
First Report of *Asphodelus microcarpus* as a New Host of Stunt Nematode, *Tylenchorhynchus* spp., on the Western Mediterranean Coast of Egypt



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ABSTRACT

A survey of phytoparasitic nematodes was carried out in 2023 on natural habitats of some wild plants viz., *Limbarda crithmoides*, *Asphodelus microcarpus*, *Euphorbia paralias*, *Ononis vaganalis*, *Thymelaea hirsuta*, *Neurada procumbens* and *Suaeda monoica*, growing in the western Mediterranean coastal areas of Marsa Matrouh governorate, Egypt. Specimens of stunt nematode (*Tylenchorhynchus* spp.) were collected from rhizosphere soil of native plant *Asphodelus microcarpus* (Family: Asphodelaceae). Morphological characters of females were utilized for identification of stunt nematode. According to available literature, this is the first report of *Tylenchorhynchus*' presence on this wild plant native in Egypt. Other plants collected in this survey did not host plant parasitic nematodes either in their roots or in their rhizosphere soil. Further surveys should be conducted to identify phytonematodes genera that might occur in Egypt, including wild plants in desert areas. Pathogenicity of *Tylenchorhynchus* genus on *Asphodelus microcarpus* will be investigated, also the spreading of stunt nematode should be explored as well as other nematode taxa to enrich the biodiversity index.

Keywords: First report, Stunt nematode, *Tylenchorhynchus*, *Asphodelus microcarpus*.

INTRODUCTION

Historically, plant parasitic nematodes (PPNs) have been discovered in Egypt since 1901 by Preyer, who reported a nematode disease (He referred to the causative agent of the disease as *Tylenchus*) in some banana plantations in Alexandria. Ibrahim et al. (2000) reported about fifty four genera of plant parasitic nematodes associated with various plants whether cultivated or naturally growing in Egypt. The abundance of information concerning phytonematodes incidence, host plants and diversity is necessary since nematodes, such as root-knot nematodes, lesion nematode and others are serious plant pathogens. Additional benefit of these surveys is enriching the biodiversity index with undiscovered organisms (Abou-Elnaga, 1979 and 1989; Ibrahim, 1990; Ibrahim and El-Sharkawy, 2001; El-Nuby, 2020).

Stunt nematodes is the common name of the members under the genus *Tylenchorhynchus* Cobb, 1913, members of this genus represent one of the hugest groups of phytonematodes. The stunt nematode attacks the roots of many plant hosts, external parasitism, mainly they are considered ectoparasites and rarely endoparasitic, including cultivated and natural plants (Siddiqi, 2000; Handoo et al., 2014). These nematodes usually occupy the rhizosphere soil of plants, all mobile juveniles and adults feed on epidermal cells of roots using their stylet. They are obligate parasites, whether migratory or ectoparasites of plant roots (Maggenti, 1981; Mai et al., 1996). Plant harm

caused by increased populations of *Tylenchorhynchus* spp. may be more serious and affecting plant production in small areas and/or crowded plants like nurseries than the damage in large areas like cultivated fields. It is difficult to assess crop injury in fields due to *Tylenchorhynchus* spp. because of its interaction with other nematode genera or more than one stunt nematode species (Koenning et al., 1999). However, *Tylenchorhynchus claytoni* could be reported to cause significant economic damage on tobacco (Mai et al., 1996).

Egyptian desert areas are subjected only to few nematological surveys, accordingly, the aim of this study is to detect phytonematodes presence associated with some wild plants inhabiting Mediterranean coastal regions of Egypt, also to refer to the impact of plant parasitic nematode as a challenge to wild plants establishment, survival and diversity in coastal areas and the cultivated plants in adjacent locations.

MATERIALS AND METHODS

Sampling

Plants with their rhizosphere soil were collected during the year of 2023. A total of fifty six soil and root samples were collected, about 1kg of root-associated soil samples were taken in clean plastic bags then labeled and preserved in ice box till reach to the Laboratory of Nematology, Desert Research Center (DRC) for nematodes extraction. Whole plants were also collected in a paper bags for identification in the DRC Herbarium.

Plant identification

The collected plants were authenticated by DRC Herbarium and Plant Taxonomy unit, Egypt. A voucher specimens had been deposited in the DRC Herbarium. Plants were identified to species levels (Boulos & El-Hadidi, 1994 ; Boulos, 2002).

Nematode extraction and morphological study















Nematodes were extracted from soil and root samples using Cobb's sieving and decanting technique followed by a modified Baermann technique (Hooper et al., 2005). Soil samples were thoroughly mixed and 250 cm³ aliquots were taken for nematode extraction. Single sample was put in two liters- cup filled with water, then stirred by aiding plastic rod and allowed to settle coarse soil particles and any large debris, the supernatant was poured on set composed of three sieves, the lowest one have apertures size of 45 µ this operation was repeated two times. The nematodes retained on the last sieve were collected in glass beaker then this solution was poured on Baermann tray for clarification, cleaning and migration of mobile nematodes. Plant parasitic nematodes were killed and fixed in 4% hot formalin with 1% glycerol and processed in glycerin (Seinhorst, 1959), identified to genus level and examined under a compound microscope (Hooper, 1970). Nematode identifications were based on the morphology of adult and juveniles forms (Mai & Lyon, 1975; Handoo & Golden, 1989 and Siddiqi, 2000).

RESULTS AND DISCUSSION

Collected plants were identified by the aid of taxonomy unit (Herbarium) in Desert Research Center (DRC). Each voucher representing a collected plant was deposited at DRC Herbarium. The identified plants were *Limbarda crithmoides*, *Asphodelus*

microcarpus, *Euphorbia paralias*, *Ononis vaganalis*, *Thymelaea hirsuta*, *Neurada procumbens* and *Suaeda monoica*. Photos for such plants were taken to document the survey steps (Table 1).

Table 1: Photos and Global Positioning System (GPS) coordinates of wild plants collected in a survey from western Mediterranean coast of Egypt.

N (latitude)	E (longitude)	Photo in natural habitat	Photo in Lab
31° 29' 350''	026° 37' 821''		
		<i>Limbarda crithmoides</i>	
31° 28' 966''	026° 41' 716''		
		<i>Asphodelus microcarpus</i>	
31° 28' 030''	026° 44' 967''		
		<i>Euphorbia paralias</i>	
31° 27' 478''	026° 47' 523''		
		<i>Ononis vaganalis</i>	
31° 26' 471''	026° 50' 265''		
		<i>Thymelaea hirsuta</i>	
31° 26' 381''	026° 56' 106''		
		<i>Neurada procumbens</i>	
31° 24' 614''	027° 00' 692''		
		<i>Suaeda monoica</i>	

Our investigations using compound and image microscope indicated that morphological features of isolated nematode matched *Tylenchorhynchus* spp. (Fig. 1 & 2). These features were characterized by length of body about one mm or less, with clear-annulation. The body is ventrally curved or C shaped (Fig. 1 C). There is an areolation at mid-body and tail region clearly observed in the majority of individuals. Head region is dome shaped and slightly offset from the rest of the body. The labial framework is not sclerotized. Stylet is well-developed with rounded knobs, median bulb is oval, isthmus is slender; pharyngeal basal bulb is saccate and be based on intestine (Fig. 3).

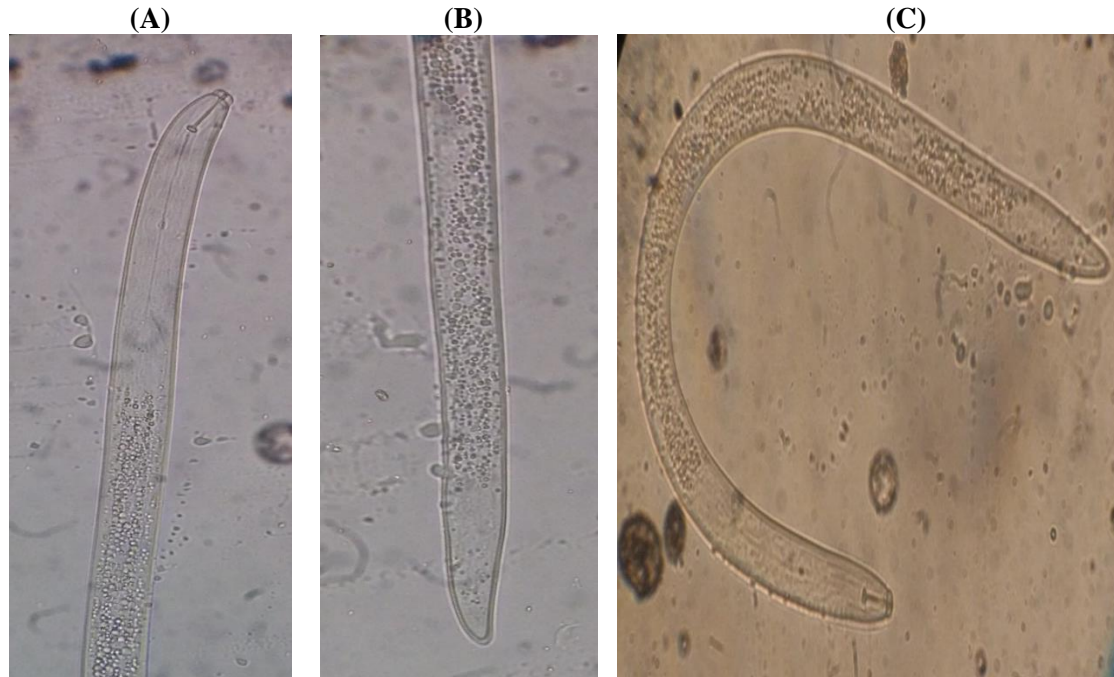


Figure 1: Anterior part of body (A), Posterior part of body or tail (B) and the whole body like C shape (C) of the genus *Tylenchorhynchus*



Arrow refers to vulval opening (A)

Strong stylet with distinct knobs (B)

Figure 2: Vulval opening in the middle of body (A), Strong stylet with distinct knobs (B) of the genus *Tylenchorhynchus*



Figure 3: Arrow refers to pharyngeal basal bulb, which is saccate and abutting intestine of the genus *Tylenchorhynchus*

Excretory pore is located at the anterior region of basal pharyngeal bulb. Vulva is a transverse slit, and vagina is v shaped comprising less than half of the corresponding diameter and the position of vulva is approximately in the middle of the body (Fig. 2A) and this is in accordance with Siddiqi (2000). The tail is elongated and conical with a terminal hyaline region, ending as bluntly pointed tip (Fig. 1). Occurrence of the nematode genus *Tylenchorhynchus* spp. associated with *Asphodelus microcarpus* (Family:Asphodelaceae –formerly placed in Liliaceae) is considered a new record in Egypt and *A. microcarpus* found to be a new host of stunt nematode. *A. microcarpus* as a plant colonizes various habitats along the western Mediterranean coast of Egypt. These habitats include saline soils, coastal dunes, inland dunes, loose soil not in the form of dunes, deep compact soil, moderately deep soil and/or shallow soil. The highest abundance of this plant was found in inland dunes and moderately deep soil (Ayyad and Hilmy, 1974). Abundance of *A. microcarpus* is affected and controlled by the moisture availability, CaCO_3 percentages and the total nitrogen in the soil. Unpalatability and growth by rhizomes may increase the abundance of the plant in grazing areas (Ayyad and Hilmy, 1974). The medical uses of *A. microcarpus* forced us to preserve it from extinction as well as in good health. This plant is used in traditional medicine for treating abscesses by local application of the powder, relieving ear pain and treating white spots. Many studies revealed that its aerial part, root, flower, leave and seed are used, other reported medicinal uses like Rheumatoid arthritis, Bronchitis, asthma, diuretic, otitis, toothache, jaundice, psoriasis, lung diseases, skin diseases and recently its anti-inflammatory properties have been proven (Mayouf et al., 2019)

More studies on the same host are needed to determine the adverse impact of various populations and inoculum load of stunt nematode on *A. microcarpus* growth. Also reproductive index of this nematode genus, besides its ability to infect with root-knot nematode, the most dominant genus in Egypt, should be examined to know if this plant is favored host of this nematode or not and determining its degree of susceptibility to avoid nematode damage.

CONCLUSION

The finding of the genus *Tylenchorhynchus* help in enriching biodiversity index of nematode fauna and adding new hosts for specific nematode genera. Depending on the available literature and knowledge, this is the first record of occurrence of stunt nematode *Tylenchorhynchus* spp. associated with *Asphodelus microcarpus* in Egypt.

Results reported herein represent a valuable database that open the way for further research to study the life cycle of stunt nematodes on *A. microcarpus* and examine other wild plants as new hosts. This study also draws attention to identify means of phytonematode transmission in these coastal areas. Additionally, the presence of plant parasitic nematodes associated with any plant may be transmitted and affect other neighbor plants whether wild or cultivated; so preventing their transmission must be considered.

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الملخص العربي

تقرير أول عن تواجد نيماتودا التقزم علي نبات *Asphodelus microcarpus* في الساحل الشمالي الغربي، مصر

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أجريت هذه الدراسة المسحية للتعرف علي مجتمع نيماتودا النبات المرافق لبعض النباتات البرية، (*Limbarda crithmoides*, *Asphodelus microcarpus*, *Euphorbia paralias*, *Ononis vaganalis*, *Thymelaea hirsuta*, *Neurada procumbens* and *Suaeda monoica*)، في موائها الطبيعية في المناطق الساحلية لغرب البحر الأبيض المتوسط التابعة لمحافظة مرسى مطروح، مصر. أظهرت النتائج وجود نيماتودا حرة المعيشة متنوعة في كل العينات، أما بالنسبة للنيماتودا المتطفلة علي النبات فقد تم تسجيل جنس واحد هو *Tylenchorhynchus* (نيماتودا التقزم) مرافق نبات بري سائد في الساحل الشمالي الغربي وهو *Asphodelus microcarpus* (بروق-عنصل) التابع للعائلة البروقية *Asphodelaceae*. ووفقاً للمراجع المتاحة يعد هذا التقرير أول تسجيل لنيماتودا التقزم *Tylenchorhynchus* المتطفلة نباتياً علي عائل جديد (بري) وفي بيئة صحراوية تحت الظروف المطرية بالساحل الشمالي الغربي بمصر. هذه النتيجة تفتح الباب لمزيد من الدراسات الخاصة بإجراء المزيد من المسوحات لتحديد أجناس النيماتودا المتطفلة نباتياً التي قد تتواجد في مصر، سواء مرافقة لنباتات منزوعة أو النباتات البرية في المناطق الصحراوية. وكذلك دراسة القدرة المرضية لجنس *Tylenchorhynchus* علي نبات العنصل، كما ينبغي البحث في مدى إنتشار هذه النيماتودا بالإضافة إلى أنواع النيماتودا الأخرى لإثراء مؤشر التنوع البيولوجي