutrients from the soil resulting in reduced functional metabolism. Visible symptoms of nematode attack often include reduced growth of individual plants. Furthermore, damaged and weakened roots by nematodes are easy prey to many types of fungi and bacteria, which invade the roots and accelerate root decay. These deleterious effects on plant growth result in reduced yields and poor quality of crops. To overcome this effect, management of nematode is, therefore, important for higher yield and quality that are expected from the higher cost of crop production.[7,8] Once nematodes are established in the field, the possibilities of complete eradication are exceeding remote and impractical on field scale due to soil inhabitant nature of nematodes. However, several measures are adopted to decrease nematode population up to an acceptable level. The main objectives of phytonematodes management are usually a matter of reducing the nematode population by one or more methods or integration of one or more methods to a low level so that the damage is negligible or at an economically acceptable level.[9]

METHODOLOGY

Soil physico-chemical analysis

The soil pH was determined in soil water suspension (1:2:5) using a pH meter[10], Electrical conductivity was determined 1:2 ratio of soil water suspension by conductivity meter, nitrogen by kjeldahl method using Kjeltech autoanalyser 1030 and phosphorus by calorimetrically employing vanado- molybdate method. Potassium was estimated by using flame photometer with determined with neutral normal ammonium acetate solution (Stanford and English, 1949). Calcium, magnesium, iron, zinc, sodium and copper were determined with neutral normal ammonium acetate solution by versenate method. Organic carbon was estimated by Walkey and Black wet digestion method.

Physiochemical parameters of soil and water are analyzed by following the methods described in the respective Indian Standards. The soil specimen obtained from field was

prepared in accordance with IS: 2720 (part 1)–1983 before performing test for physiochemical parameters.

Measurement of pH

General pH meter setup

All pH measurements were performed using an Orion model SA 720 pH meter calibrated using pH 4.0 and 7.0 buffer solutions. The buffer solutions were prepared by dissolving 1 buffer tablet in 1 litre deionised water. A gel-filled combination electrode (Thermo Electron Corporation) was used for all measurements.

Soil pH measurement

Soil: water 1: 2.5 (v/v)

A 30 ml scoop was overfilled with sample and struck level avoiding further compaction of the material. The sample was transferred to a 100 ml glass jar and 75 ml deionised water added by dispenser. The samples were shaken on an endover-end shaker for 15 minutes then mixed briefly prior to pH measurement.

Measurement of conductivity

All conductivity measurements were carried out using a Jenway 4070 conductivity meter and glass conductivity probe.

Soil conductivity

Soil: Water 1: 2.5 (v/v) A 30 ml scoop was overfilled with sample and struck level avoiding further compaction of the material. The sample was transferred to a 100 ml glass jar and 75 ml deionised water added by dispenser. The samples were shaken on an endover-end shaker for 15 minutes then filtered through Whatman No.2 filter paper prior to conductivity measurement.

Soil moisture content

Fresh soil

Approximately 20 g of fresh soil was weighed into a pre-weighed empty porcelain basin. The sample was then dried in the oven at 110 °C for 48 hours. Once dry, the samples were cooled in a desiccator for 30 minutes before reweighing.

Air dry soil

Approximately 10 g of air dry soil was weighed into a pre-weighed empty porcelain basin. The sample was then dried in the oven at 110°C for 48 hours. Once dry, the samples were cooled in a desiccator for 30 minutes before reweighing.

Soil organic carbon content

Loss on ignition provides an estimate of soil organic matter and has been carried out at a variety of temperatures by different workers. Ball (1964) compared two ignition techniques: 850°C for half an hour and 375°C for 16 hours with organic C values (using the dichromate method). He found that the 375°C method gave greater accuracy compared to the 850°C method as there were fewer non-organic material losses. Non-organic material losses can be due to: loss of carbonates in calcareous soils; loss of elemental carbon; and loss of structural water in clay minerals. The influence of loss on ignition temperature and heating time on the ash content of compost and manure was investigated by Matthiessen et al. (2005) who found that temperatures lower than 550°C could underestimate LOI (%). Increasing heating time at 400°C lead to increases in LOI (%) but could not compensate for the low temperature used. At 550°C heating times between 8 – 24 h did not lead to any significant differences in LOI (%) for compost or manure samples. The heating time of 16 h as required in the recommended soil methods in Ball (1964) and Matthiessen et al. (2005) with low temperatures of 375 – 400°C were not suitable due to safety concerns. As a compromise, organic matter content of soil samples was measured using the loss on ignition method at 550°C for 8 hours. Approximately 10 g oven dry soil was weighed into a pre-weighed silica or porcelain basin. The samples were placed in a muffle furnace and the temperature set to 550°C. The furnace was switched on for 8 hours then left to cool overnight.

Soil textural classification

particle size fractionation by mechanical analysis The soil structural peds were broken down to release the primary particles by destroying the organic matter then deflocculated and dispersed to break down the bonds causing aggregation.

METHOD

The following particle size distribution method was modified from BS 7755-5.4.[11]

Soil dispersion

10 g air dry soil (< 2 mm sieved) was accurately weighed into a 400 ml beaker. 50 ml 6% hydrogen peroxide was added by tilt dispenser followed by two drops of anti-foaming agent. Once any initial reaction subsided, the beakers were heated gently on a steam bath with occasional stirring using a glass rod. If the reaction became too vigorous, the beaker was removed from the heat and more anti-foam added as required. Heating continued until the reaction ceased. Once cool, a further 50 ml 6% hydrogen peroxide was added and the beaker was washed down with distilled water. The beaker was further heated until the reaction subsided. When no further reaction occurred with further portions of hydrogen peroxide, and to ensure complete destruction of the organic matter, a final 15 minutes of heating was carried out. The dispersing agent was prepared by dissolving 57g of sodium hexametaphosphate in 1 litre deionised water. Once the soil/peroxide mixtures were cool, 10 ml 5.7% Sodium hexametaphosphate was added and the beakers placed in an ultrasonic bath for 10 minutes to disperse the soil.

Nitrate-nitrogen

This automated system was based on the method of Best (1976) and involves a modification of the Greiss-Ilosvay method whereby NO3–N is reduced to NO2–N followed by diazotization and coupling with an aromatic amine, forming an azo dye (Keeney and Nelson, 1982). The pH was closely controlled between 9.5 and 9.7 (by use of a buffer solution) to prevent precipitation of calcium and magnesium salts which inhibit the reduction[12] The absorbance of the azo dye could be measured colorimetrically at 520 nm. The method measured the combined total of nitrate plus nitrite. Nitrite could be measured on the same manifold by omitting the reduction reagents; although levels in well aerated systems were likely to be very low. Nitrate-N was determined by difference. Organic carbon is known to interfere with the reduction stage in this method[13] However, these workers were able to alleviate the problem by ensuring the final Cu2+ concentration in the catalyst solution was at least 1 mg l-1 and by running samples with a high dilution. The combination of these two measures was enough to improve the reduction efficiency of the system. Rowland et al. (1984) also suggested that the organic carbon interferences could be minimized by using a high solution to soil ratio and using a salt solution rather than water as the extractant. High levels of calcium and magnesium were shown by Ananth and Moraghan (1987) to interfere with nitrate reduction in the hydrazine sulphate method. They recommended use of a cation exchange resin or switching to the copperizedcadmium method since these methods gave

good agreement with steam distillation techniques. Other workers have also used a copperized-cadmium column for reducing nitrate to nitrite. Henriksen and Selmer-Olsen (1970) showed a good recovery of added NO3-N in soils containing humic acids using copperized cadmium as the reductant rather than hydrazine. However, turbid or highly coloured samples required the use of a dialyser to prevent clogging of the reductor column. Hydrazine was preferred since a liquid reductant could be added in the same state of activity for all samples (Best, 1976) and because preparation of copperized cadmium columns was time consuming. Lambert and DuBois (1971) explained that a uniform copperized cadmium column was difficult to prepare and that too much precipitated Cu could lead to low nitrate results as it could be reduced to a lower oxidation state than nitrite. They also showed that chloride could have a significant effect on the reduction step although there was no effect on diazotization or coupling stages in the colorimetric determination of nitrite. The precision of the cadmium-reduced system was shown by Adamsen et al. (1985) to be slightly lower than expected due to sample crossover in the reductor column. High samples were shown to affect low samples that followed. There was however, good agreement between the system and the traditional steam distillation method. Other methods for measuring nitrate in soil extracts include steam distillation (Keeney and Nelson, 1982) and dual-wavelength UV spectrophotometry (Norman et al., 1985). UV spectrophotometry involves measuring absorbance of soil extracts at 210 nm and 270 nm with and without removal of nitrate using Raney Nickel treatment. Norman et al. (1985) advised caution when using this method for soils high in heavy metals or those treated with manure or sewage sludge since the background intensity would be high. Hence this method was not employed for measurement of nitrate.

The solutions were analysed for nitrate along with standards, blanks and zeros. The samples were run at the rate of 50 per hour or 40 per hour where in-line dilution was required. Nitrate was reduced to nitrite by adding the reducing agent and catalyst solution as it passed through a water bath set at 37°C. The colour intensity was measured at 530 nm. This was followed by a run to measure nitrite only where the reducing reagents were replaced with deionised water containing 1 ml l-1 15% Brij-35 solution.

Microbial analysis

These are the tests or steps which were followed for analysis and identification of bacteria.

Isolation and Total Bacterial Count

This was achieved by serial dilution or log dilution method. Five test tubes containing 9ml of sterile distilled water were taken. One test tube containing 10 ml of sterile distilled water was taken.1 g of soil was added to the test tube with 10 ml of sterile distilled water. Mixing was done properly. Then 1 ml of microbial suspension was added to another test tube containing 9ml of sterile distilled water. Again mixing was performed properly.1ml of microbial suspension was added to another test tube containing 9ml sterile distilled water. This step was repeated serially for other test tubes. In this way the microbial suspension get 10 fold serially diluted. 100 µl of diluted suspension was poured into the surface of Nutrient agar plate and spread by „L" shaped spreader. The bacteria can thus be isolated and counted by C.F.U i.e. Colony Forming Unit. C.F.U=No. of colonies/inoculum size (g) X Dilution Factor Pure culture was isolated from this plate by streak plate method using inoculation loop.

Quantitative estimation of the microorganisms

Dilution plating method was employed for the enumeration of microbial population in the soil samples. N-free semi solid malate medium was used for Azospirillum (Dobereiner et al., 1960), Pikovaskaya s medium was used for Phosphobacteria[13], Kings B medium was used for Pseudomonas[14], Kuster s Agar medium was used for Actinomycetes and total bacteria and total fungi in the soil samples.

RESULTS AND DISCUSSION

Physical parameters of the soil in the selected areas

The physical parameters of the soil in control and selected leafy vegetables grown soil were studied and tabulated in table 7 and 8. The physical parameters selected for the study were colour, texture and salinity which are very important for the growth of the phytonematodes (Table 1).

Table 1: Physical properties of the selected leafy vegetables grown soil in Godhumakunta village.

Control	Spinach	Coriander	Amaranthus	Mentha
Color Brown	Blackish brown	Blackish brown	Blackishbrown	Blackish brown
Textural class clay loam	Clay loam	Clay loam	Clay loam	Sandy clay
Salinity (%) 4.0	4.1	3.2	5.4	3.8

Among the soils choosen most of the soil colours were blackish brown, texture was clay loam and salinity was between 3.2 to 5.4 (Table 2).

Table 2: Physical properties of the selected leafy vegetables grown soil in Peerjadiguda village.

Control	Spinach	Coriander	Amaranthus	Mentha
Color Brown	Blackish brown	Blackish brown	Blackishbrown	Blackish brown
Textural class clay loam	Clay loam	Clay loam	Clay loam	Sandy clay
Salinity (%) 3.8	4.5	3.6	5.1	4.0

Physico chemical properties and Microorganisms were identified in the soils of these vegetables. Among the soils selected, Spinach showed high diversity of microorganisms followed by Mentha. Micrococcus roseus, Bacillus cereus, Cellulomonas terrae, Pseudomonas fluorescens, Azospirillum brasilense, Rhizopus microspores, Aspergillus niger, Curvularia clavata, Fusarium oxysporum, Penicillium chrysogenum and *Arbuscular Mycorrhizal* Spore were found in all the different soils of leafy vegetables.

Co-relation studies of Phytonematodes

Radopholus and Criconemella

Radopholus and Criconemella were specific in Spinach grown soil and Micrococcus roseus, Bacillus cereus, Cellulomonas terrae, Pseudomonas fluorescens, Azospirillum brasilense, Rhizopus microspores, Aspergillus niger, Curvularia clavata, Fusarium oxysporum, Penicillium chrysogenum and *Arbuscular Mycorrhizal* Spore were also found along with these phytonematodes in the spinach grown soil of Godhumakunta village and Peerjadiguda village (Table 3).

Table 3: Bacteria found in soil of Spinach.

S. No	Bacterial and fungal species	CFU (106 /g) dry soil
1	Micrococcus roseus	702±15.5
2	Bacillus cereus	804±14.32
3	Cellulomonas terrae	1012±18.2
4	Pseudomonas fluorescens	303±12.2
5	Azospirillum brasilense	203±11.5
6	Rhizopus microsporus	602±16.2
7	Aspergillus niger	802±11.2
8	Curvularia clavata	302±13.2
9	Fusarium oxysporum	208±14.2
10	Penicillium chrysogenum	202±13.5
11	Arbuscular Mycorrhizal Spore	08/100 gram soil

Rotylenchulus

Rotylenchulus was specific in Mentha grown soil and the microorganisms like Micrococcus roseus, Bacillus cereus, Azospirillum brasilense, Rhizopus microspores, Aspergillus niger, Fusarium oxysporum, Penicillium chrysogenum and *Arbuscular Mycorrhizal* Spores were found along with the phytonematode of Godhumakunta village and Peerjadiguda village (Table 4).

Table 4: Bacteria found in soil of Mentha.

S.No.	Bacterial and fungal species	CFU (106 /g) soil
1	Micrococcus roseus	708±16.2
2	Bacillus cereus	813±13.2
3	Azospirillum brasilense	206± 18.3
4	Rhizopus microsporus	608±14.7
5	Aspergillus niger	804±14.3
6	Fusarium oxysporum	206±16.3
7	Penicillium chrysogenum	205±16.3
8	Arbuscular Mycorrhizal Spore	08/100 gram soil

Hemicycliophora and Caloosia

Hemicycliophora and Caloosia were specific in Coriander grown soil and Bacillus cereus, Pseudomonas fluorescens, Azospirillum brasilense, Rhizopus microspores, Curvularia clavata, Fusarium oxysporum, Penicillium chrysogenum and *Arbuscular Mycorrhizal* Spore were also found along with these phytonematodes in the Coriander grown soil of Godhumakunta village and Peerjadiguda village (Table 5).

Table 5: Bacteria found in soil of Coriander.

S. No.	Bacterial and fungal species	CFU (106 /g) drysoil
1	Bacillus cereus	812±15.2
2	Pseudomonas fluorescens	315±18.3
3	Azospirillum brasilense	203±12.5
4	Rhizopus microsporus	605±13.2
5	Curvularia clavata	308±15.3
6	Fusarium oxysporum	203±17.3
7	Penicillium chrysogenum	208±17.3
8	*Arbuscular Mycorrhizal* Spore	08/100 gram soil

Trichodorus

Trichodorus was specific in Amaranthus grown soil and Micrococcus roseus, Cellulomonas terrae, Azospirillum brasilense, Rhizopus microspores, Aspergillus niger, Fusarium

oxysporum and *Arbuscular Mycorrhizal* Spore were also found along with these phytonematodes in the Amaranthus grown soil of Godhumakunta village and Peerjadiguda village (Table 6).

Table 6: Bacteria found in soil of Amaranthus.

S. No.	Bacterial and fungal species	CFU (106 /g) soil
1	Micrococcus roseus	712±16.3
2	Cellulomonas terrae	1023±22.34
3	Azospirillum brasilense	208±17.3
4	Rhizopus microsporus	603±11.4
5	Aspergillus niger	806±16.3
6	Fusarium oxysporum	205±14.3
7	*Arbuscular Mycorrhizal* Spore	08/100 gram soil

Among the selected leafy vegetable crops, Spinach grown soil showed high diversity, In Coriander grown soil *Micrococcus roseus*, *Cellulomonas terrae* and *Aspergillus niger* were not seen. In Amaranthus grown soil, Bacillus cereus, Curvularia clavata and Penicillium chrysogenum were not seen. *Cellulomonas terrae* was not seen in Mentha soil (Table 7).

Table 7: Comparision of microbial growth studies in selected test vegetable crops.

	Micrococcus roseus	Bacillus cereus	Cellulomonas terrae	Pseudomonas fluorescens	Azospirillum brasilense	Rhizopus microsporus	Aspergillus niger	Curvularia clavata	Fusarium oxysporum	Penicillium chrysogenum	Arbuscular Mycorrhizal Spore
Spinach	702±15.5	804±14.32	1012±18.2	303±12.2	203±11.5	602±16.2	802±11.2	302±13.2	208±14.2	202±13.5	08/100 gram soil
Coriander		812±15.2		315±18.3	203±12.5	605±13.2		308±15.3	203±17.3	208±17.3	08/100 gram soil
Amaranthus	712±16.3		1023±22.34		208±17.3	603±11.4	806±16.3		205±14.3		08/100 gram soil
Mentha	708±16.2	813±13.2			206±18.3	608±14.7	804±14.3		206±16.3	205±16.3	08/100 gram soil

Phytonematode Population studies at Godhumakunta village (20cm and 40cm)

The population studies were performed at a distance of 20cm from the root-knot of the selected leafy vegetables. In this studies when compared to control more number of phytonematodes were observed. Among the leafy vegetables Spinach showed less phytonematode population and Mentha showed high phytonematode population. *Hoplolaimus indicus* was found to be in low number and *Aorolaimus perscitus* was found to be in high number in leafy vegetables. Less nematodes were observed in spinach and high number of nematodes observed in Mentha (Table 8).

Table 8: Mean numbers of nematodes per 473 cc (1 pit) soil-20cm.

Soil Type	*Hoplolaimus indicus*		*Aorolaimus perscitus*	*Belonolaimus*
longicaudatus	*Psilenchu*		*Tylenchorhynchus brevilineatus*	
Control	04	06	08	15	10
Spinach	06	08	10	26	15
Coriander	15	30	20	76	37
Amaranthus	11	85	20	96	9
Mentha	35	98	10	17	20

At a distance of 40cm from the root-knot the phytonematode population was decreased when compared to 20cm distance of root-knot (Table 9).

Table 9: Mean numbers of nematodes per 473 cc (2 pit) soil-40cm.

Soil Type	*Hoplolaimus indicus*		*Aorolaimus perscitus*	*Belonolaimus*
longicaudatus	*Psilenchu*		*Tylenchorhynchus brevilineatus*	
Control	04	03	4	10	10
Spinach	04	07	5	15	10
Coriander	10	10	10	20	30
Amaranthus	10	50	15	50	10
Mentha	30	20	10	86	15

CONCLUSION

For the present investigation, Soil samples of Spinach, Mentha, Amaranthus and Coriander were collected from the different parts of Godhumakunta village Keesara Mandal (M) and Peerjadiguda village, Uppal mandal in Telangana. In this work, identified several nematodes in the soil of leafy vegetable crops Spinach, Amaranthus, Coriander and Mentha. *Psilenchus dunensissps, Tylenchorhynchus brevilineatus, Belonolaimus longicaudatus, Hoplolaimus indicussps, Peltamigratus indicussps, Peltamigratus indicussps and Aorolaimus perscitussps* were found in all four leafy vegetables grown soil. The nematodes *Radopholus and Criconemella* were specific in *Spinach soil, Hemicycliophora* and *Caloosia* were specific to

Coriander, Trichodorus was specific to Amaranthus and Rotylenchulus was specific in Mentha soil. Soil analysis of these vegetable crops was studied and the pH was found to be 7.5 in spinach grown soil, 7.7 in coriander grown soil, 7.6 in Amaranthus grown soil and 7.8 in mentha grown soil. The physical parameters selected for the study were colour, texture and salinity which are very important for the growth of the phytonematodes. Physico chemical properties and Microorganisms were identified in the soils of these vegetables. Among the soils selected, Spinach showed high diversity of microorganisms followed by Coriander, Mentha and Amaranthus. *Micrococcus roseus, Bacillus cereus, Cellulomonasterrae, Pseudomonas fluorescens, Azospirillumbrasilense, Rhizopus microspores, Aspergillus niger, Curvulariaclavata, Fusarium oxysporum, Penicilliumchrysogenum* were found in all the four different soils of leafy vegetables. *Micrococcus roseus* was not seen in the Coriander soil, *Bacillus cereus* was not seen in *Amaranthus and Cellulomonasterrae* was not seen in Mentha soil. *Hoplolaimus indicus* was found to be in low number and *Aorolaimus perscitus* was found to be in high number in leafy vegetables. Less number of nematodes were observed in spinach and high number of nematodes observed in Mentha.

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