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Evaluating the Pathogenicity of Nematodes Infecting Roses at Taif Governorate, KSA.

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ABSTRACT

Roses continue to be one of the most popular flowers in the garden landscape and have pharmaceutical uses. In Taif, cultivation of Taify rose creates more income than the cultivation of vegetables or crops. In Saudi Arabia, nematodes are becoming a real threat to almost all crops and they have been considered as limiting factors in crop production. There is no information on the nematode communities infecting rose plants and its pathogenicity at present. In this study, the distribution of nematodes infecting roses and the pathogenicity of the most important nematode species on rose cultivars were investigated at Taif governorate. Results indicated the presence of seven genera of plant parasitic nematodes, belonging to 7 families in soil samples collected from three localities of rose farms. Among soil samples collected from the three localities, the plant parasitic nematode genera, *Meloidogyne, Rotylenchulus*, *Xiphinema* and *Pratylenchus* seemed to be the most prevailing pests. None of the three tested rose cultivars was immune to *M. incognita* infection. Sultany cultivar can be classified as resistant host, whereas Taify rose was rated as susceptible host and Gory cultivar as tolerant one.

Keywords: Pathogenicity, Survey, Nematodes, Roses.

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INTRODUCTION

Roses are best known as ornamental plants grown for their flowers in the garden and sometimes indoors. They have been also used for commercial perfumery and commercial cut flower crops. Some are used as landscape plants, for hedging and for other utilitarian purposes such as game cover and slope stabilization. They also have minor medicinal uses. Yet, Taif is known for the famous Wardh Taifi, the Rose of Taif. The suburbs of Taif and its valleys Huda, al Shafa, al Ghadeerayn and Wadi Mahram, are known for cultivation of this rose, which creates more income than the cultivation of vegetables or crops. Taif Rose, Wardh Taifi (*Rosa damascena trigintipetala*) belongs to the species Damask Rose. There are several varieties of Damask Rose, such as Autumn Damask Rose (*R. damascena sempervirens*) and Damask Rose Kazanlik (also *Rosa damascena trigintipetala*).

Unfortunately, there are several diseases that commonly occur on hybrid teas, floribundas, moss roses, or whatever type of rose you are growing. To produce top quality roses, these diseases must first be identified and controlled. Plant parasitic nematodes are worldwide an economically important agric-pest, reducing the yield and quality of crops especially roses plants. Several plant parasitic nematodes were recorded as pathogens of roses in many parts of the world [1]. Four genera of these plant-parasitic nematodes have been identified and classified as important pathogens that can cause severe damage to some important crops in the world: *Meloidogyne* spp., *Rotylenchulus* spp., *Tylenchulus* spp. and *Pratylenchus* spp.

Rose plants are suspected to infection with a great number of plant parasitic nematodes. Worldwide, crop loss attributed to these pests could be estimated by 20.6 % [2]. The root-knot nematodes, *Meloidogyne* spp. and *Pratylenchus* spp. are the economically important parasites of rose cultivars. When plants are severely infected by root-knot nematodes (RKN) *Meloidogyne* spp., the normal root system is reduced to a limited number of severely galled roots with a completely disorganized vascular system. Rootlets are almost completely absent. The roots are seriously hampered in their main functions of uptake and transport of water and nutrients [3]. One of the root-knot nematodes, *Meloidogyne hapla* Chitwood, is a serious pest of roses. Roses grown on infested land are likely to be marred by galls resulting from attack by this nematode, rendering them unfit for export [4].

In Taif, during several field trips, there is high numbers of nematode infestations in rose commercial farms with reduced plant growth and flowers production as well as there is no information on the nematode communities infecting roses and its pathological effects at present. Therefore, the focal target of the present work is to understand the distribution of nematodes infecting roses and evaluate the pathogenicity of the most important nematode species on rose cultivars at Taif governorate.

MATERIALS AND METHODS

Survey study

A total of 400 soil samples were randomly collected from the rhizosphere of roses grown in different localities of Taif governorate during the period from April to August. The surveyed localities and number of representative soil samples were as follows: Al-Shafa (150), Al-Hada (150) and gardens of Taif University (100). Samples were obtained by digging the soil to a depth of about 15-30 cm from the rhizosphere of growing plants. Soil samples of about 1 kg each were placed in plastic bags and sent directly to the laboratory and kept in the refrigerator at 4 °C until nematode extraction. Observations concerning locality, plant age, cultivar and general condition of the trees were recorded at time of sampling.

Soil samples were thoroughly mixed and a volume of 250 gm soil was used to extract nematodes according to sieving and modified Baermann-pan technique [5]. Each soil sample was soaked in tap water for 20 minutes, and then the mixture was agitated by hand. Direct sieving through 60 and 400 mesh sieves was employed. Resulting suspension was transferred on a soft tissue paper fitted on the Baermann-pan for separating active nematodes from soil particles [5]. After 48 hours, water in the plate containing nematodes was transferred to a plastic cup. Identification of nematode genera in repeated aliquots (1 ml each) in each soil sample was based on the morphological characters of the adult and larval forms according to [6]. The Hawksely counting slide under x 40 magnification was used for determining the number of each nematode genus and recorded.

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Culturing of root-knot nematode

The root-knot nematode *Meloidogyne incognita* were established as a pure culture from single egg-masse of *Meloidogyne incognita* that was identified according to the characteristics of its perineal pattern [7] and reared on tomato plants grown in greenhouse of Science Faculty, Taif University. This nematode was used as inocula for the next pathogenicity experiment.

Pathogenicity study

This study was carried out in the greenhouse of Biology department, Faculty of Science, Taif University, Saudi Arabia to screen different economic cultivars of rose plants to root-knot nematode, *Meloidogyne incognita* infection as common and dangerous nematode pests of roses which indicated from the survey study. Three cultivars of the commercial cultivated roses i.e. Taify, Gory and Sultany were used. Plastic bags 15 cm diam. filled with 1.5 kg steam sterilized clay loamy soil (1:1) (v:v) each were separately planted with one rose seedling 30 days old from each tested cultivar. Thirty plastic bags were used in this experiment, ten for each rose cultivar where five bags of them was inoculated with 3000 freshly hatched second stage juveniles of *M. incognita* seven days after seedling transplanting while other five plastic bags left free of nematodes inoculum and served as control treatment. All plastic bags received water as needed. All bags were arranged in complete block design system and agronomically treated the same under greenhouse conditions at (25±2° C). Forty five days after nematode inoculation, plants were uprooted and root systems were washed from adhering soil. Plants growth parameters i.e. length and fresh weight of shoot, root and numbers of leaves for each replicate were measured and percent reduction in such growth parameters were determined and recorded in relation to healthy plants (un-inoculated ones).

The infected roots/ cultivar were stained in acid fuschin [8], washed in tap water and placed in pure cold glycerin [5]. After clearing, number of *M. incognita* galls and egg-masses per root system of each plastic bag/ rose cultivar were counted and recorded. Root gall index (RGI) and egg-mass index (EI) were rated on a scale of 0 to 5 where 0= no galling or egg-masses, 1= 1-2 galls or egg-masses, 2= 3-10 galls or egg-masses, 3= 11-30 galls or egg-masses, 4= 31-100 galls or egg-masses and 5= more than 100 galls or egg-masses [7]. Data were subjected to analysis of variance (ANOVA) [9], followed by Duncan's multiple range tests to compare means [10].

RESULTS AND DISCUSSION

Data in table (1) recorded the presence of seven genera of plant parasitic nematodes, belonging to 7 families in soil samples collected from three localities of rose farms in Taif governorate. These nematode genera were *Criconemoides* (Criconematidae), *Meloidogyne* (Meloidogynidae), *Pratylenchus* (Pratylenchidae), *Rotylenchulus* (Rotylenchulidae), *Trichodorus* (Trichodoridae), *Tylenchorhynchus* (Tylenchorhynchidae) and *Xiphinema* (Longidoridae).

Table 1: Frequency of occurrence and population density of nematode genera associated with rose plants at three localities of Taif governorate.

Nematode genera	Occurrence of nematode genera per 250 g soil within each locality of rose plants			Total	Frequency of occurrence %	No. of infested localities
	Al-Hada (n=150)	Al-Shafa (n=150)	Taif University (n=100)			
Criconemoides	5 (560)*	--	2 (150)	7	1.8	2
Meloidogyne	60 (4200)	29 (2100)	31 (1630)	120	30.0	3
Pratylenchus	30 (710)	--	15 (200)	45	11.3	2
Rotylenchulus	47 (1320)	25 (850)	45 (1685)	117	29.3	3
Trichodorus	16 (861)	7 (250)	5 (200)	28	7.0	3
Tylenchorhynchus	20 (400)	10 (460)	9 (220)	39	9.8	3
Xiphinema	18 (740)	48 (1500)	--	66	16.5	2
Total occurrence	196	119	107			
Nematode genera / locality	7	5	6			

*Numbers between parentheses are the population densities of nematode genera recovered from 250 g soil sample.

Among soil samples collected from rose farms of the three localities, the plant parasitic nematode genera, *Meloidogyne, Rotylenchulus*, *Xiphinema* and *Pratylenchus* seemed to be the most prevailing pests as they occurred at rates of 120, 117, 66 and 45 times with percentage of 30 %, 29.3 %, 16.5 % and 11.3 %, respectively.

These findings are in according with the results of [4] who reported that root-knot nematode, *Meloidogyne hapla* Chitwood, is a serious pest of roses. In Florida, at least 30 species of plant parasitic nematodes have been recovered from soil around rose roots [11]. Studies have shown that four species of nematodes are capable of causing diseases on roses. Most easily recognized are diseases caused by root-knot and dagger nematodes. Lesion nematodes are also known to cause poor growth and serious decline of roses.

Soil samples recovered from Al-Hada district revealed the presence of the 7 nematode genera in the surveyed localities of rose farms. The plant parasitic nematode genera, *Meloidogyne, Rotylenchulus* and *Pratylenchus* seemed to be the most prevailing pests as they occurred at rates of 60, 47 and 30 times with an average of 4200, 1320 and 710 individuals per 250 gm soil, respectively.

The nematode genera, *Tylenchorhynchus, Xiphinema* and *Trichodorus* showed moderate distribution as they occurred at rates of 20, 18 and 16 times, respectively. On the other hand, the genus, *Criconemoides* was less common as it occurred at 5 times only with an average of 560 individuals per 250 gm soil. Concerning Al-Shafa district, 5 nematode genera were observed in the collected soil samples. The digger nematode, *Xiphinema* seemed to be the most prevailing pest as it occurred at rate of 48 times with an average of 1500 individuals per 250 gm soil. The nematode genera, *Meloidogyne* and *Rotylenchulus* showed moderate distribution as they occurred at rates of 29 and 25 times with an average of 2100 and 850 individuals per 250 gm soil, respectively. On the other hand, the genera, *Tylenchorhynchus* and *Trichodorus* were less common as they occurred at 10 and 7 times with an average of 460 and 250 individuals per 250 gm soil, respectively.

Soil samples collected from gardens of Taif University revealed the presence of six nematode genera. These nematode genera were *Criconemoides*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, *Trichodorus* and *Tylenchorhynchus* (Table 1). The plant parasitic nematode genus, *Rotylenchulus* seemed to be the most prevailing pest as it occurred at rate of 45 times with an average of 1685 individuals per 250 gm soil whereas, the nematode genus, *Meloidogyne* ranked the second in its distribution as it occurred at rate of 31 times. Its average was 1630 individuals per 250 gm soil.

Table 2: Frequency of occurrence and population density of nematode genera associated with two cultivars of roses in Taif governorate.

Nematode genera	Occurrence and population density of nematodes per 250 g soil in two rose cultivars		Total	Frequency of occurrence %	No. of infested cultivars
	Taify Rose (n=300)	Sultany Rose (n=100)			
Criconemoides	5 (560)	2 (150)	7	1.8	2
Meloidogyne	89 (6300)	31 (1630)	120	30.0	2
Pratylenchus	30 (710)	15 (200)	45	11.3	2
Rotylenchulus	72 (2170)	45 (1685)	117	29.3	2
Trichodorus	23 (1111)	5 (200)	28	7.0	2
Tylenchorhynchus	30 (860)	9 (220)	39	9.8	2
Xiphinema	66 (2240)	--	66	16.5	1
Total occurrence	315	107			
Nematode genera / cultivar	7	6			

*Numbers between parentheses are the population densities of nematode genera recovered from 250 g soil sample.

Among two rose cultivars surveyed, Taify rose cultivar encountered the largest number of nematode genera (7) than Sultany rose (6) (Table 2). It can also be noticed that no digger nematode, *Xiphinema* was recorded on Sultany cultivar. Data also revealed that the highest densities of nematode individuals per 250 gm soil averaged 6300, 2240 and 2170 for the root-knot nematode, *Meloidogyne* spp., the digger nematode, *Xiphinema* spp., and the kidney nematode, *Rotylenchulus* spp. in the rhizosphere of Taify rose cultivar and

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occurred at rates of 89, 66 and 72 times, respectively. Moreover, an average of 1685 and 1630 individuals were recorded for the kidney nematode, *Rotylenchulus* spp. and the root-knot nematode, *Meloidogyne* spp. in the rhizosphere of Sultany rose cultivar and occurred at rates of 45 and 31 times, respectively.

Data in Tables (3 and 4) show the impact of *M. incognita* infection on plant growth and nematode parameters of three rose cultivars seedlings i.e. Taify, Gory and Sultany under greenhouse conditions. Results reveal that all growth parameters of such rose cultivars were obviously affected by *M. incognita* infection to certain extent. It was evident that treatment with 3000 second stage juveniles of *M. incognita* per pot greatly reduced the root and shoot length; and fresh weight of whole plant as well as number of leaves with values of 26.9, 40.0 and 36.5 % for Taify rose, followed by Gory rose with values of 22.5, 30.5 and 34.2 %, respectively, whereas Sultany cultivar showed the least percent reduction, especially for the fresh weight of whole plant (12.9 %) and plant length (13.0 %) comparing to nematode alone (Table 3).

Table 3: Plant growth response of three rose cultivars as influenced by *Meloidogyne incognita* infection under greenhouse conditions.

Rose cultivars	Treatments	*Plant Growth Response									
		Length (cm)		Total Length (cm)	Red. %	Fresh weight (g)		Total F.W. (g)	Red. %	No. of Leaves	Red. %
		Shoot	Root			Shoot	Root				
Taify	Infected	31.3 b	34.3 b	65.6 b	26.9	4.36 b	7.26 b	11.62 b	40.0	4.0 b	36.5
	Uninfected	48.5 a	41.3 a	89.8 a	--	10.37 a	9.01 a	19.38 a	--	6.3 a	--
Gory	Infected	43.3 b	36.0 b	79.3 b	22.5	8.32 a	9.55 b	17.87 a	30.5	4.8 b	34.2
	Uninfected	59.0 a	43.3 a	102.3 a	--	12.13 a	13.59 a	25.72 a	--	7.3 a	--
Sultany	Infected	35.0 a	25.0 b	60.0 b	13.0	14.25 a	14.12 b	28.37 a	12.9	8.8 a	20.0
	Uninfected	37.0 a	32.0 a	69.0 a	--	15.76 a	16.8 a	32.56 a	--	11.0 a	--

*Each value is the mean of five replicates.
Means in each column followed by the same letter (s) did not differ at P< 0.05 according to Duncan multiple- rage test.

Table 4: Host suitability of rose cultivars to the infection of *Meloidogyne incognita* under greenhouse conditions.

Rose cultivars	No. of galls *	RGI	No. of Egg-masses	EI
Taify	14.8 a	3.0 a	4.5 a	2.0 a
Gory	8.0 b	2.0 b	1.5 b	1.0 b
Sultany	2.0 c	1.3 c	0.0 c	0.0 c

*Each value is the mean of five replicates.
Means in each column followed by the same letter (s) did not differ at P< 0.05 according to Duncan multiple- rage test.

Data in Table (4) on host suitability of the tested rose cultivars reveal that none of these cultivars was immune to *M. incognita* infection since galls and egg-masses were found on roots of all the tested cultivars depending upon their degree of resistance. It was found that two galls and no egg-masses were formed on root system of Sultany cultivar with values of RGI and EI averaged 1.3 and 0.0, respectively. On the other hand, the highest values of RGI and EI (3.0 and 2.0) were obtained from Taify cultivar, whereas, Gory cultivar was moderately infected with nematode since RGI and EI values amounted to 2.0 and 1.0, respectively.

These findings are disagreed with the results of [12] who recorded that root-knot and lesion nematodes are the most damaging plant parasitic nematodes in roses. Although their varietal differences in tolerance towards these nematodes there are no resistant rootstocks. In 1982, [11] mentioned that rose rootstocks vary in host suitability for various species of pathogenic nematodes; as well as rootstocks that are regionally adapted and resistant to a broad spectrum of pathogenic nematodes have not been developed at present.

CONCLUSION

Apparently, we can concluded from the present study that among soil samples collected from rose farms of the three localities, the plant parasitic nematode genera, *Meloidogyne, Rotylenchulus*, *Xiphinema* and *Pratylenchus* seemed to be the most prevailing pests. Soil samples recovered from Al-Hada district revealed the presence of the highest number of nematode genera in the surveyed localities of rose farms, followed by gardens of Taif University, then Al-Shafa district. Among two rose cultivars surveyed, Taify rose cultivar encountered the largest number of nematode genera. Regarding the screening study, results showed that none of the three tested rose cultivars was immune to *M. incognita* infection, while Sultany cultivar can be classified as resistant host, whereas Taify rose was rated as susceptible host and Gory cultivar as tolerant one.

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REFERENCES

[1] Fox JA. Mississippi State University Extension Service Publication 2001; 483.
[2] Sasser JN, Freckman DW. Vistas on Nematology, edited by J. A. Veech and D. W. Dickson, Hyattsville, MD, 1987; pp. 7–14.
[3] Netscher C, Sikora RA. Plant parasitic nematodes in subtropical and tropical agriculture. M. Luc, R. A. Sikora and J. Bridge, eds., CAB International, 1990; pp. 237-284.
[4] Dale PS. New Zealand J Exp Agr 1973; 1: 121-122.
[5] Goodey JB. Laboratory methods for work with plant and soil nematodes. Tech. Bull. No.2 Min. Agric. Fish Ed. London, 1957; pp. 47.
[6] Mai WF, Lyon HH. Pictorial key to genera of plant-parasitic nematodes. Ithaca, NY: Cornell University Press; 1975.
[7] Taylor AL, Sasser JN. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Coop. Publ., Dep. Plant Pathol., North Carolina State Univ., and U.S. Agency Int. Dev., Raleigh, NC.; 1978: pp. 111.
[8] Franklin MT. J Helminthol. 1949; 23: 175-178.
[9] Gomez KA, Gomez AA. Statistical Procedures for Agricultural Research. 2nd Ed., John Wiley&Sons. Inc. New York; 1984: pp. 680.
[10] Duncan DB. Biometrics 1955; 11: 1-42.
[11] Lehman PS. Nematology Circular 1982; No.92.
[12] Ploeg A. Research Updates: UC Riverside Nematodes in Roses Grown in Rockwool. CORF News 2007; 11 (1): pp. 15.