

# Survey of Phytonematodes Associated with *Juniperus phoenicea* L. and Identification of *Xiphinema pachtaicum*. In Al-Jabal Al-Akhdar Region, Libya

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**Abstract** – Survey was carried out during 2013 – 2014, covering Al-Jabel Al-Akhdar region in Libya. To determine the frequency and abundance of plant-parasitic nematodes associated with wild trees *Juniperus phoenicea* L. A total of 68 compost soil and roots samples were collected from seventeen different locations in this region. Nematodes were extracted by sieving, Bearman funnel and root soaking, and identified to genus or species level. The results indicated that the common eight genera of plant-parasitic nematodes were identified as: *Aphelenchus* spp., *Aphelenchiodes* spp., *Helicotylenchus* spp., *Pratylenchus* spp. *Paratylenchus* spp., *Tylenchorhynchus* spp. *Tylenchus* spp., *Xiphinema* sp. The genera of *Aphelenchus* spp. was found in 52.49% of the soil samples. The dagger nematode species was identified as *X. pachtaicum* using morphology and morphometrics of adult females, and this is the first record of this species on *Juniperus phoenicea*.

**Keywords** – Digger Nematodes, Libya, Arar, Nematodes and Forest Tree.

## I. INTRODUCTION

Al-Jabel Al-Akhdar (local name mean Green Mountain) is located in north east Libya. Contains the largest natural wild forest in Libya. The *Juniperus phoenicea* L. is a species of tress occurring naturally in southern Europe, south Asia and northern Africa. In Libya, it distributes in Al-Jabel Al-Akhdar and it constitutes approximately 80% of the total vegetation of this region [1]. Recently Phoenician juniper (local name is ArAr) suffered from dieback by a high percentage. Twenty five plant parasitic genera have been reported to be associated with *Juniperus phoenicea* in Saudi Arabia by several authors which summarizes in (Table 1).

Table 1. Plant parasitic nematodes associated with Arar recorded in Saudi Arabia (Adopted from [2]).

Nematodes	References			
	[3]	[4]	[5]	[6]
<i>Aphelenchoides</i> spp.	+	+	+	-
<i>Aphelenchus</i> spp.	+	+	+	-
<i>Cacopaurus</i> spp.	+	-	-	-
<i>Criconemella</i> spp.	+	+	+	-
<i>Ditylenchus</i> spp.	+	+	+	-
<i>Helicotylenchus</i> spp.	-	+	+	-
<i>Hemicriconemoides</i> spp.	+	-	-	-
<i>Hoplolaimus</i> spp	-	+	+	-
<i>Longidorus</i> spp	+	+	+	-
<i>Merlinius</i> spp	+	+	+	-

Nematodes	References			
	[3]	[4]	[5]	[6]
<i>Paratrichodorus</i> spp.	+	+	+	-
<i>Paratylenchus</i> spp.	+	+	+	-
<i>Pratylenchoides</i> spp.,	+	+	-	-
<i>Pratylenchus</i> spp	+	+	+	-
<i>Rotylenchus</i> spp.	+	+	+	-
<i>Scutellonema</i> spp.	+	+	+	-
<i>Subanguina</i> spp.	+	+	+	-
<i>Telotylenchus</i> spp.	+	-	-	-
<i>Tetylenchus</i> spp.	+	+	-	-
<i>Trichodorus</i> spp.	+	+	+	+
<i>Tylencholaimus</i> spp	+	+	+	-
<i>Tylenchorhynchus</i> spp.	+	+	+	+
<i>Tylenchus</i> spp.	+	+	+	+
<i>Xiphinema</i> spp.	+	+	+	-
<i>Zygotylenchus</i> spp.	+	+	-	-

Digger nematode is one of the most important ecto-plant parasitic nematodes, it includes more than 260 species [7]. Besides the direct damage caused by them, some of these species are plant viruses vector. *Xiphinema* genera is divided into two groups, *Xiphinema americanum* group and non-*Xiphinema americanum* group. *Xiphinema pachtaicum* is a member of *Xiphinema americanum* group which includes 51 species. This species has a wide host range of plants including vegetables, fruits, weeds and forest trees. *X. pachtaicum* is a widely distributed species in the Mediterranean region [7, 8]. This species has not been recorded as a vector of plant viruses [9]. *X. pachtaicum* had been recorded in Al-Jabel Al-Akhdar, Libya on apple [10], on grape [11] and on cucumber [12] and on fig and several ornamental plants (unpublished Data). Other member of this group is *Xiphinema diffusum* which recorded on Mango trees at El-kufra region of Libya [13]. *X. index* member of non - *Xiphinema americanum* group found on grape in Al-Jabel Al-Akhdar region of Libya [14]. The objective of this study was identifying of plant parasitic nematodes associated with *Juniperus phoenicea* trees in Aljabel Alahkther region in Libya and identify the *Xiphinema* species.

## II. MATERIAL AND METHODS

### *Samples Collection:*

A total of 68 soil and root samples were collected from seventeen different locations in this region at deep of 50cm. Three samples from each location, Size of the sample was about one Kg soil including roots.

### Nematodes Extraction

**From soil:** Nematodes in soil were extracted from 250cc soil using Modification of sieves and Bearman funnel. The extracted nematodes were counted in Hawksly slide and identified under light microscope 100X magnification.

**From roots:** Roots of plant were gently washed by tap water. Then, roots were cut into small pieces and an aliquot of 5 g roots were extracted by incubation method according to [15].

### Nematode Identification:

The surveyed nematodes were identified to generic level according to [16]. Species of digger nematodes were identified depending morphological characteristic and morphometrics of female according to [17, 18]. Nematodes were killed and fixed in TAF and mounted in glycerin following the Seinhorst slowing method [19]. For morphological study, specimens were photographed using Lica microscope DM 1000 LED (Microsystem CMS, GmbH).

### Nematode Estimation:

Population density (PO) (Mean no. of a given genus in the samples) and frequency of occurrence% (PD) (No. of samples containing a given genus/no. of whole samples collected X100) were calculated for each nematode genus.

## III. RESULTS AND DISCUSSION

Results indicated that eight style-bearing nematodes were recorded from the seventeen locations in the sample regions of Al-Jabel Al-Akhdar of Libya (Table 1). The findings was summarized as following; *Aphelenchus* spp. (Bastain, 1865), *Aphelenchoides* spp. (Fischer, 1894), *Helicotylenchus* spp. (Steiner, 1945), *Paratylenchus* spp. (Micoletzky, 1922), *Pratylenchus* spp. (Fillipjev, 1936), *Tylenchorhynchus* spp. (Cobb, 1913), *Tylenchus* spp. (Bastian, 1936) and *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951.

The highest percentage of frequency was recorded *Aphelenchus* spp. (52.49) with average number of 5.38 nematodes per sample. Followed by The spiral nematode *Helicotylenchus* spp. (48.52) and average number 33.30 nematodes per sample. Stunt nematode *Tylenchorhynchus* spp. (30.88%) with average number of 4 nematodes per sample. The spin nematode *Paratylenchus* spp. and *Tylenchus* spp. was found at the same frequency with (25%) and with average of 6.11 and 16.64 nematodes per sample respectively. The lesion nematode *Pratylenchus* spp. (19.11%) and average of 13.61 nematodes per sample. Followed by the digger nematode *X. pachtaicum* (13.23%) and abundant of 2.66 nematodes per sample. Finally *Aphelenchoides* spp (8.82%) and average number of 8.32 nematode per sample.

The funding results agree with other research work conducted in Al-Jabel Al-Akhdar region where these genera were recorded on fruit crops [10], Solanaceae [20] and on some Cucurbitaceae crops [21] and on some ornamental plants [22]. Moreover the most important plant parasitic nematodes with high frequency and abundant recorded in this study was *Helicotylenchus* spp. support

the previously researches on different hosts [10, 20, 21].

Table 1. Frequency occurrence and population density of nematode genera linked with associated with *Juniperus phoenicea* L. in Al-Jabel Al-Akhdar region, Libya.

Nematodes	% frequency	No of nematodes / sample
<i>Aphelenchus</i> spp.	52.94	5.38
<i>Aphelenchoides</i> spp.	8.82	8.33
<i>Helicotylenchus</i> spp.	48.52	33.30
<i>Paratylenchus</i> spp.	25.00	6.11
<i>Pratylenchus</i> spp.	19.11	13.61
<i>Tylenchorhynchus</i> spp.	30.88	4.00
<i>Tylenchus</i> spp	25.00	16.64
<i>X. pachtaicum</i>	13.23	2.66

Also this results agree with plant parasitic nematode on associated with ArAr in Saudi Arabia by several authors which summarizes in (Table 1).

### *Xiphinema pachtaicum*

Description of *Xiphinema pachtaicum* female body C shaped after fixation. Lip region distinctly off set by constriction (Fig. 1). Body length 1.87-2.07 mm. Odontostyle length 82.9-90.5µm. Odontophore length 50.4-55 µm. Total length of style is 130.7-140 µm. oral aperture guide ring 79.7-81.9 µm. Tail length 28.2-32 µm. Value of a ratio 60.9-71.8; Value of c ratio 59.9-70.1, Value of c' ratio 1.5-1.8; value of V% 55-58; Oral aperture guide ring 79.7-81.9 µm; Body diameter at mild body 27.6-32.9 µm; Body diameter at lib region 7.5-10.9 µm (Table 3). Tail short, conical with narrow rounded end (Fig. 1). Male. Not found.

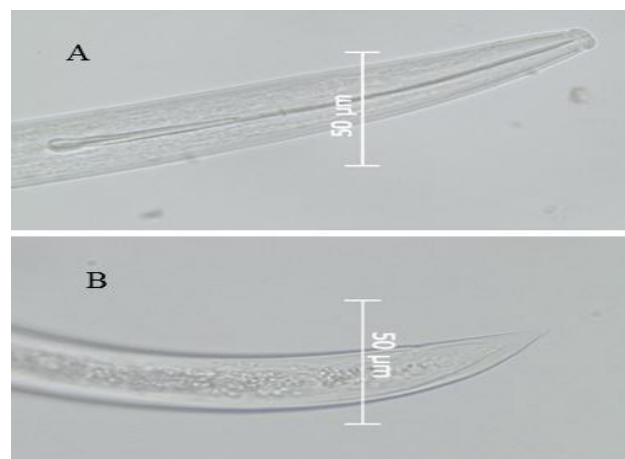


Fig. 1. *Xiphinema pachtaicum*, A, anterior region of female; B female tail. Scale bar: 50µm.

The alpha-numeric polytomous identification key codes as developed by OPPE [18] to be applied for the studied *X. pachtaicum* of the *Xiphinema americanum* group are agree with the studied population as described in table 3.

*Xiphinema* species is identify as *Xiphinema pachtaicum* based on morphology and morphometric study (Table 2 and 3). This species is for the first time recorded on

*Juniperus phoenicea* in the world which grow naturally in Al-Jabel Al-Akhdar region of Libya. This study is agree with reported of distribution of *X. pachtaicum* in Mediterranean region [8]. In Libya *X. pachtaicum* had been identified in Al-Jabel Al-Akhdar region on apple [10], on grape [11] and on cucumber [12] and on fig and several ornamental plants (unpublished Data).

**Morphometric:**

Table 2. Morphometric measurements of *Xiphinema pachtaicum*. All measurements are in  $\mu\text{m}$ , measurements except for length (mm) presented as (range) and mean  $\pm$  standard deviation.

Character	(Range) Average $\pm$ SD
n	10
L (mm)	(1.87-2.07) 1.97 $\pm$ 0.56

Character	(Range) Average $\pm$ SD
a	(60.9-71.8) 64.3 $\pm$ 4.4
c	(59.9-70.1) 64.3 $\pm$ 3.8
c'	(1.5-1.8) 1.7 $\pm$ 0.13
V%	(55-58) 56.2 $\pm$ 1.3
Odontostyle	(82.9-90.5) 86.7 $\pm$ 4
Odontophore	(50.4-55) 51.9 $\pm$ 2.6
Style	(130.7-140) 136.8 $\pm$ 3.8
Oral aperture guide ring	(79.7-81.9) 80.8 $\pm$ 1
Tail	(28.2-32) 31.1 $\pm$ 2.3
Body diameter at mild body	(27.6-32.9) 30.7 $\pm$ 1.7
Body diameter at lib region	(7.5-10.9) 9.3 $\pm$ 1.2

n = number of specimens; a = body length/greatest body diameter;; c = body length/tail length; c' = tail length/anal body diameter; V% = distance of vulva from anterior end.

Table 3. Alpha-numeric codes of the polytomous identification key for *Xiphinema americanum sensu lato* species by Bulletin OEPP/EPPO Bulletin (2017) of *Xiphinema pachtaicum* and the studied specimens.

Characters	A	B	C	D	E	F	G	H	I	J
<i>Xiphinema pachtaicum</i>	2	1	1	12345	234	1234	123	123	123	123
studied specimens	2	1	1	34	3	3	2	23	12	2

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