

# Physico-Chemical And Biological Components Of Rhizosphere Soil And Relevance To Phytonematode Prevalence Their Control On Spicy Crops

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## Abstract

It is a changing and complex habitat that occurs around the roots of plants and other organisms over time and space. All plant-parasitic nematodes must pass through the rhizosphere to reach their host, but the length of time they stay depends on their parasitic behaviour. As the soil around the roots compacts, the rhizosphere develops its own set of biochemical, physical, and biotic characteristics distinct from the bulk soil. As cells die in the epidermal and cortical layers, soluble exudates are discharged further down the root, and complex carbon-rich compounds are released. Plant parasites and nematodes that feed on bacteria are more abundant in the rhizosphere than in the surrounding soil. Rhizobia, mycorrhizas, and plant pathogens have contributed significantly to our understanding of the interactions of microorganisms and nematodes in the rhizosphere, and they continue to do so. Thytrophic interactions in the rhizosphere have been demonstrated in a number of studies, including this one, in which nematodes and bacteria cooperate or compete to affect the plant's host. The goal of this study is to look into the prevalence and control by using cow dung against phytonematode infestations in spicy crops in relation to known physicochemical and biological rhizosphere soil components.

**Keywords:** Physico-chemical; biological components; rhizosphere soil; Phytonematode; spicy crops

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## Introduction

Plant-parasitic nematodes are estimated to have caused crop yield losses of \$100–157 billion USD worldwide, accounting for 15% of total annual crop yield. Obtaining statistics on yield loss can be extremely difficult due to the complicated interrelationships between plants, nematodes, soil organisms, and soils (1). A variety of biotic and abiotic variables, including climate change, are threatening black pepper production in particular. Plant parasitic nematodes are one of the most significant limiting factors among biotic stressors, causing production losses ranging from 15% to 35% in some cases. The presence of nematodes has been linked to a decrease in black pepper bug productivity in its early stages. According to experts, slow wilt is caused by a combination of nematode fungal infection and nutritional deficiencies in many pepper-growing areas (2).

According to the researchers, rhizosphere nematodes that feed on bacteria account for more than 60% of the worm population. Despite this, plant-parasitic species have dominated research on nematode-natural enemy interactions due to their economic importance as agricultural pests (3).

When the number of nematodes in the soil exceeds a certain threshold, the rhizobial nodulation of clover plants is severely restricted. In the rhizosphere, interactions between nematodes and bacteria are complex and typically one-of-a-kind (4).

In addition to the direct feeding and migratory effects of nematode feeding, this practise has the potential to worsen secondary illnesses such as fungus and bacterium in the host animal (5). Even though nematode-caused agricultural losses account for only 0.2 percent of total agricultural losses, this amount is critical in funding nematological research. The spicy crops were found to be infested with a large number of nematodes. This nematode-fungus relationship to 'wilt disease' was first recognised in 1906, along with the discovery of the root-knot nematode at the same time (6). We suspected that low decrease infection was caused by worm and fungal illnesses, as well as nutritional deficiency. When nematodes and other diseases were present in the same environment, they worked together to cause plant withering and growth reduction (7).

## **Material and methods**

### **Sample collection**

Spicy crop names that have been selected are now frequently referred to just by their common names in the scientific literature. Soil samples from Telangana's Zahirabad (Sangareddy district), Kamepalli (Khamamm district), and Peerzadiguda, Uppal mandal near the Moosi river were collected. The plants studied were *Allium cepa* (onion), *Allium sativum* (garlic), and *Zingiber Officinale* (ginger).

### **Soil physico-chemical analysis**

A pH metre was used to determine the pH of the soil water suspension (1:2:5). (8). To determine the 1:2 ratio of the soil water suspension, an electrical conductivity metre was required (9). Kjeltech autoanalyser 1030 was used to determine the nitrogen content. (10) A flame photometer was used to measure potassium, and an ammonium acetate neutral solution was used as the standard (11). An ammonium acetate solution was used to determine the concentrations of calcium, magnesium, iron, zinc, sodium, and copper (12). The Walkey and Black wet digestion method was used to assess organic carbon (13). The physiochemical parameters of soil and water are analysed using the same procedures, according to Indian Standards.

### **Identification of Nematodes**

Jenkins' method is used to extract nematodes from soil in the traditional way (14). Nematodes in soil can be homogenised by thoroughly mixing the sample while tossing it with a gentle twirl. In a large pitcher containing 100 to 250 cubic centimetres of soil, the soil is rinsed through a 20-mesh sieve. To fill the pitcher, a pressurised stream of water is sprayed into the filtrate. After the water and dirt have settled for 20 seconds, the suspension is poured at a 45° angle over a 38 m (400 mesh) screen. The centrifuge tube containing the sieved material is spun at 1,700 rpm in the centrifuge machine for 3 minutes. The supernatant is removed by centrifuging the pellet at 1,700 rpm for 3 minutes and suspending it in a 1.328 M sucrose solution (specific gravity = 1.10). To remove the sucrose from the mixture, the supernatant is filtered through a 25 m (500 micron) filter and rinsed with water. It is possible to determine the identity and quantity of the sieved material using a light microscope.

When centrifugation is not available, a modified Baermann tray or funnel can be used to separate soil from water. To ensure that the required amount of soil is cleaned through, a 38 m sieve is used in conjunction with an 864 m sieve. After washing, the material is placed in a coffee filter supported by a screen, and then in a plastic bowl or funnel. For 24 hours, the water level must be at least one centimetre above the coffee filter. After the water has been filtered through the filter and screen and withdrawn from the bowl, the remaining water in the bowl or funnel base is filtered using a 25-meter sieve. A light microscope can be used to observe nematodes in their natural environment.

#### Maintenance of nematode culture and control

Egg masses of the root-knot nematode were collected from extensively infected spicy crops, using a tiny course sieve (1mm pore size) lined with tissue paper and then placed in a 10 cm diameter Petri dish filled with double-distilled water for further analysis. In a Petri plate, young adults in their second stage of development were collected along with their mother's milk. This pattern lasted for several days afterwards. This second stage juvenile (J2) of root-knot nematode was used as the starting inoculum in in vitro and glass house investigations. The nematode species can be determined by observing the female's persistent pattern in the first egg masses recovered. Additionally, a comparable investigation using female inoculum produced from previously created inoculum corroborated the prior evaluation's findings. It was revealed that nematodes could be collected from soil (250 g) using the Cobb's screening and decanting method, followed by the Baermann's funnel approach, by keeping *Rotylenchulus reniformis* stock populations on cowpea under glass house conditions (15). Cow dung was used to control phytonematodes.

#### Results and Discussion

Furthermore, because nematodes are more prevalent in the rhizosphere than in the bulk soil, their obligatory parasites are likely to be more prevalent in the rhizosphere as well. Unlike saprophytic pathogens and antagonists, nematode parasites that cannot feed saprophytically in soil, such as obligatory parasites, develop much more slowly outside of their worm hosts and are less vulnerable to natural world rhizosphere impacts. In the rhizosphere, there are subtle interactions between parasites that are not always visible (16).

#### Comparison of Soil parameters of selected spicy crops

##### Soil parameter of Zahirabad

	Soil pH	Temp	Moisture content (%)	Electrical conductivity (dSm-1)	Organic carbon (%)	Nitrogen (Kg/ha)	Phosphorus (Kg/ha)	Potassium (Kg/ha)	Iron (mg/g)	Manganese (mg/g)	Zinc (mg/g)	Copper (mg/g)	Calcium (mg/g)	Sodium (mg/g)
Onion	7.5	25°C	36.25	0.26	1.56	48	33	125	6.23	7.48	0.76	0.67	1.58	1.48

<b>Garlic</b>	7.7	27°C	34.76	0.37	1.58	46	42	103	6.25	7.48	0.38	0.47	1.53	1.48
<b>Ginger</b>	7.6	26°C	35.71	0.43	1.68	53	46	128	5.54	7.94	0.84	0.42	1.62	1.5

**Soil parameter of Kameppali**

	Soil pH	Temp	Moisture content (%)	Electric conductivity (dSm-1)	Organic carbon (%)	Nitrogen (Kg/ha)	Phosphorus (Kg/ha)	Potassium (Kg/ha)	Iron (mg/g)	Manganese (mg/g)	Zinc (mg/g)	Copper (mg/g)	Calcium (mg/g)	Sodium (mg/g)
<b>Onion</b>	7.4	24°C	36.75	0.24	1.56	42	30	121	6.15	7.21	0.71	0.61	1.51	1.41
<b>Garlic</b>	7.1	27°C	35.76	0.32	1.51	44	41	101	6.21	7.28	0.54	0.45	1.49	1.43
<b>Ginger</b>	7.4	25°C	34.72	0.40	1.62	51	43	125	5.68	7.54	0.81	0.41	1.35	1.38

**Soil parameter of Peerzadiguda**

	Soil pH	Temp	Moisture content (%)	Electric conductivity (dSm-1)	Organic carbon (%)	Nitrogen (Kg/ha)	Phosphorus (Kg/ha)	Potassium (Kg/ha)	Iron (mg/g)	Manganese (mg/g)	Zinc (mg/g)	Copper (mg/g)	Calcium (mg/g)	Sodium (mg/g)
<b>Onion</b>	7.7	25°C	37.25	0.29	1.61	51	36	130	7.23	8.41	1.16	0.71	1.84	1.82
<b>Garlic</b>	7.9	25°C	35.76	0.31	1.64	49	44	121	6.69	8.45	1.38	0.82	1.73	1.63
<b>Ginger</b>	7.8	26°C	36.71	0.39	1.72	56	49	148	7.54	8.99	1.24	0.62	1.82	1.81

Plant-parasitic nematodes have a direct impact on crop yield by altering the structure of the root system as a result of feeding on or invading plant tissues. Nature's enemies must be able to kill or alter the behaviour of active nematodes if they are to be effective in reducing the vast majority of agricultural damage caused by migratory stages of both ectoparasitic and endoparasitic nematodes

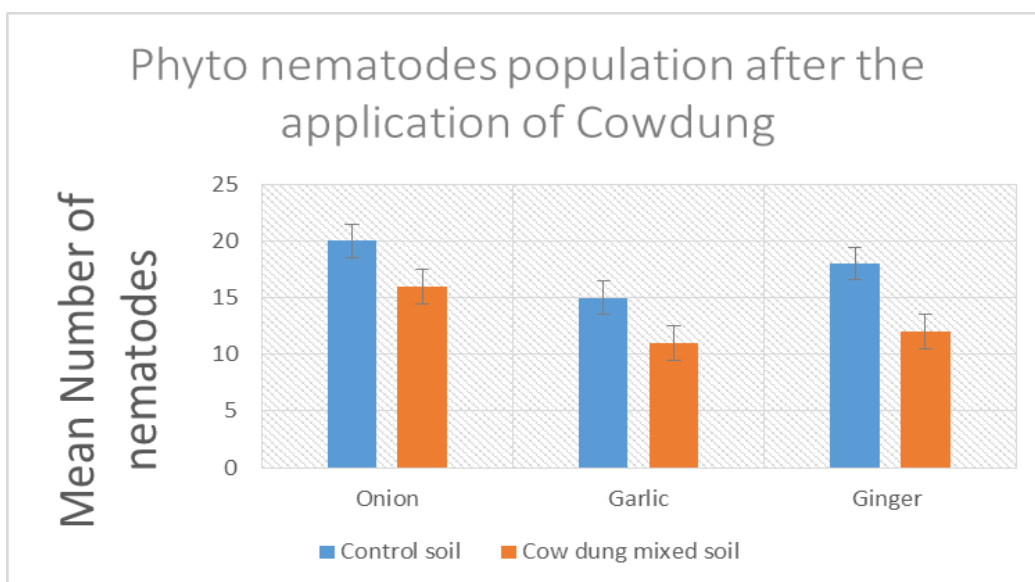
(17). Because managing end parasitic cyst and root-knot nematode infective stages is difficult, effective natural enemies that are active at this stage must be capable of reducing nematode populations to levels that are at least below the economic impairment threshold. Based on nematode count 3 were selected from spicy crops for further studies

1. *Rotylenchulus reniformi* for Ginger
2. *Ditylenchus dipsaci* for Garlic
3. *Scutellonema bradys* for Onion

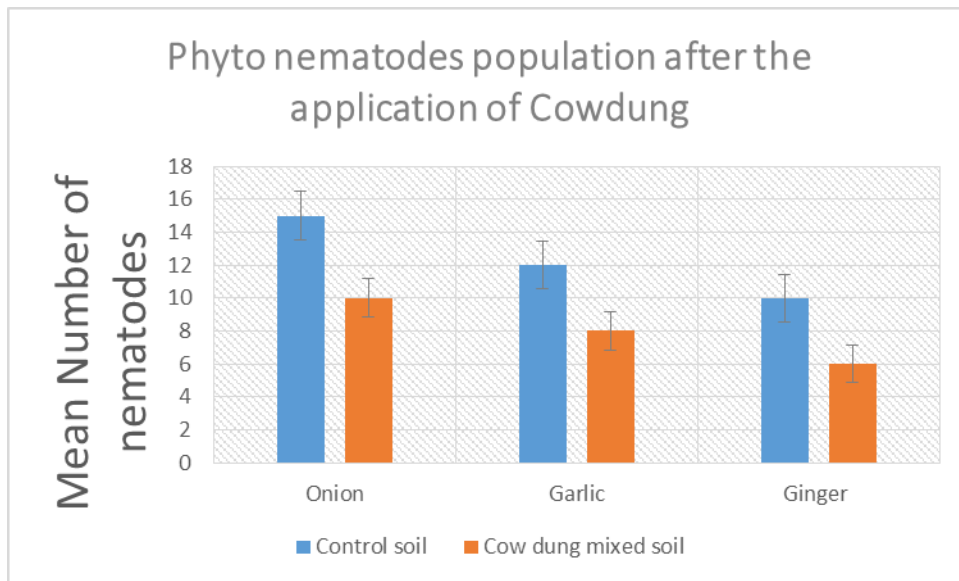
#### Phytonematode Population control studies at zahirabad village (20cm and 40cm)

It was decided that the population control research would be conducted at a distance of 20cm from the root knot of the selected spice crops... In this study, the amount of phytonematodes found in the Cow dung mixed soil was lower than in the control soils.

#### Mean numbers of nematodes per 473 cc (1 pit) soil-20cm before and after the treatment of Cow dung

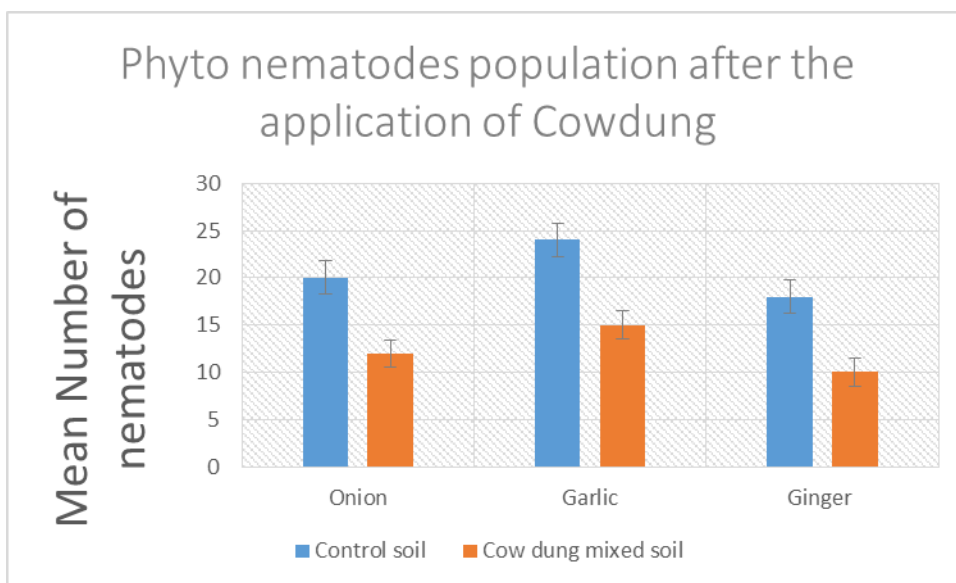


#### Mean numbers of nematodes per 473 cc (2 pit) soil-40cm

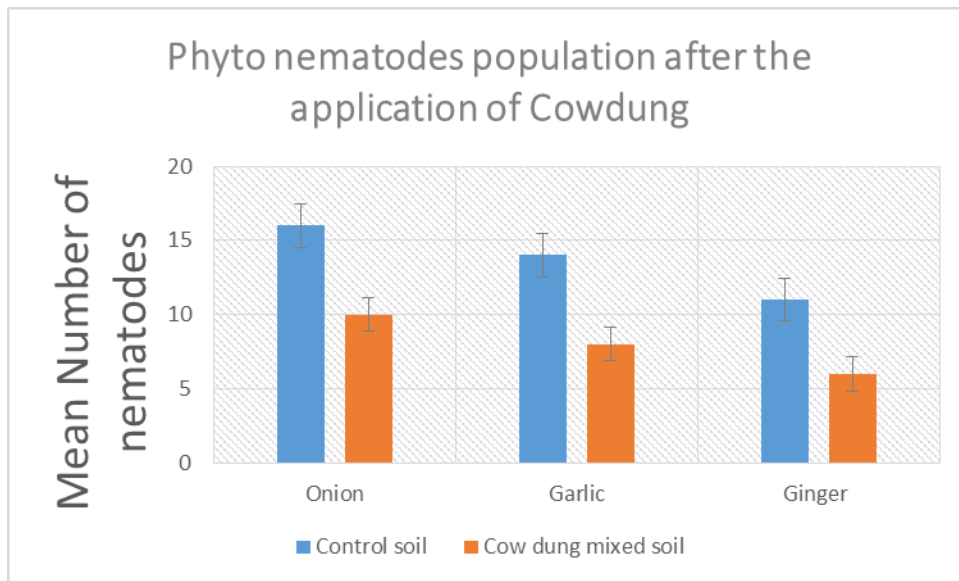


**Phytonematode Population control studies at kamepalli village (20cm and 40cm)**

**Mean numbers of nematodes per 473 cc (1 pit) soil-20cm before and after the treatment of Cow dung**

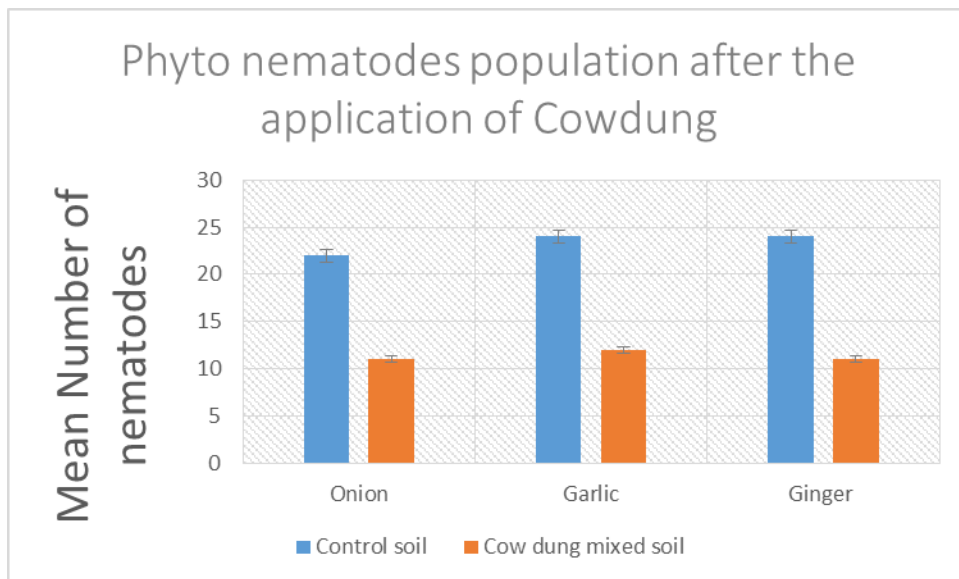


**Mean numbers of nematodes per 473 cc (2 pit) soil-40cm**

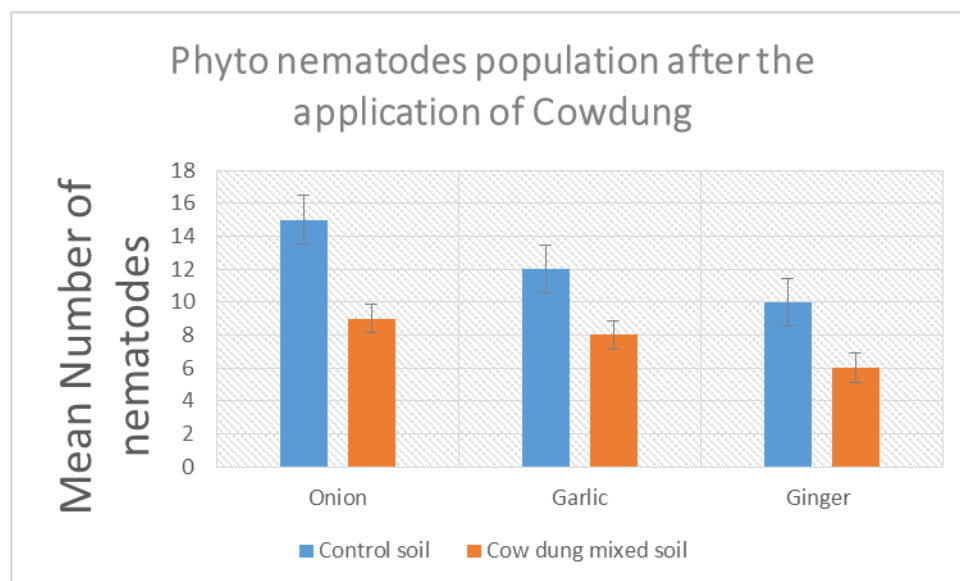


**Phytonematode Population control studies at Peerzadiguda village (20cm and 40cm)**

**Mean numbers of nematodes per 473 cc (1 pit) soil-20cm before and after the treatment of Cow dung**



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



Additional research in labs, greenhouses, and fields on the use of cow dung as a bio control agent for rootknot nematodes is urgently needed to better understand how it works (18-20).

### Conclusion

Because of the potential for negative environmental and human health consequences, the options for managing root knot nematodes are becoming increasingly limited. It is possible to control plant parasitic nematodes with a variety of synthetic compounds; however, the majority of these treatments have been withdrawn from the market due to significant off-target effects. Although nematode-controlling nematicides can be used on spice crops, doing so is discouraged due to the high costs and environmental contamination they cause. Nematodes, which pose a serious threat to spice production in countries such as India, necessitate a specific and comprehensive label claim. In the near future, nematode management will need to be more cost-effective and environmentally friendly, which means farmers will have to accept it. To effectively combat the parasite, comprehensive nematode management techniques are urgently required.

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**Conflict of Interest:** The authors declare that they don't have any conflict of interest.

### References

1. Abdelmoneim T, Massoud S (2009) The effect of endotoxin produced by *Bacillus Thuringiensis* (Bt.) against *Meloidogyne incognita*. *Egy J Nat Toxins* 6:83–93.
2. Awad HM, Germoush M (2017) Molecular and morphological identification of *Streptomyces* sp. NRC-88 nova species as  $\beta$ -lactamase inhibitor for pharmaceutical application. *Asian J Pharm Clin Res* 10(10):376–386
3. Baker GC, Smith JJ, Cowan DA (2003) Review and re-analysis of domain-specific 16S primers. *J Microbiol Methods* 55:541–555



4. Cetintas R, Kusek M, Fateh A (2018) Effect of some plant growth-promoting rhizobacteria strains on root-knot nematode, *Meloidogyne incognita*, on tomatoes. *Egypt J Biol Pest Co* 28:7
5. Coombs JT, Franco CMM (2003) Isolation and identification of actinobacteria isolated from surface-sterilized wheat roots. *Appl Environ Microbiol* 69:5603– 5608.
6. Coyne, D.L., Plowright, R.A. 2000. *Heterodera sacchari*: field population dynamics and damage to upland rice in Cote d'Ivoire. *Nematol.* 2,541-550.
7. Da Silva Campos, M. A., 2020. Bioprotection by arbuscular mycorrhizal fungi in plants infected with *Meloidogyne* nematodes: A sustainable alternative. *Crop Prot.* 135,105203.
8. Daulagala, P. W. H. K. P., 2021. Chitinolytic Endophytic Bacteria as Biocontrol Agents for Phytopathogenic Fungi and Nematode Pests: A Review. *Asian J. Res. Botany*, 14-24.
9. A.L., Millner R.H., Keeney D.R., 1982. *Method of Soil Analysis, Part 2: Chemical and Microbiological Properties*, 2nd ed. ASA and SSSA, Madison, Wisconsin.
10. Aguiar, G. P., Moraes, W. B., 2021. Occurrence of *Meloidogyne arenaria* in black pepper (*Piper nigrum* L.) in the extreme south of the State of Bahia, Brazil. *Helminthologia*, 58(2), 213-216.
11. Dong, L.Q., Zhang, Q., 2006. Microbial control of plant parasitic nematodes: a five party interaction. *Plant and Soil* 288, 31-45.
12. Eapen, J.S., Beena, B., Ramana, K., 2009. Field evaluation of *Trichoderma harzianum*, *Pochonia chlamydosporia* and *Pasteuria penetrans* in a root knot nematode infested black pepper (*Piper nigrum* L.) garden in India. *J. Plant. Crops* 37(3),196-200
13. Eissa MFM, Abd-Elgawad MMM (2015) Nematofagous bacteria as biocontrol agents of phytonematodes. In: Askari TH, Mertinelli PRP (eds) *Biocontrol agents of phytonematodes*. CABI, UK, pp 217–243
14. El-Hadad ME, Mustafa MI, Selim SM, El-Tayeb TS, Mahgoob AEA, Abdel-Aziz NH (2011) The nematicidal effect of some bacterial biofertilizers on *Meloidogyne Incognita* in sandy soil. *Braz J Microbiol* 42:102–113
15. Hegazy MI, Salama ASA, El-Ashry RM, Othman AEI (2019) *Serratia marcescens* and *Pseudomonas aeruginosa* are promising candidates as biocontrol agents against root-knot nematodes (*Meloidogyne* spp.). *Middle East J Agric Res* 8(3): 828–838
16. Holterman M, van der Wurff A, van den Elsen S, van Megen H, Bongers T, Holovach of O BJ, Helder J (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown Clades. *Mol Biol Evol* 23(9):1792–1800
17. Hugh R, Leifson E (1953) The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. *J Bacteriol* 66:24–26
18. Hussey RS, Janssen GJW (2002) Root-knot nematodes: *Meloidogyne* species. In: Starr JL, Cook R, Bridge J (eds) *Plant resistance to parasitic nematodes*. CABI, Wallingford, pp 43–70
19. Kassab SA, Eissa MFM, Badr UM, Ismail AE, Abdel Razik AB, Gaziea MS (2017) Nematicidal effect of a wild type of *Serratia Marcescens* and its mutants against *Meloidogyne incognita* juveniles. *Egypt J Agronematol* 16(2):95–114
20. Lamovšek J, Urek G, Trdan S (2013) Biological control of root-knot nematodes (*Meloidogyne* spp.): microbes against the pests. *Acta Agric Slov* 101:263–275