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Prevalence of plant parasitic nematodes associated with grapevine in Minia Governorate, Egypt

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Abstract

Grapevine orchards were surveyed for the prevalence of plant parasitic nematodes in Maghagha, Samallot, Minia and Abokorkas Districts of Minia Governorate. Soil and root samples were collected during fruiting seasons of grapevine in 2012 and 2013. The nematodes encountered were identified as *Meloidogyne* spp., *Helicotylenchus* spp., *Longidorus* spp., *Pratylenchus* spp., *Tylenchulus semipenetrans* and *Hoplolaimus* spp. Community analysis of these plant parasitic nematodes showed variability in nematodes population densities, frequencies, biomass and importance values in different Districts during 2012 and 2013. Root-knot nematodes *Meloidogyne* spp. was the most prevalent genus in the entire plant parasitic nematode community having highest relative frequency (0.78 and 0.83) in both years, respectively. The lowest relative frequencies (0.31 and 0.40) were recorded for *Hoplolaimus* spp., and *Longidorus* spp., in 2012 and 2013, respectively, *Helicotylenchus* spp. (0.44) and *Meloidogyne* spp. (0.42) were found with highest relative density in 2012 and 2013, respectively while the lowest relative densities (0.01 and 0.02) was recorded for *Hoplolaimus* spp. in 2012 and 2013, respectively. The highest biomass (1.25) and the highest importance value (1.69) recorded for needle nematodes, *Longidorus* spp., during 2012 and 2013, respectively. The lowest relative biomass (0.056) in both years was observed for *Meloidogyne* spp. while *Hoplolaimus* spp. exhibited the least importance value (0.64 and 0.79) in 2012 and 2013, respectively.

Keywords: Plant parasitic nematodes, community analysis, *Meloidogyne* spp., grapevine, Minia Governorate, Egypt.

Nematodes are hidden enemies that threaten majority of crops specially the perennial fruit crops. Apart from the infestation 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* L. appear to be less susceptible to particular nematode species than others (Ferris *et al*., 1982). Nematode population density is another important factor in grapevine growth and its productivity (Nicol *et al*., 1999). The present study aims to conduct survey for the prevalence of plant parasitic nematodes associated with grapevine in Minia Governorate and to determine the community analysis (relative frequency, relative density, relative biomass and importance value) of each nematode species.

Materials and Methods

Soil texture of the surveyed locations: Soil samples collected from grapevine of the surveyed locations were transferred to Soil Analysis Laboratory, Faculty of Agriculture, Minia University to determine the texture of the soil by hydrometer method (Gee & Bauder, 1979).

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Collection of soil samples from grapevines in Minia Governorate: Collection of soil samples from grapes (Red Roomy) was carried out during 2012 and 2013 seasons. The surveyed localities in Minia Governorate were Maghagha, Samallot, Minia and Abo-Korkas. Thirty composited samples (1kg) were collected from the rhizosphere at the surveyed localities. The composited samples composed of four sub-samples (250 g), were taken from four directions around the tree. Samples were taken in fruiting period and placed in labeled polythelene bags and were transferred to the laboratory. Adequate mixing of composite soil samples before drawing of an aliquot (200 g) for nematode extraction was ensured. Extraction of nematodes was done as mentioned below.

Extraction of nematodes: aliquot sample of 200 g was drawn/taken from the mixed composite sample for nematode extraction. Extraction of nematodes was done 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 runs with a current of water at a rate of 1000 cm^3 per minute against gravity. After elutriator application the suspension of soil and nematodes was transferred and sieved by using a 400 mesh sieve. Then the contents transferred to funnels and nematodes extraction procedure was followed as described in Baermann funnel technique. Nematodes were collected after an incubation period of 48 hrs at 21-24 ºC. For extraction of slowly moving nematodes such as *Criconemoides*, soil that remained on the screens, was taken out and soaked in water to be examined directly under stereoscopic microscope.

Extraction of root-knot nematodes: Roots from samples were separated and cut into 1-2 cm segments. These pieces were put in a Petri dish containing water and observed under the microscope. Egg-masses and root-knot females were pulled out of the galls with dissecting needles. Recovered females were transferred to glass cavity blocks with the help of a dropper for identification, based on perineal patterns (Eisenback *et al.*, 1981).

Processing of nematodes: Nematodes were killed by gentle heat on a hot plate and transferred into 5% formalin solution for fixation. These nematodes were infiltrated with glycerin by the method developed by Seinhorst (1956). Nematodes were microscopically examined and measured by the aid of micrometric eye lens. Identification of nematodes was done according to Siddiqi (2000). Greatest widths and lengths of the genera were measured and the mean was calculated. Body Mass of nematodes may be calculated using the Andrassy (1956) formula $W= (L*D^2)/(1.6*10^6)$; where W is the mass (as fresh weight µg) per individual, L is the nematode length (µm) and D is the greatest body diameter (µm).

Community analysis: Community analysis of plant parasitic nematodes from four Districts viz., Maghagha, Samallot, Minia and Abo-Korkas of Minia Governorate, Egypt was calculated according to Norton (1978). The nematode community structure was characterized by using the different parameters viz., relative frequency of occurrence, relative density (RF% and RD), relative biomass and importance value (RB and IV%).

Results and Discussion

Soil texture: Results showed that soil types at Maghagha, Samallot, Minia and Abo-Korkas were clay, loamy sand, clay loam and clay, respectively.

Encountered nematodes: The encountered plant parasitic nematodes during 2012-2013 in Minia Governorate were identified up to genus level viz., needle nematode (*Longidorus* spp.), spiral nematode (*Helicotylenchus* spp.), root-knot nematode (*Meloidogyne* spp.), lesion nematode (*Pratylenchus* spp.), citrus nematode (*Tylenchulus semipenetrans*) and lance nematode (*Hoplolaimus* spp.).

Community analysis of nematodes: The occurrence of plant parasitic nematodes associated with grape-vine in fruiting period at Minia Governorate during 2012 is shown in Fig. 1. Root-knot nematode *Meloidogyne* spp. surpassed other nematodes in occurrence whereas it was present in 93 out of 120 samples with relative frequency of 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 orchard soil of Samallot has high sand content. Jaraba *et al*., (2009) reported that soil with high content of sand would be more conducive to greater populations of root-knot nematodes. Lewis & Smith (1976) explained that root-knot and lance nematodes exhibited a strong preference for soils with high sand content. Scott & Kathy (2013) reported that population density of *Rotylenchulus 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 estimated relative density, relative biomass and importance of root-knot nematode from all the surveyed Districts were 0.36, 0.056 and 1.2, respectively. The lowest relative frequency (0.31) and relative density (0.01) was estimated for *Hoplolaimus* spp. that was present in 37 of the total of 120 samples though this nematode gave relative biomass (0.338) more than that recorded for *Meloidogyne* spp. This result may be attributed to the greater size of *Hoplolaimus* spp. than larval size of *Meloidogyne* spp. Needle nematode *Longidorus* spp., was recorded with the highest importance value (1.41) and low relative density (0.02).

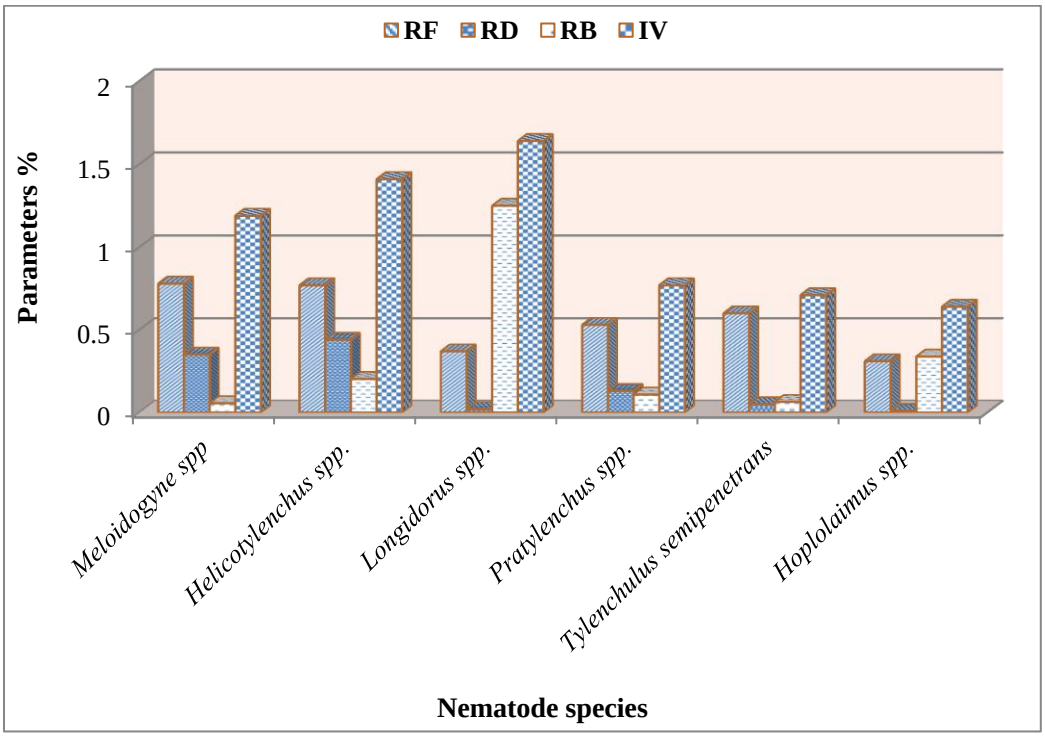

Fig. 1. Community analysis of plant parasitic nematodes at Minia Governorate during 2012.

Survey results of the year 2012 for the occurrence of nematodes are presented in Table (1). *Helicotylenchus* spp., was found in highest numbers in 2012 (9660 per 92 samples, each sample of 200 g soil), while the lowest number of nematodes was recorded of *Hoplolaimus* spp. (175 per 92 samples). In Abokorkas District, root-knot, spiral and lesion nematodes were observed in 24, 20 and 19 samples whereas *Hoplolaimus*, *Longidorus* and *Tylenchulus* were observed in 9, 7 and 6 samples, respectively. In Samallot District *Helicotylenchus* occurred in all samples followed by root-knot and citrus nematodes in equal number of samples (27) then *Pratylenchus*, *Longidorus* and *Hoplolaimus* recorded in 24, 17 and 16 samples, respectively. In Maghagha District, citrus nematode *Tylenchulus* spp. was prevalent in 25 more samples than other nematodes. This result may be attributed to the probability of planting citrus trees earlier in the place of the grapevine orchard. Lance nematodes *Hoplolaimus* spp., were recorded in lowest samples (two samples) from the surveyed orchards in Maghagha. In Minia District *Hoplolaimus* was found in 10 samples while *Meloidogyne* was recorded in 23 samples (Table 1).

Survey results of community analysis of nematodes of the year 2013 were presented in Fig. (2). Results showed that phytonematode species were more prevalent in Samallot District than other Districts and ranged between 19 (*Longidorus* spp.) and 29 (*Meloidogyne* spp.) samples out of a total of 30 samples. This result as mentioned in the previous year may be attributed to the soil of this District that contained high sand content. Koenning *et al*., (1996) mentioned that high reproduction of *R. reniformis* and *M. incognita* nematodes increased in the sandy soils. As mentioned in 2012, Maghagha District in 2013 also showed that *Hoplolaimus* spp., was recorded in lowest samples (five samples) among the collected samples. Root-knot nematode was the lowest in relative biomass (0.056) but has the highest relative density (0.42) and occurrence % (0.83). The occurrence of nematodes for the year 2013 results (Table 1) show that highest mean number of nematode species (7474) was *Meloidogyne* spp., in 120 samples while *Hoplolaimus* spp. attained the lowest number (408) in the total samples during 2013. The results are in accordance with the earlier researchers (Jaraba *et al*., 2009; Lewis & Smith, 1976; Scott & Kathy, 2013; Koenning *et al*., 1996).

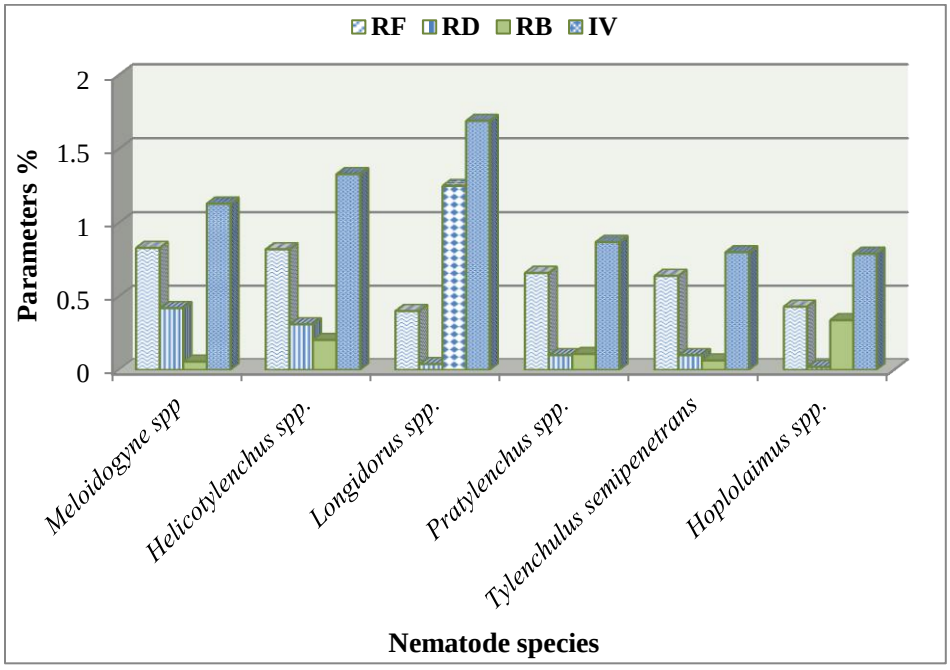

Fig. 2. Community analysis of plant parasitic nematodes at Minia Governorate during 2013.

Table 1. Occurrence of plant parasitic nematodes associated with grapevines in fruiting period at Minia Governorate during 2012 and 2013.

Nematodes Mean greatest width × length µm	Minia Districts								Total samples		Total no. of nematodes	
	Maghagha		Samallot		Minia		Abokorkas					
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
	30*	30	30	30	30	30	30	30	120	120	22063	17707
Meloidogyne spp. 15 ± 0.8 × 400 ± 16	19**	21**	27	29	23	27	24	22	93	99	7812	7474
Helicotylenchus spp. 18 ± 0.9× 1000 ± 28	24	26	30	28	18	21	20	23	92	98	9660	5488
Longidorus spp. 20 ± 0.7 × 5000 ± 408	9	11	17	19	11	10	7	8	44	48	528	720
Pratylenchus spp. 17 ± × 600 ± 19	11	13	24	27	20	19	19	20	64	79	2880	1817
Tylenchulus semipenetrans 16 ± 0.8 × 400 ± 16	25	27	27	25	14	18	6	7	72	77	1008	1800
Hoplolaimus spp. 30 ± 0.9 × 600 ± 18	2	5	16	20	10	14	9	12	37	51	175	408

*Number of samples collected from each District; **Number of positive sample for each genus
Relative frequency = No. of samples containing a genus / No. of samples collected x100
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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