

# The First Study of The Nematode Communities Associated With Medicinal Plants In Algeria

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## ABSTRACT

Nematodes play a major role in decomposition and nutrient cycling in soil food webs. But, the plant parasitic nematodes can coexist in the plant's rhizosphere and cause serious damages. For this, an inventory was made on nematode species associated with five medicinal plants (*Thapsia garganica*, *Artemisia herba alba*, *Artemisia campestris*, *Ziziphus lotus* and *Peganum harmala*) covering an area of 200 ha in the natural reserve of Mergueb (35°35'39"N 03°56'45"E) located in the north of Algeria: M'sila-Ain Elhadjel. During the year 2013, one soil sample with a weight of 01 kg, formed by 10 sub-samples, was taken monthly per plot using a soil auger approximately 30 cm deep. In the laboratory, each soil sample was thoroughly mixed and nematodes were extracted from 250 cm<sup>3</sup> soil using flotation and sedimentation technique described by Dalmasso (1966). Under the dissecting microscope, 09 genus of nematodes were identified: Aphelenchoides, Cephalobus, Chiloplacus, Discolaimus, Dorylaimus, Ditylenchus, Tylenchus and Tylenchorhynchus belonging to seven families: Aphelenchoididae, Dorylaimidae, Cephalobidae, Dorylaimidae, Anguinidae, Tylenchidae, Belonolaimidae and four trophic groups: plant-parasitic nematodes, fungi vorous nematodes, bacterivorous nematodes and omnivorous predator nematodes. The result showed that the most dominating group was the omnivorous predator nematodes (Discolaimus and Dorylaimus) followed by the bacterivorous (Cephalobus and Chiloplacus) and plant-parasitic nematodes (Tylenchorhynchus) while those of fungivorous species (Aphelenchus, Aphelenchoides, Ditylenchus and Tylenchus), which belong also to the last group, reached the least value. The results showed also that the genus Discolaimus, Ditylenchus and Tylenchus were mainly present in the rhizosphere of *Peganum harmala* and only the plant – parasitic nematode Tylenchorhynchus was observed highly in the rhizosphere of *Artemisia herba alba*, *Artemisia campestris* and *Ziziphus lotus*. This study express that these medicinal plants can offer treatment helminthic methods that are more environmentally benign, since they tend to be less toxic.

**KEYWORDS:** inventory, natural reserve, trophic groups, pine wood nematode.

## INTRODUCTION

In an ongoing effort to preserve from diseases or to find methods to relieve pain, Natural products have contributed greatly to the development of modern therapeutic drugs over the years which are frequently considered to be less toxic and induce fewer side effects than synthetic ones. Recently, the greater demand on medicinal plants throughout the world in medicine, phytochemicals, nutraceuticals, cosmetic and other products, they have become a major sector of commerce [16].

In Algeria, vegetation is diverse flora and medicinal plants that form the canopy, is the genus: *Thapsia*, *Artemisia*, *Ziziphus* and *Peganum*. These plants are widely distributed especially in semi-arid regions. Many species of this genus are used in traditional medicine because they contain many molecules with therapeutics' activities, and its biological properties.

*Artemisia herba-alba* (white wormwood, "Chih") and *Artemisia campestris* (Tgouft) are a medicinal plants belonging to family of Asteraceae; the first plant rich in essential oils, the leaves of this species are used in the Middle East traditional medicine to treat diabetes [2]. Herbal infusions from this species have been used as analgesic, antibacterial, and hemostatic agents [33]. While the second plant used in the treatment of many infections, such as urinary infections and hepatitis [29]. Sefi et al. [30] found that the leaves aqueous extract of *A. campestris*, lowers glucose levels in rat plasmas companied by a decrease in triglyceride and low density lipoprotein (LDL), and by an increase in the level of insulin. *Zizyphus lotus* (Sedra) is a medicinal plant belonging to the family of Rhamnaceae used in decoction as pectoral, emollient, sedative and diuretic. Tunisian traditional pharmacopoeia, used *Z. lotus* for treatment of pulmonary bronchitis, diabetes, such as Anti-inflammatory and analgesic [28,7]. *Peganum harmala* (El-harmel) is a medicinal plant belonging to the Zygophyllaceae family used among societies to treat hypertension, cardiac disease worldwide [32] and some nervous system disorders such as Parkinson's disease [21] in psychiatric conditions [18] such as nervosity [1] and to relieve rigorous pain [11]. Various studies have shown different antiparasidal [3], antifungal, antibacterial [26] and insecticidal [19].

Today, much research on nematological problems of these crops has been done and published. However, problems of medicinal plant don't pay sufficient attention in Algeria. For this, The aim of the present study was to analyze the biodiversity of plant-associated nematodes communities recovered from five medicinal plants (*Thapsia garganica*, *Artemisia herba alba*, *Artemisia campestris*, *Zizyphus lotus* and *Peganum harmala*) and further to describepopulation patterns of different trophicgroups of these plants located in the natural reserve of Mergueb (Ain Elhadjel-M'sila).

## MATERIEL AND METHODS

### 1. Site description and climate:

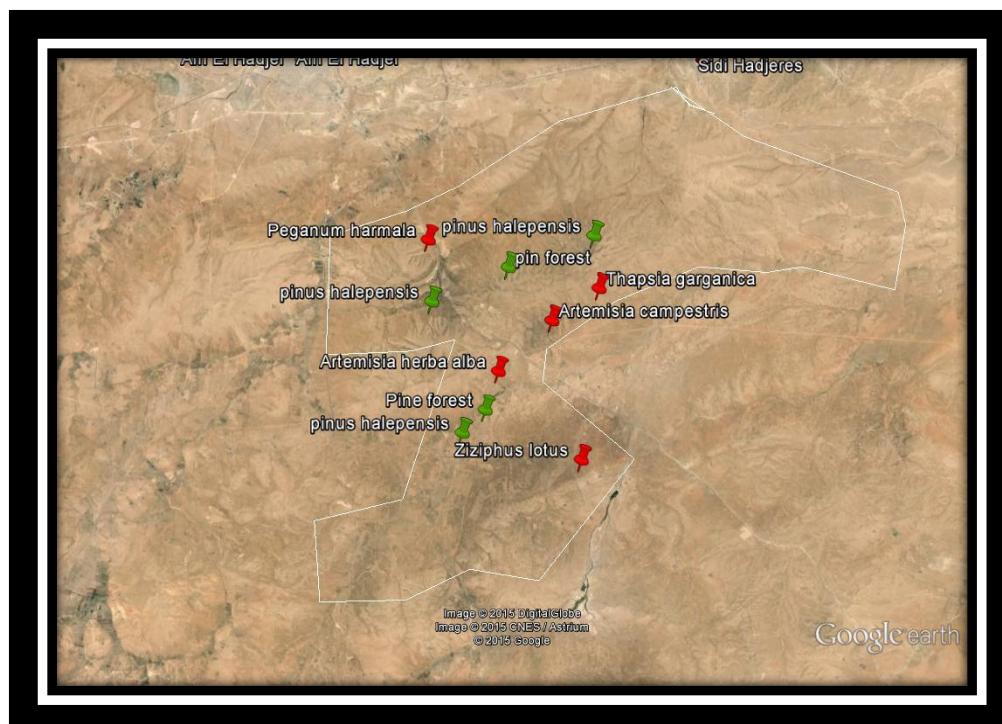
A survey on nematode genera has been carried out for five medicinal herbs (*Thapsia garganica*, *Artemisia herba alba*, *Artemisia campestris*, *Zizyphus lotus*, *Peganum harmala*) covering an area of 200 ha near the pine's forest (2000 ha) fig (2). The fallow land used in this study was located in the north of Algeria: M'sila- Natural reserve of Mergueb (35°35'39''N 03°56'45''E) at a distance of 180 km to south of Algiers, 55 km North of Boussaâda and 10 km South of Ain el Hadjel [6]. This reserve is spread overran area of 16,481ha and 43 acres. Its environment belongs to Hodna plain bounded by the Tell Atlas in the north and Saharan Atlas in the south and characterized by Mediterranean climate (sub-continental) [31] fig. (1). Rainfall is mainly in winter and spring, where annual rainfall on average is 155, 4 mm.



**Fig. 1:** Geographic Situation of Ain El-Hadjel area and the natural reserve of Mergueb.

## 2. Methodology:

The soil's samples were withdrawn from five stations in the natural reserve of Mergueb during the months of 2013. These stations are chosen according to the dominance of plant of white wormwood, *Artemisia fields*; *rue de Syrie*, *Jujubier* and *Thapsia garganica*)fig. (2). The samples made consist of sub-samples with a weight of 80g to 100g of soil of rhizosphere's plant due to sample all the ten meter on diagonal projection with an area of ten hectares. These samples are placed in bags bearing referenced the date, the place and the dominant plant. The method's extraction of nematodes used is that buckets Dalmasso [9], named methods of flotation and sedimentation. It is based on density differences between the nematodes and the different parts of the ground. It allows us to extract the nematodes in different sizes of soil by layering different sieve mesh. These nematodes were identified and counted under binocular microscope and with the key of morphological determinations of Mai and Lyon [22], Yeates and et al. [38] and Brzeski [8]. The abundance of nematodes was expressed as the number of nematodes per  $\text{dm}^3$  ( $N / \text{dm}^3$ ) [35].



**Fig. 2:** Repartition of plants in the natural reserve of Mergueb.

## 3. Statistical analysis:

Statistical analysis was performed using software SYSTAT vers. 12, SPSS 2009 and Excel <sup>TM</sup>. The means were separated by global linear model (G.L.M) at a level of significance  $\alpha = 5\%$ . The correlation between abundance and distribution of nematodes was analyzed by DCA (Detrended Correspondence analysis), and was followed by CAH (Hierarchical classification analysis) with the PAST logiciel - Palaeontological Statistics, ver. 1.81. [14].

## Results:

### 1. Nematodes taxa found in the natural reserve of Mergueb:

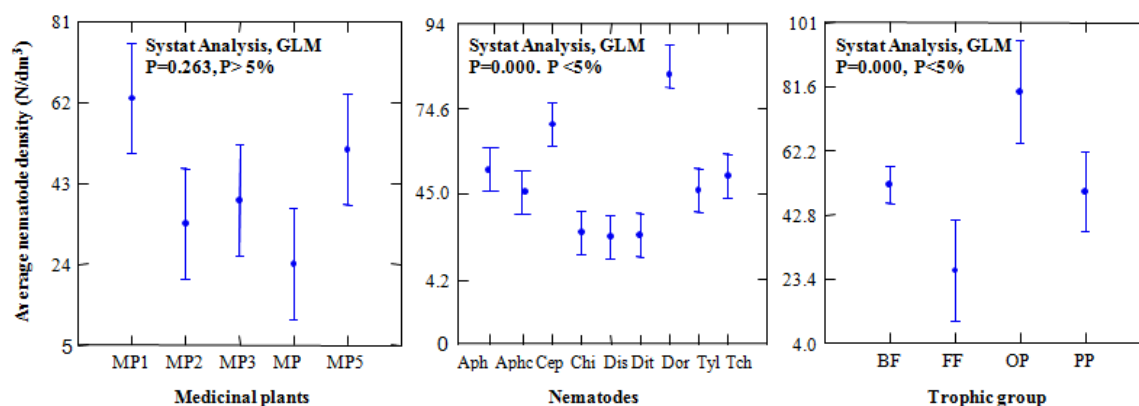
The survey of nematode of North Algeria revealed the presence of 09 genus of nematodes represented by: Aphelenchoides, Cephalobus, Chiloplacus, Discolaimus, Dorylaimus, Ditylenchus, Tylenchus, Tylenchorhynchus and belonging to seven families (Aphelenchoididae, Dorylaimidae, Cephalobidae, Dorylaimidae, Anguinidae, Tylenchidae et Belonolaimidae). The dominant taxa was represented by Dorylaimus and Cephalobus genus with mean relative abundances were 38.27% and 27.27% respectively (Table no.1). Similar tends of average abundance were notes for the other nematode populations.

**Table 1:** Frequency/ Abundance of nematode populations found in spatial sampling on the study areas.

Genus	Family	Trophic group	C-P	Frequency (%)	Absolute Abundance (N/dm <sup>3</sup> )	Relative Abundance (%)
<i>Dorylaimus</i>	Dorylaimidae	OP	4	100	400	38.27
<i>Discolaimus</i>	Dorylaimidae	OP	5	20	5	0.47
<i>Cephalobus</i>	Cephalobidae	BF	2	60	285	27.27
<i>Chiloplacus</i>	Cephalobidae	BF	2	20	40	3.83
<i>Aphelenchus</i>	Aphelenchidae	FF-PP	2	60	130	12.44
<i>Aphelenchoides</i>	Aphelenchoididae	FF-PP	2	80	35	3.35
<i>Tylenchus</i>	Tylenchidae	FF-PP	2	40	30	2.87
<i>Ditylenchus</i>	Anguinidae	FF-PP	2	40	30	2.87
<i>Tylenchorhynchus</i>	Belonolaimidae	PP	2	60	90	8.61
Total					1045	100

## 2. The nematode's fauna associated with the medicinal plants:

The total average number of nematodes associated with five medicinal herbs (*Thapsia garganica*, *Artemisia herba alba*, *Artemisia campestris*, *Ziziphus lotus*, *Peganum harmala*) represented by abundance of 1045 N/dm<sup>3</sup> (313.500 nematodes per m<sup>2</sup>). The results showed a significant differences between Trophic groups ( $P < 0.05$ ,  $p = 0.000$ ) under the medicinal plants hosts where the most abundant group is the nematodes of predatory-omnivorous (OP) compared to bacterivores's nematode (BF) and plant-parasitic's nematodes (PP) by using global linear model (G.L.M.). While, fungivorous nematodes which belong also to plant-parasitic nematodes species reached the least value. A significant difference was also observed between average nematode density ( $P < 0.05$ ,  $p = 0.000$ ) which the omnivorous nematode *Dorylaimus* was the dominant nematode species studied in this areas. Whereas, the nematodes's densities were not varied according to the medicinal plant host (Graph no. 1).



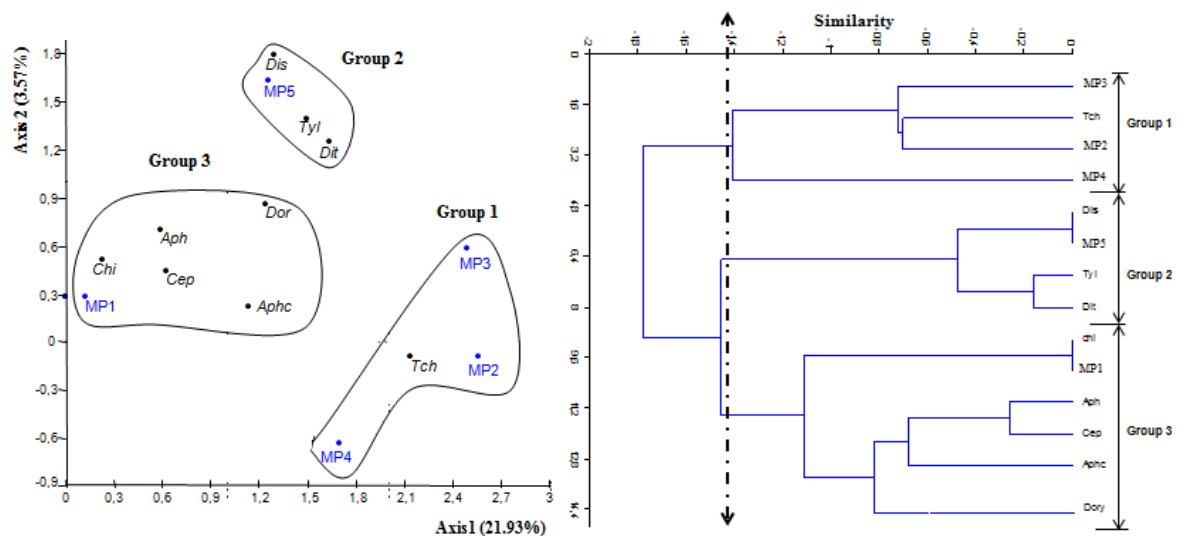
MP1: *Thapsia garganica*, MP2: *Artemisia herba alba*, MP3: *Artemisia campestris*, MP4: *Ziziphus lotus*, MP5: *Peganum harmala*, Aph: *Aphelenchus*, Aphc: *Aphelenchoides*, Cep: *Cephalobus*, Chi: *Chiloplacus*, Dis: *Discolaimus*, Dor: *Dorylaimus*, Dit: *Ditylenchus*, Tyl: *Tylenchus*, Tch: *Tylenchorhynchus*, BF: Bacterial feeders, FF: Fungal feeders, OP: Omnivorous- predator nematodes, PP: Plant parasitic feeders.

**Graph 1:** G.L.M. Model applied to the global average density (N/dm<sup>3</sup>) of diverse nematodes found in the study areas.

## 3. Spatial distribution of nematode's genus:

The analysis of the nematode's densities explains the affinity of some taxa compared to the prospected study areas by using the DCA (Detrended Correspondence analysis). The Euclidean's distance on similarity basis (- 1.4) showed the presence of three mixed groups (Graph no. 2) in which the first group gathers the taxa of *Tylenchorhynchus* in the rhizosphere of *Artemisia herba alba*, *Artemisia campestris* and *Ziziphus lotus*. The second group included *Discolaimus*'s, *Ditylenchus*'s and *Tylenchus*'s genus in the rhizosphere of *Peganum harmala*. The third group gathers *Aphelenchus*'s, *Aphelenchoides*'s, *Cephalobus*'s, *Chiloplacus*'s and *Dorylaimus*'s genus in the rhizosphere of *Thapsia garganica*.





A. Homogenous groups of nematodes B. Ascending hierarchical classification

**Graph 2:** The main component analysis (DCA) of the distribution of nematode genera, in the study areas.

#### Discussion:

The nematode analysis within five plants of rhizosphere showed that *Thapsia garganica*, *Artemisia herba alba*, *Artemisia campestris*, *Ziziphus lotus* and *Peganum harmala* reveal a wealth of 09 genera (Cephalobus, Chiloplacus, Discolaimus, Dorylaimus, Aphelenchus, Aphelenchoides, Ditylenchus, Tylenchus and Tylenchorhynchus). Similar results have been reported in other genres of plants are confirmed by the work of Haseeb and Pandey [16] on nematodes associated with medicinal plant *Artemisia pallens* L.

The identified genre of nematode is represented by the abundance of 1045 N/dm<sup>3</sup> (313,500 nematodes per m<sup>2</sup>) less to that found by Franz [12] and counted in calcareous soil and dry wood (800 000 nematodes per m<sup>2</sup>), but is different to those found by Banage [4] who counted 2 to 3 Million m<sup>2</sup> nematodes in soil heath (*Juncus squarrosus*). The low presence of nematodes in our soils is closely related to environmental conditions. This result is confirmed by Bachelier [5] which showed that the nematodes are important in sod and grassland soils which are rich in organic matter, satisfactory water balance and good structure.

Analysis of the results by the General Linear Models (G.L.M.) reveals very highly significant differences ( $P < 0.05$ ,  $P = 0.000$ ) between the densities of trophic groups and nematodes. The group of omnivorous predators (Discolaimus and Dorylaimus) is the most abundant followed by the bacterivorous group (Cephalobus and Chiloplacus) and herbivores (Tylenchorhynchus) but the fungivorous group (Aphelenchoides, Ditylenchus and Tylenchus) which are classified in the previous group, detect the smaller presence. These results are comparable to several research works which showed that predatory nematodes and omnivores are more abundant in natural areas than in agricultural fields due to their greater sensitivity to soil disturbance [24]. Ou et al. [27] reported that the number of total nematodes, bacterivorous and plant-parasitic nematodes were positively correlated with the contents of total organic carbon. So, within soil animals, nematodes have been broadly used as ecosystem health indicators at very different scales [10, 24, 23]. Nematode faunas are usually richer in non-cultivated lands, and nematode diversity increases within a soil type when arable fields are abandoned [15].

The DCA statistical analysis revealed the presence of three homogeneous groups following the spatial distribution modality of genres identified in the five study sites:

The first group showing the phytophagous *Tylenchorhynchus* highly present in the rhizosphere plant: *Ziziphus lotus*, *Artemisia herba alba* and *Artemisia campestris*. The absence of fungivorous and bacterivorous in the rhizospheres of *Artemisia campestris* and *Artemisia herba alba* are closely related to non-food availability due to antibacterial and anti-fungal of this plant [34, 20, 33].

The second group included the *Discolaimus*'s, *Ditylenchus*'s and *Tylenchus*'s genus which are abundant in the rhizosphere of *Peganum harmala* plant characterized by different antiparasidal [3], antifungal, antibacterial [26] and insecticidal activities [19].

However, the third group represents a generic diversity (*Aphelenchus*, *Aphelenchoides*, *Cephalobus*, *Chiloplacus* and *Dorylaimus*) is high in the rhizosphere of *Thapsia garganica* plant compared to medicinal plants mentioned in the preceding.

#### Conclusion:

Nematodes are the most abundant and ubiquitous metazoans in soil, which have a great effect in the functioning of soil food webs. In ecosystems nematodes interact directly as herbivores on plants and indirectly as

consumers of micro-flora and fauna, this has a good effect on regulating primary production, on predation, on energy transfer decomposition of organic matter, and on nutrient cycling in soil ecosystems. In agro-ecosystems Nematodes have beneficial and harmful roles to crop production.

In the present work, Total population abundance nematode represented 1045 Nematodes/dm<sup>3</sup> (313.500 nematodes per m<sup>2</sup>), formed by 09 genus, and according to their feeding habits, can be classified into four major groups: plant parasitic (*Tylenchorhynchus*), bacterial (*Cephalobus* and *Chiloplacus*), fungal (*Aphelenchus*, *Aphelenchoides*, *Ditylenchus* and *Tylenchus*), predators and omnivores feeders (*Discolaimus* and *Dorylaimus*) which present a higher number of the predatory-omnivorous nematodes compared to phytophagous and bacterivorous nematodes.

Using the detrended correspondence analysis, the spatial distribution of nematodes genus in the study areas and according to the medicinal plant species showed that in one hand the genus of *Aphelenchus*, *Aphelenchoides*, *Cephalobus*, *Chiloplacus* and *Dorylaimus* were the most abundant in the rhizosphere of *Thapsia garganica*. In the other hand, the species of the genus *Discolaimus*, *Ditylenchus* and *Tylenchus* were mainly present in the rhizosphere of *Peganum harmala*. However, only the plant – parasitic nematode *Tylenchorhynchus* was observed highly in the rhizosphere of *Artemisia herba alba*, *Artemisia campestris* and *Ziziphus lotus*.

Thus, the medicinal plants described in this study, can contain a secondary metabolic in merit further study for evaluation of their anthelmintic activities against the most damaged phytophagous nematodes (*Meloidogyne*, *Heterodera*, *Globodera*, *Pratylenchus*, *Ditylenchus*, *Longidorus* and *Xiphenema*).

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