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Population estimates for the *Heterodera avenae* (Wollenweber, 1924) cereal cyst nematode in the Chlef region (Algeria) based on two simple soil levels

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Abstract
The study aimed to determine the infestation level of the cereal cyst nematode *Heterodera avenea* (Wollenweber, 1924) in the Chlef area. Nematological analysis, considered the most reliable diagnostic method, was carried out as it enables both identification and quantitative estimation of the nematode populations in the soil. *H. avenae* detection surveys were conducted in six cereal-growing areas of the Chlef region. The sampling conducted in the 18 cereal plots based on two depths of the ground, 0 to 10 cm and 10 to 30 cm. The results revealed that 8 to 21% and 8 to 30% of the plots surveyed were infested. The infestation rate varies between plots in the same region according to the number of eggs, the estimated second larval stage per gram of soil, and rooting depth. Variance Analysis (ANOVA) showed that the infection rates at the two depths were the same. This is most likely a result of the type of tillage that most cereal producers use, which is essentially repetitive superficial disking in plots to prepare the soil, which leaves cyst concentrations at the same depth of 0 to 30 cm.
Keywords: *Heterodera avenae*; Nematode; Cereals; Chlef; soil depths

Introduction

Grain cereals, such as wheat, barley, rice, and maize are the nutritional basis of humans and animals worldwide. Therefore, these crop plants are essential in terms of global food security (Grote et al., 2021). Wheat (*Triticum spp.*) and barley (*Hordeum vulgare L.*) are two cereal crops that belong to the *Poaceae* family (Giraldo et al., 2019). Globally, about 17% of cereal production is traded internationally, with individual commodities ranging from 9% for rice to

25% for wheat. In 2020, Algeria's total cereal production amounted to approximately 4.4 million metric tonnes, a decrease from the previous year. Cereal production in the country fluctuated from 2010 to 2018, peaking at approximately 6.06 million metric tons. It is projected that total world cereal production will increase by 18% by 2030 (OECD/FAO, 2021). Cereals face a myriad of challenges, including both biotic and abiotic stresses. Plant parasitic nematodes, among other biotic stresses, have a significant impact on crop yield. In many parts of the world, cereal cyst nematodes are known to be a major issue in wheat production. Substantial losses have been reported due to cereal cyst nematode attacks (Mokrini et al., 2017). Diagnosis and accurate species identification of cereal cyst nematodes are the keys to effective nematode management.

The genus *Heterodera*, belonging to the *Heteroderidae* family, plays a crucial role as obligate parasites of various crops, causing significant economic losses up to 100% in severely infested crops (Riley et al., 2009; Mokrin et al., 2017). Many studies have reported that *H. avenae* (Wollenweber, 1924) is widely spread in the main cereal crop areas of Algeria, its presence extends from the coastal to the desert regions (Mokabli et al., 2016; Righi et al., 2019).

These nematodes have been shown to have a major impact on wheat production, particularly in regions characterized by drought conditions and a prevalent practice of cereal monoculture practices (Nicol and Rivoal, 2008). The genus is unique among nematode genera due to the female's ability to transform into a tough brown cyst that protects the eggs formed within her body (Mitiku, 2018).

According to Djetti et al. (2018), the first record of a species from the *H. avenae* group on wheat in Algeria was reported by Scotto La Massese (1962). Since then, *H. avenae* has become more dominant in northern Algeria, particularly in cereal-producing regions such as Tissemsilt (Djetti et al., 2014), Aïn Defla (Tirchi et al., 2016), Algiers (Haddadi et al., 2013), and Tiaret (Mokabli et al., 2002; Haddadi and Mokabli, 2015). This study aims to provide valuable insights into the spatial distribution patterns of *H. avenae* in the Chlef area, which can aid in implementing effective management strategies. Additionally, understanding the infestation rate at different soil depths (0-30 cm) will help in determining the nematode's preferred habitat and potential impact on cereal crops.

2. Materials and Methods

2.1 Soil Sampling Localities

Chlef is the capital of Algeria's Chlef Province (Latitude: 36.1653, Longitude: 1.3345, GPS Coordinates: 36° 9' 54.9000" N, 1° 20' 4.2720" E). It is located 200 kilometers (120 mi) west of the capital, Algiers. The sampling of cereal soil naturally infested by *H. avenae* is carried out in six regions representing different bioclimatic stages: Tadjna (36° 07′ 00″ N, 5° 59′ 00″ E), Abou El Hassen (36° 25′ 04″ N, 1° 11′ 42″ E), Sendjas (36° 04′ 00″ N, 1° 24′ 00″ E), El Karimia (36° 06′ 58″ N, 1° 33′ 00″ E), Ouled Fares (36° 13′ 58″ N, 1° 14′ 25″ E) and Oued Fodda (36°11'N, 1°32'E) (Figure 1).

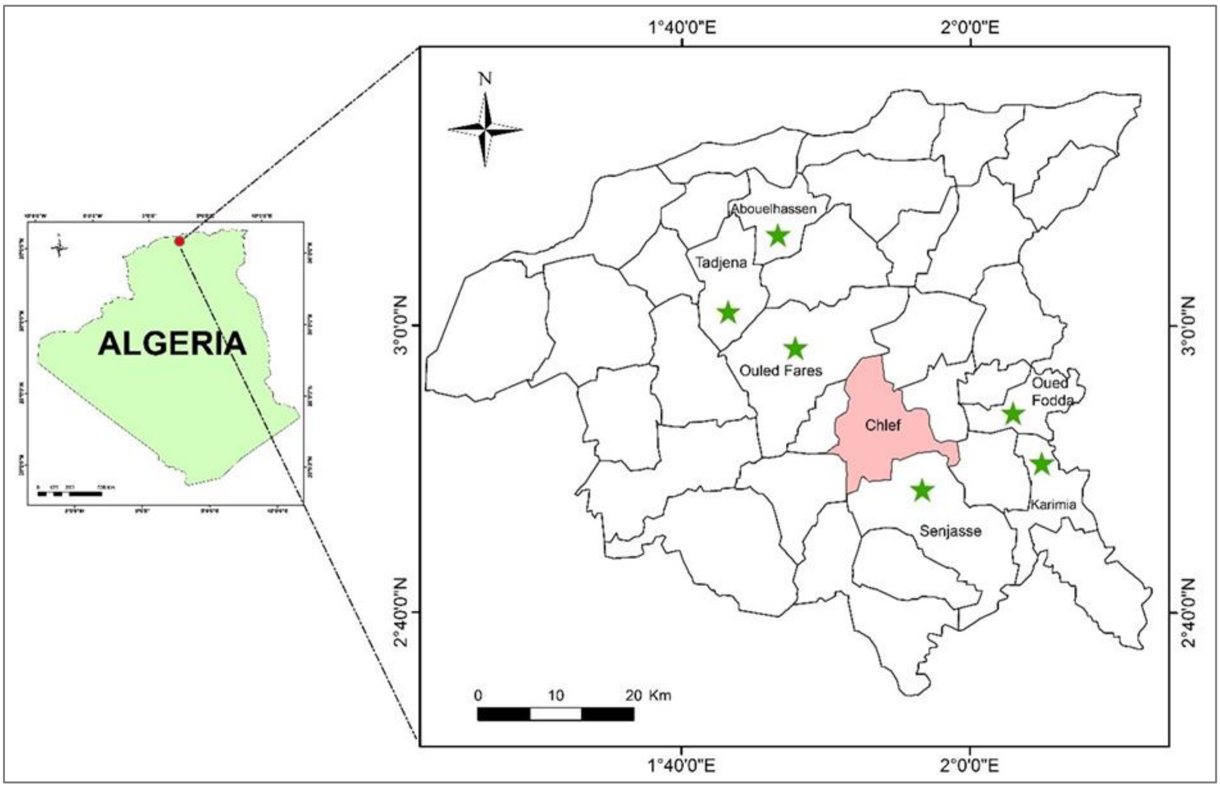

Figure 1. *The location sites of the samples in the representative case study.*

Cereal soil samples (durum wheat, common wheat, and barley) are collected from eighteen plots between mid-December and the end of April, with three plots in each of the six sampling localities. To collect samples, an auger is used at two depths of 0-10 cm and 10-30 cm. Three soil samples of 100 g each are taken from every plot, with each sample being composed of five increments. All samples are properly labelled and stored at 4°C in labelled plastic bags, including the location and plot number. The soil samples were first dried under the ambient conditions of the laboratory. The Fenwick apparatus is utilized for cyst extraction (Camacho et al., 2018).

2.2 Nematode Extraction and Counting

Fenwick's technique, initially developed in 1940 and subsequently modified by Oosten Brink in 1960, was employed for cyst separation from other soil materials. It is fundamentally based on the cyst flotation principle (based on the density of the cysts compared to that of the water). Dry cysts, whether empty or full, process a density of less than 1, causing them to float on the surface, facilitating their retrieval. The moist cysts constituted by the endured body of the female of *Heterodera*, have, according to Coyne et al. (2010), a density greater than 1.08; that is to say: higher than that of other nematodes and therefore sediment more quickly.

Each soil sample was mixed thoroughly. Cysts were counted in 100 g of soil. Cleaned and sterilized cysts were transferred into an Eppendorf and stored at 4°C until use.

2.3 Recovery and counting of cysts of *Heterodera avenae*

Using a syringe under a binocular magnifying glass with a magnification of 10 x 1.6, the cysts mixed with organic matter are isolated and then placed in a Petri dish lined with filter paper and labelled. The number of full and empty cysts was assessed.

We separate the full cysts from the empty cysts with a brush and a binocular magnifying glass. Their counting is done later. Different characteristics of the cysts can be observed, including their colour, shape, and, most importantly, the presence or absence of eggs inside the decreased female's body. Full cysts are distinguished by their dark colour, whereas empty cysts are characterized by their light colour, and appear devoid of eggs and larvae when in contact with a water drop (Figure 2).

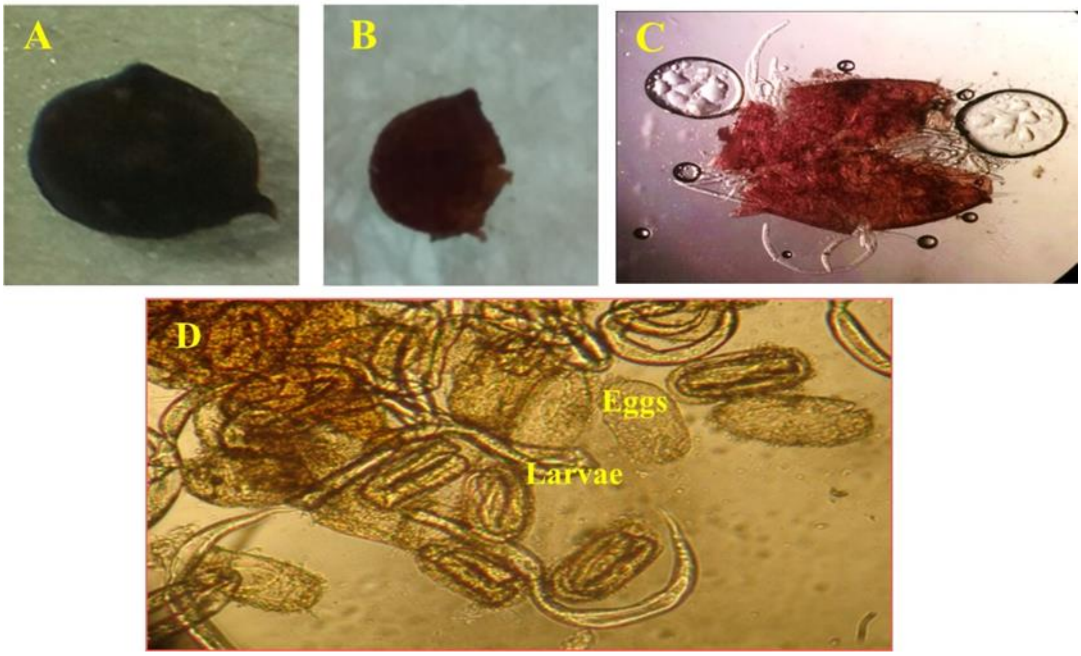

Figure 2. *Photomicrographs of H. avenae*

A) *Full cyst;* ***B)*** *Empty cyst;* ***C)*** *Ruptured cyst with eggs and juveniles;* ***D****) Eggs hatching*

2.4 Data analysis

The average number of cysts was calculated based on the five subsamples and expressed per 100 g of soil; the number of eggs in each cyst was estimated for two depths, specifically 0-10 cm and 10-30 cm. The data on infestations was first subjected to a thorough analysis to identify any patterns or trends. This analysis included examining the frequency and severity of infestations, as well as any factors that may contribute to their occurrence. Additionally, the data was cross-referenced with other relevant information, such as geographical location and environmental conditions, to gain a comprehensive understanding of the infestation patterns. to determine the correlations (Namouchi-Kachouri et al., 2009).

Data were analyzed using Analysis of Variance with SPSS software *v* 25. The distribution used was based on two variables, namely soil depth and the number of *H. avenae* cysts. Differences were tested using Analysis of Variance (ANOVA) followed by the Newman-Keuls test to compare means if the F-value was significant.

3. Results and Discussion

3.1 The Occurrence of the Infestation

The calculation of the average density for each plot or the locality is based on the relationship between the number of full cysts (FC) extracted and the total weight of soil the samples collected. In practice, the degree of soil contamination by nematodes of the genus *Heterodera* is often expressed as the number of cysts per unit volume of soil.

Figure 3 illustrates the correlation between the total cyst count and the count of full and empty cyst at different depths. Figure 3a shows that there is a weak relationship (r = 0.17) between empty cysts and the total number of cysts, conversely, a considerable portion of the total number of cysts discovered at this level are empty cysts (Fig. 3b). There is a 0.97 correlation between the total number of cysts and empty cysts. The two cyst types (FC and EC) significantly correlate with the total cyst count at soil depths between 10 and 30 cm (Fig. 3c and 3d). This result indicates a high level of root contamination at this depth. At this depth, this suggests a significant amount of root contamination. The reason for this outcome is the tillage that was done before seeding.

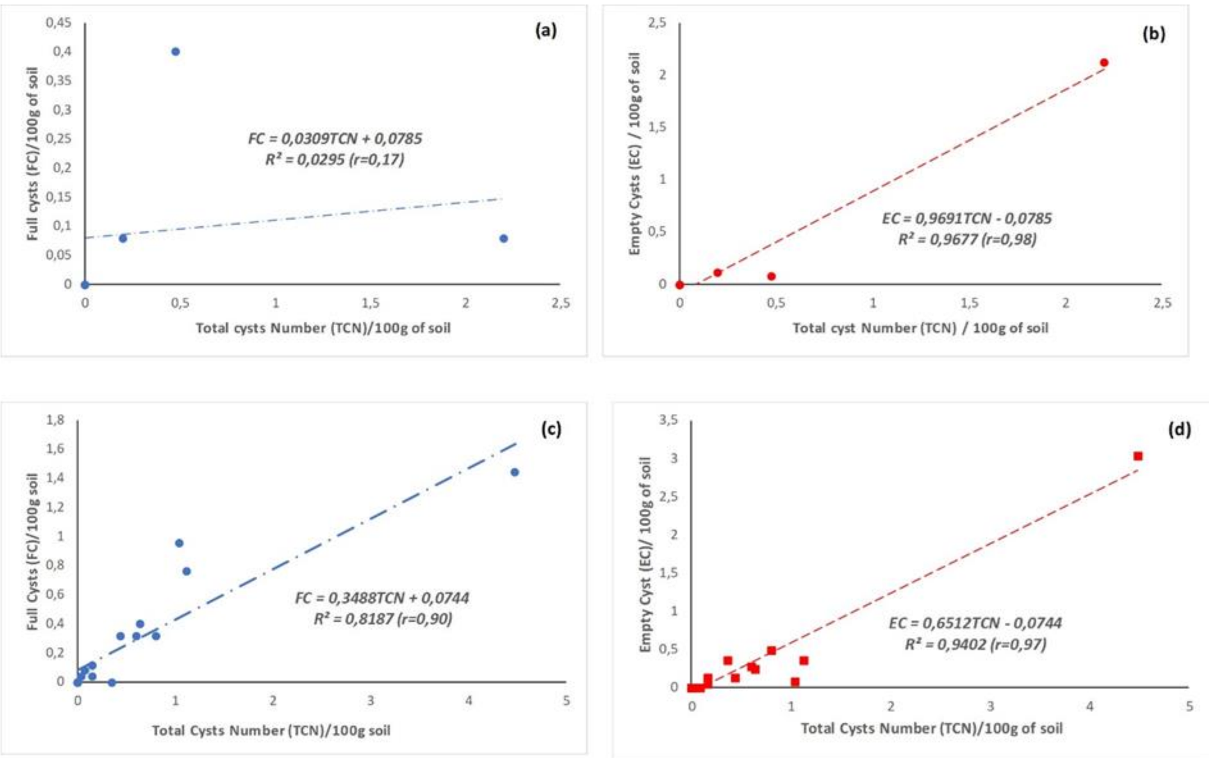

Figure 3. *The Relationships at depth 0-10 cm between Depth 0-10 cm: Full cyst vs Total cysts Number **(a)**; Empty cysts vs Total Cysts Number **(b)**. Depth 10-30 cm: Full cyst vs Total cysts Number **(c)**; Empty cysts vs Total Cysts Number **(d)**.*

Aubert (1986) suggests that this rather crude indication can be refined by specifying the number of full and empty cysts (EC), or even more effectively, by counting the juveniles and live viable eggs inside the cysts (expressed as the number of individuals per gram of soil). The same author notes that the size of the cysts varies greatly. As a result, the infectious potential represented by the cyst contents must vary depending on the individual. However, the embryonic development of eggs within a cyst may not be homogeneous.

These different reasons pushed us to investigate the content of the cysts of the various populations, particularly focusing on the rate of eggs in diapause and the mortality rate. Another significant point is the viability of the eggs in the cyst, which involves the number of eggs in diapause and mortality.

According to Rhigi *et al.* (2019), *Heterodera spp*. cyst nematodes are cosmopolitan parasites and their infestation rates vary from one plot to another, and from one region to another. Indeed, the frequency of *Heterodera ssp*. infestation varies according to the region and cereal species. The recorded infestation rates along two depths of 0 - 10 cm are around 50% depending on the region and reach 66.7% in a few localities such as Tadjena and Abou El Hassen (Figure 4, 5).

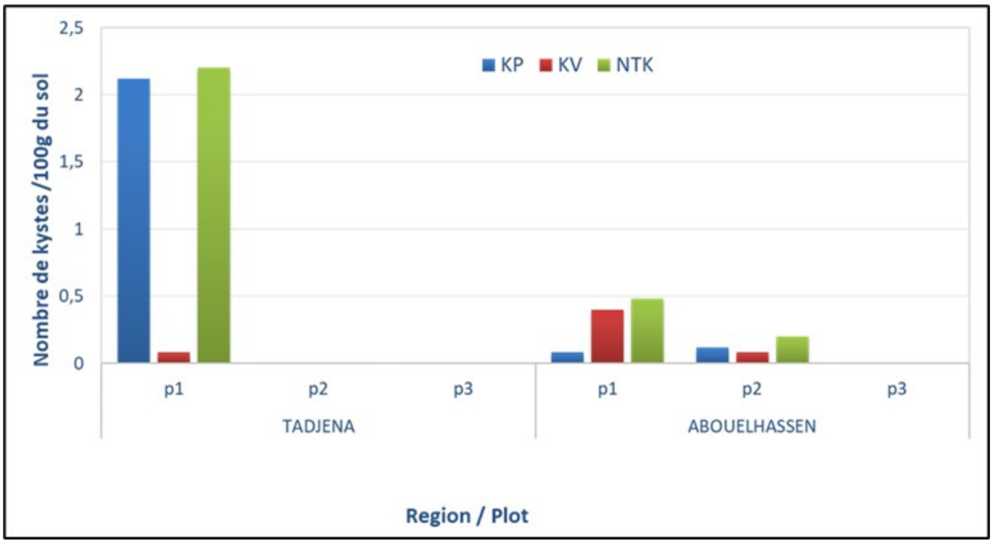

Figure 4. Average cyst density of *Heterodera avenae* at depth (0 to 10 cm)
EC: Empty cyst**; FC**: Full cyst; **TCN:** Total cyst number

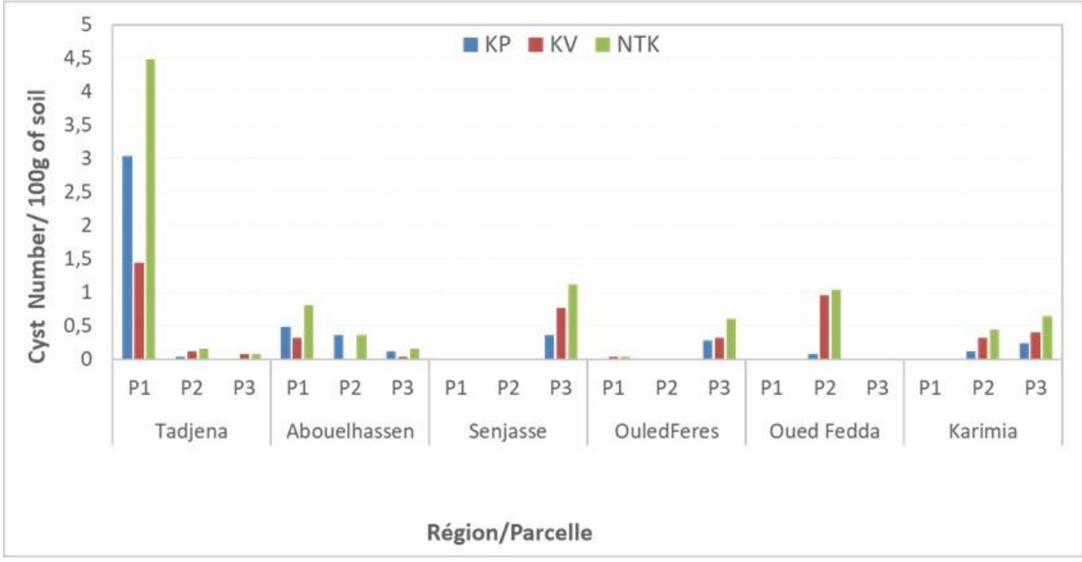

Figure 5. *The average number of Heterodera avenae cysts /100 g of soil at a depth of 10 to 30 cm according to the Commons surveyed.*

These fluctuations in infestation levels are the result of the interaction of many factors such as the climate, the rotation system, the varieties cultivated of the soil type, and the use of chemical inputs (pesticides, fertilizers, etc.), which affect the *Heterodera spp.* populations (Arjoune, 2022). The same analysis was reported in the previous studies on the distribution and infestation of the different species of *Heterodera* in certain regions of western Algeria (Righi *et al.* 2019).

3.2 Enumeration of Eggs and Larvae of *Heterodera avenae* in the Plots Studied

The degree of infestation is calculated considering on the number of eggs and infesting the second larval stage in one gram of soil (Mugniery, 1975 and Mugniery, 1982).

To determine the degree of the infestation, the contents of the removed cyst are analysed as well as the viability of the eggs/larvae they contain. According to the findings, the heterogeneous distribution is influenced by soil texture, cultivar variety, and previous crops. The infestation rate varies from one plot to another in the same region depending on the number of eggs and L2 larvae estimated in one gram of soil, as well as depending on the rooting depth (Figure 3, 4).

Nematological analysis has revealed differences in values for the studied variables, including the number of cysts (full, empty, and total) and the number of eggs plus larvae per gram of soil in each sampled plot. The Analysis of Variance (ANOVA) has shown a highly significant effect of sampled plots on all nematological parameters ($p<0.05$) (Table 1). LSD test grouped means into two homogenous groups, with the first including P2, P3, P4, P5, and P6, while the second group includes a single plot, P1. Unlike horizontal analysis, when assessing the vertical distribution of *H.avenae* between the two depths, the ANOVA test has revealed that there are no significant differences between the two depths, allowing us to retain the null hypothesis and conclude that all variables are equal (p>0.05). It is suspected that the interaction of various factors causes the variation in the states of infestation and the heterogeneous distribution of this nematode, including (edaphic, climatic, and human factors).

Table 1. *Repeated-measures ANOVA for the number of cysts (full, empty, and total) effects on the different soil depth.*

	Soil Depth 0 – 10 cm		**Soil Depth 10 – 30 cm**	
	F	*P*[a]	*F*	*P*
EC	66.05	***	56.09	***
FC	433.75	***	57.71	***
TCN	2.51	ns	79.22	***

*[a]Probability level : n.s. P > 0.05; ***P < 0.001; **P < 0.01; *P < 0.05.*

Although close monitoring is advised to prevent any risk of outbreaks and dissemination, the monitoring of all the infested plots has revealed an economic threshold of less than 10 L2/g of soil, indicating that this nematode does not pose an immediate risk to cereals in these areas. However, the rate of L2 per g of soil was lower than the harmfulness threshold of 12 L2/g of soil established by Mokabli (2002).

According to the two depths (0 to 10 cm and 10 to 30 cm), the vertical distribution of *H. avenae* in the six plots studied was found to be insignificant, due to the type of tillage used. Indeed, Foxi *et al* (2017) reported the impact of tillage on certain cereal pathogens and parasites, where the effects vary depending on species, soil type, climate, cereal variety, tillage depth, and intensity, in addition to other management practices.

4. Conclusions

Heterodera avenae is one of the most harmful pests on cereals and whose yield losses are difficult to assess because their symptoms are not very specific.

The presence of this parasite in cereal plots is a real threat. This is the reason why we decided to conduct this survey to detection of this nematode and establishment of a map of its distribution in the areas classified as potential cereal crops in the wilaya of Chlef. The nematological analysis allowed us to show the presence of this parasite in all the surveyed communes but with rates of infestation that vary from one parcel to another.

Plot 1 in the locality of Tadjena exhibited the highest degree of infestation (4.53 (egg +L2/g soil), whereas the lowest degree of infestation was recorded in Plot 2 in the region of Abu El Hassen.

Additionally, the plots (P1, P2) in Sendjas and Ouled fares as well as P1 in Oued Fodda and Karimia and P3 in Tadjena and Oued Fodda were found to be non-infested. It is assumed that this difference in infestation states and the heterogeneous distribution of this nematode are due to the interaction of different including edaphic, climatic, and human factors.

Monitoring of infested plots revealed a damage threshold below 10 L2/g of soil, which means that this nematode does not pose an immediate risk to cereals in these areas. However, continuous monitoring is recommended to avoid any risk of proliferation or dissemination. The vertical distribution of *H. avenae* in the six plots studied according to both depths (0 to 10 cm and 10 to 30 cm) was not significant; this may be due to the nature of the tillage.

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