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STUDIES ON PHYTONEMATODE AND MICROBIAL DIVERSITY OF CORIANDER AND AMARANTHUS LEAFY VEGETABLE CROPS

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ABSTRACT: Nematodes feed from plants in a variety of ways, but use a specialized spear called a stylet. Identified several nematodes in the soil of leafy vegetable crops such as Coriander and Amaranthus. The nematodes *Hemicycliophora* and *Caloosia* were specific to *Coriander*, *Trichodorus was specific to Amaranthus*. Soil analysis of these vegetable crops was studied and the pH was found to be 7.7 in coriander grown soil, 7.6 in Amaranthus grown soil. Among the soils selected, coriander had more of microorganisms followed by Amaranthus. Micrococcus roseus, Bacillus cereus, Cellulomonasterrae, Pseudomonas fluorescens, Azospirillumbrasilense, Rhizopus microspores, Aspergillus niger, Curvulariaclavata, Fusarium oxysporum, and Penicillium chrysogenum 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 30th 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.
Key Words: Phyto Nematodes, Coriander, Amaranthus, Soil microorganisms,Caloosia and Micrococcus roseus.

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INTRODUCTION

On a global scale, the distribution of nematode species varies greatly. Some are cosmopolitan, such as certain Meloidogyne spp., while others are particularly restricted geographically as Nacobbus spp. or are highly host specific, such as Heteroderacarotae which attacks only carrots. Some crops may have very few nematode pests, while others have a particularly wide range of genera and species associated with them leading to difficulties for nematode management strategies. Distribution maps and host range data are available and updated regularly as a useful source for determining nematode damage potential. One difficulty with assessing nematode impact is that damage resulting from nematode infection is often less obvious than that caused by many other pests or diseases [1-3]. Losses that result from nematode attack may not necessarily be as a consequence of direct cell death, necrosis, or "diseased" tissue, but may derive from other more insidious aspects, such as interference with the root system absorption, growth and development such as, reducing their efficiency in terms of access and uptake of nutrients and water; to the unaware, nematode-affected plants present typical drought and nutrient stress symptoms, which are easily and often misdiagnosed. Many plant-parasitic nematodes feed on the roots of plants [4-6]. The feeding process damages the plant's root system and reduces the plant's ability to absorb water and nutrients. Typical nematode damage symptoms are a reduction of root mass, a distortion of root structure and/or enlargement of the roots. Nematode damage of the plant's root system also provides an opportunity for other plant pathogens to invade the root and thus further weakens the plant [7].

Direct damage to plant tissues by shoot-feeding nematodes includes reduced vigor, distortion of plant parts, and death of infected tissues depending upon the nematode species [8-10]. Most plant parasitic nematodes are soilborne root pathogens, but a few species feed primarily upon shoot tissues. The majority of plant parasitic nematode species are in the class Chromodorea, order Rhabditida.

METHODOLOGY

Soil collection

Soil samples were collected from the different parts of Godhumakunta village Keesara Mandal (M), Peerjadiguda village, Uppal mandal near Moosiriver in Telangana.

Identification of Nematodes

The classic method of extraction of nematodes from soil is conducted following the method of Jenkins (1964). The soil sample is mixed thoroughly, but gently when tumbling, to homogenize the nematodes within the soil. A measured volume of soil (either 100 cm3 or 250 cm3) is rinsed through a 864 μm (20 mesh) sieve into a large pitcher. The filtrate is mixed with a pressurized water spray to fill the pitcher. After allowing the water and soil in the pitcher to settle for 20 seconds, the suspension is poured over a 38 μm (400 mesh) sieve held at a 45° angle. Material captured on the sieve is rinsed into a 100 mL centrifuge tube and centrifuged for 3minutes at 1,700 rpm. The supernatant is poured off and the pellet is resuspended in a 1.328 M sucrose solution (specific gravity = 1.10) before a repeated centrifugation at 1,700 rpm for 3 minutes. Following centrifugation, the supernatant is poured over a 25 μm (500 mesh) sieve and rinsed with water to remove any traces of sucrose. The resulting material captured on the sieve can be examined under a light microscope for identification and quantification. An alternative method to centrifugation of the soil sample is a modified Baermann tray or funnel. In this case, the required volume of soil is rinsed through a 864 μm sieve and over a 38 μm sieve, just as with the centrifugation method. The captured material is rinsed into a coffee filter placed within a plastic bowl or funnel and supported by a screen. The water level is brought up to at least 1.0 cm above the coffee filter and allowed to incubate for 24 hours. Following incubation, the filter and screen are removed from the bowl and the water left in the bowl or funnel base is poured over a 25 μm sieve. The material contains only live, mobile nematodes and can be observed under a light microscope.

Soil physico-chemical analysis

The soil pH was determined in soil water suspension (1:2:5) using a pH meter, Electrical conductivity was determined 1:2 ratio of soil water suspension by conductivity meter, nitrogen by kjeldahl method using Kjeltechautoanalyser 1030 (Piper, 1966) and phosphorus by calorimetrically employing vanado- molybdate method. Potassium was estimated by using flame photometer with determined with neutral normal ammonium acetate solution. 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.

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 Azospirillum0), Pikovaskaya s medium was used for Phosphobacteria, Kings B medium was used for Pseudomonas, Kuster s Agar medium was used for Actinomycetes (Subbarao, 1986) and total bacteria and total fungi in the soil samples.

Quantitative estimation of Arbuscular Mycorrhizal spore

Arbuscular Mycorrhizal spore density in each soil samples was estimated by a modified wet sieving and decanting technique as described by Gerdemann and Nicolson (1963).

Statistical analysis

The data were statistically analyzed by analysis of variance (ANOVA) and treatment means were separated using Duncan s Multiple Range Test.

RESULTS AND DISCUSSION

Nematodes found in all two different plant soils

In this work, we have identified several nematodes in the soil of leafy vegetable crops like Amaranthus and Coriander. *Psilenchus sps, Tylenchorhynchus sps, Belonolaimus sps, Hoplolaimus sps, Peltamigratus sps, Peltamigratus sps and Aorolaimus sps* were found in two leafy vegetables grown soil (Fig 1-9).

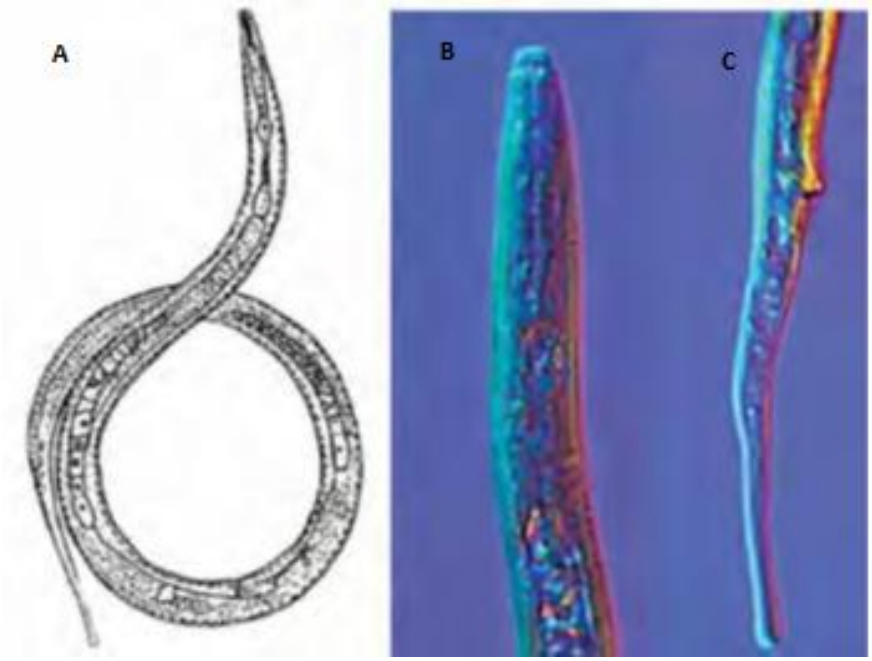

Fig 1: *Psilenchus* full body (A), head region (B), and male tail (C).

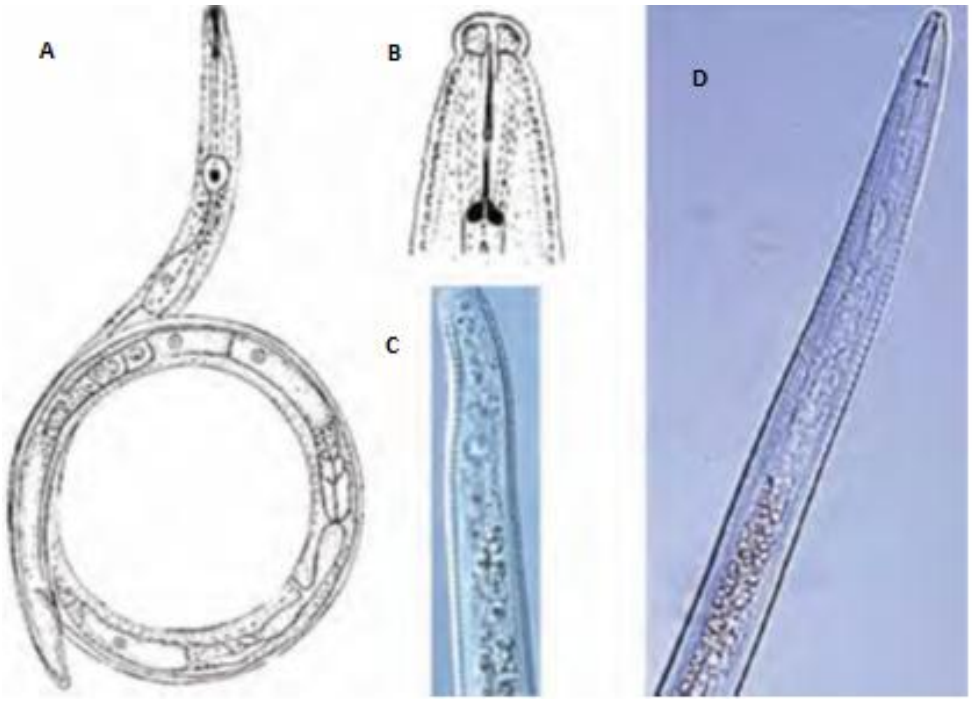

Fig 2: *Tylenchorhynchus* full body (A), short stylet (B), round tail tip (C), basal bulb not overlapping intestine (D).

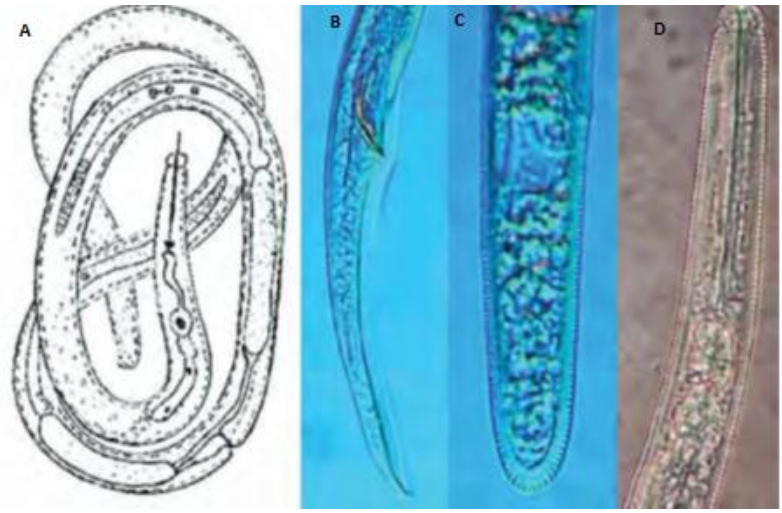

Figure 3: *Belonolaimus* full body length (A), male tail region (B), female tail region (C), and head region (D).

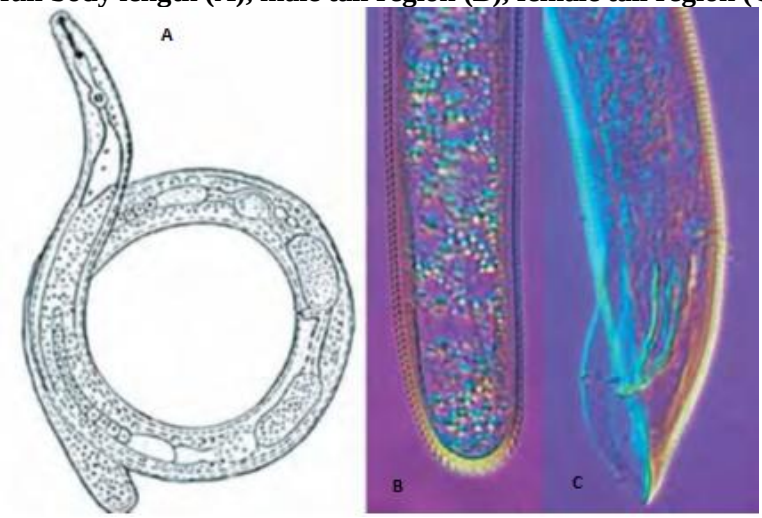

Figure 4: *Hoplolaimus* full body (A), female tail (B), and male tail (C).

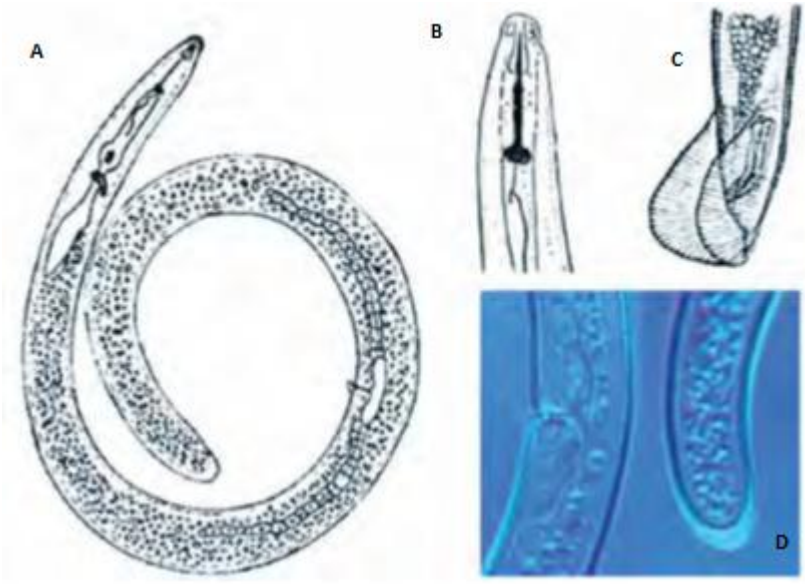

Figure 5: *Peltamigratus* female full body with epitygma [e] (A), stylet region (B), male tail (C), epiptygma (D), and female tail region (E).

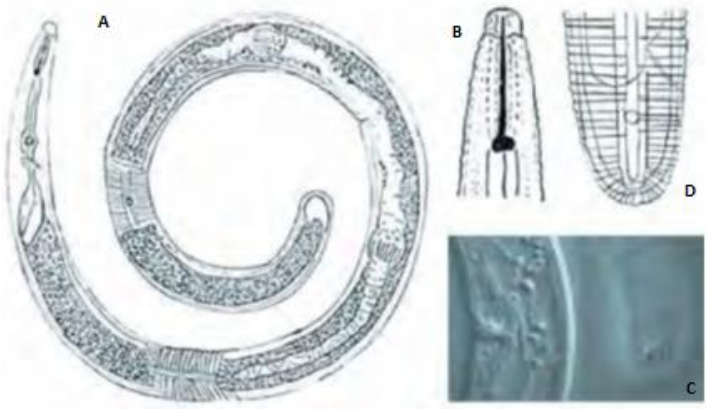

Figure 6: *Aorolaimus* female body with plasmid [p] midbody (A), stylet region of *Aorolaimus* and *Scutellonema* (B), tail region of *Scutellonema* with scutellum [s] (C), vulva [v] without epitygma (D), and scutellum [s] near tail tip of *Scutellonema* (E). *Hemicycliophora* and *Caloosia* were specific to *Coriander*, *Trichodorus* was specific to Amaranthus

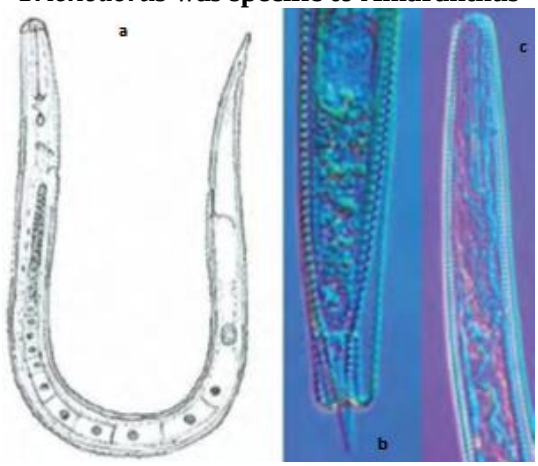

Figure 7: *Hemicycliophora* female full body (A), tail (B), and head (C).

Figure 8: *Caloosia* female body (A), tail (B), and head (C).

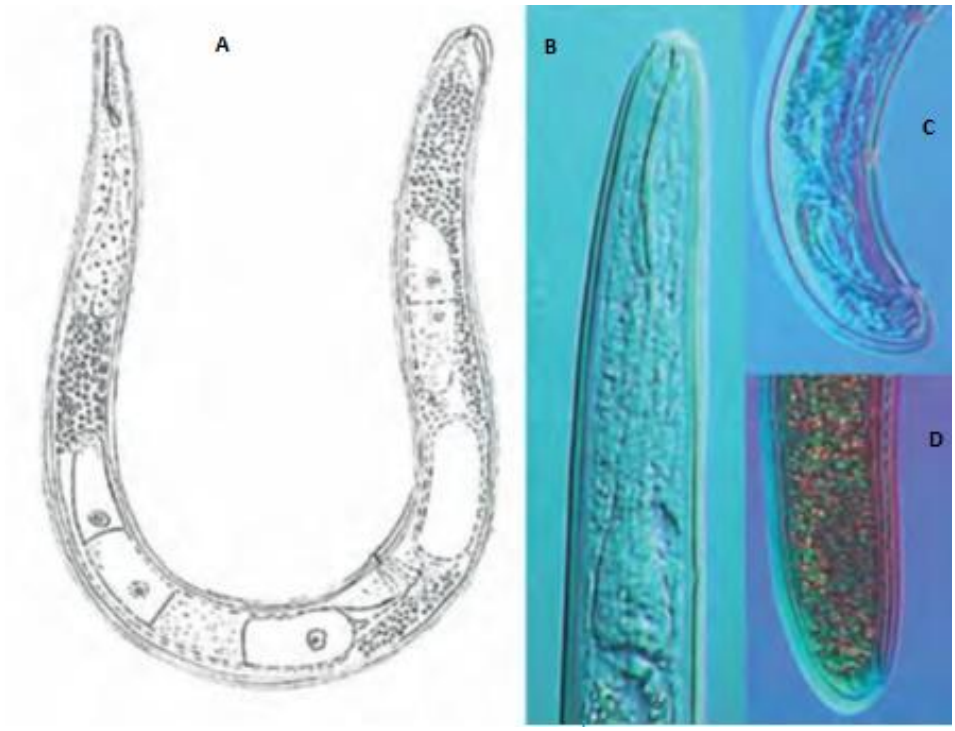

Figure 9: *Trichodorus* full body (A), lip region with stylet (B), male tail region (C) and female tail region (D).

Soil analysis

Soil analysis of these vegetable crops was studied and the pH was found to be 7.7 in coriander grown soil, 7.6 in Amaranthus grown soil. The temperature was found to be 27^0C for Coriander, 26^0C for Amaranthus soil (Table 1, 2).

Table-1: Soil analysis of Coriander

S. No	Soil Parameters	Measurements
1	Soil pH	7.7
2	temp	27^oC
3	Moisture content (%)	34.76
4	Electrical conductivity (dSm^{-1})	0.37
5	Organic carbon (%)	1.58
6	Nitrogen (Kg/ha)	46
7	Phosphorus (Kg/ha)	42
8	Potassium (Kg/ha)	103
9	Iron (mg/g)	6.25
10	Manganese (mg/g)	7.48
11	Zinc (mg/g)	0.38
12	Copper (mg/g)	0.47
13	Calcium (mg/g)	1.53
14	Sodium (mg/g)	1.48

Table-2: Soil analysis of Amaranthus

S. No	Soil Parameters	Measurements
1	Soil pH	7.6
2	temp	26^{o}C
3	Moisture content (%)	35.71
4	Electrical conductivity (dSm^{-1})	0.43
5	Organic carbon (%)	1.68
6	Nitrogen (Kg/ha)	53
7	Phosphorus (Kg/ha)	46
8	Potassium (Kg/ha)	128
9	Iron (mg/g)	5.54
10	Manganese (mg/g)	7.94
11	Zinc (mg/g)	0.84
12	Copper (mg/g)	0.42
13	Calcium (mg/g)	1.62
14	Sodium (mg/g)	1.50

Physico chemical properties and Microorganisms were identified in the soils of these vegetables. Among the soils selected, Coriander showed high diversity of microorganisms. Micrococcus roseus, Bacillus cereus, Cellulomonasterrae, Pseudomonas fluorescens, Azospirillumbrasilense, Rhizopus microspores, Aspergillus niger, Curvulariaclavata, Fusarium oxysporum, Penicilliumchrysogenum and AM Spore were found in all the soils of leafy vegetables. *Micrococcus roseus* was not seen in the Coriander soil, Bacillus cereus was not seen in *Amaranthus* grown soil. *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 30th day in all the four leafy vegetable crops.When compared to control (without leafy vegetables), the soil which contain with leafy vegetables have high nematode number (Table 3-6).

Table-3: Physiological properties

	Coriander	Amaranthus
Color	Blackish brown	Blackish brown
Textural class	Clay loam	Clay loam
Salinity (%)	3.2	5.4

Table-4: Bacteria found in soil of Coriander

S. No	Bacterial and fungal species	CFU (106 /g) dry soil
1	Bacillus cereus	8^{d}
2	Pseudomonas fluorescens	3^{b}
3	Azospirillumbrasilense	2^{a}
4	Rhizopusmicrosporus	6^{c}
5	Curvulariaclavata	3^{b}
6	Fusarium oxysporum	2^{a}
7	Penicilliumchrysogenum	2^{a}
8	AM Spore	08/100 gram soil

Table-5: Bacteria found in soil of Amaranthus

S. No	Bacterial and fungal species	CFU (106 /g) soil
1	Micrococcus roseus	7^{c}
2	Cellulomonas terrae	10^{e}
3	Azospirillumbrasilense	2^{a}
4	Rhizopusmicrosporus	6^{c}
5	Aspergillus niger	8^{d}
6	Fusarium oxysporum	2^{a}
	AM Spore	08/100 gram soil

Table-6: Mean numbers of nematodes per 473 cc (1 pit) soil

Soil Type	*Hoplolaimus*	*Aorolaimus*	*Belonolaimus*	*Psilenchu*	*Tylenchorhynchus*
Coriander	15	530	20	276	37
Amaranthus	11	385	20	196	9

Table-7: Mean numbers of nematodes per 473 cc (1 pit) soil

Soil Type	*Hoplolaimus*	*Aorolaimus*	*Belonolaimus*	*Psilenchu*	*Tylenchorhynchus*
Coriander	10	510	10	220	30
Amaranthus	10	350	15	150	10

Table-8: Relationship between nematode population density (individual g-1 dry soil) and fertilization in rhizosphere

Days	Control	Coriander	Amaranthus
1	12.8 c	13.4 b	13.0 ns
10 Days	14.0 c	15.3 c	15.7 ns
20 Days	16.6 c	16.2 a	16.2 ns
30 Days	18.7 c	19.5 bc	19.0 ns

CONCLUSION

In this work, we have identified several nematodes in the soil of leafy vegetable crops like Amaranthus and Coriander. The nematodes *Hemicycliophora* and *Caloosia* were specific to *Coriander*, *Trichodorus* was specific to *Amaranthus* grown soil. Soil analysis of these vegetable crops was studied and the pH ranges between 7.5-7.8, temp 25-27°C, and the amounts of NPK as well as micronutrients were found in all the four soils. All the soils used in this study were blackish brown with clay loam texture. Physico chemical properties and Microorganisms were identified in the soils of these vegetables. Among the soils selected, coriander showed high diversity of microorganisms followed by Amaranthus. *Micrococcus roseus* was not seen in the Coriander soil, Bacillus cereus was not seen in *Amaranthus*. *Hoplolaimus* was found to be in low number and *Aorolaimus* was found to be in high number in the soil. The nematode population density was increased from first day to 30th day in all the four leafy vegetable crops. When compared to control (without leafy vegetables), the soil which contain with leafy vegetables have high nematode number. Physico-chemical studies of Roots of leafy vegetables were studied. Relationship between nematode population density and fertilization in rhizosphere. The variations in soil physicochemical and microbial diversity is probably based on host plant. Coriander is a spicy crop and Amaranthus is a vegetable crop without any volatile compounds.

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