Results and Discussion

I- Occurrence and Distribution of Plant Parasitic Nematodes Associated with Some Vegetable Crops.

A total of 500 soil samples were collected from different counties cultivated with certain vegetable crops in Sharkia and Qalubia Governorates. Samples were extracted and nematodes were recovered according to **Christie and Perry (1951).**

Table (1) and (2) showed the frequency occurrence (FO) of plant parasitic nematodes recovered from certain counties; Abu-Hammad, Abu-Kabir, El-Hussania, Fakous, Hehia, and Zagazig (Sharkia Governorate); Abu Zaabal, El-Amar , Kafr-Shebin and Moshtohor (Qalubia Governorate) . Data revealed that thirteen plant parasitic nematode genera : *Criconemella, Helicotylenchus, Heterodera, Hoplolaimus, Longidorus , Meloidogyne , Paratylenchus , Pratylenchus , Rotylenchulus , Trichodorus, Tylenchorhynchus , Tylenchulus and Xiphinema,* were occurred. Highest parameters of FO were 88.5% & 85.5% for the stunt nematode, *Tylenchorhynchus* spp. in Sharkia and Qalubia Governorates, and 72.8% & 65.7% for the root-knot nematode, *Meloidogyne* spp. respectively.

Genera; *Helicotylenchus, Pratylenchus* and *Rotylenchulus* come next in the same parameters of FO. *Meloidogyne* spp. was the most distributed in Fakous and Abu-Hammad (Sharkia Governorate); El-Amar and Abu Zaabal (Qalubia Governorate)

Table (1): Percentage of frequency occurrence of plant parasitic nematodes associated with some vegetable crops cultivated in Sharkia Governorate.

Area / Nema. Genera	Abu-Hammad N=39	Abu-Kabir N=30	El- hussania N=25	Fakous N=120	Hehia N=35	Zagazig N=50	Av.
Criconemella	8.2	0	0	0.5	0	0	1.45
Helicotylenchus	40.8	55.3	3.3	11.4	12.2	23.1	24.35
Heterodera	20.4	0	0	0.8	8.6	0	4.96
Hoplolaimus	30.6	0	0	0	5.7	16	8.7
Longidorus	12.2	0	0	51.4	0	24	14.6
*Meloidogyne**	98.9	33.3	64	99.5	86	55	72.8
Paratylenchus	3.7	4.7	4.2	9.3	0	1.8	3.95
Pratylenchus	38.8	36.7	4.2	75	51.4	34	40.01
*Rotylenchulus**	20.2	20	45	33.3	11.4	50	29.9
Trichodorus	6.1	0	0	5.3	7.1	0	3.08
Tylenchorhynchus	73.5	90	72	100	100	96	88.5
*Tylenchulus**	10.2	0	0	29.3	8.6	3.1	8.53
Xiphinema	12.2	0	0	0	5.7	5	22.11

N= Number of samples
*Larvae only

Table (2): Percentage of frequency occurrence of plant parasitic nematodes associated with some vegetable crops cultivated in Qalubia Governorate.

Area / Nema. Genera	Abu- Zaabal N=38	Al-Amar N=100	Kafr-Shebin N=30	Moshtohor N=100	Av.
Criconemella	71.1	50	30.6	21	43.17
Helicotylenchus	59	77	44	50	57.5
Heterodera	55.3	63	0	35.1	38.35
Hoplolaimus	65.8	72	0	31.6	42.35
Longidorus	63.2	60	6.1	26.3	38.9
*Meloidogyne**	82.5	86	31.2	63.2	65.7
Paratylenchus	6.3	4.4	0	0	2.67
Pratylenchus	65.8	47	65.8	63.2	60.45
*Rotylenchulus**	52.6	55	60	31.1	49.67
Trichodorus	6.5	8.8	0	8.8	20.65
Tylenchorhynchus	100	66.6	75.2	100	85.5
*Tylenchulus**	34.2	40	40	1.5	28.92
Xiphinema	23.7	35	0	17.5	19.05

N= Number of samples
*Larvae only

counties since FO were 99.5 & 98.9% and 86 & 82.5% respectively.

Various nematode genera and species found to be associated with each vegetable crops of greatest consequence to temperate-zone vegetable production are *Meloidogyne* , *Pratylenchus* , *Heterodera* , *Globodera* and *Ditylenchus.* **(Goodey et al., 1965).**

Genera of lesser significance and of sporadic distribution of vegetables include *Aphelenchoides, Criconemoides, Helicotylenchus, Hemicycliophora,Longidorus, Paratrichodorus, Pratylenchus, Trichodorus, Tylenchorhynchus, Tylenchus, and Xiphinema.* These genera may be of local importance, but in most cases have been associated with one of the vegetables, damaging to specific vegetable crops (**Barker and Olthof, 1976).**

In Egypt, nematological studies have shown that three root-knot nematodes; *M. javanica*, *M. incognita* and *M. arenaria* are of widespread occurrence and are becoming a real threat to agriculture especially in localities with light sandy soil **(El-Gindi and Mousa, 1979 & Ibrahim et al, 1976).** The first two species are widely distributed while *M. arenaria* is of limited occurrence **(Ibrahim,1983).** He demonstrated the existence of two races (Races 1 & 3) in M. incognita and only one race (Race 1) in *M. arenaria*. host range investigation revealed that about 115 plant species were recognized as host of the root-knot nematodes. *M. incognita* was isolated from 103 host plants while *M. javanica* and *M.arenaria* were found on 65 and 20 host plants, respectively.

El-Shinnawy et al. (1999) , carried out a survey in newly reclaimed lands throughout different regions of Egyptian Governorates cultivated with certain vegetable crops. The results revealed the presence of *Meloidogyne* , *Pratylenchus* , *Tylenchorhynchus* and *Helicotylenchus* in high densities and frequencies on tomato , cucumber , soybean , potato , eggplant ,Pepper and cantaloupe. *Hoplolaimus* and *Heterodera* were found with low densities.

(Sikora 1978 & 1979 and Abu Ghabieh, 1978 & 1983) Observed a severe damage by root-knot nematodes on tomato, potato, eggplant, pepper and squash. Results of this survey study indicated that root-knot nematodes were present in about 30% of 110 field samples. Two species, *M. incognita* and *M. javanica*, were identified. *M. incognita*, the most common, was found in 60% of the tested populations, followed by *M. javanica*. Tomato seed beds were often severely infested with both *M. incognita* and *M. javanica.* It was evident that certain adverse factors, in addition to severe *M. incognita* attack, were responsible for heavy losses .

II-Laboratory Experiments

a – Some Factors Affecting Myceliogenic Germination of Certain Antagonistic Fungi:

1- Effect of Temperatures on Fungal Growth :

To study the effect of temperature on rate and amount of growth of the antagonistic fungi *Arthrobotrys oligospora* *Dactylella brochopaga* , *Nematoctonus concurrens* and *Trichoderma harzianum*, four different temperature regimens were tested . Data in Table (3) show that ,the four tested fungi were varied in their rate of growth according to tested temperature regimens after 11 days (time of the experiment).

T. harzianum showed the best growth rates with all treatments compared to the other tested antagonistic fungi. The fungus achieved (100%) of its growth rate at 30, 25,20 and 15°c after 2,3,4 and 5 days respectively. Data revealed that the range between 25-30°c is the most suitable for the growth rate of *T. harzianum*.

A. oligospora followed *T. harzianum* in its growth rates , the fungus achieved the best growth rate (100%) at 30 and 25°c in 7 days , while by using 20°c the petri-dishes were filled during 10 days. At 15°c the fungus growth reached to its least value 85.56% at the end of the experiment(11 days).

N. concurrens achieved the best growth rate (100%) at 25°c after 11 days , while it achieved 77.3,76.4 and 62.7% of its growth rates at 20,30 and 15°c, respectively, at the time end of the experiment.

D. brochopaga achieved the least growth rates at 15,20,25 and 30°c , since it achieved 40, 57.7,42.7 and 42.7% respectively after 11 days.

Generally, it was observed that , the least growth rates were obtained by using 15°c in all tested fungi .

Data in Table (4) show that, the highest amount of growth was found with *A. oligospora* at 20,25 and 30°c after 13 days. The best amount of growth(0.879 g) was achieved at 30°c . At 25 and 20°c, the amount of growth decreased to (0.878 & 0.868 g), respectively.

D. brochopaga followed *A. oligospora* in weight of growth. The best growth (0.781 g) achieved by 25°c, while at 30 and 20°c the amount of growth decreased to (0.778 & 0.774 g), respectively.

T. harzianum achieved the best growth weight (0.753 g) at 30°c while at 20 and 25°c the amount of growth decreased to (0.661 and 0.693) respectively.

N. concurrens gave the lowest value in this respect . The best amount of growth (0.723 g) was gained at 25°c followed by(0.693 and 0.661) at 30 and 20°c respectively.

In all tested fungi it was observed that temperature degree 15°c recorded the least values of growth weights.

From the above mentioned results it can be concluded that the Egyptian soil has the same temperature range which favorable for the best growth of the concerned fungi , so they can be applied successfully in Egypt.

Table (3) Effect of certain temperature degrees on the rate of growth of some antagonistic fungi

Antagonistic fungi	Temp.	Rate of growth (%)										
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day
Arthrobotrys oligospora	15°c	5.6	5.6	8.5	17.4	27.8	36.7	49.2	57.4	67.8	78.9	85.6
	20°c	7.8	13.3	25.9	35.9	45.9	56.7	67.8	79.2	91.1	100	
	25°c	8.1	23.7	34.0	46.3	68.9	93.7	100				
	30°c	7.8	21.1	37.8	51.1	69.2	90.0	100				
Dactylella brochopaga	15°c	7.99	9.33	11.5	13.3	20.8	24.8	28.8	29.7	31.1	33.3	40.0
	20°c	11.1	12.0	16.4	22.1	28.8	36.4	38.7	41.7	44.4	47.1	57.7
	25°c	5.7	9.33	13.7	17.7	35.1	38.1	41.7	44.0	47.1	50.7	60.8
	30°c	6.0	17.7	21.3	24.4	27.5	28.8	31.5	33.3	35.1	36.4	42.7
Nematoctonus concurrens	15°c	6.67	20.0	21.0	23.1	28.4	32.4	37.3	41.7	46.7	50.1	62.7
	20°c	9.33	18.7	25.7	34.1	36.0	50.7	55.5	60.8	66.7	73.7	77.3
	25°c	10.8	24.8	35.5	48.0	56.8	65.3	73.3	78.1	82.1	86.6	100
	30°c	9.60	21.3	28.0	35.1	42.1	50.7	56.0	60.8	64.8	72.0	76.4
Trichoderma harzianum	15°c	24.0	44.0	71.0	89.3	100						
	20°c	37.7	71.0	96.4	100							
	25°c	48.0	90.6	100								
	30°c	48.0	100									

Table (4): Effect of different temperature degrees on amount of growth of certain antagonistic fungi

Antagonistic Fungi	Amount of growth (g)			
	15°c	**20°c**	**25°c**	**30°c**
Arthrobotrys oligospora	0.706	0.868	0.878	0.879
Dactylella brochopaga	0.708	0.774	0.781	0.778
Nematoctonus concurrens	0.612	0.661	0.723	0.693
Trichoderma harzianum	0.688	0.700	0.728	0.753
L.S.D (at 0.05)	-	0.119	0.119	-

Soil temperature is perhaps the most obvious physical variable of biological significance and it should be taken into consideration when systems of biological control are being developed. On a global scale, nematodes and their natural enemies are likely to be adapted to the temperature regimes of a particular climatic zone and specific isolates or populations of an antagonist may not always be suited for use in other area. At a local level, the temperature of soil fluctuates diurnally and also varies with depth and season of the year. A biological control agent must be able to cope with this variation in temperature and, in particular, must be active at the temperatures which occur at the time target nematodes are susceptible to antagonism.

Since all organisms have specific maximum, minimum and optimum temperatures for activity, interactions between nematodes and their antagonists are likely to be affected by temperature. This has been confirmed for some nematode-parasite combinations. For example, the optimum temperatures for hatch of *Meloidogyne incognita* eggs and growth of the fungal egg parasite *Dactylella oviparasitica* were similar, but the growth of the fungus was not affected by low temperatures to the same extent as was developed and hatch of eggs **(Stirling,1979).**

Invasion of egg-masses by the fungus and penetration and growth in eggs took longer as temperatures decreased, but the slower rate of production, development and hatch of eggs more than compensated for the decreased growth of the fungus. Consequently, *D. oviparasitica* was more efficient parasite as temperature decreased from 27 to 15° C.

The actual of parasitism may have been much higher because the fungus (*Dactylella oviparasitica*) destroyed eggs of root-knot nematode , *Meloidogyne sp.* in less than nine days at 27°c **(Stirling, 1979).**

Since pathogenesis by *D. oviparasitica* , *Pasteuria penetrans* , *Paecilomyces lilacinus* and probably most other antagonists is affected by temperature **(Stirling 1979, and Cabanillas *et. al.* , 1989)** , the effectiveness of many biological control agents could be expected to improve if soil temperature regimes were altered to favour the antagonist . Evidence that such a strategy might be feasible was obtained by **Walker and Wachtel (1988)** , who observed that soil solarization using clear polyethylene in a commercial vineyard increased the rate of infection of *Meloidogyne javanica* by *P. penetrans* for at least ten months . During the period when the polyethylene was present, the increased infection probably resulted from an increased mobility of nematodes at higher soil temperatures and the consequent increased probability of contact between nematodes and spores . The higher rate of infection that was maintained after the sheeting was removed possibly occurred because higher soil temperatures increased the rate of development of *M. javanica* females and the production of *P. penetrans* spores . This would have resulted in higher densities of spores in soil .

One of the main problems involved in estimating the levels of natural control of nematodes in soil is the rapid disintegration of diseased individuals . For example , the contents of *Meloidogyne* eggs are completely consumed by

Dactylella oviparasitica in nine days at 27°c **(Stirling , 1979)** , while females of *Heterodera avenae* are unrecognizable after four days at 13°c **(Kerry and Crump , 1980).** *Criconemella xenoplax* parasitized by *Hirsutella rhossiliensis* disappear from soil in about 15 days , with the rate of degradation varying with soil temperature and nematode life stage **(Jaffe et al., 1988)** . Consequently , an accurate census of level of parasitism by such organisms can only be obtained if samples are collected frequently and data on rates of disintegration of nematodes are available .Such studies are both time-consuming and labour intensive .

Part of research on nematode-capturing fungi has focused on the transition from saprophytic to predacious behavior and factors that induce trap formation. For many species trap structure development appears conditioned by environmental factors. The observation that nematode-fungus attachment is not only realized by complex capture structures, but can also be accomplished by not visibly differentiated vegetative hyphae **(Eefje and Ellis, 1994)** raised the question whether adhesive hyphae are active in a temperature range or under nutrient conditions,different from those that induce formation of adhesive networks.

The same authors demonstrated that while temperature between 5°C and 30°C clearly affected ring structure development, attachment of nematodes to hyphae of *A. oligospora* (CBS 289.82) was not affected. All active second stage juveniles of *M. hapla* were attached to hyphae within a very short time after addition to fungal colonies , irrespective of

the temperature even through the nematode mobility at 5°C is one third of that at 25°C. At the first observation, one hour after the addition of the nematodes, all juveniles observed were already attached to the hyphae at 5, 10 as well at 15°C. This confirms earlier observations at 25°C in which 28 out of 30 juveniles became attached to hyphae within 45 min.

Several authors reported that the nematode capturing fungi they studied , including other isolates of *A. oligospora* , did not respond to nematodes at low temperatures **(Soprunov, 1966 ; Cayrol & Brun, 1975)**. Studies on the induction of adhesive networks in *A. oligospora* (ATCC 24927) showed a total failure at 5, 30 and 35°C **(Gronvold, 1989).** Nevertheless at temperatures below 15°C the development was significantly slower than at 15 to 30°C. At temperature occurring in the field in temperature regions this fungal isolate and the juveniles of the nematode species are both significantly active to ensure capture of contact occurs.

Little is known about the influence of abiotic and biotic conditions such as temperature on the formation of the adhesive hyphae in nematophagous fungi and on subsequent capture and infection of nematodes. **Gronvold (1989)** found a significant effect of temperature on the adhesive network development in *Arthrobotrys oligospora* (ATCC 24927) : mycelium did not respond to juveniles or responded only slowly with the development of adhesive networks at temperature below 15°C. Also *Dactylella* spp. captured larger proportion of nematodes between 20 and 24°C than at lower temperatures **(Feder, 1963).**

2- Effect of Different Relative Humidity Percentage on Fungal Growth Rates :

This experiment was conducted to study the relationship between humidity percentage and growth rate of certain antagonistic fungi.

Growth rates of each fungus were reported along the period of eleven days by using four different humidity percentages (14.5 , 50 , 75 and 100%) . Data in Table (5) showed that there were marked variation in linear growth of mycelium as affected by the four relative humidity (14.5 , 50 , 75 and 100%) after 11 days.

T. harzianum, gave the best growth rate under the four used humidity percentages compared to the other tested fungi. At 14.5% Relative Humidity, the rate of growth reached 100% after only eight days. Also higher growth rates were achieved at humidity percentage (50 , 75 and 100%) after 9 , 10 and 11 days respectively , data revealed that the growth rate was decreased with the increase of relative humidity.

Referring to *A. oligospora,* which followed *T. harzianum* in its growth rates, reached to 100% by using 14.5% R.H., then markedly decrease with the increase of relative humidity percentage to reach (59.2%) with 100% R.H.

N. concurrens had the same trend which exhibited its higher growth rate (46.2%) under 14.5% humidity percentage , then markedly decreased with the increase of relative humidity to reach (42.8%) with 100% relative humidity.

In *Dactylella brochopaga,* it was noticed that the growth rate was increased with the increase of the humidity percentage and reaches its maximum (41.4%) at 75% humidity percentage , followed by rates of growth at 50 and 14.5% R.H. respectively , whereas the growth was declined to (21.1%) at 100% relative humidity.

It could be concluded that there were no gross differences in growth rates with humidity percentages ranging from 14-75%. The Egyptian soil humidity, which lies in this range is suitable for growth rates of the concerned fungi, because they require a water film in which to move.

In soil with small particles, maximum mobility occurs when pores are full of water, while in other soils it occurs at a potential corresponding to the stage when pores are draining of water **(Wallace,1968).** As the soil continues to drain, 30 μm and 15 μm diameter pore necks are just emptied at potentials of –0.1 bar and –0.2 bar, respectively. Hence nematodes are immobilized at relatively slight potential in the range of –0.1 to –0.2 bar **(Jones,1978).** Although egg hatch occurs at soil moistures which inhibit movement, it decreases rapidly as soil dries to moisture potentials of about –3 bar **(Baxter & Blake, 1969).**

Thus the response of nematodes to increasing soil moisture contrasts sharply with that of some other soil organisms, which tend to remain active at low soil moisture potentials. Because of their filamentous habit, fungi can continue to ramify in relatively dry soil. They are not confined to water-filled pores

Table (5) Effect of different humidity percentages on rate of growth of certain antagonistic fungi

Antagonistic fungi	Hum.%	Rate of growth %										
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day
Arthrobotrys oligospora	**14.5%**	5.9	20.2	41.2	67.6	76.4	83.2	85.8	91.2	95.1	98.0	100
	50%	5.8	18.0	38.6	56.4	60.2	65.7	71.1	73.7	76.8	78.3	79.8
	75%	5.55	15.9	36.2	42.1	46.4	52.9	59.0	61.1	64.5	66.5	68.7
	100%	5.55	15.9	32.3	40.1	44.1	49.8	52.4	54.3	56.1	58.8	59.2
Dactylella brochopaga	**14.5%**	5.55	7.77	14.4	18.4	20.7	22.2	26.2	28.9	30	31.4	31.4
	50%	5.55	7.77	18.4	24	27.8	30	32.9	34	34.8	35.9	36.7
	75%	5.55	6.66	17.8	23.7	26.2	30	34.4	36.2	38.4	41.4	41.4
	100%	5.55	8.88	13.3	17.8	17.8	18.9	20.7	21.1	21.1	21.1	21.1
Nematoctonus concurren	**14.5%**	5.55	15.1	31.1	42.2	42.2	42.9	44	44.4	45.1	45.5	46.2
	50%	5.55	16.7	30.3	36.7	42.9	44.4	45.9	45.9	45.9	45.9	45.9
	75%	5.55	14.4	27.3	36.2	37.3	39.2	41.4	42.2	42.9	43.3	43.3
	100%	5.55	15.9	26.7	36.7	41.1	42.2	42.8	42.8	42.8	42.8	42.8
Trichoderma harzianum	**14.5%**	20	64.8	83.3	94.4	95.5	96.2	98	100			
	50%	18.9	72.9	81.8	90.7	93.7	95.9	96.2	98	100		
	75%	16.7	64	70	75.5	82.9	83.7	86.7	94.4	99	100	
	100%	16.7	63.9	77.2	81.7	86.1	88.3	90	92.2	95.5	97.8	100

because their hyphae can bridge air spaces and can spread along the walls of drained pores. The growth of most fungi is not restricted until the water potential drops to between –30 and –60 bar, and some fungi can germinate and grow at potential as low as –200 to –400 bar **(Cook & Papendick, 1972 ; Griffin, 1972 and Harris, 1981).**

The above consideration suggest that soil moisture potential is likely to affect of level of antagonism by fungi towards nematodes. Because of their filamentous habit, fungi are likely to be the main antagonists in drier soils.

Such a contention is supported by observations on the infection of *Heterodera avenae* by *Nematophthora gynophila* and *Catenaria auxiliaris.* Levels of parasitism by *N. Gynophila* in England increased in heavily water pots **(Kerry et al.,1980)** and in the field, infection was closely related to soil moisture levels **(Kerry et al., 1982a,b).** In South Australia, *C. auxiliaris* was restricted to the wettest cereal growing districts and was most active during wet seasons **(Stirling & Kerry. 1983).**

3- Effect of Different Grades of Lighting on Fungal Growth:

In this experiment ,the effect of three different grades of lighting (Light "320 Lux", Dark "0.02 Lux" and Light / Dark) were studied on the growth rate of the tested fungi (*Arthrobotrys oligospora* , *Dactylella brochopaga* , *Nematoctonus concurrens* and *Trichoderma harzianum*) .

Data in Table (6) show that , the growth rates of *T. harzianum* reached its maximum (100%) in the second day with light/dark system whereas, it reached its maximum (100%) in the

third day with both light and dark systems thus, the light/dark system gives the best growth rate with this fungus.

A. oligospora achieved the best growth rate with dark system in the 10^{th} day, while the same percentage was achieved with both light and light/dark systems at the end of the experiment.

In *N. concurrens* , the highest measure was (86.2%) with both light and dark and the lowest growth rate (81.4%) gained with light/dark system.

D. brochopaga reached the highest growth rate (53.3%) with dark followed by light/dark (51.4%) and the lowest value in this concern(51.1%) was obtained by light system after 11 days.

Data in Table (7) cleared that , *A. oligospora* reached to the highest weight (0.796 g) under dark system followed by (0.709 & 0.646 g) with light/dark and light systems respectively.

D. brochopaga recorded the highest weight (0.780 g) with dark system followed by (0.730 and 0.631 g) with light/dark and light systems, respectively.

N. concurrens recorded the best amount of growth (0.706 g) with dark system followed by (0.632 g) with light/dark system and the lowest weight (0.616 g) was resulted by light system .

In *T. harzianum* it was observed that dark treatment achieved the highest weight (0.673 g) , followed by light/dark and light (0.659 and 0.641 g) after 13 days respectively.

Table (6) Effect of different lighting grades on rate of growth of certain antagonistic fungi

Antagonistic fungi	Light grade	Rate of growth (%)										
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day
Arthrobotrys oligospora	L	5.55	20.5	31.2	55.6	69.1	75.3	83.2	93.7	97.2	99.1	100
	D	5.86	23.4	35.3	60.7	75.2	81.2	94.0	97.1	99.2	100	
	L/D	5.55	20.4	31.1	55.0	67.2	74.3	81.1	92.5	96.8	98.7	100
Dactylella brochopaga	L	5.55	4.22	18.2	21.4	26.2	27.3	36.1	33.3	36.7	40.0	51.1
	D	5.55	9.55	13.7	20.7	25.8	27.3	32.9	35.9	38.1	41.1	53.3
	L/D	5.55	8.88	15.6	21.4	27.3	29.6	33.7	37.3	40.0	43.3	51.4
Nematoctonus concurrens	L	5.55	18.1	28.1	41.1	48.1	48.9	58.1	60.3	66.2	71.8	86.2
	D	5.55	16.2	25.6	35.1	44.0	49.6	55.5	60.3	66.2	71.8	86.2
	L/D	5.55	14.0	26.7	35.6	43.3	49.2	54.0	58.1	61.1	62.9	81.4
Trichoderma harzianum	L	28.1	80.0	83.3	100							
	D	23.3	75.9	98.9	100							
	L/D	20.3	71.1	100								

Table (7): Effect of different lighting grades on amount of growth of certain antagonistic fungi.

Antagonistic Fungi	Amount of growth (g)		
	L	L/D	D
Arthrobotrys oligospora	0.646	0.709	0.796
Dactylella brochopaga	0.631	0.730	0.780
Nematoctonus concurrens	0.616	0.632	0.706
Trichoderma harzianum	0.641	0.659	0.673

It could be noticed that, there were no clear differences in rates and amount of growth with the tested fungi by using three lighting grades, but the dark system was observed to be the best condition for fungi growth.

From the above mentioned results, it could be concluded that these fungi could be used in biological control of nematode by their application on the soil surface and/or under the soil surface. Also this fungi could act in day light and dark (during the whole day).

Morphological responses to light have been described for many fungi. **Gronvold (1989)** reported that light supresses development of adhesive networks in *A. oligospora* (ATCC 24927), whereas **Olthof and Estey (1965)** did not observe any influence of light on the vegetative growth of another isolate of this fungus , they also added that light, whether continuous or alternating with darkness , had little effect on mycelial growth of *A. oligospora, A. conoides* and *A. brochopaga.* **Eejie and Ellis (1994)** have placed three petri-dishes of the fungus *A. oligospora* in the dark and three petri-dishes were incubated under constant artificial light , they noticed that light did not affect nematode hyphae attachment or ring structure development of the fungus.

4- Effect of pH Values on the Amount of Fungal Growth :

This experiment was done to study the effect of pH value on the amount of growth of some antagonistic fungi . Four different values of pH were used with the concerned fungi.

Data in Table (8) revealed that *Arthrobotrys oligospora* , *Dactylella brochopaga* and *Nematoctonus concurrens* show

Table (8): Effect of pH values on amount of growth of some antagonistic fungi.

Antagonistic fungi	Amount of growth in grams			
	pH 5.6	pH 6.4	pH7.2	pH 7.5
A. oligospora	0.819	0.796	0.769	0.737
D. brochopaga	1.012	1.004	0.990	0.942
N. concurren	0.968	0.925	0.913	0.898
T. harzianum	0.949	0.977	0.966	0.957
L.S.D (at0.05)	0.167	0.154	0.089	0.088

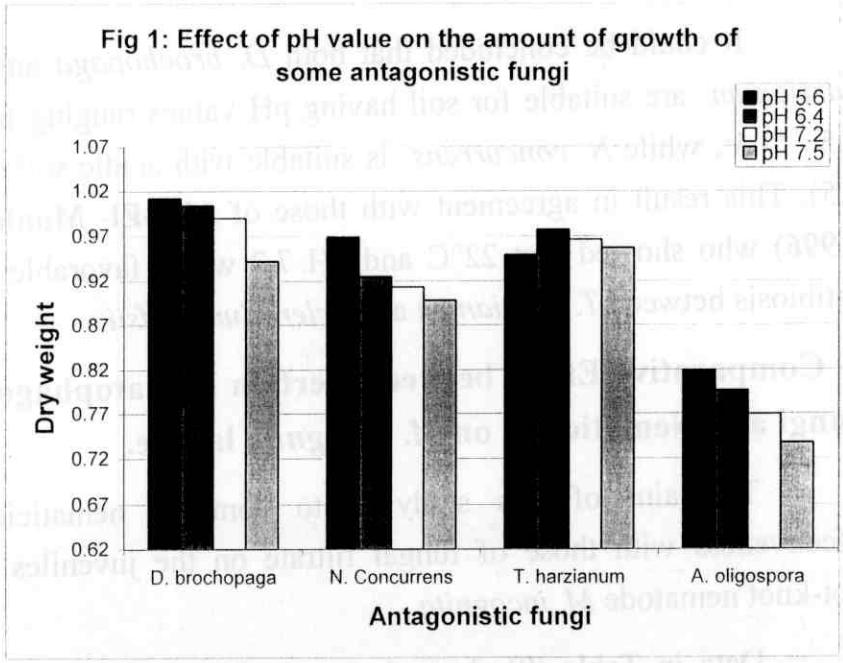


Fig 1: Effect of pH value on the amount of growth of some antagonistic fungi
pH 5.6
pH 6.4
pH 7.2
pH 7.5
1.07
1.02
0.97
0.92
0.87
0.82
0.77
0.72
0.67
0.62
Dry weight
D. brochopaga
N. Concurrens
T. harzianum
A. oligospora
Antagonistic fungi

increase in their amount of growth with the decrease of pH value, their best growth (0.819 , 1.012 and 0.968 g) respectively, were detected with pH 5.6 (acidic), while the fungus *Trichoderma harzianum* recorded its best amount of growth (0.977 & 0.960 g) with pH 6.4 and 7.2 respectively, (nearly neutral) then decrease with both increase or decrease in pH values (acidic or alkaline) .

It could be concluded that both *D. brochopaga* and *T. harzianum* are suitable for soil having pH values ranging from 5.5 – 7.5 , while *N. concurrens* is suitable with acidic soil (pH 5.5). This result in agreement with those of **Abd-El- Moniem, (1996)** who showed that 22°C and pH 7.2 were favorable for antibiosis between *T. harzianum* and *Sclerotium rolfsii*.

b- Comparative Effect between Certain Nematophagous Fungi and Nematicides on *M. incognita* larvae.

The aim of this study is to compare nematicidal effectiveness with those of fungal filtrate on the juveniles of root-knot nematode *M. incognita* .

Data in Table (9) show that a significant reduction of nematode were obtained by using Oxamyle and Fenamiphos , i.e., the percentages of nematode mortality ranged from 25.3 to 97.7% according to the tested concentration levels. It was observed that the nematicide Fenamiphos gave better results than Oxamyle at the same concentration levels , the highest percentage of nematode mortality was (97.7 %) by using Fenamiphos at 16 p.p.m. , while it was (89.7%) by using Oxamyle .

Data in Table (10) , show that all antagonistic fungi significantly reduced the percentage of active juveniles . The highest percentage of mortality was obtained by the filtrate of *Trichoderma harzianum* (98.3%) , when the fungus disk grew on 50 ml. of Gliotoxin fermentation medium. Filtrate of *Nematoctonus concurrens* and *Arthrobotrys oligospora* achieved good results when they were propagated on 50 ml. medium , the percentage of nematode mortality reached 92.7 and 85.7 % respectively .

The least result was obtained by the filtrate of *Dactylella brochopaga* , the percentage of nematode mortality ranged from 7.3 to 30.7 % according to increasing of concentration . Data generally revealed that the effect of any of these fungi increases by increasing the concentration of the filtrate.

Although the nematicides achieved good results , filtrate of some fungi were more effective than nematicides . Filtrate of *Trichoderma harzianum* when the fungi was propagated on 50 ml. of medium gave better results than both used nematicides in all concentrations and gave the best results at all. The next fungi in acting is *Nematoctonus concurrens* which was better than the nematicides "Oxamyle" in killing the nematode . Filtrate of *Arthrobotrys oligospora* comes after *Nematoctonus concurrens* in reducing nematode population and its results were close to those results obtained by nematicides. The least results were obtained by the filtrate of *Dactylella brochopaga* .

From the previously data , there are no clear differences between using fungi filtrates or nematicides.

Table (9) : Effect of different concentrations of certain nematicides on larvae mortality of *M. incognita*.

Treatments	Nematicide conc. (p.p.m)	% Nematode mortality
Oxamyle	2.4	25.3
	4.8	58.7
	9.6	73.3
	19.2	89.7
Fenamiphos	2	39.3
	4	63.7
	8	85.3
	16	97.7
Control	Distilled water	0.0

Table (10): Effect of different concentrations of certain fungi filtrates on larvae mortality of *M. incognita*.

Antagonistic fungi	Conc. of fungal filtrate	% Nematode Mortality	
		With fungi	Without fungi
Arthrobotrys oligospora	A	42.7	0
	B	69.3	0
	C	85.7	0
Dactylella brochopaga	A	7.3	0
	B	25.3	0
	C	30.7	0
Nematoctonus concurrens	A	36.7	0
	B	68.3	0
	C	92.7	0
Trichoderma harzianum	A	41.3	0
	B	53.7	0
	C	98.3	0

A = Fungi propagation in 200 cm^3 of medium.
B = Fungi propagation in 100 cm^3 of medium.
C = Fungi propagation in 50 cm^3 of medium.

II) Greenhouse Experiments:

1- Effect of Fungal Filtrate in Controlling *M. incognita* in Tomato Plants.

Data in Table (11) show that filtrate of the tested antagonistic fungi gave a high effect in reducing root galls , egg masses , root developmental stages and juvenile population without any harmful to the host plant (Table 12). When filtrate of *N. concurrens* , *A. oligospora* and *T. harzianum* were used on tomato plants infected with *M. incognita* , it was found that the number of juveniles were declined to "nil" while in using filtrate of *D. brochopaga,* the number of juveniles were significantly decreased. Calculating the value of "R" gave a clear idea about the effect of the fungi used which was "zero" with *N. concurrens*, *A. oligospora* and *T. harzianum* filtrates and "6.25" with *D. brochopaga* filtrate compared to its value when no fungi were used "14.58" .

Number of galls , egg-masses , mature female and root developmental stages/root were declined to "nil" by fungal filtrate of the examined fungi, except of *D. brochopaga* which was lesser to some extent others fungi used (Table 11).

Data in Table (12) and Fig (2) show that no significant differences were found in shoot measurements when filtrates of *N. concurrens* , *A. oligospora* and *T. harzianum* were used as compared to that of the untreated plants , moreover it was found that the use of *N. concurrens* and *T. harzianum* filtrates gave better measures in shoot weight compared to that of the free plant application . Significant decrease was observed in shoot

Table (11) : Effect of certain nematophagous fungal filtrates on *Meloidogyne incognita* in tomato plants under greenhouse conditions.

Treatments	No. of nematode	No. galls	No. eggmasses	No. mature female	No. R.D.S	R
N. concurrens filtrate + *M. incognita*	0	0	0	0	0	0
D. brochopaga filtrate + *M. incognita*	7500	119	56	56	96	6.25
A. oligospora filtrate + *M. incognita*	0	0	0	0	0	0
T. harzianum filtrate + *M. incognita*	0	0	0	0	0	0
M. incognita only	17500	283	205	205	165	14.58
L.S.D. (at 0.05)	4104.87	29.17	18.23	18.23	28.42	

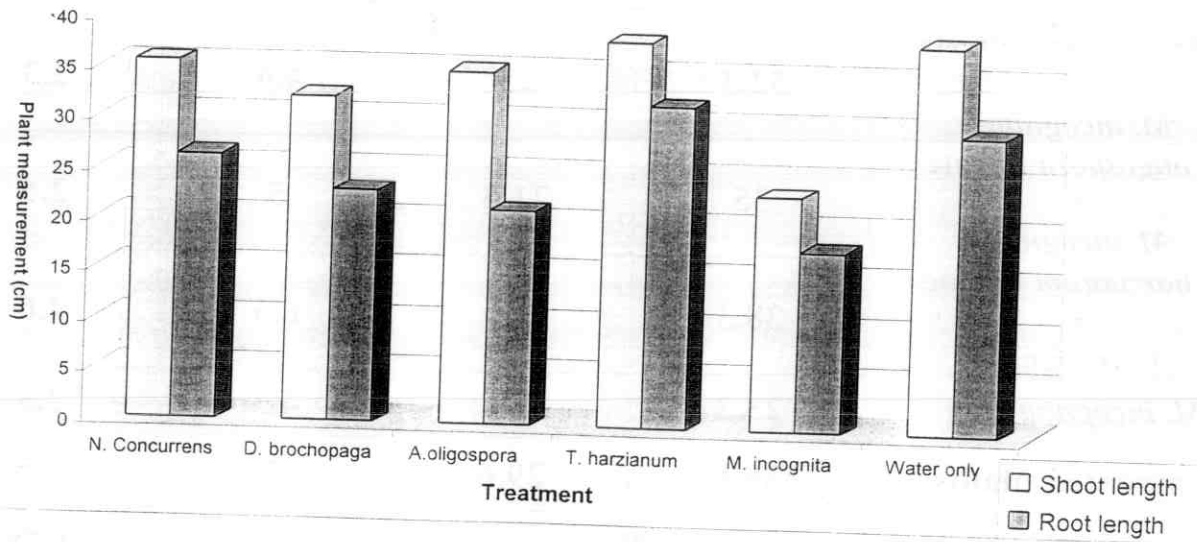
$$R = \frac{\text{Final population of larvae}}{\text{Initial population of larvae}}$$

R.D.S. = Root developmental stages

Table (12) : Effect of certain nematophagous fungal filtrates on growth measurements of tomato plants infected by *M. incognita* under greenhouse conditions.

Treatments	Shoot length	Root length	Shoot weight	Root weight
N. concurrens filtrate + *M. incognita*	35.6	26.3	5.6	3.6
D. brochopaga filtrate + *M. incognita*	32.3	23	4.6	2.3
A. oligospora filtrate + *M. incognita*	35	21.3	5	2.6
T. harzianum filtrate + *M. incognita*	38.3	32	6.3	4.6
M. incognita only	23.3	18	4.3	2.3
Untreated plants	38.6	29.6	5	3.3
L.S.D. (at 0.05)	4.89	6.14	2.21	1.78

Fig 2: Effect of certain nematophagous fungi filtrates on plant growth measurements of tomato plants affected by root- knot nematode *Meloidogyne incognita* .



length in infected plants with nematode alone treatment compared to both of the untreated plants and all fungi filtrate treatments . Significant decrease was observed in shoot length with the use of *D. brochopaga* filtrate compared to T. harzianum and free plant application.

In root measurements there were no significant differences with the use of *N. concurrens* and *T. harzianum* compared to the untreated plant. Moreover, filtrate of *T. harzianum* gave better measures in root length compared to free plant application and all treatments (fungi + nematode and nematode only).nematode only treatment achieved significant decrease in root length compared to the *N. concurrens* and *T. harzianum* and untreated plants . In root weight there were no significant differences among the untreated plants , and other treatments , moreover *T. harzianum* and *N. concurrens* gave better measures in root weight compared to *D. brochopaga* , *A. oligospora* and nematode only.

2- Effect of Some Antagonistic Fungi Spores in Controlling *M. incognita* on Tomato Plants.

This experiment was carried out to determine the effect of some fungi on *Meloidogyne incognita* by using their spores on tomato plants, under greenhouse conditions.

Data in Table (13) show that the better results were detected by using fungal spores than other treatments. Fungal spores treatments succeeded in reducing number of galls, eggmasses , mature females, root developmental stages and

juvenile populations without any harmful to the host plant. (Table 14 and Fig 3).

The best results were obtained by adding spores of *D. brochopaga* and *A. oligospora,* to nematode infected plants, declined the number of nematode to "nil" and there were no symptoms on plants neither on roots nor on shoots. While by using spores of *N. concurrens* and *T. harzianum* the number of juveniles, significantly decreased compared with nematode only treatment which achieved the highest number of juveniles (17500 ind./kg soil).

Calculating value of "R" showed clearly the differences between treatments. The highest value obtained by nematode only treatment while in *T. harzianum* and *N. concurrens* it was 7.36 and 2.77 respectively. The least value "zero" was obtained by using spores of *D. brochopaga* and *A. oligospora* also, water only treatment achieved the least value of "R".

Number of galls, egg-masses, mature females and root developmental stages were declined to "nil" by using spores of *D. brochopaga* and *A. oligospora* . Although, *N. concurrens* and *T. harzianum* measures were lesser, but they were significant when compared with nematode only treatments.

Data in Table (14) showed that no significant differences in shoot measurements with the use of all fungi treatments compared to the untreated plants, however it was found that the use of *D. brochopaga* and *A. oligospora* achieved better measures of shoot length compared to that of check control . In addition, the use of *N. concurrens* and *A. oligospora* achieved

Table (13) : Effect of certain nematophagous fungal spores on *M. incognita* in tomato plants under greenhouse conditions.

Treatments	No. of nematode	No. galls	No. eggmasses	No. mature female	No. R.D.S	R
N. concurrens spores + *M. incognita*	3333	56	35	35	34	2.77
D. brochopaga spores + *M. incognita*	0	0	0	0	0	0
A. oligospora spores + *M. incognita*	0	0	0	0	0	0
T. harzianum spores + *M. incognita*	8833	74	64	64	63	7.36
M. incognita only	17500	283	205	205	165	14.58
L.S.D. (at 0.05)	2850.65	31.97	23.98	23.98	28.71	

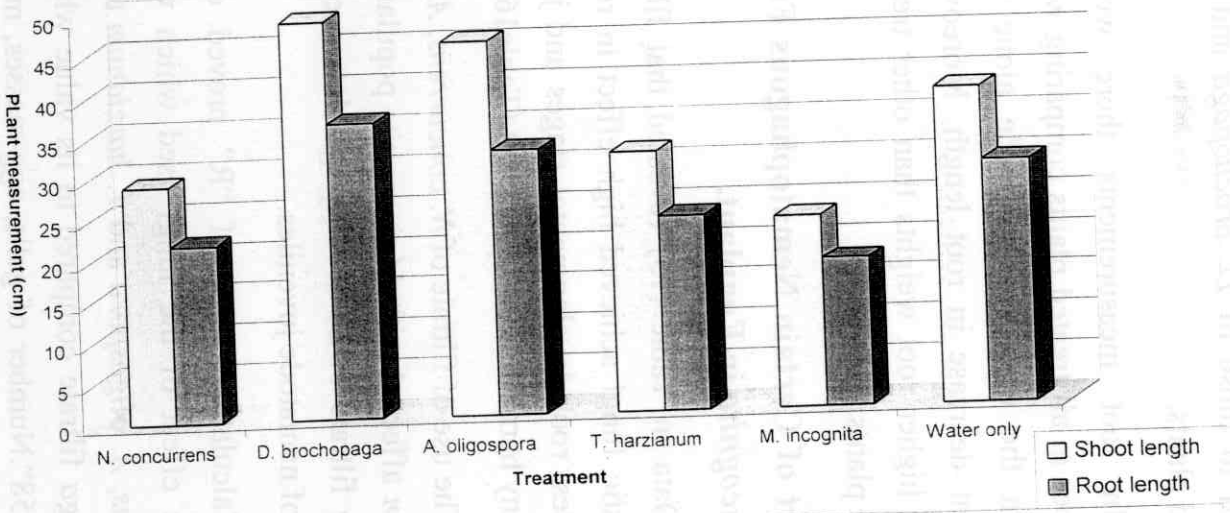
$$R = \frac{\text{Final population of larvae}}{\text{Initial population of larvae}}$$

R.D.S. = Root developmental stages

Table (14) : Effect of certain nematophagous fungal spores on growth measurements of tomato plants infected by *M. incognita* under greenhouse conditions.

Treatments	Shoot length	Root length	Shoot weight	Root weight
N. concurrens spores + *M. incognita*	29	21.66	5.33	2.66
D. brochopaga spores + *M. incognita*	48.6	36	4.66	3.66
A. oligospora spores + *M. incognita*	45.6	32.33	6	3.33
T. harzianum spores + *M. incognita*	31.6	23.66	3.33	2.66
M. incognita only	23.3	18	4.3	2.33
Untreated plants	38.6	29.6	5	3.3
L.S.D. (at 0.05)	13.08	9.38	2.12	1.38

Fig 3: Effect of certain nematophagous fungi spores on plant growth measurements of tomato plant affected by root-knot nematode *Meliodgyne incognita*



higher measures in shoot weights. Significant decrease was observed in shoot length with the nematode only treatment as compared to those of *D. brochopaga* and *A. oligospora* and untreated plants.

In root measurements there were no significant differences in all treated plants comparing with untreated plants except in the case of nematode alone which exhibited a significant decrease in root length. Moreover *D. brochopaga* achieved higher root weights than other treatments as well as untreated plants.

3- Effect of Certain Nematophagous Fungi Filtrates on *M. incognita* in Eggplant.

Data in Table (15) revealed that filtrate of the tested antagonistic fungi achieved high effect in reducing root galls, egg-masses, root developmental stages and juvenile population without any harmful to the host plant.(Table16 and Fig.4).

The use of filtrate of *N. concurrens*, *A. oligospora* and *T. harzianum* affected with *M. incognita* population to "nil", while when use filtrate of *D. brochopaga* decreased significantly the numbers of nematode juveniles.

Calculating value of "R" proved clear informations about the effect of the fungi used which was "nil" with *N. concurrens, A. oligospora* and *T. harzianum* and "7.91" with *D. brochopaga* filtrate compared to its value when no fungi were used "14.58".Number of galls, egg-masses, mature females and root developmental stages declined to "nil" by using the filtrates

Table (15) : Effect of certain nematophagous fungal filtrates on *Meloidogyne incognita* on Egg plant under greenhouse conditions.

Treatment	No. of nematode	No. galls	No. eggmasses	No. mature female	No. R.D.S	R
N. concurrens filtrate + *M. incognita*	0	0	0	0	0	0
D. brochopaga filtrate + *M. incognita*	9500	122.33	68	68	69.66	7.91
A. oligospora filtrate + *M. incognita*	0	0	0	0	0	0
T. harzianum filtrate + *M. incognita*	0	0	0	0	0	0
M. incognita only	22500	437.33	288.3	288.3	223.33	18.75
L.S.D. (at 0.05)	2423.64	56.93	34.61	34.61	43.59	

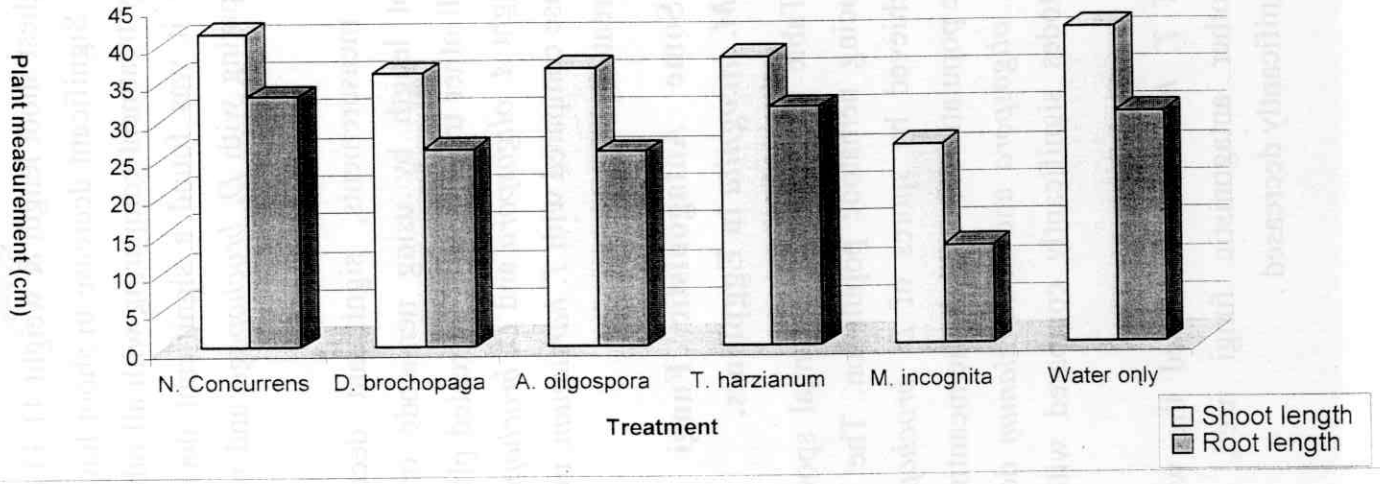
$$R = \frac{\text{Final population of larvae}}{\text{Initial population of larvae}}$$

R.D.S. = Root developmental stages

Table (16) : Effect of certain nematophagous fungal filtrates on growth measurements of Egg plant infected by *M. incognita* under greenhouse conditions.

Treatment	Shoot length	Root length	Shoot weight	Root weight
N. concurrens filtrate + *M. incognita*	41.33	33	7	4
D. brochopaga filtrate + *M. incognita*	36	26	4.33	2.66
A. oligospora filtrate + *M. incognita*	36.66	25.66	5	2.33
T. harzianum filtrate + *M. incognita*	38	31.33	5.33	6
M. incognita only	26.33	13	4	5.33
Untreated plants	41.66	30.33	5.66	4.33
L.S.D. (at 0.05)	9.68	9.7	2.6	1.92

Fig 4: Effect of certain nematophagous fungi filtrates on plant growth measurements of egg plant affected by root-knot nematode *Meloidogyne incognita*



of examined fungi except those of *D. brochopaga* which also achieved a significant nematode inhibition.

Data in (Table 16 and Fig 4) show that, *N. concurrens* achieved the highest shoot length & weight 41.33 cm length and 7 g dry weight . Significant decrease in shoot length was found in nematode only treatment comparing with all other treatments. In shoot weight it was found a significant decrease with *N. concurrens* comparing with *D. brochopaga* and nematode only treatment.

In root measurements, significant decrease were observed in root length by using nematode only treatment compared with all other treatments & untreated plant treatment, while in root weight *A. oligospora* and *D. brochopaga* achieved significant decrease compared with *T. harzianum,* nematode only treatment and untreated plants.

4- Effect of Some Antagonistic Fungi Spores in Controlling *M. incognita* in Eggplants.

Data in Table (17) show that fungal spores achieved high rate in reducing nematode population. The best rate of reduction was achieved by spores of *D. brochopaga,* which declined nematode population to "nil", in the meantime spores of *N. concurrens, A. oligospora* and *T. harzianum* decreased the number of nematodes significantly compared with nematode check.

Spores of *T. harzianum* achieved the worst results compared with other antagonistic fungi used, number of nematodes was significantly decreased .

Calculating value of "R" show clear idea of the effect of antagonistic fungi used, which was "zero" with *D. brochopaga* and free plant application, while it was"18.75" with nematode only treatment by using *N. concurrens, A. oligospora* and *T. harzianum* value of "R" was "22.2", "2.08" and "5.27" respectively.

The number of galls, egg-masses, mature female and R.D.S. were declined to "nil" by using *D. brochopaga* in addition the other antagonistic fungi caused a significant nematode decrease when compared with those of nematode check treatment.

Data in Table (18) show that, in shoot measures were significantly increase in their length under all examined fungi compared to nematode check treatment. The highest root and shoot length was obtained by using *D. brochopaga* spores, In shoot weights, there were no significant differences among treatments except in *D. brochopaga* which exhibited a significant increase overall treatments.

In root measurements a significant increase was observed in root length in all treatments that used fungi as compared to those of nematode check treatment. In root weights there were no significant differences among all treatments except in *A. oligospora* treatment which achieved a significant decrease as compared with nematode check treatment.

Data presented in this study showed no pathogenic symptoms on treated plants due to the use of the treated

antagonistic fungi. Data also revealed that the use of concerned antagonistic fungi as filtrate was better than spores.

The best results were obtained by using filtrates of *N. concurrens, A. oligospora* and *T. harzianum* which declined nematode populations to "NIL" , while the least values were achieved by filtrates of *D. brochopaga* .

In spores treatments , *D. brochopaga* was the best in reducing nematode populations to "NIL", followed by *A. oligospora* , then *N. concurrens* and the least values were obtained by spores of *T. harzianum* .

Table (17) : Effect of certain nematophagous fungal spores on *M. incognita* on Eggplant under greenhouse conditions.

Treatments	No. of nematode	No. galls	No. eggmasses	No. Mature female	No. R.D.S	R
N. concurrens spores + *M. incognita*	2667	42	23	23	31	2.22
D. brochopaga spores + *M. incognita*	0	0	0	0	0	0
A. oligospora spores + *M. incognita*	2500	50	16	16	45	2.08
T. harzianum spores + *M. incognita*	6333	70	29	29	62	5.27
M. incognita	22500	437	288	288	223	18.75
L.S.D. (at 0.05)	2881.12	56.79	31.71	31.71	47.5	

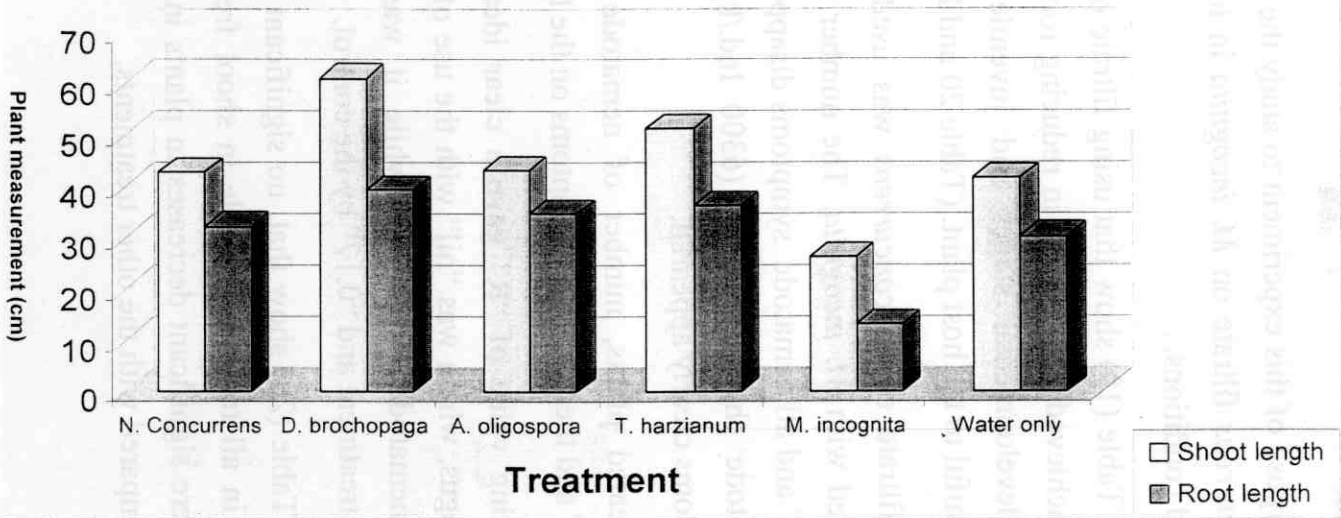
$$R = \frac{\text{Final population of larvae}}{\text{Initial population of larvae}}$$

R.D.S. = Root developmental stages

Table (18) : Effect of certain nematophagous fungal spores on growth measurements of Egg plant infected by *M. incognita* under greenhouse conditions.

Treatment	Shoot length	Root length	Shoot weight	Root weight
N. concurrens + *M. incognita*	43.00	32.33	5.66	3.00
D. brochopaga + *M. incognita*	61.33	39.33	7.66	4.00
A. oligospora + *M. incognita*	43.33	34.66	4.66	2.66
T. harzianum + *M. incognita*	51.33	36.33	5.00	4.33
M. incognita alone	26.33	13.00	4.00	5.33
Untreated plants	41.66	30.33	5.66	4.33
L.S.D. (at 0.05)	12.51	12.08	2.11	2.52

Fig 5: Effect of certain nematophagous fungi spores on plant measurement of eggplant affected by root-knot nematode *Meliodgyne incognita*



D) Semi-Field Experiment:

Effect of the Filtrate of *N. concurrens* on *M. incognita* in Tomato Plants.

The purpose of this experiment to study the effect of the fungus *N. concurrens* filtrate on *M. incognita* in tomato plants under semi-field conditions.

Data in Table (19) show that using filtrate of the fungus *N. concurrens* achieved high effect in reducing root galls, egg-masses, root developmental stages and juvenile population without any harmful to the host plant. (Table 20 and Fig 6 &7).

When filtrate of *N. concurrens* was used on tomato plants or infected with *M. incognita,* The number of juveniles declined to "nil" and all nematode symptoms disappeared, while by using nematode check treatment (6300 Ind./kg soil) and nematode symptoms clearly appeared.

In untreated plants, number of nematode population declined to "nil" and there were no symptoms on the host plant.

Calculating value of "R" gave a clear idea about the effect of the fungus, which was "nil" with the use of fungi only and "fungi + nematode" treatment, while it was "4.2" by nematode check treatment and "0.17" by the control.

Data in Table (20) show that, no significant differences in shoot length in all treatments while in shoot fresh and dry weights there were significant decreases in plants infected with nematodes as compared with the other treatments.

There were significant decreases in root length and root fresh weights in nematode check treatment comparing with all other treatments. In root dry weight there were a significant decrease in nematode check treatment comparing with those of fungi check and "fungi + nematode" treatments.

It was observed that the highest measures of shoot fresh and dry weights were obtained in fungi only treatment.

Moreover, we can see that "fungi + nematode" treatment achieved better results than untreated plants in root measurements and dry weight.

In case of flower and fruit measurements, a significant decrease was found in all these measurements with nematode check treatment comparing with the other treatments, however the highest measurements in all characters were obtained by using fungi check treatment.

Mean of yield/plant can be used as an indicator for the fungi success, which was the highest (4381.8 g/plant) with fungi only treatment, the least (660.83) with nematode check treatment and (3593.1 & 3940 g/plant) with "fungi + nematode" treatment as well as in untreated plants.

(Jatala, 1985) reported that a strong movement to determine the potential of biological control agents in nematode management has occurred over the past several years. The impetus behind this movement has been largely due to the recent advances in the use of toxic pesticides and unawareness of their danger, the time required for development of resistant cultivars, and the economic pressure on land use which limits the use of

Table (19): Effect of filtrate of nematophagous fungus *N. concurrens* on *M. incognita* on tomato plants under semi-field conditions.

Treatment	No. of nematode	No. of galls	No. of eggmasses	No. of mature females	No. of R.D.S	R
A	0	0	0	0	0	0
B	0	0	0	0	0	0
C	6300	102	51.5	51.5	82.4	4.2
D	0	0	0	0	0	0.17
L.S.D (at 0.05)	97.026	0.894	0.894	0.894	0.894	

A: Fungi only B: Fungus + nematode C: Nematode only D: Untreated plants

Table (20): Effect of filtrate of nematophagous fungi *N. concurrens* on growth measurements of tomato plants infected by *M. incognita*.

Treatment	Shoot L. (cm)	Shoot F.W (g)	Shoot D.W (g)	Root L. (cm)	Root F.W (g)	Root D.W (g)	No. of flowers/ plant	No. of fruits/ plant	Mean of fruit weight	Mean of yeild/P.
A	69.06	410	12.49	54.4	30.03	7.34	58	54	80.5	4381.8
B	67.3	373.3	11.93	51.16	26.16	6.2	57.3	49.3	72.73	3593.1
C	43.3	156.6	5.91	23	12.46	3.3	17	13.3	48.6	660.83
D	73.26	385	11.71	46.4	22.56	5.42	58	50.66	78.46	3940.0
L.S.D (at 0.05)	43.53	221.46	4.79	13.7	8.56	2.78	26.90	21.80	12.72	1983.5

A: Fungi only B: Fungus + nematode C: Nematode only D: Untreated plants

Fig 6: Effect of nematophagous fungus *Nematoctonus concurrens* on flower and fruit numbers of tomato plants affected by root-knot nematode *Meloidogyne incognita* under semi-field conditions

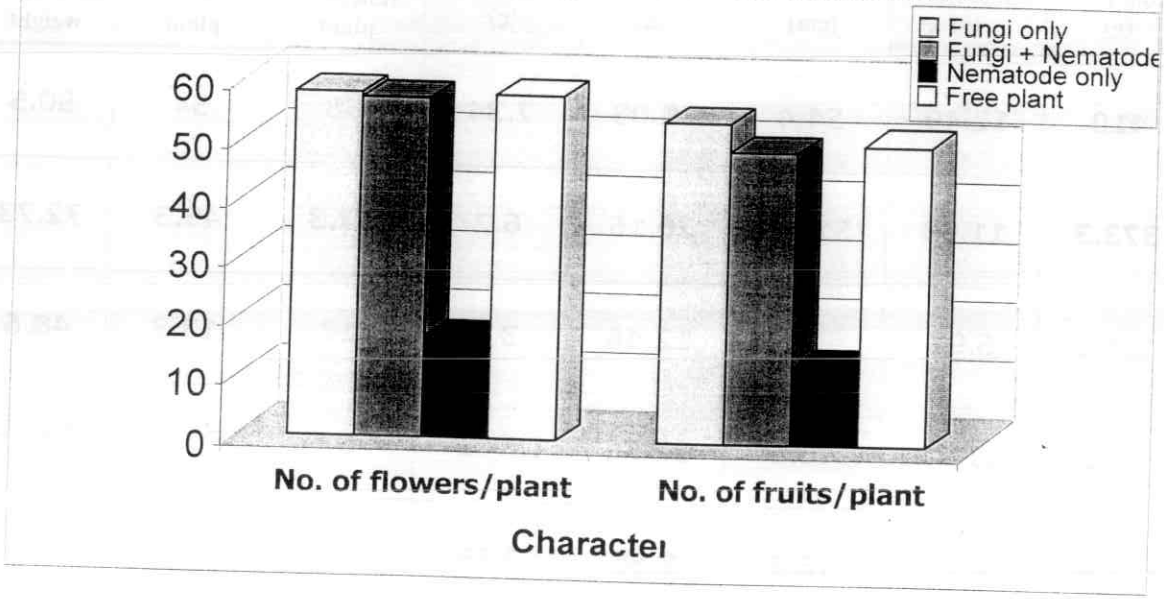
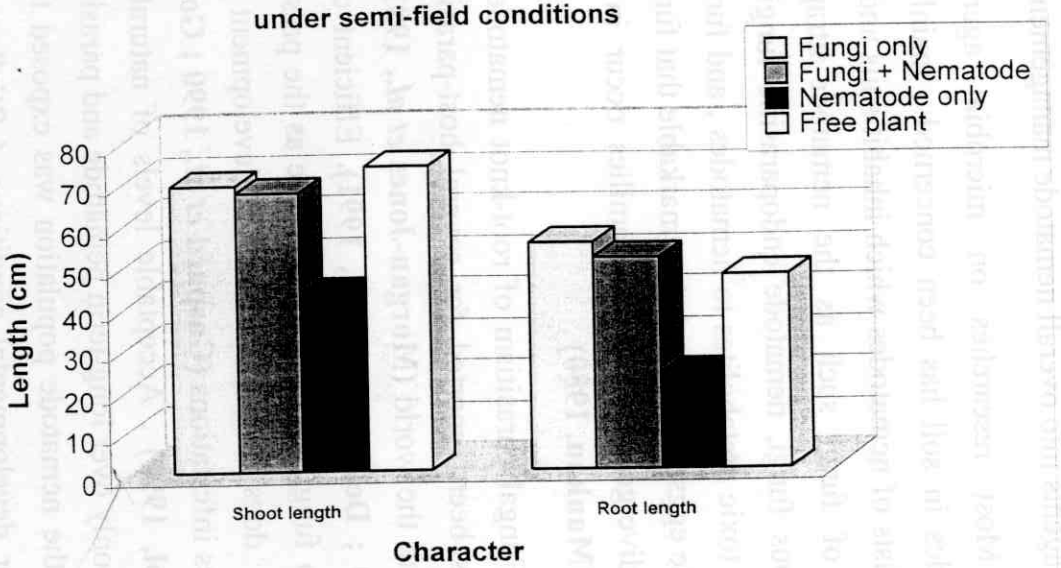


Fig7: Effect of nematophagous fungus *Nematoctonus concurrens* on root and shoot length of tomato plants affected by root knot nematode *Meloidogyne incognita* under semi-field conditions



rotation and other cultural methods. Since many of the most commonly used nematicides are expensive and being taken out of the market because of their harmful effect on humans as well as their persistence in the soil or contamination of the water table, efforts are concentrating on integration of biological control agents into overall nematode management strategies.

Most researches on microbial agents that attack nematodes in soil has been concerned , mainly of the fungal antagonists of nematodes which inhabit soils and include a great variety of fungi such as the nematode-attrapping fungi or predacious fungi, nematode endoparasitic fungi , fungi which produce toxic metabolites to nematodes , and fungal parasites of nematode eggs and cysts. It is remarkable that fungi belonging to widely divergent orders and families occur in each of these groups. **(Mankau, 1980)** .

Fungal parasitism of root-knot nematode (*Meloidogyne* spp.) has been reported for several host-parasite associations throughout the world **(Morgan-Jones *et al.*, 1981 ; Gaspard *et al.*, 1990 ; Deleij & Kerry , 1991).** Efficient control appears related to fungi density and virulence as the presence of fungal parasites does not prevent the development of root-knot nematodes infestations **(Gaspard *et al.*, 1990 ; Gomes Carneiro & Cayrol, 1991)** . Acceptable levels of natural control were observed only over long-term nematode and parasite associations or when the nematode population was exposed to non-optimal feeding or developmental conditions. **(Stirling *et al.*, 1979 ; Kerry, 1990).**

The identification of *A. oligospora* was shown by **(Aboul-Eid *et al.*, 1997).** He indicated that the mycelium is of branched , septated hyphae , and the trap mechanism is an adhesive network that grows extensively . This fungus is easily recognized by elongated , slender , simple , septated and unbranched conidiophores . the average measurements of the conidiophores (n=10) are 380 X 10u. Conidia in clusters , hyaline , unequally two celled, ovate-oblong , smooth , pyriform and distinctly shorter and wider than those of *A. conoides* ; the upper cell being larger than the basal cell . The average measurements of conidium (n=10) are 28 X 16 u.

Arthrobotrys (Hyphomycetes) has been tested against *Meloidogyne* since 1975, by **Uladova. Zopf (1888)** first demonstrated the capture of living eelworms by *Arthrobotrys oligospora*. The fungus attracts the nematodes by a mechanism, which is not fully understood. Nematode excretion products and direct contact with hyphae play a role in the initiation of trap formation which is, also dependent on environmental conditions **(Nordbring- Hertz , 1977)**. Ones traps are present the nematodes are captured within one hour **(Nordbring-Hertz and Stalhammar-Carlemaln , 1978).** Trap formation induced by nematodes has been correlated with motility of nematodes **(Jansson and Nordbring-Hertz , 1981)**.

Arthrobotrys irregularis has been tested for the control of *M. incognita* on tomato. A commercial product, Royal 350, based on A. irregularis was used in pot and field experiments **(Cayrol, 1983 ; Pelagatti *et al.*, 1986)** and in the laboratory **(Cayrol , 1983).** In the pot experiment , sterilized soil was used

and *Arthrobotrys* was applied 40 days before planting and inoculation with *Meloidogyne* juveniles . In field experiments the fungus was applied in *Meloidogyne* infested soil . Both investigators used the same dose (140 g/m^2) and they obtained significant control.

Pelaggatti et al., (1986) reported good results by adding nematodes in tomato plants two days after the fungus incorporation into the soil.

Ali (1994), tested *Trichoderma harzianum* , *Arthrobotrys oligospora* and *A. conoides* under greenhouse conditions. He found that :

1- All used antagonistic fungi have no pathogenic effect on host plants.

2- *Arthrobotrys oligospora* and *A. conoides* showed high effect on activity of the second stage juvenile of *Meloidogyne incognita* (J_2) compared with other antagonists or control treatment .

3- *Arthrobotrys oligospora* and *A. conoides* showed the highest effect during the first 4 weeks whereas *Trichoderma harzianum* gave there maximum effect after 8 weeks . A clear reduction in their efficacy was noticed during period from 8th – 12th week.

The effect of antagonistic fungi on nematode population during growing season was investigated by the same author, to evaluate duration of different antagonists . Data indicated that *A. oligospora* showed the highest effect during the first 4 weeks whereas *T. harzianum* was the most durable one and gave the

highest percentage of reduction in root gall index after 12 weeks. This phenomenon may be due to the fact that *A. oligospora* act through traps developed from special hyphae , development of such hyphae needs special chemical substances in the environment. Shortage in these substances does not allow antagonist to develop these hyphae consequently fail to capture nematodes and appear as reduction in its efficacy. On the other hand, *T. harzianum* was reported as high saprophytic active fungus and act through different mechanism , so this fungus can establish and effect plant parasitic nematodes during a long period of the growing season.

In *Nematoctonus* spp., nematoxic compounds secreted by germinating spores cause rapid immobilization and death of nematodes **(Giuma & Cooke , 1971 ; Giuma *et al.*, 1973)** . However , in all other respects , the infection process is similar to that of other endoparasites with adhesive spores. The cuticle is penetrated by a germ tube , a bulbous infection hyphae forms within the nematode and then assimilative hyphae rapidly fill the body cavity. Ones the body contents have been completely destroyed , hyphae break through the cuticle and conidia are borne on these conidiiferous hyphae.

All the endoparasites with adhesive conidia have small spores , which have limited food reserves and just enough energy to germinate and penetrate a host, but insufficient reserves to establish an extensive mycelium in soil. These fungi therefore tend to be dependent on nematodes as a food source and can be difficult to cultural *in vitro*. **(Gray, 1983)**

The genus *Nematoctonus* has characteristic , non-detachable , hourglass shaped knobs which are engulfed in a larger, spherical ball of viscous material **(Barron , 1977)** .

Dactylella gephyropaga is easily recognized by its conidioform and its peculiar capturing mechanism of nematodes. A conidiophore is well developed , slender , simple , septated and unbranched. The measurements of conidiophores (n=10) are 324 X 9 u . Conidia single, spindle-shaped, hyaline , 3 or more celled. The average measurements of the conidium are 41 X 16 u . The trapping mechanism is adhesive globose cells in a scaliroform shape which hold nematodes by adhesion.

D. oviparasitica was responsible for the low populations of root-knot nematode on peach trees in California , they did not explain why the fungus had no apparent effect in adjacent vineyards where the nematode and parasite were also present. Greenhouse experiments then showed that , *M. incognita* produced relatively small eggmasses containing a maximum of 350 eggs on peach , whereas eggmasses on grape contained more than 1600 eggs. *D. oviparasitica* invariably parasitized most of the eggs in the small eggmasses on peach but on hosts such as grape only about 50% of the eggs were parasitized and many viable eggs remained **(stirling *et al.*, 1979).** The authors therefore suggested that successful suppression by *D. oviparasitica* depended as much on the inability of the nematode to produce large numbers of eggs on the host plant as it did on the activity of the parasite.

Unfortunately , the limited studies of *D. oviparasitica* carried out in the field by **Stirling *et al.*, (1979)** have not been

pursued . Evidence for the involvement of the fungus in the suppression of root-knot nematode in California peach orchards therefore remains largely circumstantial and is based mainly on the results of greenhouse studies and observations that high levels of parasitism occur in the field.

Cayrol (1989) , found that *Trichoderma* sp. produced toxins in culture media . These toxins were active against larvae and adults of *Meloidogyne* sp.

Certain toxic metabolites producing fungi were isolated from the rhizospheres of the surveyed plants and were identified as *Trichoderma harzianum* and *T. viride* . These fungi were effective to a certain degree in reducing the reproduction of *Meloidogyne incognita* **(Aboul-Eid et al., 1997).**

T. harzianum acted through production of some toxic substances such as gliotoxin **(Turner , 1971)** which affect the respiratory sites in the living organism and destroy it **(Abd El-moity *et al.*, 1985).**

The genus *Trichoderma* is known to produce toxic and antibiotics as malformin , hadacidine , gliotoxin , virdin and penicillin and the effects of the filtrates of these fungi against nematodes were previousely reported by several authors **(Mankau, 1969).**

T. harzianum acted through production of large quantities of extra cellular B- (1,3)- glucanase and chitinase , **(Chet *et al.*, 1979)**. This chitinase dissolve the egg layer of nematode which contains chitin as its major components **(Stirling 1991)** .

Dennis and Webster (1971), tested *T. harzianum* and proved that it coiled around other hyphae of all fungi tested.

Zhong *et al.*, (1990) studied the mechanism of hyperparasitism of *T. harzianum* , they found that the fungus formed bulbular or hook-like structure parasited on the host hyphae or penetrated into and grew within them . they concluded that the hyperparasitism is one of the most important mechanism of antagonism.

Chao et al. (1986) who showed that *Trichoderma harzianum* had only a limited capacity to grow in the rhizosphere, as they were unable to colonize more than 2 cm from the seed on which inoculum had been placed. **Ahmed and Baker (1987)** confirmed that *T. harzianum* was not rhizosphere-competent and then produced mutants that could multiply at the root tip to population densities of 10^6 colony-forming units per gram rhizosphere soil.

Fungi such as *Trichoderma* , whose conidia fail to germinate in soil because they are susceptible to fungistasis , have been established in natural soils by incubating conidia in sterile moist bran for 1-3 days and using the germinated spores and young mycelium as inoculum **(Lewis & Papavizas, 1984)**

Effect of these different antagonists on vigor of treated plants was also studied. Percentage of increase in shoot and root weight , due to biological treatment ranged from 40.9% in case of adding *T. harzianum* to 7.2% in case of *A. oligospora* . This effect may be attributed to that *T. harzianum* produced some growth regulators **(Baker, 1988 ; Kleifeld and Chet, 1989)** . On

the other hand , *A. oligospora* effect may be due only to partial protection of root of host plants from damage by nematodes. **(Cayrol and Fran Kowski , 1979 ; Al- Hazmy *et al.*, 1982).**

Chemical Analysis of Tomato Plants as Influenced by *M. incognita* :

1- Phenol Contents:

One) In Shoot

Results in Table (21) show that phenol contents in the dry weight of tomato shoots were increased by the infection of each of fungi alone , M. incognita and more increased content for fungi + nematode.

Relatively low parameter was noticed in non inoculated plants . The highest total phenol contents was obtained with the infected plants inoculated with fungi + nematode , followed by fungi alone , while the lowest were obtained with nematode alone and control.

B) In Roots

Results in Table (22) show the same trend that with that of the shoots except in the control treatment which was approximately equal with fungi + nematode treatment.

2- Sugar Contents:

1st) In Shoots

Results in Table (21) show that the preparation of total sugar was increased by the infection of each of

fungi alone followed by nematode alone then fungi + nematode . No differences were obtained between fungi alone and control treatment , the lowest total sugar content was obtained with fungi + nematode treatment , while nematode alone gave the lowest content for reducing sugar.

2nd) In Roots

Results in Table (22) show that the proportion of total sugar were increased by the infection of each of fungi + nematode followed by fungi alone then nematode alone. No differences were obtained between fungi + nematode and control treatments. The lowest contents of total and reducing sugar was obtained for nematode alone treatment.

3- Amino Acid:

One) In Shoots

Data presented in Table (21) show that amount of amino acids was increased with the infected plant compared to control treatment. Nematode alone and fungi + nematode gave higher increase than fungi only.

2nd) In Roots

Data presented in Table (22) show that inoculation of each of fungi + nematode , fungi alone and nematode alone increased the total amino acids contents than control treatment.

Table (21): Effect of nematophagous fungus filtrate *N. concurrens* on chemical determination of shoot dry weight of tomato plants.

Treatment	Total phenol (%)	Total soluble sugar (%)	Red. sugar (%)	Non red. sugar (%)	Total free amino acid (%)
A	0.5288	2.3987	1.5296	0.8691	0.1686
B	0.5351	1.9347	1.4274	0.5073	0.207
C	0.5007	2.0483	1.1137	0.9346	0.212
D	0.4960	2.3443	1.5884	0.7559	0.1398
L.S.D. (at 0.05)	0.157	0.0210	0.021	0.020	0.0421

A: Fungi only B: Fungi + Nematode C: Nematode only D: Untreated plants

Table (22): Effect of nematophagous fungus filtrate *N. concurrens* on chemical determination of root dry weight of tomato.

Treatment	Total phenol (%)	Total soluble sugar (%)	Red. sugar (%)	Non red. sugar (%)	Total free amino acid (%)
A	0.4423	2.2999	1.0578	1.2421	0.1578
B	0.4654	2.5697	1.5596	1.0101	0.1746
C	0.4881	1.8692	1.0293	0.8399	0.1492
D	0.4649	2.6440	1.1233	1.5207	0.114
L.S.D. (at 0.05)	0.0421	0.0431	0.0468	0.0665	0.0615

A: Fungi only B: Fungi + Nematode C: Nematode only D: Untreated plants

4- Total Soluble Solids in Fruits of Tomato T.S.S. :

Results in Table (23) show that T.S.S. in tomato fruits were decreased in the presence of fungi . The highest T.S.S. was obtained with control fruits followed by nematode check treatment.

5-Acidity:

Data presented in Table (23) show that the highest acidity value was recorded with fungi only treatment followed by control treatment , whereas the lowest acidity value was recorded with nematode only treatment.

Osman (1974) found that great increase in reducing sugars in watermelon plants was achieved when the plants infected with *M. incognita* than those of the control plants.

Total amino acids increased in roots of infected plants as a result of nematode infection. These results are in agreement with **Myuge (1956)** ; who found that higher concentrations of amino acids in galls of tomato roots infected with root-knot nematodes than in non-galled roots. The much greater concentration amino acids in nematode galls , may probably responsible for such root swellings **(Myuge , 1956)** ; presence of a growth promoting substance was not found in healthy roots **(Bird , 1962)** . **Bumbu (1972)** thought that the protein changes in infected cucumber may due to local nematode secretion the waste products of their protein metabolism (ammonia and amino acids). The same results also obtained by **Osman (1974).**

Table (23): Effect of nematophagous fungus filtrate *N. concurrens* on chemical determination of (Acidity & T.S.S) on tomato fruits as influenced by *M. incognita*.

Treatment	Titrable acidity %	T.S.S.
N. concurrens	0.7704	4.00
N. concurrens + *M. incognita*	0.5246	4.00
M. incognita	0.3772	5.20
Untreated plants	0.7163	5.60
L.S.D.(at 0.05)	0.0635	0.429

Determination of phenol contents, showed that total and free phenols in *M. incognita* infected roots were higher than non-infected controls. This increase might be a defense reaction of infected plants. These results agree with those reported by **Osman (1974)** with infected watermelon roots by *M. incognita* , **Allam (1980)** with infected broad bean roots by *M. javanica* and **Khalil (1981)** with onion roots infected by *M. incognita*.