ejpmr, 2017,5(02), 355-361

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

POPULATION STUDIES OF PHYTONEMATODES AND EFFECT OF COWDUNG ON THE PLANT GROWTH

Datrika Swapna*, Geeta Rajalingam and P. Nagaraja Rao

Department of Zoology, Osmania University, Hyderabad.

***Corresponding Author: Datrika Swapna**

Department of Zoology, Osmania University, Hyderabad.

Article Received on 10/12/2017 **Article Revised on 31/12/2017** **Article Accepted on 21/01/2018**

ABSTRACT

The phytonematode diversity changes were studied by mixing the cow dung into the all soil samples collected and checked for the population of nematodes. After the application of Cow dung into the soil, the growth of the phytonematodes were decreased when compared to the test crops grown soil. Based on the phylogenetic studies it was identified that species *Psilenchus dunensis, Tylenchorhynchus brevilineatus,* more related than the *other species Belonolaimus longicaudatus, Hoplolaimus indicus, Peltamigratus indicus and Aorolaimus perscitus.* Thus, our present studies revealed that there is a diversity changes of phytonematode based on type of the plant. This diversity is more at root level as it is effecting the soil physicochemical nature of the root zone. Hence, based on these results, one can develop phytonematode control methods.

KEYWORDS: Diversity, phytonematode, *Psilenchus dunensis, Tylenchorhynchus brevilineatus,*

INTRODUCTION

Established populations of plant parasitic nematodes are very persistent in the soil. Their density may vary with the host plants and with climatic, soil and other factors. Under favourable conditions, nematodes move, feed and reproduce; under unfavourable conditions they become inactive and may be damaged or die, but there are always enough survivors to restore the population promptly when circumstances improve.[1-3] Most nematode parasites of aerial plant tissues, such as Ditylenchus, Anguina, and Aphelenchoides species, have certain stages which may become quiescent and extraordinary resistant under adverse conditions. The cyst-forming and root-knot nematodes have special protecting mechanisms against environmental stresses.[4-6] Their eggs and unhatched larvae are covered by a cyst wall or a jelly matrix and the greater part of the life cycle occurs inside the host plant. Many species of ectoparasitic root infecting nematodes, however, do not have apparent mechanisms for protection; their entire life cycle occurs in the soil and all developmental stages are exposed to a perpetually changing environment.[7-9] Populations of ectoparasites are nevertheless persistent. Such nematodes have been chosen for further investigation. Soil inhabiting nematodes need an aerobic, aquatic milieu for their activities, and the moisture content of the soil, which may fluctuate widely, is therefore of great importance.[10]

The length of a nematode is usually less than 1 mm and its weight less than 2.10- 7 g. Even large nematodes are unable to move anything but the film of water around their bodies and an occasional minute soil particle and they do not change the structure of the soil.[11] This can be seen in vitro by watching nematodes moving in thin layers of soil under a microscope, and can also be noticed from a recent time-lapse photographic study on nematode activity in glass-panelled observation chambers. Nematodes do require space and must have some influence on the quality of soil water and soil air. The biomass, calculated from the data above, however, is only some 20 kg per ha, with perhaps 100 kg per ha or 0.003 % by weight of the tilth as a maximum, and must therefore be négligeable as a factor in soil formation or as a constituent of the soil. This calculation has, of course, no bearing on the significance of nematodes in biological processes, i.e. the causation of plant diseases and interactions with the micro-flora and fauna in the soil.[12] Several studies, reviewed in detail demonstrate that solid soil, soil water and soil air affect the behavior of nematodes. The information, however, is too scanty and erratic to draw a clear picture about the relationship between soil moisture and nematodes, also because the soil phases are complex and interrelated. It has, for example, been established beyond doubt that certain nematode species thrive only in certain types of soil, but it is not known in how far this is determined by the soil structure, the solid materials, the moisture, the dissolved materials, the biotic elements or other components.[13-15]

METHODOLOGY

The soil is alluvial in nature and affected by salts. Average annual rainfall is 662 mm and temperature ranges from $4^{o}C$ – $47^{o}C$. The average relative humidity

ranges from 32 to 82%. The chosen site was naturally infested with phytonematodes viz., stunt nematode (Tylenchorhynchus brassicae Siddiqi), reniform nematode (Rotylenchulus reniformis Linford and Oliveira), filiform nematode (Tylenchus filiformis Butschli), lance nematode (Hoplolaimus indicus Sher), spiral nematode (Helicotylenchus indicus Siddiqi) and root-knot nematode (Meloidogyne incognita, (Kofoid and White) Chitwood. The field was divided into two parts, one part received normal ploughing (20 cm deep), whereas the other part received deep ploughing (40 cm deep). Both parts were further divided into 3×3 m 2 beds and a buffer zone of 0.25 meter left between the beds. Different beds, which were replicated five times and this experiment was carried out using the experimental design of Randomized Complete Block Design (RCBD) and made them as the plots for detailed study.

1). Control soil with out plants (CSP)
2). Test soil samples with plants (TSSP)
3). Organic amendment treated soil (Cowdung) (OASC)

Immediately after the treatment, the beds were watered for ensuring proper decomposition of the organic additives. Five replicates for each treatment were arranged in a random manner according to the table of Khan and Khanum (2004). Three week old seedlings of Spinach, Mentha, Amaranthus and Coriander were raised in pots filled with autoclaved soil and transplanted after two week of the treatment. Spinach were sown directly and necessary caring such as watering, weeding etc. were also done throughout the experiment which was terminated after three months of the treatment. Soil sampling was taken at the depth of 0-15 cm both prior and after termination of the experiment from each beds with the help of a soil sampler to count the initial and final population of phytonematodes. These samples were collected and brought to the laboratory in polythene bags and were mixed thoroughly. A representative soil sub-sample of 250 g was used for isolating the nematodes with the help of Cobb's sieving and decanting method along with modified Baermann funnel technique. The nematodes were counted with the help of counting dish. For each sample aliquots of 100 ml were used for counting and from this, 5 ml suspension was used after thorough stirring. Plant growth (fresh weight of shoot and root in terms of gram) and yield was also measured.

Residual effect of different treatments

For these experiments, different beds were maintained as such and were thoroughly prepared for the next growing season. Three week old seedlings of Spinach, Mentha, Amaranthus and Coriander were raised in autoclaved soil and transplanted without giving any further treatment to the beds except the ploughing treatment (normal and deep ploughing). The seeds of Spinach, Mentha, Amaranthus and Coriander were sown directly. The final data including nematode population, plant growth (length of shoot and root in terms of centimeters (cm), fresh weight of shoot and root and fruit weight in terms of grams) were determined after three months at the termination of the crop.

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.

Maintenance of nematode culture

Healthy eggmasses of root-knot nematode, M. incognita was collected from the heavily infected roots of eggplant field near G.T Road, Aligarh-Delhi and placed on a small course sieve (1mm pore size) lined with tissue paper and then placed in 10 cm diameter Petri dish containing double distilled water. The second stage juveniles, which hatched out, were collected along with water from the Petri dish. This process was repeated for several days. These second stage juveniles (J2) of root-knot nematode served as the initial inoculum in glass houses and in vitro experiments. The nematode species were asserted by close examination of the perennial pattern of the female from the first eggmasses collected. It was confirmed by the similar examination of the randomly collected females from the inoculum raised as above. Stock populations of Rotylenchulus reniformis were maintained on cowpea under glass house conditions and mixed vermiform (4th stage) of the nematodes were extracted by processing the soil (250 g) with the Cobb's sieving and decanting method followed by Baermann's funnel technique.

RESULTS AND DISCUSSION

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 1).

Table 1: 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 2).

Table 2: 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

Phytonematode Population studies at Peerjadiguda 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 3).

Table 3: Mean numbers of nematodes per 473 cc (1 pit) soil-20cm

Soil Type	*Hoplolaimus indicus*	*Aorolaimus perscitus*	*Belonolaimus*	
longicaudatus	*Psilenchu*	*Tylenchorhynchus brevilineatus*		
Control	03	05	07	12	09
Spinach	04	07	09	23	05
Coriander	12	20	15	72	30
Amaranthus	10	75	16	94	08
Mentha	32	78	09	12	14

At a distance of 40cm from the root-knot the phytonematode population was decreased when compared to 20cm distance of root-knot (Table 4).

Table 4: Mean numbers of nematodes per 473 cc (2 pit) soil-40cm.

Soil Type	*Hoplolaimus indicus*	*Aorolaimus perscitus*		*Belonolaimus*
longicaudatus	*Psilenchu*	*Tylenchorhynchus brevilineatus*		
Control	02	03	04	10	10
Spinach	03	07	05	12	10
Coriander	11	10	11	16	20
Amaranthus	09	40	12	38	10
Mentha	28	10	10	76	13

Phytonematode Population studies at Godhumakunta village (20cm and 40cm) during 30 days period

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 5).

Table 5: Relationship between nematode population density (individual g-1 dry soil) at 20cm distance from Root-Knot.

Days	Control	Spinach	Coriander	Amaranthus	Mentha
1	12.8±0.12	13.2 ±0.13	13.4± 0.11	13.0±0.12	13.2±0.14
10DAYS	14.0±0.21	15.2± 0.24	15.3±0.26	15.7±0.26	15.7± 0.28
20DAYS	16.6± 0.16	16.1± 0.15	16.2±0.18	16.2±0.18	16.1± 0.14
30DAYS	18.7± 0.22	19.3± 0.23	19.5± 0.18	19.0± 0.18	19.0± 0.22

The nematode population density was decresed from first day to 30th day in all the four leafy vegetable crops at 40cm distance from root -knot. When compared to control (without leafy vegetables), the soil which contain with leafy vegetables have less nematode number (Table 6).

Table 6: Relationship between nematode population density (individual g-1 dry soil) at 40cm distance from Root-Knot.

Days	Control	Spinach	Coriander	Amaranthus	Mentha
1	11.8±0.12	12.2 ±0.13	11.4± 0.11	11.0±0.12	11.2±0.14
10DAYS	10.0±0.21	11.2± 0.24	10.3±0.26	12.7±0.26	10.7± 0.28
20DAYS	09.6± 0.16	10.1± 0.15	09.2±0.18	11.2±0.18	08.1± 0.14
30DAYS	08.7± 0.22	09.3± 0.23	08.5± 0.18	10.0± 0.18	07.0± 0.22

Phytonematode Population studies at Peerjadiguda village village (20cm and 40cm) during 30 days period

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 7).

Table 7: Relationship between nematode population density (individual g-1 dry soil) at 20cm distance from Root-Knot

Days	Control	Spinach	Coriander	Amaranthus	Mentha
1	12.3±0.12	13.5 ±0.13	13.8± 0.11	13.5±0.12	13.5±0.14
10DAYS	14.5±0.21	15.9± 0.24	15.9±0.26	15.8±0.26	15.8± 0.28
20DAYS	16.8± 0.16	16.8± 0.15	16.8±0.18	16.8±0.18	16.5± 0.14
30DAYS	18.9± 0.22	19.5± 0.23	19.8± 0.18	19.4± 0.18	19.5± 0.22

The nematode population density was decresed from first day to 30th day in all the four leafy vegetable crops at 40cm distance from root-knot. When compared to control (without leafy vegetables), the soil which contain with leafy vegetables have less nematode number (Table 8).

Table 8: Relationship between nematode population density (individual g-1 dry soil) at 40cm distance from Root-Knot.

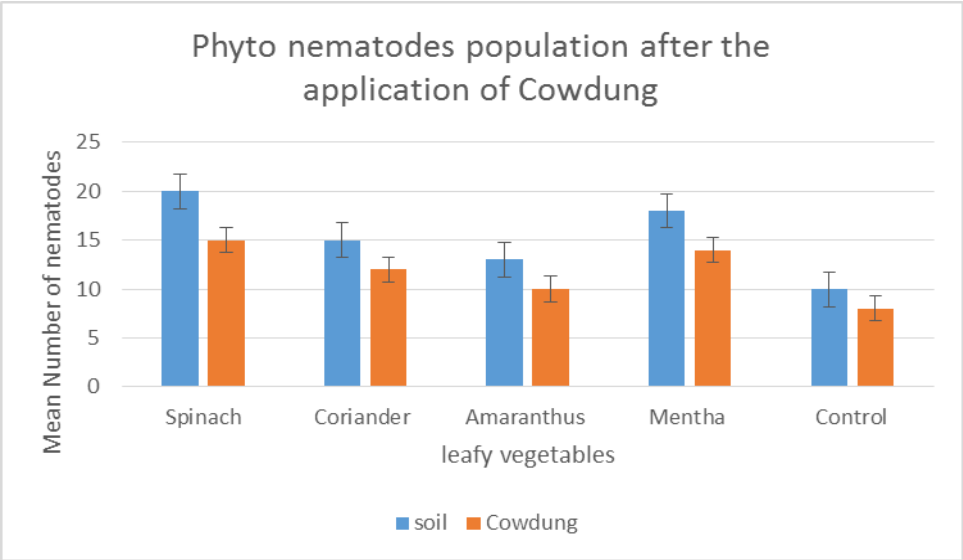
Days	Control	Spinach	Coriander	Amaranthus	Mentha
1	11.4±0.12	12.0 ±0.13	11.2± 0.11	11.0±0.12	11.0±0.14
10DAYS	10.0±0.21	11.0± 0.24	10.1±0.26	12.3±0.26	10.4± 0.28
20DAYS	09.4± 0.16	10.5± 0.15	09.1±0.18	11.6±0.18	08.0± 0.14
30DAYS	08.4± 0.22	09.0± 0.23	08.2± 0.18	10.2± 0.18	07.5± 0.22

Controlling of Phytonematode growth using Cow Dung

The phytonematode control studies were performed by mixing the cow dung into the all soil samples collected and checked for the population of nematodes. After the application of Cow dung into the soil, the growth of the phytonematodes were decreased when compared to the test crops grown soil.

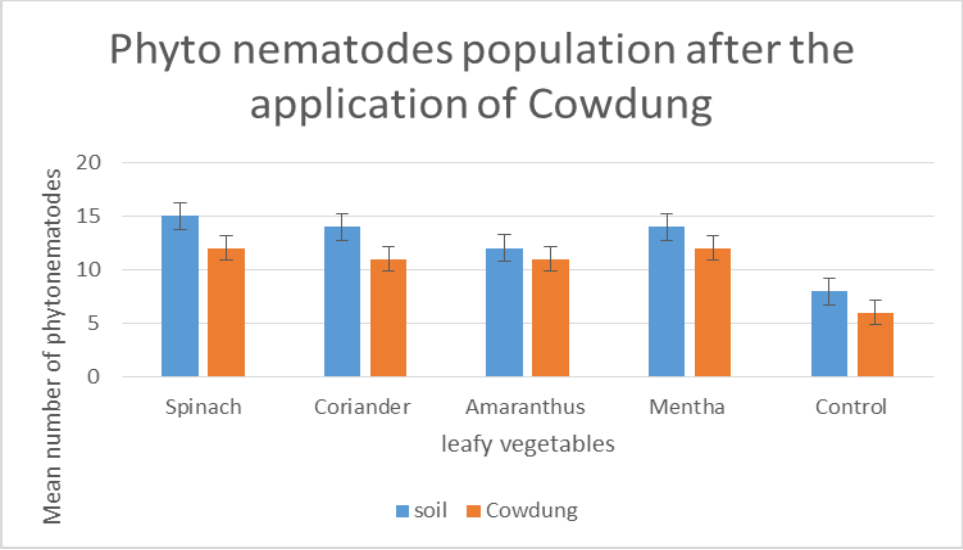
Phytonematode Population control studies at Godhumakunta village (20cm and 40cm)

The population control studies were performed at a distance of 20cm from the root-knot of the selected leafy vegetables. In this studies when compared to vegetable grown soils, the Cow dung mixed soil showed less number of phytonematodes (graph 1).



Graph 1: Mean numbers of nematodes per 473 cc (1 pit) soil-20cm before and after the treatment of Cowdung.

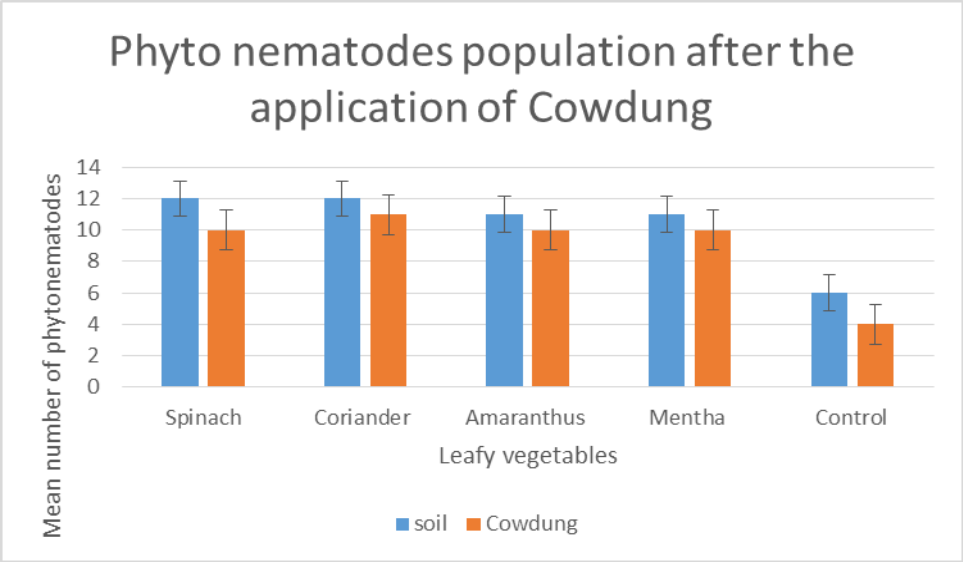
At a distance of 40cm from the root-knot the phytonematode population was decreased when compared to 20cm distance of root-knot (Graph 2).



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

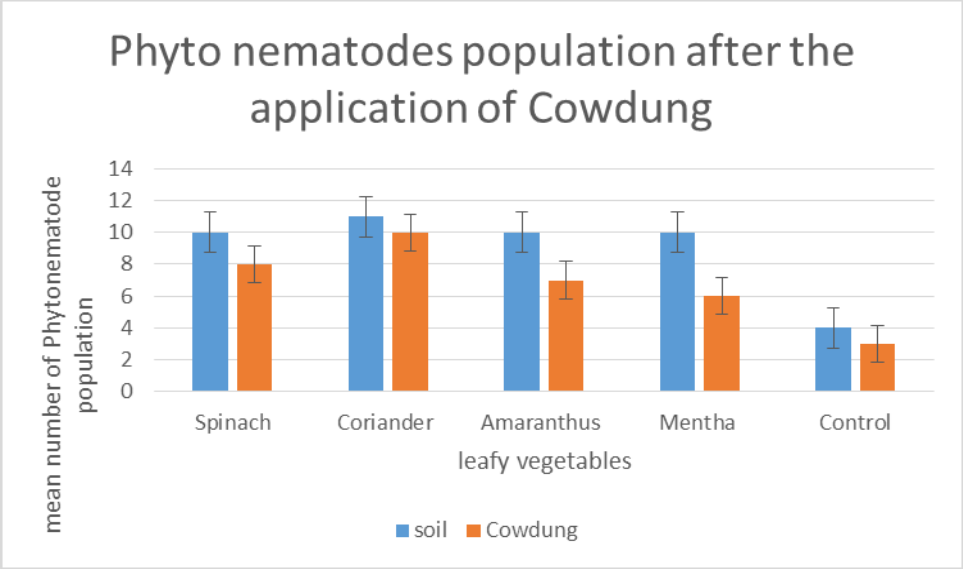
Phytonematode Population control studies at Peerjadiguda village (20cm and 40cm)

The population control studies were performed at a distance of 20cm from the root-knot of the selected leafy vegetables. In this studies when compared to vegetable grown soils, the Cow dung mixed soil showed less number of phytonematodes (Graph 3).



Graph 3: Mean numbers of nematodes per 473 cc (1 pit) soil-20cm.

At a distance of 40cm from the root-knot the phytonematode population was decreased when compared to 20cm distance of root-knot (Graph 4).



Graph 4: Mean numbers of nematodes per 473 cc (2 pit) soil-40cm.

CONCLUSION

Among the selected areas Godhumakunta village, Keesara Mandal (M) and Peerjadiguda village, Uppal mandal of Telangana state, Godhumakunta Village is suitable for the growth of Spinach, Mentha, Coriander and Amaranthus. Also the phytonematodes has more suitable conditions in Godhumakunta when compared to Peerjagiguda village. The nematode population density was increased from first day to 30th day in all the four leafy vegetable crops along with the growth of the crop plant. When compared to control soil (without leafy vegetables), the soil which contain with leafy vegetables have high nematode number. The nematode population density was decresed from first day to 30th day in all the four leafy vegetable crops at 40cm distance from root - knot. When compared to control (without leafy vegetables), the soil which contain with leafy vegetables have less nematode number. The population studies were performed at a distance of 20cm-40cm from the root-knot of the selected leafy vegetables. In this studies when

compared to control more number of phytonematodes were observed. At a distance of 40cm from the root-knot the phytonematode population was decreased when compared to 20cm distance of root-knot. 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. *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.

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

REFERENCES

1. Tzortzakakis, E.A. and Gowen, S.R. (1994). Evaluation of *Pasteuria penetrans* alone and in combination with oxamyl, plant resistance and solarization for control of *Meloidogyne* spp. on vegetables grown in greenhouses in Crete. *Crop Protection*, 13(6): 455-462.
2. Uma Shankar, Kumar, N., Krishnappa, K., Reddy, B.M.R., Ravichandra, N.G. and Karuna, K. (2005). Intercropping for the management of root-knot nematode, *Meloidogyne incognita* in vegetable-based cropping systems. *Indian Journal of Nematology*, 35(1): 46-49.
3. Husan-Bano, Siddiqui, I.A., Ahmed, W. and Ehteshamul-Haque, S. (1999). Effect of some medicinal plants on egg hatching of *Meloidogyne javanica* root-knot nematode. *Pakistan Journal of Biological Science*, 2: 1364-1365.
4. Ismail, A.E., Eid, R.A. and El-Nagdi, W.M.A. (2006). Effect of different composts, biofertilizer and olive pomace as soil amendements on *Rotylenchulus Reniformis*, growth and chemical analysis of Jasmine in Egypt. *Journal of Applied Sciences Research*, 2(11): 909-919.
5. Zareena, B.M. and Kumar, S.M. (2005). Management of disease complex involving *Heterodera cajani* Koshy, 1967 and *Macrophomina phaseolina* (Tassi.) Goid. on greengram (*Vigna radiata* L. Wilczek). *Indian Journal of Nematology*, 35(2): 192-194.
6. Widmer, T.L. and Abawi, G.S. (2000). Mechanism of suppression of *Meloidogyne hapla* and its damage by a green manure of sudangrass. *Plant Disease*, 84(5): 652-568.
7. Jada, M.Y., Gungula, D.T. and Jacob, I. (2011). Efficacy of carbofuran in controlling root-knot nematode (*Meloidogyne javanica* Whitehead, 1949) on cultivars of Bambara groundnut (*Vigna subterranea* L. Verdc.) in Yola, Nigeria. *International Journal of Agronomy*, 2011: 1-5.
8. Hussain, S.I., Kumar, R., Kahn, I.A. and Titov, A. (1984). Effect of root-dip treatments of eggplant seedlings with plant extracts, nematicides, oil cake extracts and antihelmintic drugs on plant growth and root knot development. *Pakistan Journal of Nematology*, 2(2): 79-83.
9. Faske, T.R. and Starr, J.L. (2007). Cotton root protection from plant parasitic nematodes by abamectin treated seed. *Journal of Nematology*, 39(1): 27-30.
10. Feldmesser, J., Edwards, D.I., Epps, J.M., Heald, C.M., Jenkins, W.R., Lear, B., Mc Beth, C.W., Nigh, E.L. and Perry, V.G. (1971). Estimated crop losses from plant-parasitic nematodes in the United States. Comm. Crop losses. Special publication No. 1. Soc. Nematologists, Hyattsville, Maryland.
11. Usman, A., Rehman, B. and Siddiqui, M.A. (2012). Effect of some botanical extracts for the management of *Meloidogyne incognita* infecting eggplant. *Biosciences International*, 1(1): 12-15.
12. Segers, R., Butt, T.M., Kerry, B.R. and Peberdy, J.F. (1994). The nematophagous fungus *Verticillium chlamydosporium* produces a chymoelastase-like protease which hydrolyses host nematode proteins in situ. *Microbiology*, 140: 2715-2723.
13. Vadhera, I. and Shukla, B.N. (2002). Management of *Rotylenchulus reniformis* in agriculture crops - a review. *Agriculture Review*, 23 (1): 14-22.
14. Talaveran, M., Itou, K. and Mizukubo, T. (2002). Combined application of *Glomus* sp. and *Pasteuria penetrans* for reducing *Meloidogyne incognita* (Tylenchida: Meloidogynidae) opulations and improving tomato growth. *Applied Entomology and Zoology*, 37: 61-67.
15. Tariq, I. and Siddiqui, M.A. (2005). Evaluation of nematicidal properties of neem for the management of *Meloidogyne incognita* on tomato. *Indian Journal of Nematology*, 35(1): 56-58.