Chapter 1

Evaluation of the Efficiency of Some Antagonistic *Trichoderma* spp. in the Management of Plant Parasitic Nematodes

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Additional information is available at the end of the chapter

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Abstract

Plant parasitic nematodes cause great economic losses to agricultural crops worldwide. They along, with their hosts, are not isolated in the ecological system, but are strongly influenced by antagonists, parasites and pathogens. Though pesticides appear to be the most economical and efficacious means of controlling plant pathogens, toxicological, environmental and sociological concerns have led to drastic reductions in the availability of efficient commercial nematicides. These restrictions have forced farmers to look for an integral system that makes use of other means of disease control. Species of spiral nematodes, *Helicotylenchus* and *Scutellonema*, were among the most abundant plant parasitic nematodes of the mulberry plant. Eco-friendly control of the parasitic nematodes could be achieved by means of endoparasitic fungi (like *Hirsutella*, *Meria*, *Nematophthora* and *Nematoctonus*), trapping fungi (like *Arthrobotrys* and *Duddingtonia*) or parasitic fungi (like *Paeceilomyces lilacinus*). During the course of this present work, Trichoderma Pers. Ex. Fr. was found to be one of the most effective fungi in controlling the eggs and J_2 of *Meloidogyne javanica*. The present study outlines the comparative efficacy of five *Trichoderma* species (*T. viride, T. harzianum, T. longibrachiatum, T. koningii and T. hamatum*) against *Helicotylenchus* sp. and *Scutellonema* sp. The study also outlines the effect of *Trichoderma viride* Persoon on *Scutellonema* spp. and *Helicotylenchus* sp., effect of *Trichoderma harzianum* Raifae on *Scutellonema* sp. and *Helicotylenchus* sp., effect of *Trichoderma longibrachiatum* Rifai on *Scutellonema* sp. and *Helicotylenchus* sp., effect of *Trichoderma koningii* Oudeom on *Scutellonema* sp. and *Helicotylenchus* sp., and lastly effect of *Trichoderma hamatum* (Bonord) Bainier on *Scutellonema* sp. and *Helicotylenchus* sp.

Keywords: Plant parasitic nematodes, mulberry plant, fungus, antagonistic *Trichoderma* spp, biocontrol

1. Introduction

Plant parasitic nematodes cause great economic losses to agricultural crops worldwide. They along, with their hosts, are not isolated in the ecological system, but are strongly influenced by antagonists, parasites and pathogens. Though pesticides appear to be the most economical and efficacious means of controlling plant pathogens, toxicological, environmental and sociological concerns have led to drastic reductions in the availability of efficient commercial nematicides. These restrictions have forced farmers to look for an integral system that makes use of other means of disease control. This imperative approach involves a mixture of agro-technical, biological, chemical and genetic (breeding) means of plant disease control [20, 24, 36]. Species of spiral nematodes, *Helicotylenchus* and *Scutellonema*, were among the most abundant plant parasitic nematodes of the mulberry plant. Reductions in length and weight of shoot, number and weight of leaves, and number of leaf buds were the characteristic symptoms of the infection of spiral nematodes [10]. Rao and Swarup [26] found stunting of the plants and reduction in fresh and dry weights of both shoot and root system in sugarcane due to *Helicotylenchus dihystera*. Besides chemicals, various researchers suggested other control measures in view of the need to replace highly toxic and potentially polluting chemicals used to control plant parasitic nematodes and fungi with less dangerous chemicals, or preferably with biological control agents and botanicals [21]). The discovery of new biocontrol agents and the demonstration of their value in reducing disease incidence and severity has opened promising new avenues for practical applications in agriculture as well as for promoting environmental safety [8]. Considerable efforts have been made by many researchers for the management of different plant parasitic nematodes with the use of *Trichoderma harzianum* [1 - 5, 23, 28, 33].

Eco-friendly control of the parasitic nematodes could be achieved by means of endoparasitic fungi (like *Hirsutella*, *Meria*, *Nematophthora* and *Nematoctonus*), trapping fungi (like *Arthrobotrys* and *Duddingtonia*) or parasitic fungi (like *Paeceilomyces lilacinus*). But there are problems in the culture of the fungi, such as unavailability of their host, and the generalist feeding nature of fungi that means they can become trapped on and digest beneficial as well as pest species of nematode. The general approach has been to go to locations where nematodes have reached high densities, and extract parasitized individuals from the soil. Then, the fungi were cultured and tested as parasites of the nematode pest. The mycoparasitic ability of *Trichoderma* sp. against soil-borne plant pathogens allows for the development of biocontrol strategies [11, 13, 14, 16, 24]). Windham *et al.* [40] reported reduced egg production in the root-knot nematode *Meloidogyne arenaria* following soil treatment with *Trichoderma harzianum* and *T. koningii* preparations. Combining *T. harzianum* with neem cakes reduced the population of citrus nematode, *Tylenchulus semipenetrans* [25]. Reduction of *M. javanica* infection with several isolates of *Trichoderma lingnorum* and *T. harzianum* has been reported [32]. *Trichoderma* may also promote plant growth [19].

During the course of this present work, *Trichoderma* Pers. Ex. Fr. was found to be one of the most effective fungi in controlling the eggs and J_2 of *Meloidogyne javanica*. The fungi is characterized by rapidly growing colonies bearing tufted or postulate, repeatedly branched coni-

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diophores with lageniform phialides and hyaline or green conidia borne in slimy heads. They can be cultured and isolated from any type of soil. Considering the importance of the fungal genus containing species that have the potential for economic impact, the present study was carried out to determine the comparative efficacy of five *Trichoderma* species (*T. viride*, *T. harzianum*, *T. longibrachiatum*, *T. koningii* and *T. hamatum*) against *Helicotylenchus* sp. and *Scutellonema* sp.

2. Materials and Methods

2.1. Extraction of nematodes

Soil samples from around the rhizospheric regions of mulberry plants were collected and processed through Cobb's sieving and decanting method followed by Baerman's funnel technique [38]. The nematodes were observed under stereoscopic microscope and were counted using a Syracuse counting disc.

2.2. Isolation and enumeration of *Trichoderma* sp. from soil

The fungi were isolated through the serial soil dilution plate method [39]. Then, 10 g of oven dried fungi was added to a sterile Erlenmeyer flask with 90 ml sterile water, and the mixture was stirred with a magnetic stirrer for 20-30 minutes. A blender was used for blending the samples. While the suspension was in motion, 10 ml of solution was taken and added to 90 ml sterile water in a screw-cap flask or medicine bottle. It was shaken for one minute and 10 ml of the suspension was transferred to a 90 ml sterile water blank. The process was repeated until the desired dilution was obtained. Ten millilitres of soil solution was pipetted and mixed with 90 ml distilled water and marked to 10^{-3}. From 10^{-2} and 10^{-3} test tubes about 5 ml solution was added to culture media contained in four petri dishes (two of each) and kept at laminar flow for 3-4 days.

To facilitate uniform spreading of the suspension over Czapek Dox agar surface at pH 5.5, the plate was placed on a turntable and the suspension spread with a flamed L-shaped rod with one hand, while rotating the turntable with the other. To obtain distinct colonies, plates were prepared 2-3 days before use or placed for a few hours at 35 to 40° C after pouring to ensure a dry agar when the suspension was added. A water film on the freshly poured plates caused excessive spreading of organisms. The plate was incubated for a few days at 24-30° C and colony counted.

The composition of the culture media was as follows:

1. Sodium nitrate ($Na_2 NO_3$) - 1.0 g
2. Magnesium sulphate ($Mg SO_4 \cdot 7 H_2O$) - 0.5 g
3. Potassium chloride (K Cl) - 0.25 g
4. Potassium dihydrogen phosphate (KH_2PO_4) - 0.5 g

5. Ferrous sulphate ($FeSO_4.7\ H_{2O}$) - 0.5 g
6. Sucrose - 15.0 g
7. Agar agar - 10.0 g
8. Distilled water – 500 ml

A broth media was made by mixing together the substances excepting the agar. It was kept for 24 hours to dissolve the substances completely. Five to six drops of the broth were removed with a dropper into different autoclaved cavity blocks.

2.3. Inoculation of nematode and fungi

Five to six drops of the broth were removed with a dropper into different autoclaved cavity blocks. Then, 0.1 ml of selected *Trichoderma* spp. was transferred into the cavity block containing the broth. Next, 200 female *Scutellonema* spp. and *Helicotylenchus* spp. each were also inoculated into the cavity blocks. The cavity blocks containing the whole mixture were incubated at room temperature covered upside down by autoclaved petri dishes. Uninoculated nematodes on the broth were also kept as control. Observation of the nematodes under stereoscopic binocular microscope to record their mobility and fungal infection was done at each 12-hour interval. Each treatment was replicated three times.

3. Results and Discussion

The fungus attacked the nematodes though the production of conidia, sticky spores and mycelia, which on contact adhere to the cuticle and germinate, forming germ tubes that penetrated the nematodes. Then, they extended their hyphae inside the nematodes after penetration of the cuticle by conidia formation. These hyphae multiplied profusely. They inactivated the parasitic nematodes and immobilized them due to production of certain antibiotics and compounds. Observations of the immobility and parasitism of the nematodes due to the fungi were made every 12 hours. Each observation was replicated three times and the results are represented in tables 1–10. Photographs with graphs of parasitism are also provided in figures 1-32.

3.1. Effect of *Trichoderma viride* Persoon on *Scutellonema* spp. and *Helicotylenchus* sp. (table 1 and 2)

After 12 hours of inoculation, the fungus produced mycelium and conidia. The highest immobility was found at 108 hours of inoculation. The spores attached at the middle and anterior end of the body, and made the nematode immobile in the case of *Scutellonema* spp. After 24 hours of inoculation, many mycelium were found attached to the entire body of the nematode, due to which the body of the nematode was deformed and became shrunken, killing many of the nematodes. Body constrictions of nematodes might be due to the sucking of body contents by the fungus. Fifty percent of *Scutellonema* spp. out of 200 nematodes were immo-

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bilized by the fungus at 84 hours, and complete immobilization was observed at 108 hours of inoculation. In the case of *Helicotylenchus* spp., infection started within 24 hours of inoculation, and complete infestation occurred within 4-5 days of inoculation. The conidiophores of *Trichoderma viride* were less complicated; they formed aerial hyphae and coiled around the body of the nematode, producing smaller branches and ultimately forming a conifer-like branching system.

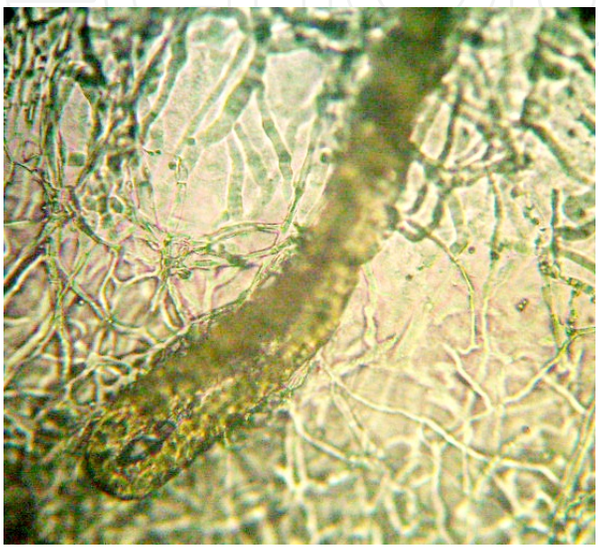

Figure 1. Effect of fungal inoculums of *Trichoderma viride* on *Scutellonema*

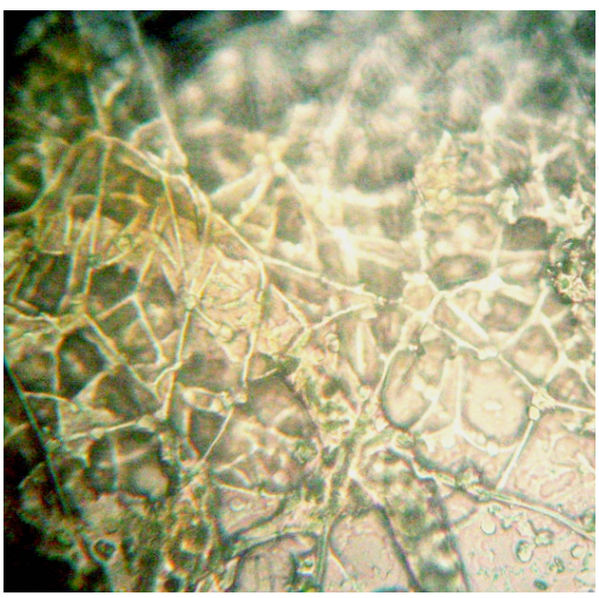

Figure 2. Effect of fungal inoculums of *T. harzianum* on *Helicotylenchus*

Observation	Active female nematodes	Non-active female nematodes	% of non-active female nematode	Control	
				Active	Non-active
12 hours	184	14.33	7.16	200	0
24 hours	174.33	25.66	12.83	200	0
36 hours	165.33	32.00	15.83	200	0
48 hours	148.66	52.66	26.33	200	0
60 hours	134.33	62.66	31.26	200	0
72 hours	120.66	84.00	42.00	200	0
84 hours	94.00	109.33	54.66	200	0
96 hours	48.66	154.0	77.0	200	0
108 hours	0.0	200.0	100	200	0
SE m±	19.33	19.67	9.84	0.0	0
C.D. at 0.05*	3.52	60.66	1.71	0.0	0

* Significant at 0.05 level of significance.

Table 1. Effect of fungal inoculums *Trichoderma viride* on the activities of *Scutellonema* spp.

Observation	Active nematode	Non-active female nematode	% of non- active nematode	Control	
				Active	Non-active
12 hours	200	0	0	200	0
24 hours	182	17	9	200	0
36 hours	172	27.66	13.83	200	0
48 hours	166	34	17	200	0
60 hours	145	54.66	27.33	200	0
72 hours	133	66.66	33.33	200	0
84 hours	126	74	37	200	0
96 hours	97	103	51.5	200	0
108 hours	46.33	153.66	76.83	200	0
120 hours	13	187	93.5	200	0
132 hours	0	200	100	200	0
SEm±	19.87	19.87	9.67	0.0	0
C.D. at 0.05*	2.760	2.760	1.38	0.0	0

* Significant at 0.05 level of significance.

Table 2. Effect of fungal inoculums *Trichoderma viride* on the activities of *Helicotylenchus* spp.

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3.2. Effect of *Trichoderma harzianum Raifae* on *Scutellonema* sp. and *Helicotylenchus* sp. (table 3 and 4)

There was no infection after 24 hours of inoculation, but 3 % of the nematodes were immobile. Infection and parasitism of the nematode occurred after 48 hours of inoculation. The highest immobility was found at 108 hours of inoculation. Many mycelia grew over the body of the nematode. The conidiophores were seen to be multiple-branched, forming loose tufts which arose in distinct and continuous ring-like zones. The main branches, mostly in groups of two or three, stood at right angles, and the length increased with the distance from the tip of the main branch, giving a conical or pyramidal appearance. The body cuticle of the nematode was suppressed. The mycelia tip ran parallel to the nematode. There was rapid and excessive coiling on the target host. The mycelium coiled with its constricting networks of loops at the anterior region of the body and the head region, making constrictions that might be due to the sucking of body contents. After 96 hours of inoculation, there was complete immobilization of the nematodes. In the case of *Helicotylenchus*, the highest percentage of infection and immobility occurred during 96th hour of inoculation.

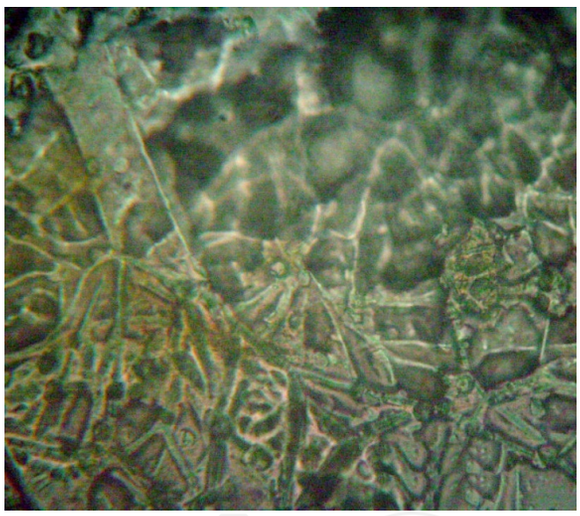

Figure 3. Effect of fungal inoculums of *T. harzianum* on *Helicotylenchus*

Observation	Active female nematodes	Non-active female nematodes	% of non-active female nematode	Control	
				Active	Non-active
12 hours	196.66	4.0	3.0	200	0
24 hours	198.66	5.0	3.5	200	0
36 hours	193.33	6.33	3.16	200	0
48 hours	161.33	36.0	17.66	200	0
60 hours	122.66	76.0	38.0	200	0
72 hours	104.0	90.0	45.0	200	0

Observation	Active female nematodes	Non-active female nematodes	% of non-active female nematode	Control	
				Active	Non-active
84 hours	89.66	104.66	52.33	200	0
96 hours	00	200	100	200	0
SEm±	22.88	22.47	11.15	0.0	0
C.D. at 0.05*	3.72	3.99	2.99	0.0	0

* Significant at 0.05 level of significance.

Table 3. Effect of fungal inoculums *Trichoderma harzianum* on the activities of *Scutellonema* spp.

Observation	Active nematode	Non-active nematode	% of non-active nematode	Control	
				Active	Non-active
12 hours	197	3	1.5	200	0
24 hours	193	7	3.5	200	0
36 hours	184.66	15.33	7.66	200	0
48 hours	162	38	19	200	0
60 hours	125.66	74.33	37.16	200	0
72 hours	108	92	47	200	0
84 hours	93	107	54	200	0
96 hours	53	147	73.5	200	0
108 hours	0	200	100	200	0
SEm±	21.34	19.32	10.67	0.0	0
C.D. at 0.05*	2.33	2.33	0.36	0.0	0

* Significant at 0.05 level of significance.

Table 4. Effect of fungal inoculums *Trichoderma harzianum* on the activities of *Helicotylenchus* spp.

3.3. Effect of *Trichoderma longibrachiatum Rifai* on *Scutellonema* sp. and *Helicotylenchus* sp. (table 5 and 6)

After 12 hours of inoculation, 4 % of the total nematode population was found to be immobile, with the highest immobility at 108 hours of inoculation. Infection started before 20 hours of inoculation. Hyphae of the fungus strain formed an appressorium-like structure in close contact with the nematode. They produced penetration holes in the cuticle of the nematode. The penetrated cuticle rapidly lost turgor and collapsed. At contact with the nematode cuticle, the hyphae branched dichotomously at the tip. The hyphae were not observed to coil around the nematode cuticle, and instead grew along the cuticle. However, penetration was not

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evident. Despite the absence of visible penetration, the nematode cuticle lost turgor pressure, wrinkled and collapsed. Finally, both the cuticle and body content of the nematode completely disintegrated. In the case of *Helicotylenchus*, the highest immobility was found at 60 hours of inoculation.

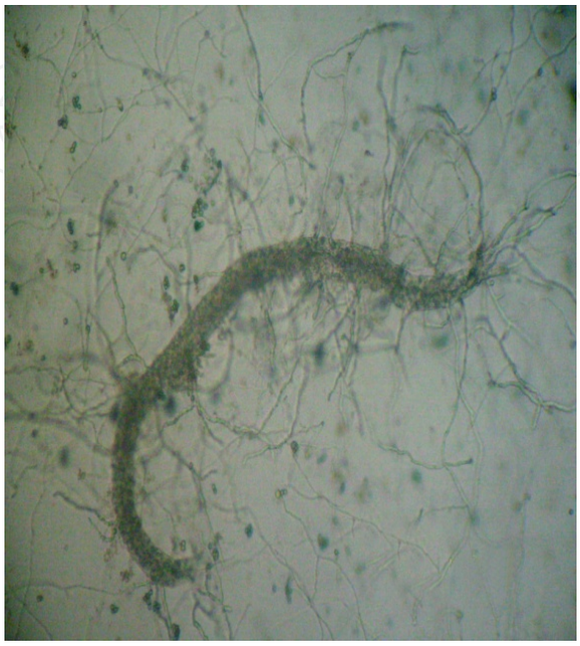

Figure 4. Effect of fungal inoculums of *T. longibranchiatum* on *Scutellonema,* whole body

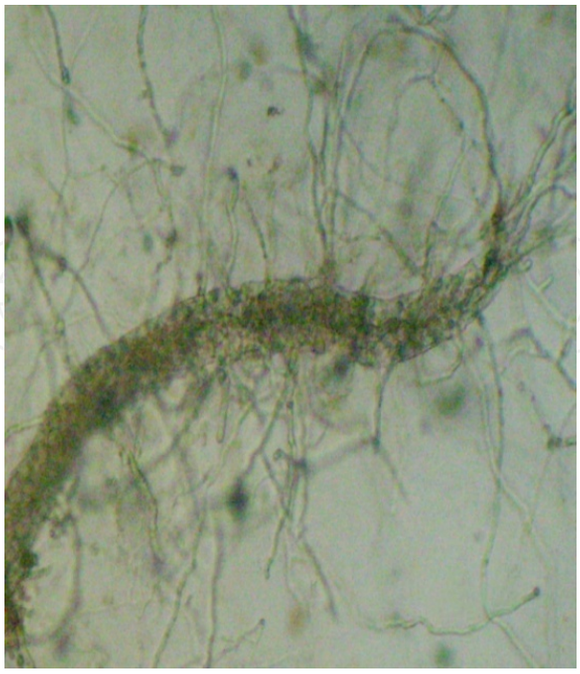

Figure 5. Effect of fungal inoculums of *T. longibranchiatum* on *Scutellonema,* head region

Observation	Active female nematodes	Non-active female nematodes	% of non-active female nematode	Control	
				Active	Non-active
12 hours	186.0	10.0	4	200	0
24 hours	180.0	18.0	9.0	200	0
36 hours	164	36	18.0	200	0
48 hours	154	46.33	22.33	200	0
60 hours	78.33	84.33	40.33	200	0
72 hours	83	121.33	60.13	200	0
84 hours	62	136.66	68.0	200	0
96 hours	27.33	173	86.53	200	0
108 hours	00	200	100	200	0
SEm±	21.70	21.81	10.97	0.0	0
C.D. at 0.05*	2.72	3.14	2.54	0.0	0

* Significant at 0.05 level of significance.

Table 5. Effect of fungal inoculums *Trichoderma longibrachiatum* on the activities of *Scutellonema* spp.

Observation	Active nematode	Non-active nematode	% of non-active nematode	Control	
				Active	Non-active
12 hours	193	7	3.5	200	0
24 hours	186	14	7	200	0
36 hours	174	26	13	200	0
48 hours	154	45	23	200	0
60 hours	83	117	58.5	200	0
72 hours	77	123	61.5	200	0
84 hours	52	147.66	83.5	200	0
96 hours	33	167	83.5	200	0
108 hours	12.66	187.33	93.66	200	0
120 hours	0	200	100	200	0
SEm±	22.24	22.05	11.02	0.0	0
C.D. at 0.05*	2.06	2.06	1.05	0.0	0

* Significant at 0.05 level of significance.

Table 6. Effect of fungal inoculums *Trichoderma longibrachiatum* on the activities of *Helicotylenchus* spp.

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3.4. Effect of *Trichoderma koningii* Oudeom on *Scutellonema* sp. and *Helicotylenchus* sp. (table 7 and 8)

There was no effect during the first 12 hours of inoculation in the case of *Helicotylenchus* spp., but 7 % of *Scutellonema* spp. were immobilized during that time. After 24 hours of exposure, conidia attachment of the nematode was found. The conidia stuck towards the cephalic region and stylet of the nematode. Maximum immobility in the case of *Scutellonema* occurred at 144 hours of nematode exposure to the fungus, while it occurred at 168 hours of exposure in the case of *Helicotylenchus* sp. At 48 hours of exposure, hyphae formation was found around the body of *Helicotylenchus* and at the anterior and posterior part of the body of *Scutellonema* spp. The hyphae penetrated towards the body cuticle of the nematode and sucked the body contents, affecting the nematode. This might be attributed to the fungus's production of endo- and exochitinases by which hyphae penetration of the nematode cuticle was made possible.

Figure 6. Effect of fungal inoculums of *T. koningii* on *Scutellonema*

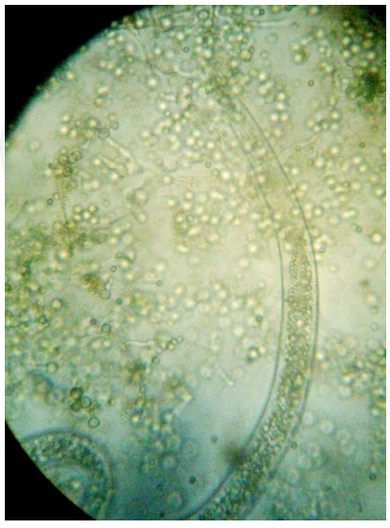

Figure 7. Effect of fungal inoculums of *T. koningii* on *Helicotylenchus*

Observation	Active female nematodes	Non-active female nematodes	% of non-active female nematode	Control	
				Active	Non-active
12 hours	186.0	13.0	7	200	0
24 hours	176.0	23	12	200	0
36 hours	166	33	17	200	0
48 hours	135	65	33	200	0
60 hours	127.0	73.0	37	200	0
72 hours	113.0	87.0	44.0	200	0
84 hours	97.0	100	50	200	0
96 hours	77	122	60	200	0
108 hours	65	132	65	200	0
120 hours	37	161	80	200	0
132 hours	13	185	92	200	0
144 hours	00	200	100	200	0
SEm±	17.269	17.192	8.483	0.0	0
C.D. at 0.05*	6.97	2.17	92.10	0.0	0

* Significant at 0.05 level of significance.

Table 7. Effect of fungal inoculum *Trichoderma koningii* on the activities of *Scutellonema* spp.

Observation	Active female nematodes	Non-active female nematodes	% of non-active female nematode	Control	
				Active	Non-active
12 hours	200	00	00	200	0
24 hours	198	1.33	0.66	200	0
36 hours	194	4.66	2.33	200	0
48 hours	179.66	16	7	200	0
60 hours	165	30	14	200	0
72 hours	133	62	30	200	0
84 hours	1235.33	70.0	34	200	0
96 hours	111	85	43.3	200	0
108 hours	95	101	51.3	200	0
120 hours	73.33	122	61.8	200	0
132 hours	60	135	68.3	200	0

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Observation	Active female nematodes	Non-active female nematodes	% of non-active female nematode	Control	
				Active	Non-active
144 hours	44.66	150	75.93	200	0
156 hours	13	183.0	92	200	0
168 hours	00	200	100	200	0
SEm±	17.67	17.55	8.88	0.0	0
C.D. at 0.05*	26.088	3.089	1.891	0.0	0

* Significant at 0.05 level of significance.

Table 8. Effect of fungal inoculum *Trichoderma koningii* on the activities of *Helicotylenchus* spp.

3.5. Effect of *Trichoderma hamatum* (Bonord) Bainier on *Scutellonema* sp. and *Helicotylenchus* sp. (table 9 and 10)

There was no effect on the nematode *Scutellonema* spp. during the first 60 hours of exposure to the fungus, or 72 hours in the case of *Helicotylenchus* sp. Immobilizations of a few *Scutellonema* sp. were found at 72 hours of exposure, while this occurred at 96 hours of exposure in the case of *Helicotylenchus* sp. Hundred percent immobility of *Scutellonema* sp. was found at 300 hours of inoculation, and in the case of *Helicotylenchus*, it was found at 444 hours of exposure. Infection of *Scutellonema* sp. started after 68 hours of exposure, and 80 hours in case of *Helicotylenchus*. Direct growths of the mycoparasite from the body of the nematodes were observed. There was spore formation inside the body of the nematode and shrinkage of body contents occurred. *Trichoderma hamatum* produced aspersoria-like structures attached to the host cell wall. Subsequently several different types of interaction occurred. The fungus either grew parallel to and along the host hyphae or coiled around the host. In *Helicotylenchus* sp., the parasite penetrated into and grew within the cuticle. The cuticle became vacuolated, shrank, collapsed and finally disintegrated. The oesophageal part of the nematode had shrunken and the tail region was disintegrated into two, as in a fork.

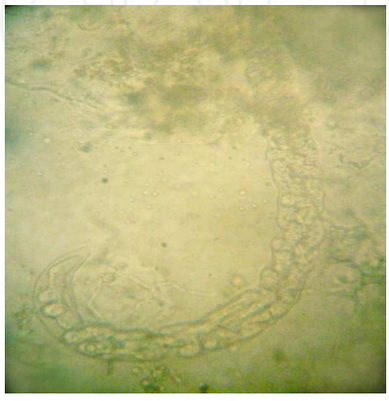

Figure 8. Effect of fungal inoculums of *T. hamatum* on *Scutellonema*

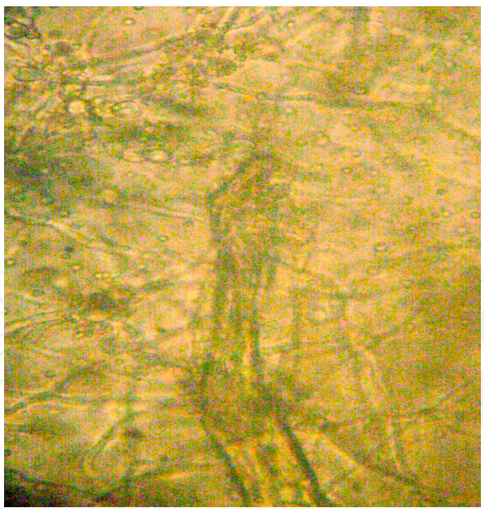

Figure 9. Effect of fungal inoculums of *T. hamatum* on *Scutellonema,* head region

Figure 10. Effect of fungal inoculums of *T. hamatum* on *Scutellonema,* tail region

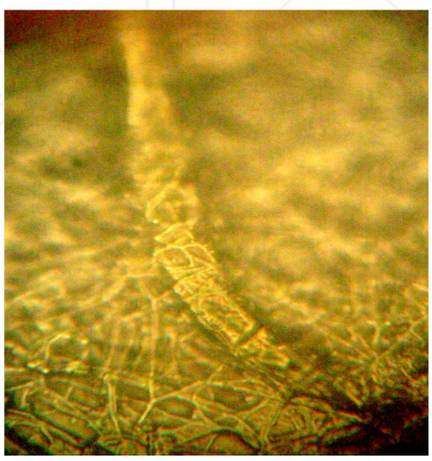

Figure 11. Effect of fungal inoculums of *T. hamatum* on *Helicotylenchus*

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Observation	Active female nematodes	Non-active female nematodes	% of non-active female nematode	Control	
				Active	Non-active
12 hours	200	00	00	200	0
24 hours	200	00	00	200	0
36 hours	200	00	00	200	0
48 hours	200	00	00	200	0
60 hours	200	00	00	200	0
72 hours	195	5	2.5	200	0
84 hours	184.33	15.66	7.8	200	0
96 hours	173.66	26.33	13.16	200	0
108 hours	165	35	17	200	0
120 hours	152	48	24	200	0
132 hours	146.33	53.66	26.83	200	0
144 hours	135.33	64.66	32.3	200	0
156 hours	123	77	38.5	200	0
168 hours	117	83	41.5	200	0
180 hours	105.33	94.66	44	198	2
192 hours	91	109	54.4	196	4
204 hours	80.33	123.66	61.83	194	6
216 hours	68.66	134.66	61.83	193	7
228 hours	54	146	73	191	9
252 hours	37	163	81.5	189	11
264 hours	26	174	87.33	187	13
276 hours	16.33	183.66	95.83	185	15
288 hours	8.33	191.66	95.83	183	17
300 hours	00	200	100	181	19
SEm±	4.907	13.98	7.14	1.25	1.25
C.D. at 0.05*	3.57	3.27	2.55	0.0	0.0

* Significant at 0.05 level of significance.

Table 9. Effect of fungal inoculum *Trichoderma hamatum* on the activities of *Scutellonema* spp.

Observation	Active female nematodes	Non-active female nematodes	% of non-active female nematode	Control	
				Active	Non-active
24 hours	200	00	00	200	0
48 hours	200	00	00	200	0
72 hours	200	00	00	200	0
96 hours	191.33	6.0	3	200	0
120 hours	180	15.66	7.66	200	0
144 hours	174.66	24	12	200	0
156 hours	150.66	50.66	25.33	200	0
180 hours	134.66	62.33	31.16	200	0
204 hours	122	77	48.5	194	6
228 hours	106	93	46.5	193	7
252 hours	85.33	119	59.16	191	9
276 hours	77	125	62.5	189	11
300 hours	63	140.66	70.33	187	13
324 hours	47	153	77.33	185	15
348 hours	30	171	85.63	183	17
372 hours	28.66	177	88.5	181	19
396 hours	17.66	186.33	94.83	179	21
420 hours	8.66	195.0	94.16	177	23
444 hours	00	200	100	176	24
SEm±	16.13	16.519	8.229	2.00	2.0
C.D. at 0.05*	2.86	3.61	7.08	0.0	0.0

* Significant at 5 % level of significance.

Table 10. Effect of *Trichoderma hamatum* on the activities of *Helicotylenchus* spp.

Several possible mechanisms have been suggested to be involved in *Trichoderma* antagonism, such as production of volatile or non-volatile antibiotics by the fungus [6], space- or nutrient-(carbon, nitrogen, iron, etc.) limiting factors that compete with the host [31], and direct mycoparasitism whereby the host cell wall is degraded by the lytic enzymes secreted by *Trichoderma* [9]. *Trichoderma harzianum* produced antibiotic 6-pentyl-α-pyrone, which had the dual effect of inhibiting pathogen growth and down-regulating genes for biosynthesis of trichothecenes, a class of mycotoxins with broad-spectrum antimicrobial activity [12]. *Trichoderma longibrachiatum* produced three main hydrolytic enzymes: protease, β-1, 3-glucanase and chitinase, which were involved in fungal cell wall degradation. *Trichoderma*

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koningii has also been found to produce cell wall degrading enzymes – chitinases, β-1, 3-glucanase and cellulose – which aid in the colonization of their host cells, while isonitrin, homothalin A, melanoxadin, trichodermin, ergokonin, viridian, viridio fungin A, B and C produced by the fungus act in antibiosis [22]. Sharon *et al*. [29] studied the mechanism involved in the attachment and parasitic processes with special emphasis on the important role of the nematode's gelatinous matrix (gm) in direct nematode-fungus interactions, and suggested that carbohydrate-lectin-like interactions might be involved in the attachment of conidia to the nematodes. The authors also found that parasitism was one of the modes of action of *Trichoderma* species against *Meloidogyne javanica*. *Trichoderma longibrachiatum* produced nematotoxic concentrations of acetic acid. Secondary metabolites from fungi also contained compounds which were toxic to plant parasitic nematodes [17, 30]. *Trichoderma* are also known to produce toxins and antibiotics like malformin, hadacidine, gliotoxin, viridian and penicillin [37], which might contribute to the inactivity and immobility of the nematodes. Parasitic interactions between *Trichoderma* and nematodes might take place in soil, on root surfaces [29] and in the rhizosphere, sites that could be colonized by these opportunistic avirulent plant symbionts [18]. The improved attachment and parasitism observed in vitro could facilitate the development of new strategies to affect interactions between the nematode, plant and fungus for successful biocontrol.

The tested species of *Trichoderma* show a significant effect on the activity of nematodes. The results indicated that *T. harzianum* followed by *T. longibrachiatum*, *T. viride*, *T. koningii* and *T. hamatum* were effective in controlling the plant parasitic nematodes *Helicotylenchus* sp. and *Scutellonema* sp. *Trichoderma* sp. was considered imperfect filamentous (Deuetromycetes, Hyphomycetes, Phialasporace, Hyphales, Dematiaceae), and was the most common saprophytic fungi in the rhizosphere found in almost any soil.

Using beneficial fungi for control of plant disease is a useful and acceptable method for farmers. As such, the fungal microbe *Trichoderma* sp. can be used in biological control in the Integrated Pest Management (IPM) programme to achieve good success. Among the tested species of *Trichoderma*, *Trichoderma harzianum* is a potential candidate. These results are in agreement with [7, 15, 27, 34, 35].

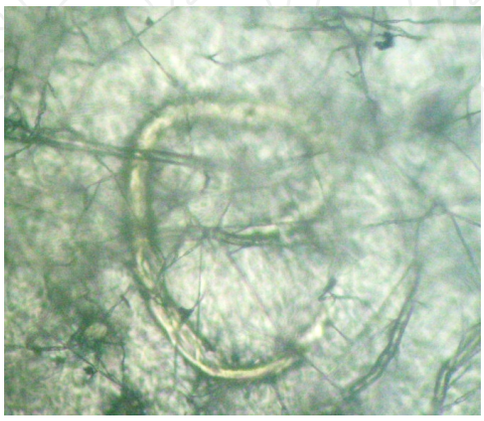

Figure 12. Effect of fungal inoculums of *Aspergillus* sp. on J_2 *M. javanica* (8 days after inoculation) 10 X 10x

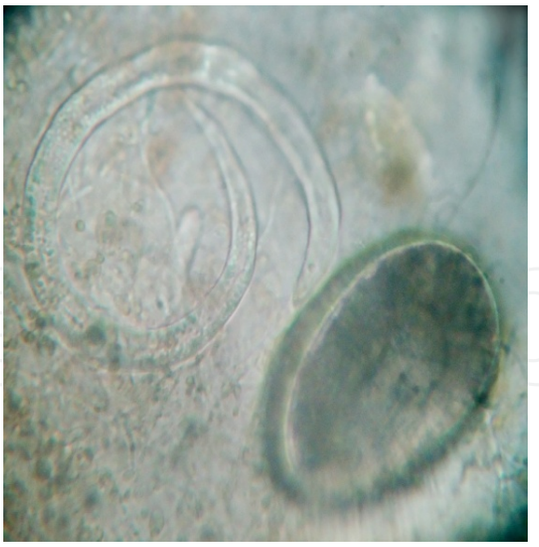

Figure 13. Effect of fungal inoculums of *Aspergillus* sp. on egg and J_2 *M. javanica* (39 days after inoculation) 10 X 10x

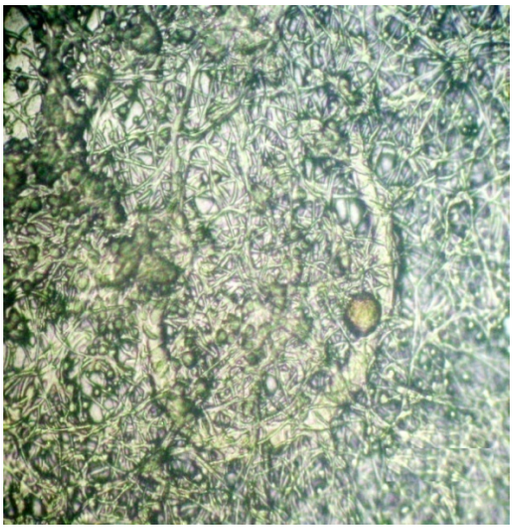

Figure 14. Effect of fungal inoculums of *Mucor* sp. on egg and J_2 *M. javanica* (39 days after inoculation) 10 X 10x

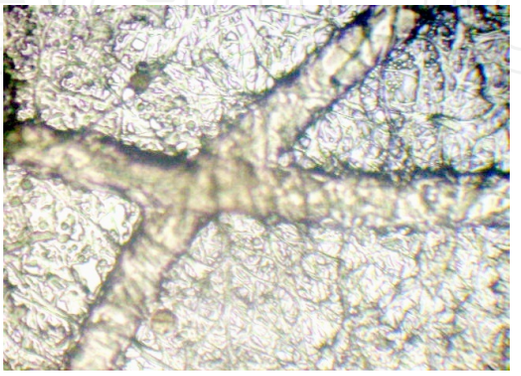

Figure 15. Effect of fungal inoculums of *Mucor* sp. on J_2 *M. javanica* (39 days after inoculation) 10 X 40x

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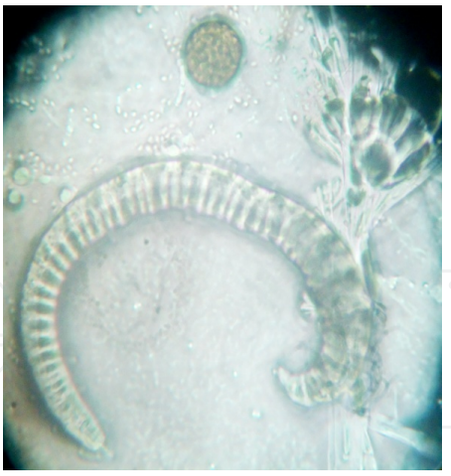

Figure 16. Effect of fungal inoculums of *Paeceilomyces* sp. on egg and J_2 *M.* (39 days after inoculation) 10 X 10x

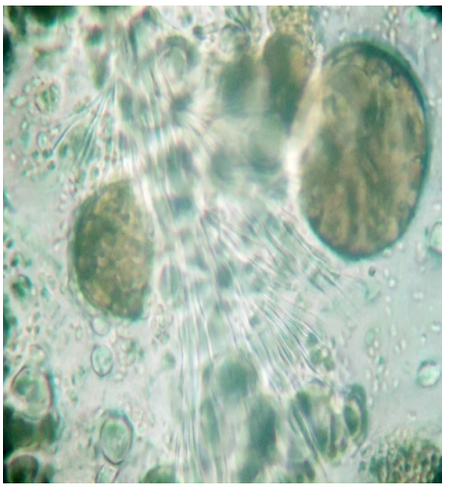

Figure 17. Effect of fungal inoculums of *Paeceilomyces* sp. on egg of *M. javanica javanica* (39 days after inoculation) 10 X 40x

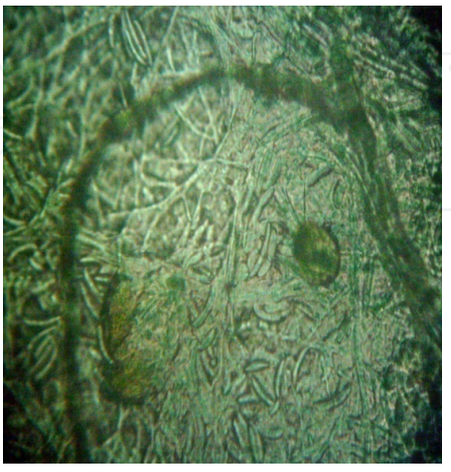

Figure 18. Effect of fungal inoculums of *Penicillium* sp. on egg and J_2 *M.javanica* (39 days after inoculation) 10 X 10x

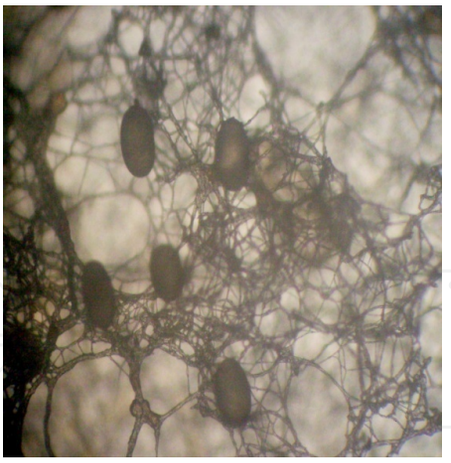

Figure 19. Effect of fungal inoculums of *Penicillium* sp. on egg of *M. javanica* (8 days after inoculation) 10 X 10x

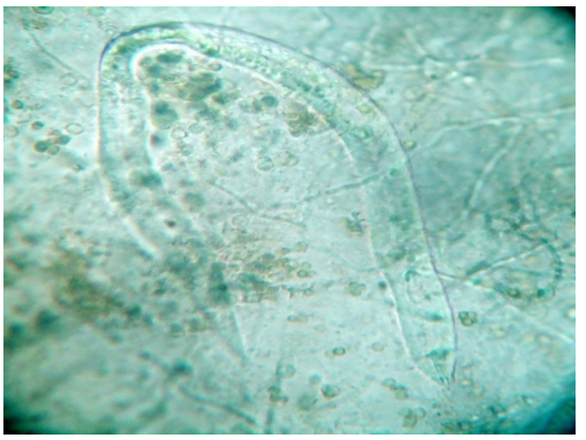

Figure 20. Effect of fungal inoculums of *Trichoderma* sp. on J_2 of *M. javanica* (39 days after inoculation) 10 X 10x

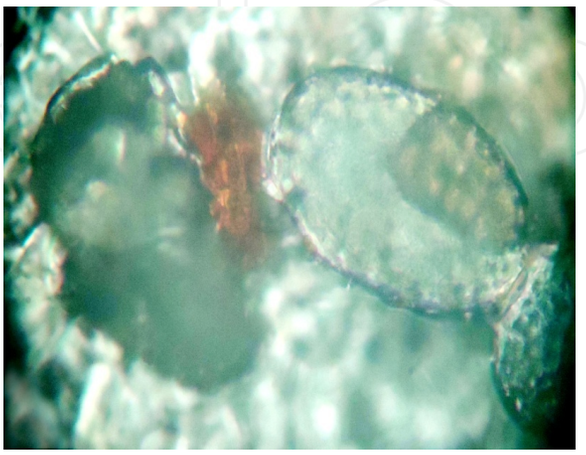

Figure 21. Effect of fungal inoculums of *Trichoderma* sp. on egg of *M. javanica* (39 days after inoculation) 10 X 10x

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Figure 22. Effect of different inoculums of J_2*Meloidogyne javanica* on mulberry plants (Var. S_{10})

Figure 23. Galled roots of mulberry plant (Var.S_{10}) due to *M. javanica*

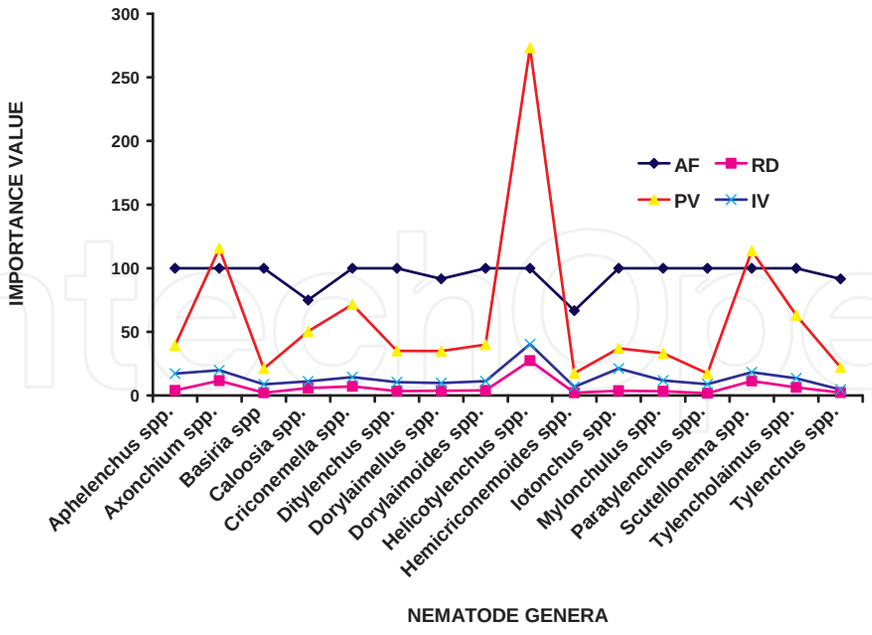

Figure 24. Graph showing absolute frequency, relative density, prominence value and importance value of soil and plant parasitic nematodes associated with mulberry plants at Govt. Silk Farm, Wangbal, Thoubal District, during the year 2006

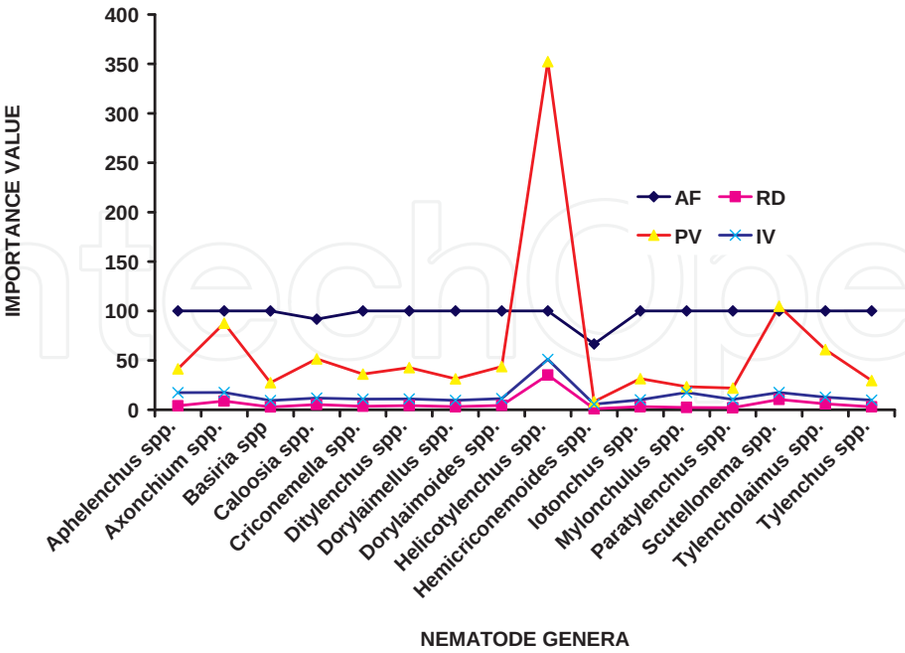

Figure 25. Graph showing absolute frequency, relative density, prominence value and importance value of soil and plant parasitic nematodes associated with mulberry plants at Govt. Silkfarm, Wangbal, Thoubal District during the year 2007

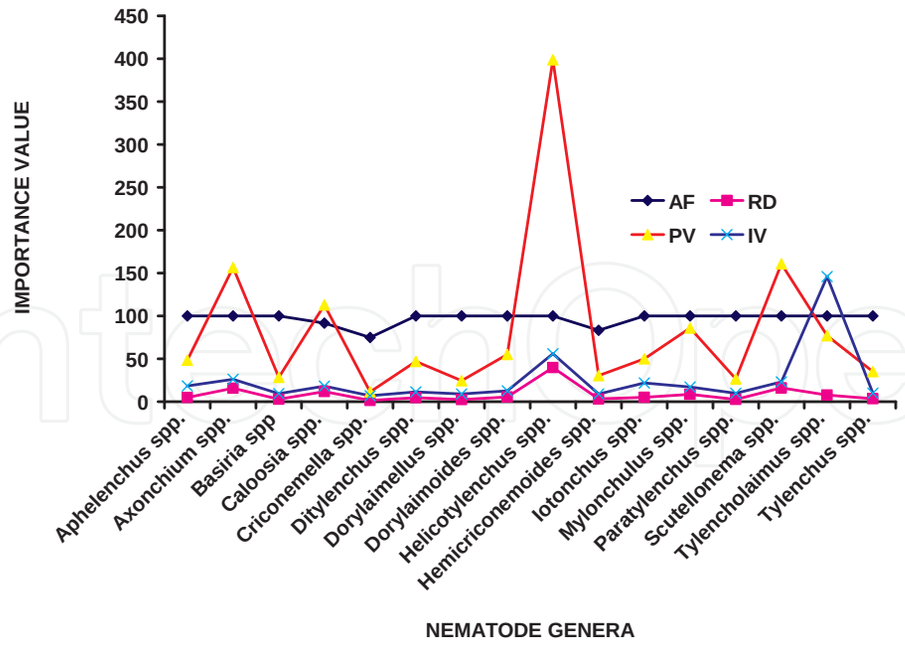

Figure 26. Graph showing absolute frequency, relative density, prominence value and importancevalue of soil and plant parasitic nematodes associated with mulberry plants at Govt. Silk Farm, Wangbal, Thoubal District during the year 2008

http://dx.doi.org/10.5772/60082

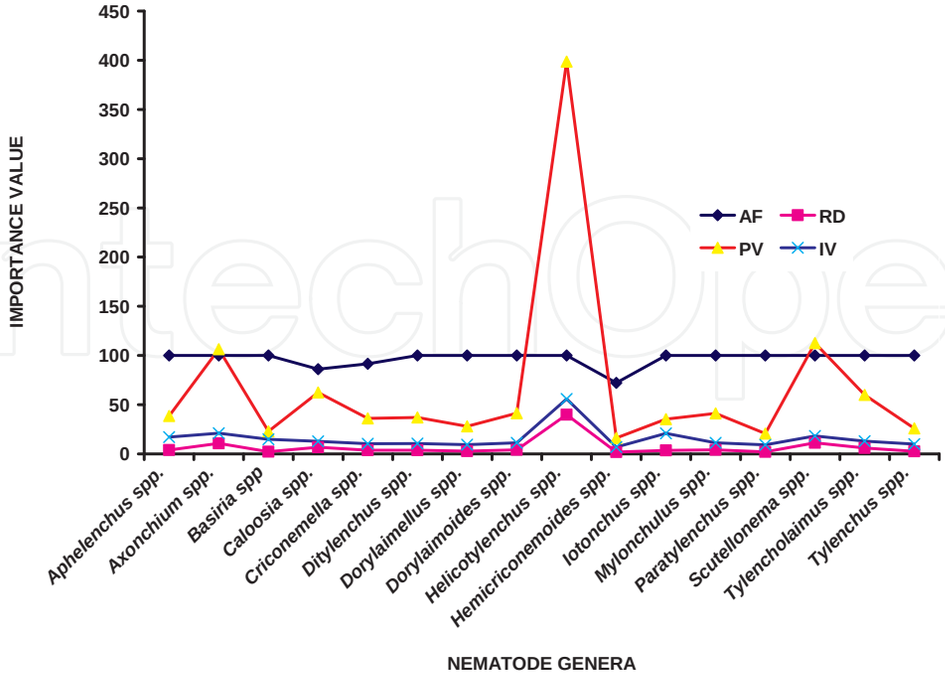

Figure 27. Graph showing absolute frequency, relative density, prominence value and importance value of soil and plant parasitic nematodes associated with mulberry plants at Govt. Silk Farm, Wangbal, Thoubal District during years the 2006–2008

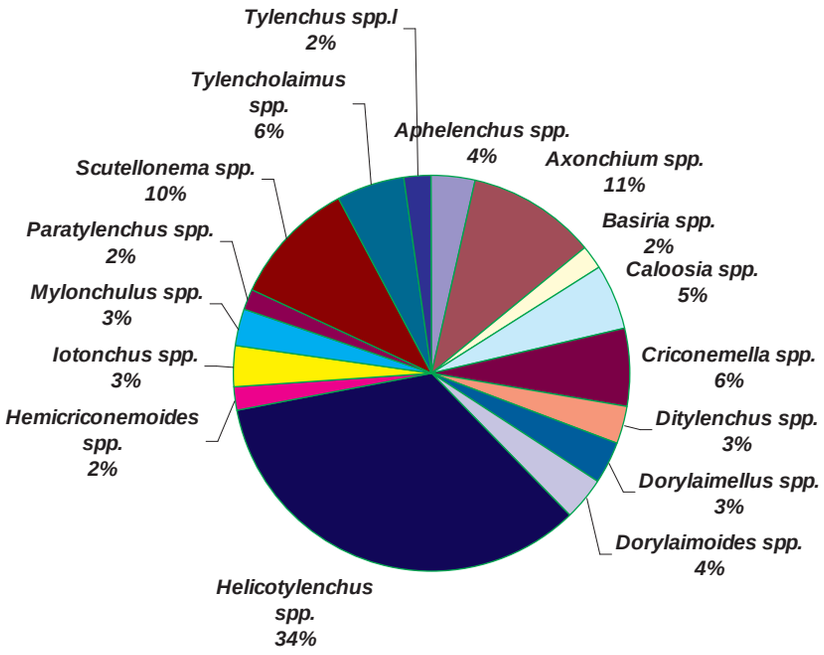

Figure 28. Pie-chart representation of the nematode genera during the year 2006

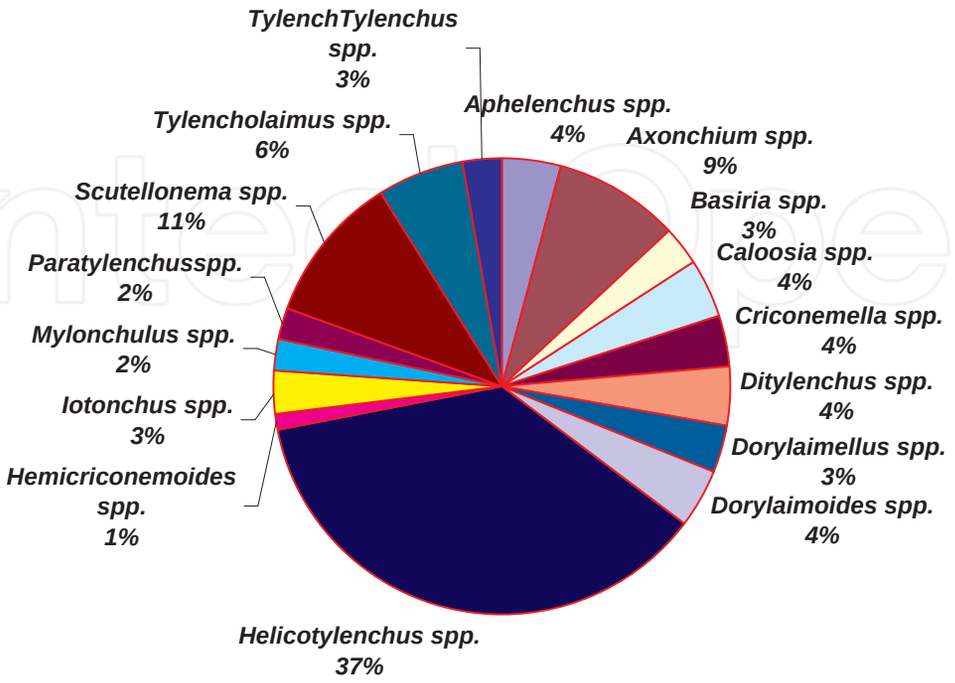

Figure 29. Pie-chart representation of the nematode genera during the year 2007

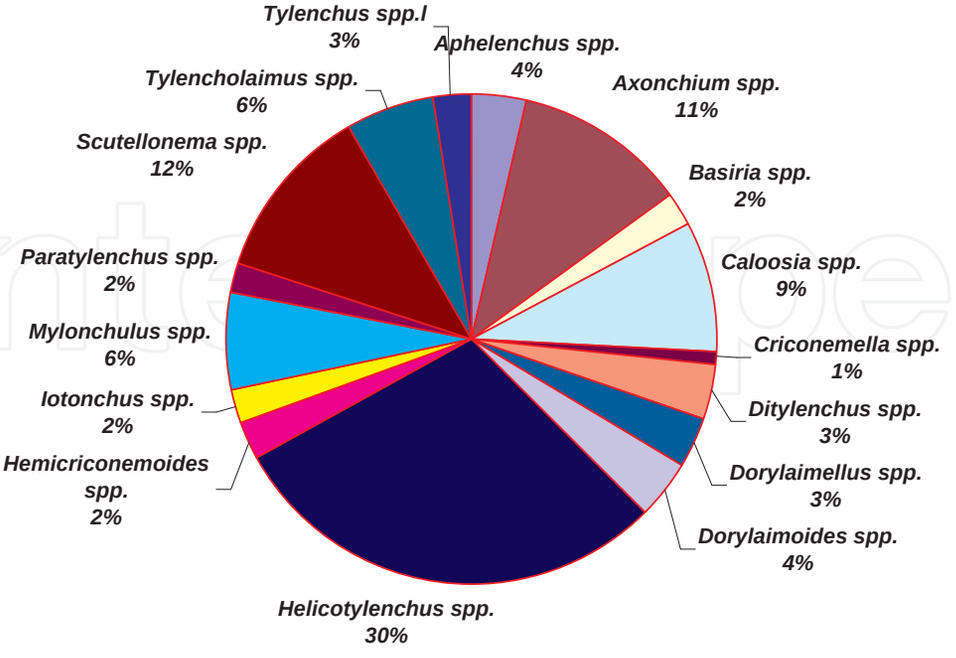

Figure 30. Pie-chart representation of the nematode genera during the year 2008

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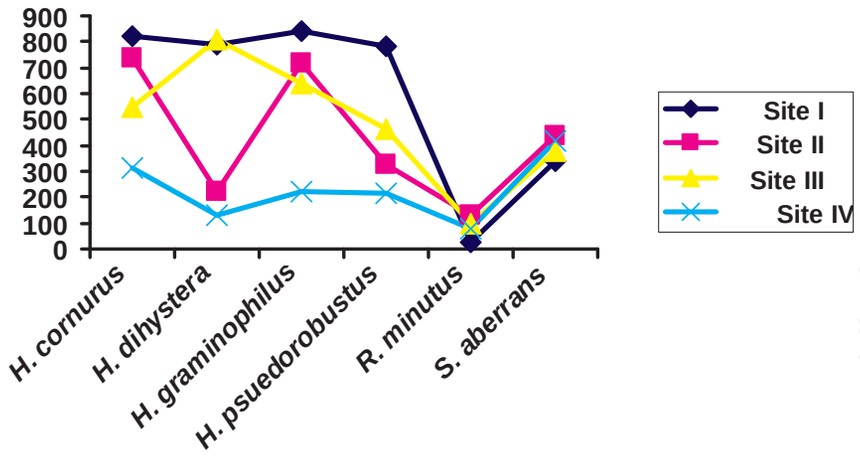

Figure 31. Total nematode population of the family Hoplolaimidae at four different sites in valley districts of Manipur

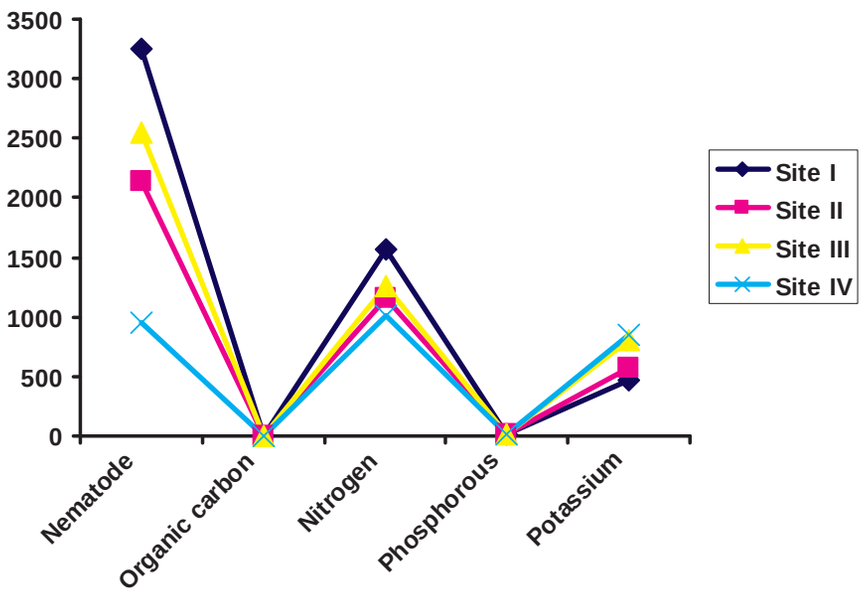

Figure 32. Relationship between the total nematode populations at four sites with their physical-chemical parameters

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