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Infestation of Cereals by Cyst Nematodes of the Genus Heterodera (Nematoda: Heteroderidae) in Chlef Area (Algeria)

Z. Rahim[1], A. Mekhaneg[2], A. Merouche[2], L. Khelifi[3]

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ABSTRACT

Background: The investigation was conducted in the wilaya of chlef, on the presence and distribution of cereal cyst nematodes of the genus *Heterodera*. The experiment carried out is intended to estimate the effect of the wheat crop on the distribution of the *Heterodera* spp.

Methods: Seventy-two (72) soil samples were collected from 4 wheat-growing regions. Through a field survey, we studied how soil nematode community densities distributed at two soil depths (0-5 cm and 5-20 cm).

Result: The results revealed that the nematodes were present in all regions and in 59 soil samples (81.94 %). The frequencies of infestations calculated are variable from plot to plot. The highest frequencies were recorded in the regions of Chetia (77.77%) and Boukadir (66.66%) and the lowest were noted in the plots of Sobha and Ouled Fares with an average of 11.11%. The cyst densities varied from one plot to another, ranging from 0 to 33 cysts/100 g of soil, which corresponds to an average range of 0.26 to 11.72 eggs and larvae/g of soil. From the infested samples, 30.55% had a population density of fewer than 10 eggs and larvae/g soil.

Key words: Algeria, Chlef, Distribution, *Heterodera* Spp. Infestation level.

INTRODUCTION

Nematodes are among the most important faunal groups in soil food webs and serve as important bioindicators in evaluating subsurface ecological processes and ecosystem functioning (Liu J *et al.,* 2022). Cereal cyst nematodes (CCNs) are known as the utmost vital nematode pathogens that reduce the growth of cereal crops. Cereal cyst nematode parasitize the newly developing roots of plants and reduce the crop vigor by producing patches of stunted plants with pale green leaves. Young plants develop less number of tillers and root system turns shallow and have a “bushy-knotted” look (Nicol *et al.,* 2010; Smiley and Nicol, 2009). In Algeria, annual production was about 4.92 million tons, including 3.3 million tons of wheat, according to the statistical services of the Algerian agricultural services directorate. In 2021, the drought episodes that hit North Africa affected grain production. As well as the biotic agents that limit cereal production, cyst nematodes of the *Heterodera* genus cause considerable losses and represent a major constraint to the intensification of cereal production (Chabert *et al.,* 2012; Dababat *et al.,* 2015; Rahim *et al.,* 2020). Our work consists in studying the state of infestation of four cereal plots in the Wilaya of Chlef as well as their distribution and their degrees of infestation.

MATERIALS AND METHODS

Study site

The study site is located in the wilaya of Chlef, in the North, on the Algiers-Oran axis. It covers an area of 4791 km2. It is bounded by the Mediterranean Sea to the north, the wilaya of Tissemsilt to the south, the wilaya of Tipaza and Ain-Defla to the east and the wilaya of Mostaganem and Relizane to the west. The geographical coordinates of the wilaya of chlef are 36°9.9152 N: 1°20.07122 E. The average temperature varies from 5°C in January to 40°C in July and the annual rainfall varies between 158.77 mm and 357.8 mm.

[1]Departement of Agronomy, Faculty of Sciences, University of Medea, Medea, Algeria.
[2]Faculty of Nature and Life Sciences and Earth Sciences, Khemis-Miliana University, Road of Theniet-El Had, Khemis-Miliana, Algeria.
[3]Department of Agronomy, University Center Morsli Abdallah-TIPAZA, Algeria. Research Laboratory Production and Protection of Crop in the Chlef Region, Chlef Algeria.

Corresponding Author: Z. Rahim, Departement of Agronomy, Faculty of Sciences, University of Medea, Medea, Algeria.
Email: z.rahim@univ-medea.dz

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Soil sampling

The study analyzed variations in the population density of *Heterodera* infesting cereal crops during the growing season (2020-2021). In September 2020, before the crop was planted, four 10,000 m^2 plots were selected in four provinces (Fig 1). In each plot, we took 18 samples divided into two depths: from 0 to 5 cm and from 5 to 20 cm at different points along the two diagonals to represent the whole area (Coyne *et al.,* 2010), with 1.8 kg of soil. This resulted in a total of 72 soil samples (4 plots × 9 sampling points × 2 depths). Each soil sample was passed through

a 2 mm sieve immediately after collection and transported to the laboratory of the National Institute for Plant Protection (NIPP), located in the wilaya of Chlef, for extraction, mounting and cyst number counting.

Analysis of the nematode community

To extract cyst nematodes, 100 g of dry soil was manipulated using one of the Fenwick (1940) methods. All cysts in the soil were counted under a binocular loupe (G: 10×2). Full cysts are characterized by a turgid appearance and dark color, while empty cysts are recognized by the light color and wrinkled appearance of their cuticle. Nematode abundance was expressed as the number of nematodes per 100 g of dry soil. Then, the solid cysts of Heterodera were crushed separately under the binocular magnifying glass (G: 10× 4) and their contents were counted (Eggs and J2) (Fig 2). The infestation frequency represents the percentage of infested samples and is calculated by the relationship:

$$\frac{\text{Number of infested samples}}{\text{Number of samples taken}} \times 100$$

This formula is adopted by many researchers in the field (Ibrahim *et al.,* 2000, 2001, 2004, 2017; Minnis *et al.,* 2002; Cunha *et al.,* 2004; Hlaoua *et al.,* 2008; Seenivasan, 2017). In order to assess the level of infestation of a plot by these nematodes, a population density is determined and is expressed by the number of cysts per 100g of soil. Similarly, it is expressed as the number of eggs and larvae per gram (g) of soil (Minnis *et al.,* 2002; Ibrahim *et al.,* 2004, 2017). The latter is compared with the threshold of harmfulness, which is determined at 10 J2/g soil.

Soil analysis

The Wilaya of Chlef is characterized by a rather great topographic homogeneity with important cultural aptitude: the soils are formed by recent deep alluvium, calcareous with clay predominance (ABH cheliff Zahrez, 2003). The physical analysis consists mainly of the determination of the granulometry and the apparent density. Referring to the textural triangle, we can say that the soil is of clay-silt texture, with an apparent density of 1.34 cm^3.

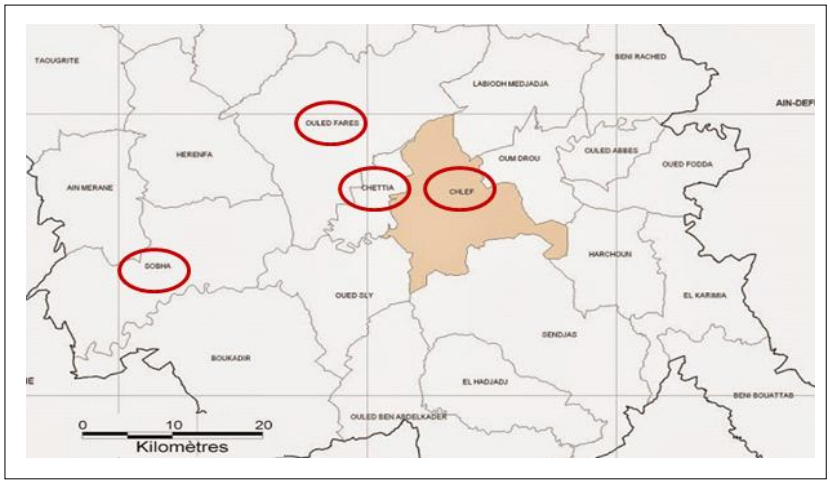

Fig 1: Geographical location of the plots studied.

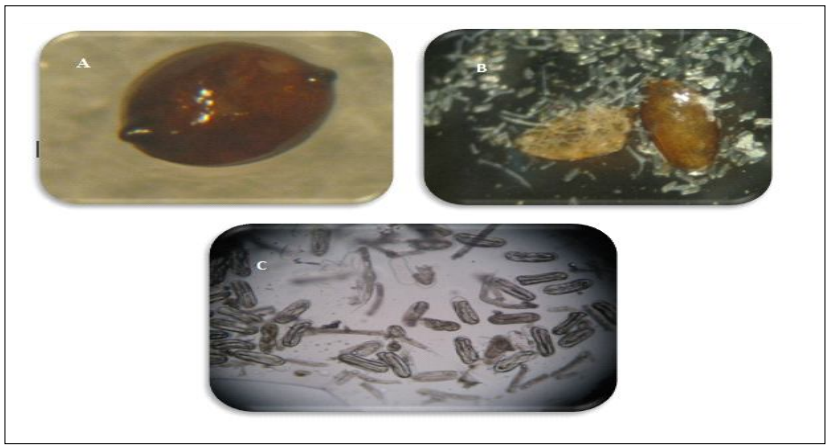

Fig 2: A: Whole Cyst; B-C: Eggs and second stage juveniles (G : 10×40) (Original).

Data analysis

A one-way analysis of variance (ANOVA) was performed to examine the effects of soil depth and their interaction on the abundance of cyst nematodes of the genus *Heterodera* spp. in all plots studied. Analyses of variance were performed using the software statistix 9.0 (Analytical, Software, Talahassee, FL, USA).

RESULTS AND DISCUSSION

The distribution and population density of *Heterodera* spp. in the wilaya of Chlef (Table 1).

Infestation rate of the surveyed plots

Across all regions, wheat cyst nematodes were present in all soil samples analyzed. Infestation rates varied between plots and even between depths. The highest infestation percentages are on average 77.77% and 66.66%, which were recorded respectively in the region of Chetia and Boukadir, while the lowest infestation frequency (11.11%) was noted in the region of Sobha and Ouled Fares (Fig 3). According to Norton (1989); Blair *et al.* (1999) ; Cadet *et al.* (2005), as with microbial communities, the spatial distribution of some nematode species is dependent on soil type and abiotic soil characteristics. The results of the analysis of variance of the infestation rates of the plots by *Heterodera* spp. in two depths, show a very highly significant difference in the Chetia region with a probability of (0.000), Significant in the plot of Boukadir (p=0.0381; p=0.0438) for solid and total cyst nematodes/100g soil. On the other hand, the plots of Sobha and Ouled Fares recorded a non-significant difference (p<0.05) (Table 2) .Symptoms of phytophagous nematode attacks on the aerial part of plants, are not very specific and result in discoloration, yellowing of the foliage and slowing of the growth of the attacked plant to Chabert *et al.* (2012).

Table 1: Population density and frequency of *Heterodera* spp.

Location	Samples (no)	Infested samples (%)		Cyst density (cyst/100 g soil)		Eggs and J2 (no/g soil)	
		0-5 cm	5-20 cm	0-5 cm	5-20 cm	0-5 cm	5-20 cm
Chetia	18	66.66	77.77	25	33	(0.91-9.82)	(1.01-11.72)
Sobha	18	22.22	11.11	01	02	(0.98-0.33)	(00-0.82)
Ouled Fares	18	11.11	33.33	01	03	(00-0.26)	(00-0.42)
Boukadir	18	44.44	66.66	09	25	(0.84-7.26)	(02.01-10.86)

Table 2: Effects of soil disturbance on reproduction of *Heterodera* spp.

Location	Chetia	Sobha	Ouled fares	Boukadir
	P value	P value	P value	P value
Cysts pleins/100 g of soil	0.3120	1.000	0.1550	0.0381*
Total cyst/100 g of soil	0.000***	1.000	1.0000	0.0438*
Egg/100 g of soil	0.000***	0.3175	0.7728	0.1579
Eggs+J2/g of soil	0.000***	0.3175	0.7728	0.1579

(p<0.05) ; Pf, Eggs+J2/100 g soil.

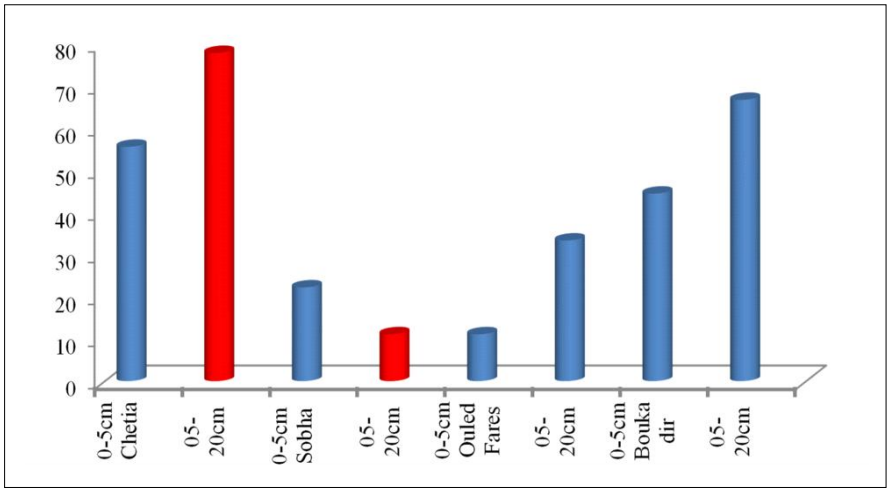

Fig 3: Infestation rate of surveyed plots by *Heterodera* spp.

Cyst density of *Heterodera* spp.

Due to the very heterogeneous distribution of nematodes in the soil, both vertically and horizontally, the highest density of *Heterodera* spp. were recorded in the Chetia and Boukadir area with a total of 33 and 25 full cysts respectively at the depth of 05 to 20 cm. While the lowest density was recorded in the region of Sobha with 01 full cyst/100 g soil (Fig 4).

This was similar to the findings of Schneider and Mygniéry (1971).According to Mugniéry *et al.* (1989), the average density for a given plot or locality is calculated by relating the number of solid cysts extracted from all samples taken in that locality to the total weight of the samples. In practice, the degree of soil contamination by cyst nematodes is often expressed as the number of cysts per unit of soil. Aubert (1986) specifies that this rather coarse indication can be refined by specifying the number of full cysts and the number of empty cysts, or better by counting the juveniles and living eggs inside the cysts (expressed in a number of individuals per gram of soil). Table 2 shows a significant difference between the population densities of nematodes expressed in number of full and total cysts/ 100g soil in the plot of Boukadir and Chetia. The difference is mainly due to the conditions that characterize the plots studied (soil depth, climate, variety of the host plant, rotation, *etc*.). In spite of their reduced presence, these cysts are a threat to subsequent crops because from a single cyst one can have many individuals due to the high fecundity rate of the females, up to 1000 eggs/cyst as reported by Chauvin *et al.* (2008). According to Brinkman *et al.* (2015), soil nematode communities are often not directly associated with plants, except for herbivorous nematodes that feed on or parasitize plant roots. In most cases, the effects of plant communities on soil nematode communities are mediated by soil properties and microbial communities to Lv *et al.* (2013) ; Liu *et al.* (2017).

***Heterodera* spp. infestation level (J2/g soil)**

Most plots surveyed were found to be contaminated, although the size of nematode populations varied among localities (Fig 5). The succession of cereals on the same plots and the favorable climatic conditions only increase the infectious potential of the soils (Labdelli *et al.,* 2017 ; Ritter, 2008) ; this can explain in a general way the high infestations recorded in the plots of Chetia and Boukadir, with a level of infestation more important than the threshold of harmfulness estimated at 10 Juvenile (J2) per gram of soil (Mokabli, 2002). For the rest of the plots (Sobha and Ouled Fares), the average infestation is low (between 0.26 and 0.82). The difference between these plots is almost

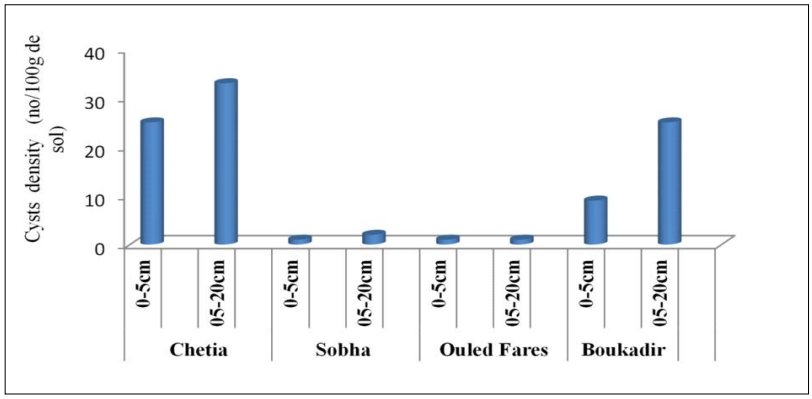

Fig 4: Densities of cyst nematodes of the genus *Heterodera* spp.

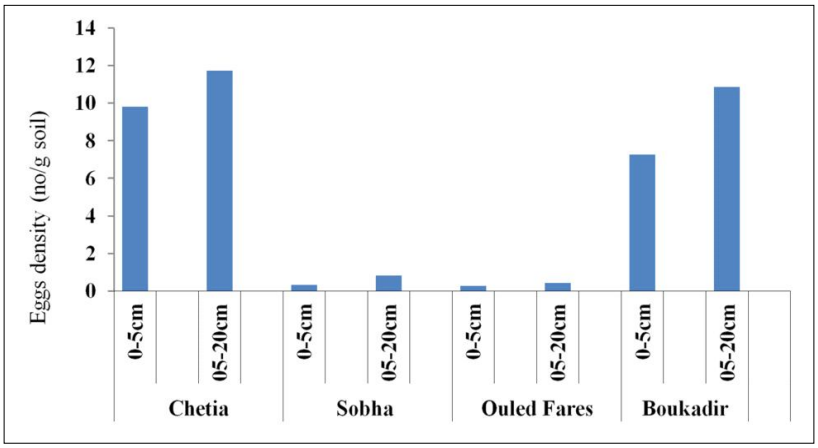

Fig 5: Egg and J2/g soil infestation levels.

insignificant compared to the results obtained by Cadet *et al.* (2003); Cadet *et al.* (2005) who noted that plant-parasitic and free-living nematode densities vary between 0 and 2480 nematodes/cm^3, depending on species, sampling and sub-plots. But low to medium infestation rates are still important and worrisome rates that should be taken seriously, as reported in many works to Smiley *et al.* (1994) that have shown that this nematode can cause significant losses, even at very low densities (1J2/g soil). The study will enable the development of resistant varieties and management strategies to improve cereal crop yields by providing data on the population and distribution of cereal cyst nematodes in wheat-growing regions (Hamid *et al.,* 2021). Our results was similar by Taya *et al.* (2000),The of population was found to be related with the age of fields. Aged fields were found to have more population density of CCNs.

CONCLUSION

In summary, nematode trophic groups showed different patterns of variation between 0 and 5 cm and soil depths of 5 to 20 cm, suggesting that wheat succession may exhibit layer-specific effects on nematode trophic groups.The cyst nematode, *Heterodera* spp. is a cosmopolitan pest; their infestation rate on wheat varies from one plot to another and from one depth to another. In the wilaya of Chlef, it constitutes a real threat to cereal crops, especially where they are unknown to farmers.The quantification of nematodes allowed us to put a variability of infestation levels between plots that corresponds to 11.72 and 10.86 eggs and J2/g of soil in the region of Chetia and Boukadir respectively. The other infested plots have population densities below the threshold of harmfulness (<10 J2/g of soil). The rotation remains the best way to fight against these pests in the wilaya of Chlef, because it is an economical method for polluting and is respectful for the environment.

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Conflict of interest

All authors declared that there is no conflict of interest.

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