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SURVEY AND ESTIMATION OF PLANT PARASITIC NEMATODES ASSOCIATED WITH GRAPEVINE IN MINIA GOVERNORATE, EGYPT

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ABSTRACT

Plant parasitic nematodes associated with grapevine in Minia Governorate during 2012 and 2013 fruiting seasons were surveyed in Maghagha, Samallot, Minia and Abokorkas districts. Relative frequency, density, biomass as well as importance of nematodes were estimated. The surveyed nematodes in Minia Governorate during 2012 and 2013 were *Meloidogyne* spp., *Helicotylenchus* spp., *Longidorus* spp., *Pratylenchus* spp., *Tylenchulus semipenetrans* and *Hoplolaimus* spp. Those recorded 0.78, 0.77, 0.37, 0.53, 0.60 and 0.31 opposite to 0.83, 0.82, 0.40, 0.66, 0.64 and 0.43 relative frequencies in 2012 and 2013, respectively.

Root knot nematodes *Meloidogyne* spp. surpassed other nematode species in occurrence whereas it was represented in 93 and 101 samples from 120 samples recording relative frequency of 0.78 and 0.83 in 2012 and 2013, respectively. Lowest positive samples of root knot nematode were recorded in Maghagha district but the highest were detected in Samallot district. The general estimated relative density, relative biomass and importance of root knot nematode from the all surveyed districts were 0.36, 0.056 and 1.20 in 2012 opposite to 0.42, 0.056 and 1.31 in 2013, respectively. The lowest relative frequencies, 0.31 and 0.40 were recorded with *Hoplolaimus* spp. and *Longidorus* spp. in 2012 and 2013, respectively, while the lowest relative densities (0.008 and 0.02) was recorded with *Hoplolaimus* spp. in 2012 and 2013, respectively that represented by 37 and 51 samples from the total of 120 samples were taken from the all surveyed districts in 2012 and 2013, respectively, but though this nematode gave relative biomass (0.338) more than that recorded with *Meloidogyne* spp. The highest biomass (1.25) and the highest

importance (1.69) were recorded with needle nematodes, *Longidorus* spp. In Maghagha district citrus nematode surpassed other nematodes where detected in 25 and 27 samples from 30 samples during 2012 and 2013, respectively. The highest nematode mean number (9660/120 samples) was with *Helicotylenchus* spp. in 2012, while in 2013 the highest nematode number (7474/120 samples) was realized with *Meloidogyne* spp. On the other hand *Hoplolaimus* spp. attained the lowest numbers 175 and 408 in the total collected samples during 2012 and 2013 seasons, respectively.

INTRODUCTION

Nematodes are hidden enemies that threaten majority of crops specially the perennial fruit crops. Plant parasitic nematodes cause mechanical damage to the roots of many plants. Their mouths have spears which can protrude to penetrate the plants to suck plant juices. Apart from the wounds causing damage, host reactions may produce gross distortion which may stunt or kill the plant. Effect of plant-parasitic nematodes on grapevine growth and productivity are influenced by a number of factors. Some varieties of *Vitis vinifera* appear to be less susceptible to particular nematode species than other (Ferris *et al*., 1982). Nematode population density is another important factor (Nicol *et al*., 1999).

The present study aims to throw the light on the following topics:-

1. Survey of plant parasitic nematodes associated with grapevine in Minia Governorate.
2. Determining the frequency of occurrence and relative biomass of each nematode species.

MATERIALS AND METHODS

I-Soil texture of the different surveyed locations:-

Soil samples of the surveyed locations were transferred to Soil Analysis Laboratory, Faculty of Agriculture, Minia University for determining the texture of the soil and results showed that Maghagha, Samallot, Minia, Abo-Korkas soil types were clay, loamy sand, clay loam and clay, respectively

II-Extraction of nematodes

Aliquot sample of 200 g was removed from the mixed composited sample for nematode extraction. Extraction of nematodes was by combination of Baermann funnels with elutriation and sieving technique (Barker *et al.,* 1985). Samples were mixed with water and added to the elutriator "Oostenbrink apparatus" which run with a current of water in a rate of 1000 cm^3 per minute against gravity. After elutriator was filled,current of water stopped and the suspension of soil and nematodes was transferred to sieved by using a 400 mesh sieve. Then the contents transferred to Baermann funnels, each consists of 2 wet facial tissues supported on a screen 8 cm in diameter

superimposed on a glass funnel 10 cm in diameter. A small hose ended with clamp was fixed in the funnel stem. Amount of water added till the level of water become 1 cm^3. upstairs the sample level. Nematodes were collected after an incubation period of 3 days at 21-24 ºC. For extraction of slowly moving nematodes such as *Criconemoides*, soil remained above screens was taken out and soaked in water to examined directly under microscope.

III-Survey of plant parasitic nematodes associated with grapevines in Minia Governorate:-

Survey of plant parasitic nematodes associated with grapes (Red Roomy) was carried out during 2012 and 2013 seasons. Thirty composited samples (1kg / each) were collected from the rhizosphere of the surveyed locality. The composited sample was composed from four sub–samples (250 g.) / each that were taken from four directions around the tree. The surveyed localities in Minia Governorate were Maghagha, Samallot, Minia and Abo–Korkas districts. Samples were taken in fruiting period and placed in labeled polyethelene bags. These samples were transferred to the laboratory. Adequate mixing of composite soil samples before removal of an aliquant (200 g.) for nematode extraction is important. Extraction of nematodes was as mentioned above.

Nematodes were killed by heating and each genus identified by the aid of classification keys (Thorne, 1961; Goodey, 1963; Mai and Lyon, 1975 and Andrassy, 1976). Each genus was separately transferred to the count slide (1 ml capacity) and examined using a light microscope for counting.

IV-Frequency of occurrence :-

For estimating occurrence % (relative frequency), number of samples containing a genus divided on the number of all collected samples. On the other hand, relative density of each genus was estimated by dividing the number of the genus individuals on the total nematode individuals in the sample

V-Relative biomass:

For estimating the relative biomass of these genera, twenty nematodes from each genus were transferred by syringe to formalin solution 5% for fixation. These nematodes were infiltrated with glycerin by the following method developed by Seinhorst (1956):-

Nematodes were transferred from fixative to a small dish containing 0.5 ml of the following 96% ethanol 20 parts; glycerin 1 part and distilled water 79 parts. This dish was placed in a desiccator containing enough 96% ethanol to saturate the atmosphere (at least one tenth the volume) and maintained at 35°C for 12 hours then dish was removed and filled with a solution of 5 parts glycerin in 95 parts of 96% ethanol, then placed in a partly closed Petri dish and maintained at 40 °C until the alcohol was evaporated (at least 3 hours).

These nematodes in pure glycerin transferred to a drop of glycerin on a slide. A few glass fibers placed in the drop as spacers. A cover glass was

added and sealed by probable sealer. Nematodes were microscopically examined and measured by the aid of micrometric eye lens. Greatest widths and lengths of the genus individuals were measured and the mean was counted.

Relative biomass (according to Andrassy1956) = Greatest width^2 × length × 1.6 × 1000000

Whereas 1.6=constant for correcting volume of nematode and 1000000 is a factor for converting µm^3 to µg

Importance of a nematode genus in a community was estimated according to Nortorn formula (1978) as follow:-

Importance value = relative frequency + relative density + relative biomass.

RESULTS AND DISCUSSION

The surveyed plant parasitic nematodes during 2012-2013 in Minia governorate arranged in descending order according to their estimated importance were, needle nematode *Longidorus* spp., spiral nematode *Helicotylenchus*, root knot nematode *Meloidogyne* spp., lesion nematode *Pratylenchus* spp., citrus nematode *Tylenchulus semipenetrans* and spiral nematode *Hoplolaimus* spp.

Data in Table (1) show the occurrence of plant parasitic nematodes associated with grape-vines in fruiting period at Minia Governorate during 2012. Root knot nematode *Meloidogyne* spp. surpassed other nematodes in occurrence whereas it was represented in 93 samples from 120 samples, recording relative frequency 0.78. Lowest positive samples of root knot nematode were recorded in Maghagha district but the highest were detected in Samallot district. This result may be attributed to the farmer habits of intercropping vegetable crops with most vine orchards in Samallot. Other interpretation is that the surveyed orchard soil of Samallot was with high sand content. Jaraba *et al.* (2009) reported that soil with high content of sand would be more conductive to greater populations of root knot nematodes. Lewis and Smith (1976) explained that root knot and lance nematodes exhibit a strong preference for soils with high sand content. Scott and Kathy (2013) recorded that population density of *Rotylenchus reniformis* was significantly influenced by soil texture and exhibited a general decrease with increasing medium soil particles (0.04 mm with clay and 0.30 mm with sandy soils). The general estimated relative density, relative biomass and importance of root knot nematode from the all surveyed districts were 0.36, 0.056 and 1.2, respectively. The lowest relative frequency (0.31) and relative density (0.01) was estimated with *Hoplolaimus* spp. that represented by 37 samples from the total of 120 samples were taken from the all surveyed districts but though this nematode gave relative biomass (0.338) more than that recorded with *Meloidogyne* spp. This result may be attributed to the great size of *Hoplolaimus* spp. than larval size of *Meloidogyne* spp. Resultant the greatest size of needle nematode *Longidorus* spp., this nematode

recorded the highest value of importance (1.41) although this nematode recorded low relative density (0.02).

The highest total number of nematodes in 2012 was noted with spiral nematode *Helicotylenchus* spp. recording 9660 per 92 samples, each sample equal 200 g soil comparing to the lowest number of nematodes, 175 per 92 samples that recorded with *Hoplolaimus* spp. In Abokorkas district root knot, spiral and lesion nematodes were plenty observed whereas represented in 24, 20 and 19 samples compared to the low represented nematodes, *Hoplolaimus*, *Longidorus* and *Tylenchulus* that observed in 9, 7 and 6 samples , respectively. In Samallot district spiral nematode frequently occurred in all samples followed by root knot and citrus nematodes in equal samples (27) then *Pratylenchus*, *Longidorus* and *Hoplolaimus* recorded in 24, 17 and 16 samples, respectively. In Maghagha district, citrus nematode was highly recorded than other nematodes where represented in 25 samples. This result may be attributed to the probability of previous citrus trees that were planted in the place of the surveyed orchard lands. Lance nematodes *Hoplolaimus* spp. were recorded in lowest samples (two samples) from the surveyed orchards in Maghagha. In Minia district the occurrence of *Hoplolaimus* was low in 10 samples with comparing to the high occurrence of *Meloidogyne* that represented in 23 samples.

Survey results of the second year 2013 were tabulated in Table (2). These results show that all phytonematode species were more represented in Samallot district than other districts in range of 19 to 29 samples from 30 samples with *Longidorus* spp. and *Meloidogyne* spp., respectively. This result as mentioned with the previous year may be attributed to the soil of this district that contains high sand content. Koenning *et al*. 1996 mentioned that high reproduction of *R. reniformes* and *M. incognita* nematodes increased in the sandy soils. Also as mentioned in 2012, Maghagha district was the minimum district in 2013 that combined *Hoplolaimus* spp. among the collected samples. In spite of root knot nematode was the lowest in relative biomass (0.056) it was the highest in relative density (0.42) and occurrence (0.84). The highest nematode mean number in 2013 was realized with *Meloidogyne* spp. recording 7474 in 120 samples. While *Hoplolaimus* spp. attained the lowest number (408 in the total samples during 2013 season)

Table (1): Occurrence of plant parasitic nematodes associated with grape-vines in fruiting period at Minia Governorate during 2012.

Nematodes Mean greatest width × length µm	Minia districts				Total Samples	Total number of nematodes	Occurrence (Relative frequency)	Relative density	Relative biomass	Importance
	Maghagha	Samallot	Minia	Abokorkas						
	30*	30	30	30	120	22063				
Meloidogyne spp. 15±0.8 × 400 ±16	19**	27	23	24	93	7812	0.78	0.35	0.056	1.19
Helicotylenchus spp. 18 ± 0.9× 1000 ±28	24	30	18	20	92	9660	0.77	0.44	0.203	1.41
Longidorus spp. 20 ± 0.7 ×5000 ± 408	9	17	11	7	44	528	0.37	0.02	1.25	1.64
Pratylenchus spp. 17 ±×600±19	11	24	20	19	64	2880	0.53	0.13	0.108	0.77
Tylenchulus semipenetrans 16± 0.8×400±16	25	27	14	6	72	1008	0.60	0.05	0.064	0.71
Hoplolaimus spp. 30± 0.9× 600± 18	2	16	10	9	37	175	0.31	0.01	0.338	0.64

*Number of samples collected from each district **Number of positive sample for each genus

Occurrence % (Relative frequency) = No. of samples containing a genus / No. of samples collected X 100

Relative density = No. of individuals of a genus in the examined samples / Total no. of individuals in these examined samples

Relative biomass= a^2b/ (1.6)(1000000)

a=the greatest width; b=body length

1.6=constant for correcting volume of nematode and 1000000 is a factor for converting μm^3 to µg

Table (2): Occurrence of plant parasitic nematodes associated with grape-vines in fruiting period at Minia Governorate during 2013.

Nematodes Mean greatest width × length µm	Minia districts				Total Samples	Total number of nematodes	Occurrence (Relative frequency)	Relative density	Relative biomass	Importance
	Maghagha	Samallot	Minia	Abokorkas						
	30*	30	30	30	120	17707				
Meloidogyne spp. 15±0.8 × 400 ±16	21**	29	27	22	99	7474	0.83	0.42	0.056	1.31
Helicotylenchus spp. 18 ± 0.9× 1000 ±28	26	28	21	23	98	5488	0.82	0.31	0.203	1.33
Longidorus spp. 20 ± 0.7 ×5000 ± 408	11	19	10	8	48	720	0.40	0.04	1.250	1.69
Pratylenchus spp. 17 ±×600±19	13	27	19	20	79	1817	0.66	0.10	0.108	0.87
Tylenchulus semipenetrans 16± 0.8×400±16	27	25	18	7	77	1800	0.64	0.10	0.064	0.80
Hoplolaimus spp. 30± 0.9× 600± 18	5	20	14	12	51	408	0.43	0.02	0.338	0.79

*Number of samples collected from each district **Number of positive sample for each genus

Occurrence % (Relative frequency) = No. of samples containing a genus / No. of samples collected X 100

Relative density = No. of individuals of a genus in the examined samples / Total no. of individuals in these examined samples

Relative biomass= a^2b/ (1.6)(1000000)

a=the greatest width; b=body length

1.6=constant for correcting volume of nematode and 1000000 is a factor for converting μm^3 to µg

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الملخص العربي

حصر وتقدير أهمية النيماتودا المتطفلة على النبات المصاحبة لأشجار العنب فى محافظة المنيا

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تم حصر وتقدير التواجد النسبى والكثافة النسبية والكتلة الحيوية النسبية وأهمية النيماتودا النباتية المتطفلة والمصاحبة لأشجار العنب فى محافظة المنيا فى مراكز مغاغة، سمالوط ، المنيا و أبوقرقاص وأوضح الحصر تواجد كل من نيماتودا تعقد الجذور *Meloidogyne* spp. ، النيماتودا الحلزونية *Helicotylenchus* spp.، النيماتودا الإبرية *Longidorus* spp. ، نيماتودا التقرح *Pratylenchus* spp. ، نيماتودا الموالح *Tylenchulus semipenetrans* والنيماتودا الرمحية *Hoplolaimus* spp. بإجمالى نسب تواجد فى محافظة المنيا 0.78، 0.77، 0.37، 0.53، 0.60و 0.31 مقابل 0.83، 0.82، 0.40، 0.66، 0.64 و 0.43 فى موسمى 2012 و 2013 على التوالى

تواجدت نيماتودا تعقد الجذور بدرجة أكبر عن باقى الأجناس النيماتودية التى تم حصرها حيث تواجدت فى 93 ، 101 عينة من 120 عينة تم جمعها بنسبة تواجد 0.78، 0.84 فى موسم الإثمار فى 2012 و2013 على التوالى وكان أقل تواجد لهذه النيماتودا تم تسجيله فى مركز مغاغة وأعلى تواجد لها سُجِل فى مركز سمالوط وقدرت الكثافة النسبية والكتلة الحيوية النسبية والأهمية لهذه النيماتودا فى المراكز التى خضعت للحصر حيث كانت 0.36، 0.056 و 1.20 فى موسم 2012 مقابل 0.42، 0.056 و 1.31 فى موسم 2013

تم حساب أقل تواجد نسبى 0.31 ، 0.43 وكثافة نسبية 0.01 ، 0.02 للنيماتودا الرمحية خلال موسمى 2012 ، 2013 على التوالى حيث تواجدت فى 35 ، 51 عينة من 120 عينة ومع ذلك فإن هذه النيماتودا سجلت كتلة حيوية نسبية 0.338 أكبر من نيماتودا تعقد الجذور. أعلى كتلة حيوية نسبية (1.25) وأكثر أهمية (1.41) تم حسابهما مع النيماتودا الإبرية

تفوقت نيماتودا الموالح فى التواجد فى مركز مغاغة على باقى الأجناس حيث تواجدت فى 25 و 27 عينة من إجمالى 30 عينة تم جمعها فى خلال موسمى 2012 و 2013 على التوالى