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Research Article

Vertical distribution and infestation dynamics of *Heterodera avenae* in cereal crops of Chlef, Algeria: Implications for nematode management

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Abstract: *Heterodera avenae*, a major cereal cyst nematode, threatens wheat and barley production in North Africa, yet its distribution in Algerian soils remains poorly quantified. This study investigated the spatial and vertical distribution of *H. avenae* across 18 cereal plots in six localities of Chlef, Algeria, focusing on two soil depths (0–10 cm and 10–30 cm). Using Fenwick flotation and microscopic analysis, we extracted and quantified cysts and second-stage juveniles (J2s). Results showed infestation rates of 8–30%, with no significant difference between soil depths ($p > 0.05$), suggesting that conventional superficial tillage homogenizes nematode distribution. The highest cyst density (4.48 ± 0.01 cysts/100 g soil) occurred in Tadjena, while the lowest (0.04 ± 0.02) was recorded in Ouled Fares. Second-stage juvenile densities stayed below the economic damage threshold of 12 J2/g soil (as noted in previous studies) in all plots, indicating a low immediate risk but underscoring the importance of continuous monitoring to prevent future outbreaks. These findings provide actionable insights for integrated pest management, emphasizing the importance of depth-inclusive control strategies in semi-arid cereal systems.

Keywords: Agroecological factors; Cereal cyst nematode monitoring; Nematode quantification; Sampling depth

Introduction

Cereal crops-particularly wheat (*Triticum* spp.) and barley (*Hordeum vulgare* L.) - form the cornerstone of global food security, providing over 50% of the world's caloric intake (FAO, 2023). In Algeria, where cereals occupy > 3 million hectares, production fluctuates significantly (4.4-6.1 million metric tonnes annually) due to biotic stresses, including devastating infestations by the cereal cyst nematode *H. avenae* (OECD/FAO, 2021; Smaha *et al.*, 2019). This pathogen causes yield losses up to 30% in Mediterranean regions, with economic impacts exceeding $80 million/year in North Africa alone (Smiley *et al.*, 2023).

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Heterodera avenae has become increasingly prevalent across Algerian cereal systems since its first report by Scotto La Massese (1961). Recent surveys confirm its expansion from coastal to semi-arid regions (Smaha *et al*., 2019; Righi *et al*., 2022), exacerbated by monoculture practices and climate variability (Nicol and Rivoal, 2008). The nematode's unique life cycle, where females transform into durable cysts reported to contain 200 to 600 eggs (Williams and Siddiqi, 1972), enables long-term soil persistence despite management.

While studies have documented *H. avenae* in regions like Tissemsilt and Tiaret (Djetti *et al.*, 2018), Previous work in Algeria characterized CCN populations for virulence on resistant varieties in Tiaret and Oued Smar (Haddadi *et al.*, 2013). Critical knowledge gaps remain regarding:1) Vertical distribution in soil profiles (0-30 cm), which dictates tillage efficacy; 2) Population dynamics in Chlef's distinct agroecosystem; 3) Evaluation of the usefulness of published economic thresholds under local soil.

Addressing these gaps is essential for developing targeted management strategies adapted to Chlef's specific edaphoclimatic conditions. This study offers the first detailed look at *H. avenae* spatial patterns across six bioclimatic zones in Chlef, Algeria. We analyze cyst densities at two soil depths, 0–10 cm and 10–30 cm, to measure depth-dependent infestation gradients and assess how tillage affects nematode distribution. Although we reference existing economic threshold values, such as those from Mokabli (2002) and Dababat *et al*. (2021), we did not use any new empirical or statistical methods to create risk thresholds specific to this region. Instead, our goal is to assess how well published thresholds work under local soil conditions. This underscores the need for more studies using robust statistical methods to develop tailored local thresholds that can inform integrated pest management.

Our findings directly inform precision agriculture strategies for Algerian cereal systems, while contributing to a broader understanding of nematode ecology in semi-arid environments.

Materials and Methods

Study Area and Sampling Design

Study Region:

Chlef Province (36°9'54.9 "N, 1°20'4.3 "E) is a key cereal production area in northwestern Algeria, characterized by a Mediterranean climate with annual precipitation ranging from 300-400 mm. The province's agricultural lands show significant variation in:

- Soil types (from sandy loams to clay-rich vertisols)
- Farming practices (irrigated vs. rainfed systems)
- Crop rotation histories

Sampling Sites:

We selected six localities representing the province's agroecological diversity (Fig. 1; Table 1):

Due to changing climate patterns and uneven rainfall in the Chlef region, most farmers use groundwater irrigation to meet their crop water needs. Although rainfed conditions occur occasionally, the six sampling locations mainly rely on borehole or well water, particularly during crucial growth stages (Bradai *et al.*, 2022).

Sampling methodology

Soil sampling was conducted during the 2022/2023 growing season (December–April), coinciding with peak cyst nematode activity (Mokabli, 2002; Nicol and Rivoal, 2008). Samples were collected from two depth strata: the surface layer (0–10 cm) and the subsurface layer (10–30 cm). The surface layer (0–10 cm), most intensively and frequently disturbed by tillage, and the subsurface layer (10–30 cm) correspond mainly to the root zone. It should be clarified that these two strata do not correspond strictly to a "tilled" versus an "untilled" layer: conventional tillage in this semi-arid cereal system (moldboard/chisel plowing followed by disc harrowing, repeated across cropping seasons) commonly disturbs the soil profile to an effective depth of 20–30 cm. Both sampled strata therefore fall within the zone cumulatively affected by tillage operations, which explains why nematode distribution was

homogenized down to 30 cm depth despite the nominal 0–10 cm tillage-pass reference. At each sampling point, five subsamples per depth were extracted using a sterile stainless-steel auger (Ø 5 cm) following standard protocols (EPPO, 2013), then homogenized into composite samples. Samples were immediately refrigerated at 4 °C and processed within 24 h to preserve nematode viability. Each sample was labeled with GPS coordinates and depth for full traceability.

Cyst isolation and processing

Cysts of *H. avenae* were isolated from soil samples using the Fenwick flotation method (Fenwick, 1940; Oostenbrink, 1960). This technique exploits the differential buoyancy of cysts: dry cysts (both empty and egg-filled) have a density lower than 1.0 g/cm³, enabling flotation, whereas hydrated cysts (intact females) have a density greater than 1.08 g/cm³, resulting in sedimentation (Coyne *et al.*, 2010). For each assay, 100 g of homogenized soil subsamples were suspended in 500 mL of tap water and passed through nested sieves of 250 µm (mesh 60) and 150 µm (mesh 100). The retained material was subsequently subjected to centrifugal flotation at 500 × g for 5 minutes, and floating cysts were collected using a Pasteur pipette under stereomicroscope guidance (10 × magnification).

After isolation, cysts were surface sterilized by immersion in a 0.5% sodium hypochlorite (NaOCl) solution for 2 minutes to reduce microbial contamination (Fenwick, 1940). They were then rinsed three times with sterile distilled water to remove residual NaOCl and transferred into 1.5 mL Eppendorf tubes at a density of 10 cysts per tube, in accordance with established protocols (Smaha *et al.*, 2019). The prepared samples were stored at 4 °C for up to 48 hours before microscopic analysis to preserve cyst integrity and viability.

Cyst extraction, enumeration, and morphological analysis

The isolation and quantification of *H. avenae* cysts were performed using standardized nematological procedures. Cysts were extracted from soil organic matter via sterile syringe aspiration under 16 × magnification (binocular stereomicroscope, Leica MZ75) and transferred to labeled Petri dishes lined with moistened filter paper (Whatman No. 1).

Cysts were classified into:

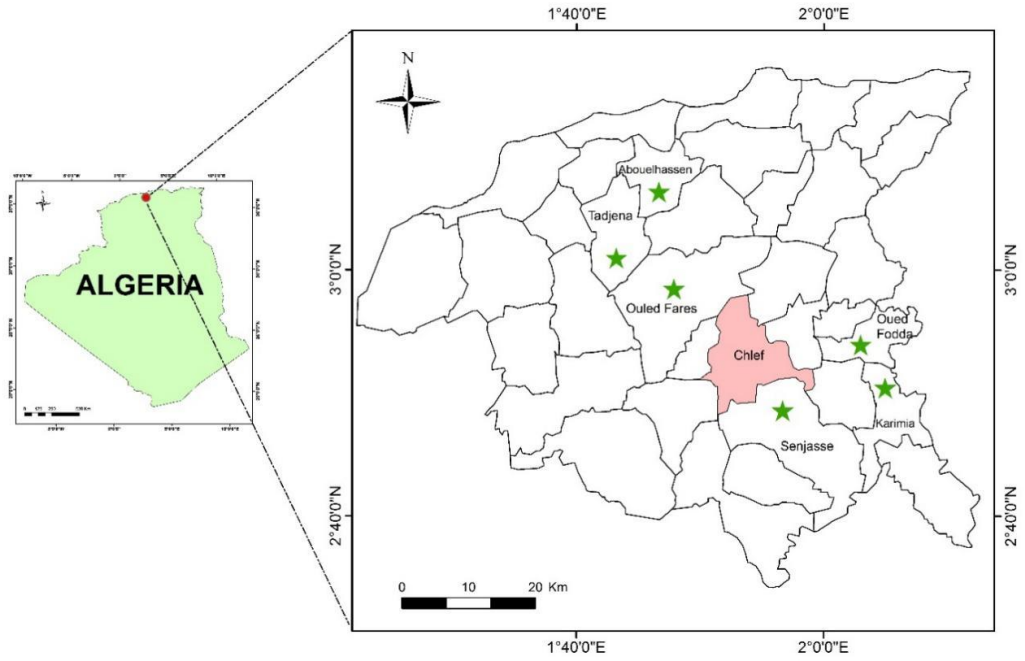


Figure 1 Geographical distribution of soil sampling sites in Chlef Province, Algeria.

Table 1 Edaphic and agronomic properties of sampling sites in the Chlef region.

Locality	Coordinates	Altitude (m)	Soil Type	Farming System	Dominant Crop
Tadjna	36°07'00"N, 5°59'00"E	420	Sandy loam	Rainfed monoculture	Durum wheat
Abou El Hassen	36°25'04"N, 1°11'42"E	180	Clay loam	Monoculture Intensive irrigation	Common wheat
Sendjas	36°04'00"N, 1°24'00"E	320	Siltyclay	Rainfed Crop rotation	Barley
El Karimia	36°06'58"N, 1°33'00"E	210	Alluvial	Rainfed/Supplemental irrigation	Wheat/Barley mix
OuledFares	36°13'58"N, 1°14'25"E	275	Loamy	Rainfed/Supplemental irrigation	Barley
Oued Fodda	36°11'N, 1°32'E	150	Clay-rich	Flood irrigation	Durum wheat

- **Full cysts:** Characterized by dark brown pigmentation and visible egg masses
- **Empty cysts:** Identified by light tan coloration and absence of internal contents.

Separation was achieved using fine-tipped brushes (size 00) with microscopic verification. Following Mulvey's (1972) methodology, vulval cone morphology was analyzed for species confirmation. For full cysts, quantitative assessment involved:

1. Bisection in water droplets using sterile scalpels.
2. Microscopic examination at 100 × magnification.
3. Manual counting of eggs and J2 juveniles using dissection pins.
4. Density calculation (eggs/J2 per 100 g soil) with conversion to g/soil for economic threshold evaluation (Mokabli, 2002).

Morphological characterization, including cyst wall integrity, vulval structure, and content viability, was systematically documented. Hydration tests confirmed viability, with intact cysts maintaining structure, whereas transparent cysts were degraded.

Data analysis

Cyst densities were calculated as mean values from five subsamples per plot and standardized per 100 g of soil, with separate quantification of eggs and juveniles for both soil depth strata(0–10 cm and 10–30 cm). Statistical analyses were performed using SPSS v25. A two-way analysis of variance (ANOVA) was conducted to assess the effects of locality (six levels) and soil depth (two levels), as fixed factors, and their interaction on cyst density. When significant effects were detected (F-test), mean comparisons were performed using the Newman–Keuls post hoc test.

Following Namouchi-Kachouri *et al*. (2009), correlation analyses were carried out to examine relationships between nematode distribution and environmental variables (soil type, moisture and geographic location) to identify potential drivers of infestation severity. Before analysis, all data were tested for normality using the Shapiro–Wilk (1965) test and for homogeneity of variances using Levene's (1960) test. Economic threshold levels were determined based on established criteria for cereal cyst nematodes (Smiley *et al.*, 2005; Brahim *et al.*, 2023).

Results and Discussion

The criteria of cyst pigmentation, egg mass content, and vulval cone structure constitute the basis for both the classification of full versus empty cysts and the subsequent morphometric measurements (Table 2).

Table 2 Morphometric characteristics of *Heterodera avenae* cysts and vulval cones from Chlef Province.

Character	Mean ± SD (µm)	Range (µm)
Cyst length (without neck)	789 ± 74.0	659-899
Neck length	32 ± 7.0	19-47
Cyst width	590 ± 44.0	500-687
Semifenestra width	20 ± 1.9	19-23
Fenestra length	45 ± 1.6	42-48
Vulvalslit length	10 ± 0.6	9-11
Vulval bridge width	5 ± 0.7	4-6

Morphometric characterization of cysts and vulval cones.

Morphological examination of *H. avenae* cysts (n = 10) revealed characteristic features consistent with species identification. Morphological characters and morphometric features are shown in Figure 2 and Table 2. Underbridge was notably absent; bullae were consistently present in all examined specimens. These morphometric parameters align with previous descriptions of *H. avenae* from Mediterranean cereal systems (Smaha *et al.*, 2019) and confirm the taxonomic identity of the populations studied in Chlef Province.

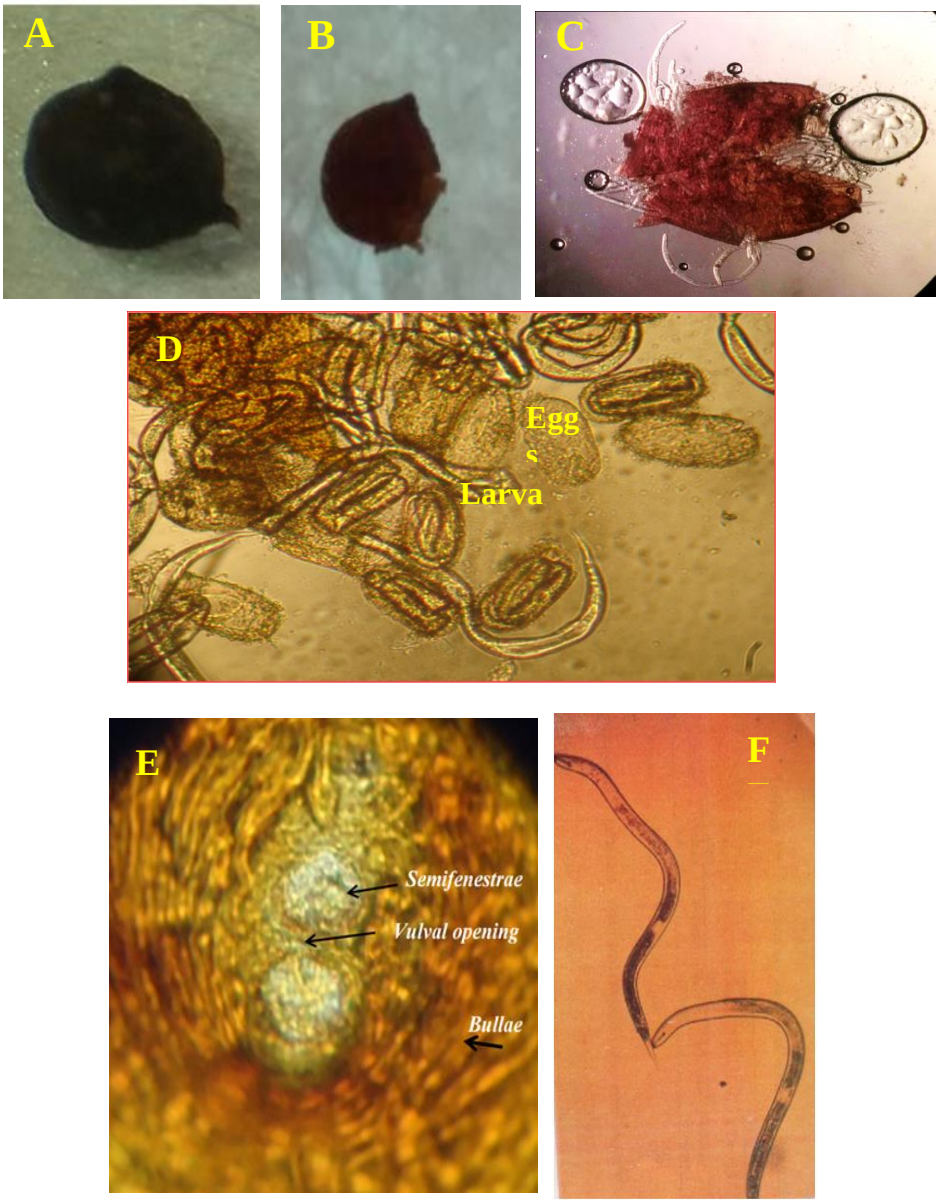


Figure 2 *Heterodera avenae* cysts, eggs, and second-stage juveniles: **(A)** Intact viable cyst with egg mass; **(B)** Degenerated empty cyst; **(C)** Ruptured cyst releasing eggs and second-stage juveniles (J2s)**; (D)** Active J2s during hatching; **(E)** Section at the vulval cone level (magnification 40×10)**; (F)** Second stage juveniles **(J2s)** (magnification 10 × 40).

Occurrence and distribution patterns of infestation
Average cyst density per plot was calculated as the ratio of the number of full cysts (FC) to the total sampled soil weight (expressed as cysts per 100 g of soil). Statistical analysis revealed significant effects of locality on cyst density for both full cysts ($F = 433.75$, $p < 0.001$ at 0–10 cm; $F=57.71$, $p < 0.001$ at 10–30 cm) and empty cysts ($F=66.05$, $p < 0.001$ at 0–10 cm; $F = 56.09$, $p < 0.001$ at 10–30 cm) (Table 3), while soil depth showed no significant effect on total cyst number at the surface layer ($F = 2.51$, $p > 0.05$) (Table 3). The interaction between locality and depth was non-significant for surface samples but significant for deeper layers ($F = 79.22$, $p < 0.001$), confirming homogeneous vertical distribution of cysts within the plow layer for most localities.

Table 3 Repeated-measures ANOVA for the number of cysts (full, empty, and total) at different soil.

	Soil depth 0-10 (cm)		Soil depth 10-30 (cm)	
Cyst	F	P	F	P
EC	66.05	***	56.09	***
FC	433.75	***	57.71	***
TCN	2.51	ns	79.22	***

Abbreviations: EC = Empty Cysts; FC = Full Cysts; TCN = Total Cyst Number
Statistical significance: ns = not significant ($P > 0.05$); *** = $P < 0.001$.

Our analysis revealed distinct patterns in cyst distribution across soil depths:
0-10 cm depth: The relationship between total cyst number (TCN) and full cysts (FC) per 100g of soil exhibited a non-linear pattern best described by a quadratic regression model ($R^2 = 0.92$, $r = 0.96$), while the linear model showed a poor fit ($R^2 = 0.0295$). The quadratic curve revealed an optimal cyst density around 1.2- 1.5 cysts per 100g of soil, where complete cyst development reached its maximum. Beyond this threshold, the proportion of fully developed cysts declined despite increasing total cyst density, suggesting density-dependent regulation mechanisms such as intraspecific competition for host resources or other limiting factors that impair cyst maturation at higher infestation levels (Fig. 3a, b).
10–30 cm depth: Strong correlations were detected between total cyst counts and both FC ($r = 0.90$) and EC ($r = 0.97$) (Fig. 3c, d), indicating significant root zone infestation.
These findings suggest that tillage-initiated redistribution of cysts within the plow layer is reinforced by complementary processes that extend this effect into the 10–30 cm zone. In the clay-rich vertisols present at several sites (e.g., Oued Fodda), shrink–swell cracking during wetting–drying cycles allows percolating water to transport cysts and eggs to greater depths; motile second-stage juveniles migrate along root channels and moisture gradients (Turner and Rowe, 2006), aided by cereal roots that commonly extend beyond 10 cm; and the multi-year monoculture history of the sampled plots suggests that this depth uniformity is a cumulative effect built up over several growing seasons rather than the outcome of a single tillage pass. No significant vertical stratification was detected ($p > 0.05$). The higher cyst density in deeper strata may reflect:

- Nematode ecology: Preferential migration toward root systems.
- Agronomic factors: Tillage-driven vertical displacement of cysts.
- Soil texture: Enhanced cyst preservation in lower horizons.

Recent advances in cyst nematode assessment have shifted from simple cyst counts (Aubert, 1986) to integrated viability analyses. Contemporary studies demonstrate that quantifying viable eggs and J2 juveniles through microscopic dissection (Shepherd, 1986) and fluorescence staining (Ferreira *et al.*, 2015; Sivasankara Pillai and Dandurand, 2019) provides more accurate estimates of infection potential than traditional full/empty cyst differentiation. Our findings reveal significant biological variability, with 15-40% of eggs in diapause (Righi *et al.*, 2022), and mortality rates influenced by the soil microclimate (Smiley *et al.*, 2023). Spatial analysis shows infestation rates ranging from 50-66.7% across sites (Fig. 3-4), consistent with global patterns of depth-dependent stratification (10-30 cm) and cultivar-specific susceptibility (Dababat *et al*., 2021; El Hassani, 2023). These results underscore the need for a multidimensional assessment of cyst populations in integrated pest management.

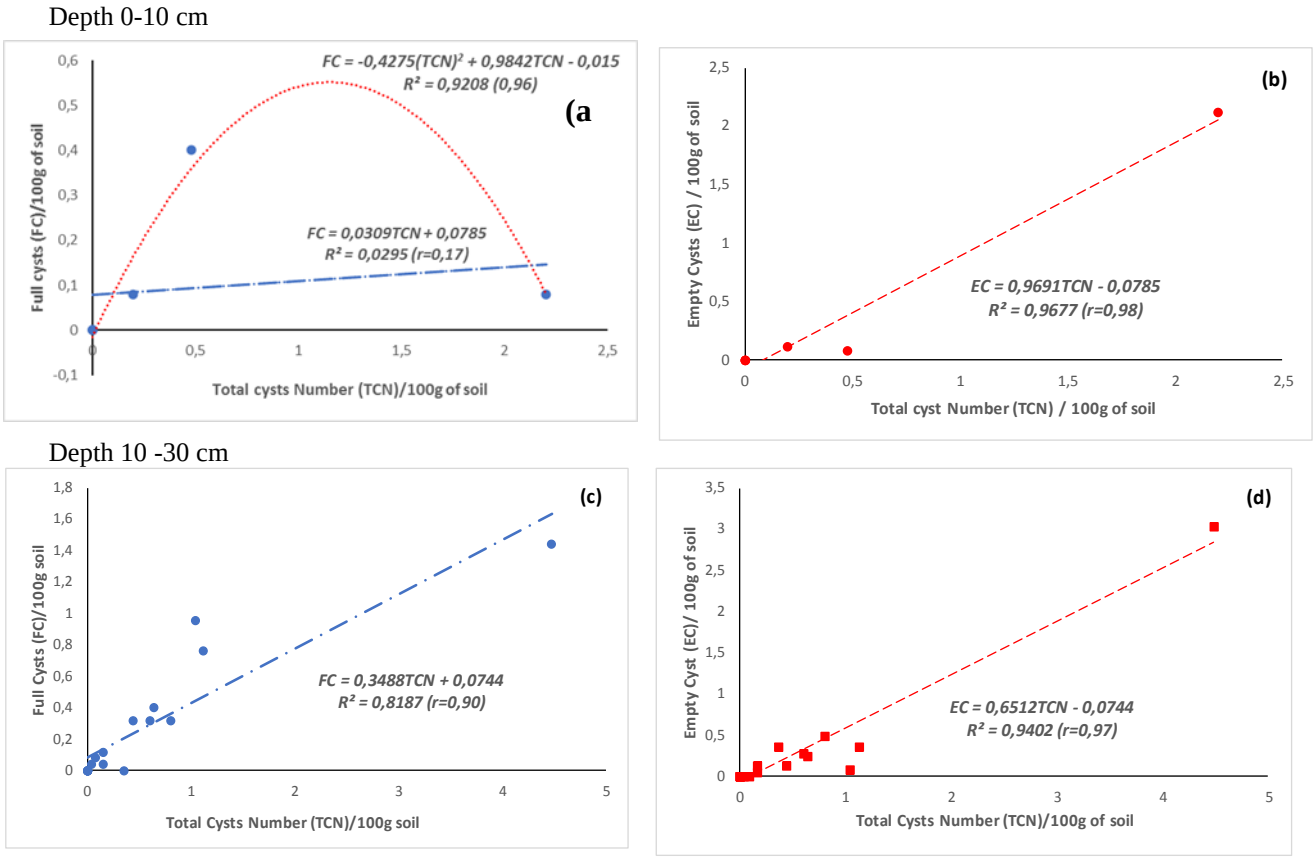


Figure 3 Depth-dependent correlations between full (FC) and empty (EC) cyst counts. (a, b) Surface layer (0-10 cm); (c, d) Subsurface layer (10-30 cm).

The observed spatial and temporal variations in *Heterodera* spp. population densities reflect differences in soil properties, agricultural practices, and climatic conditions among the surveyed fields. Climatic conditions, particularly temperature and precipitation regimes, significantly influence nematode life cycles and soil survival rates. Agricultural practices, including crop rotation systems and the selection of host versus non-host varieties, further modulate population dynamics. Soil characteristics such as texture, organic matter content, and pH also play a critical role in cyst viability and larval hatching. Additionally, chemical inputs (e.g., nematicides, nitrogen fertilizers) alter soil ecologies and directly affect nematode communities. Consistent with earlier studies on *Heterodera* distribution in North Africa, our findings support global models of nematode responses to agro-management practices, such as the one proposed by Arjoune *et al.* (2022).

Quantification of eggs and juveniles for assessing economic thresholds in field plots

The infestation severity was assessed based on the density of eggs and second-stage juveniles (J2) per gram of soil, following established protocols (Turner and Rowe, 2006). Cyst contents were extracted and examined to determine egg viability and J2 activity, revealing significant variability across plots ($p < 0.05$). Spatial analysis identified two distinct clusters: a homogeneous group (P2–P6) with moderate infestation levels (mean: 6.2 ± 1.8 J2/g soil) and an outlier plot (P1) exhibiting elevated densities (14.3 J2/g soil). These differences

were driven primarily by soil texture (clay content: $r = 0.62$, $p = 0.01$), crop genotype (resistant vs. susceptible varieties: $\Delta = 4.1$ J2/g, $p = 0.03$), and rotational history (monoculture vs. rotation: $F = 8.9$, $p < 0.01$) (Figs. 3–4).

In contrast, vertical distribution between 0–10 cm and 10–30 cm depths showed no significant differences ($p > 0.05$), consistent with tillage-induced homogenization of soil layers (Foxi *et al.*, 2017). Notably, observed J2 densities (<10 J2/g soil) remained below the conventional damage threshold (12 J2/g soil) (Mokabli, 2002), suggesting limited immediate risk. However, multivariate analysis indicated that 78% of infestation variance ($R^2 = 0.78$) was explained by interacting edaphic (soil organic matter: $\beta = 0.34$), climatic (growing-degree days: $\beta = 0.28$), and agronomic factors (nitrogen input: $\beta = 0.19$).

These edaphic and climatic gradients likely act mainly on egg hatching, J2 survival, and the proportion of full versus empty cysts, rather than on total cyst density: the surface layer undergoes more frequent wetting–drying cycles and wider temperature swings, both known to trigger earlier, more synchronous hatching in *Heterodera* species, while the more buffered 10–30 cm layer favors prolonged egg dormancy, and the higher organic matter typically found near the surface supports larger populations of nematode-antagonistic microorganisms that likely raise egg mortality in the upper horizon. Full versus empty cyst status was determined by visual inspection under a stereomicroscope (10–16 × magnification; Section 2.3), a structural proxy that does not directly measure biochemical viability; the depth-related differences in full/empty cyst proportions should therefore be read as consistent with, rather than direct evidence of, differential hatching and viability between strata. Fluorescence-based viability assays, such as SYTOX Green staining (Ferreira *et al.*, 2015), would offer more direct confirmation and represent a valuable direction for future work.

The infestation rates observed in this study are consistent with those reported in similar research conducted in dry and semi-arid regions. Mokabli (2002) documented *H. avenae* cyst densities ranging from 10 to over 100 cysts per 100 grams of soil in Algerian cereal fields. The damage threshold was estimated at 12 J2 per gram of soil, which is comparable to our findings of fewer than 10 J2 per gram. Furthermore, Smaha *et al.* (2019) reported prevalence rates of 40–66% in Algerian wheat fields, aligning with our 8–30% results.

Conclusions

This investigation demonstrates the widespread distribution of *Heterodera avenae* in Chlef cereal-growing areas, with infestation rates of 8- 30%. Cyst densities ranged from 0.04 ± 0.02 per 100 g soil in Ouled Fares to 4.48 ± 0.01 cysts per 100 g in Tadjena, while J2 densities remained below the established economic threshold of 12 J2/g soil (Mokabli, 2002). However, certain high-risk plots approached critical levels. These results validate region-specific economic thresholds under Chlef's local edaphic conditions. The absence of vertical stratification (ANOVA, $p > 0.05$) confirms tillage-induced homogenization of cyst distribution within the 0-30 cm profile, while inter-site variability reflects agro-environmental influences ($r = 0.62$ clay; $F = 8.9$ rotation). This baseline supports precision agriculture through targeted monitoring of high-risk sites, optimized crop rotations with resistant cultivars (Smiley *et al.*, 2023), and adjusted tillage for semi-arid systems (Otero *et al.*, 2023; Arjoune *et al.*, 2022). Future research should focus on long-term monitoring and validation of integrated control strategies under projected climate scenarios. Broader sampling coverage across additional sites would help confirm these relationships and better capture the environmental heterogeneity within the province. These findings provide essential data for sustainable cereal production in Algerian semi-arid regions.

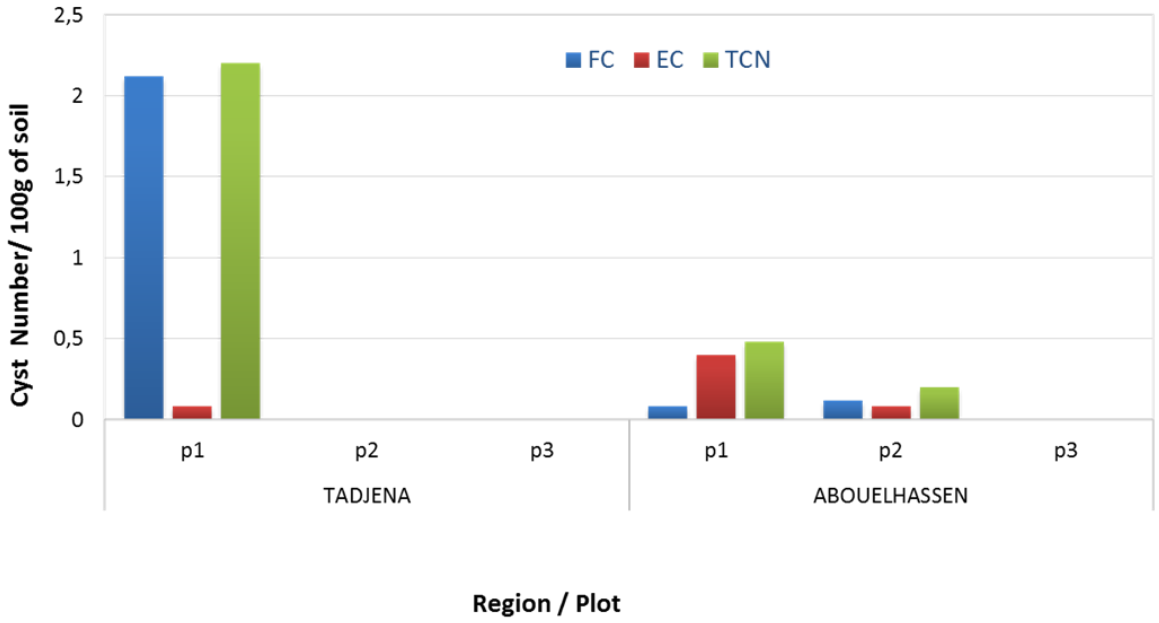


Figure 4 Vertical distribution of *Heteroderaa venae* cysts (0-10 cm): FC (full), EC (empty), and TCN (Total Cyst Number per 100 g soil).

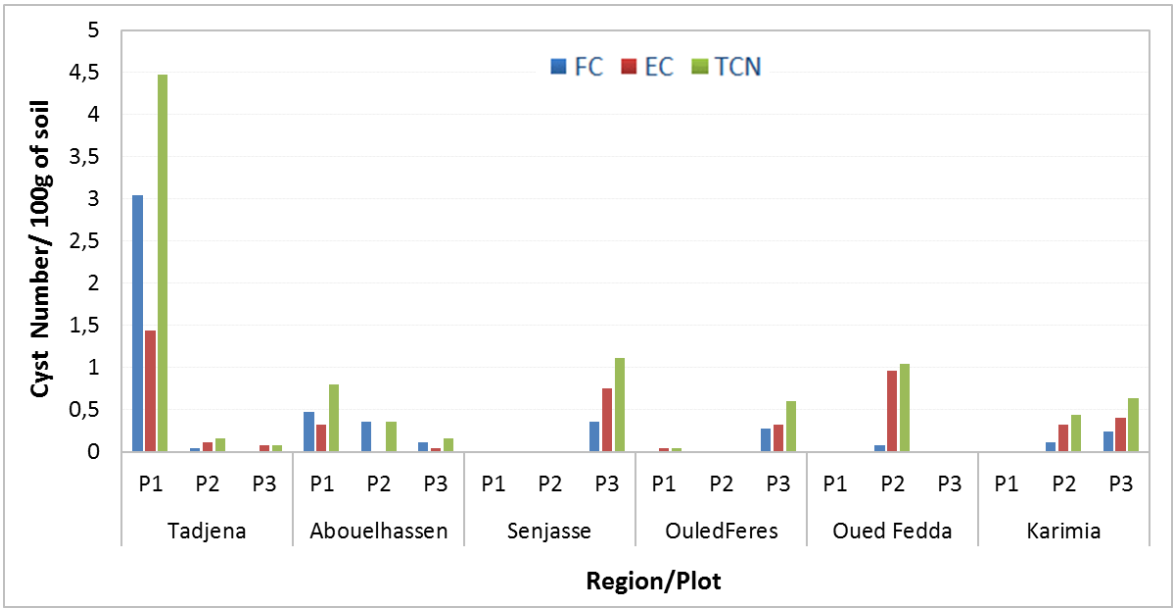


Figure 5 Density of *Heterodera avenae* cysts per 100 g soil at 10-30 cm depth across surveyed localities FC (full CYST), EC (empty CYST), and TCN (Total Cyst Number per 100 g soil).

Declaration of interest

The authors declare no conflict of interest.

Authors' Contributions: Meziane Boukhatem Malika[1], conceived and supervised the study, interpreted data, and contributed to manuscript preparation. Ali Arous Samir[1] collected samples, performed experiments and analyses, and assisted in writing. Bradai Abdelhamid[2] provided support for methodology, statistical

analysis, and critical revision. Smaha Djamel[3] contributed to cyst extraction, nematode quantification, and data processing. Mekhaneg Boualem[3] provided technical and field assistance.Rouam Djawad translated and contributed to editing and final revision. Meziane Ahmed Malika[4] contributed to editing, final revision, and approval of the manuscript.

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توزیع عمودی و پویایی آلودگی نماتد *Heterodera avenae* در مزارع غلات منطقه شلف (Chlef) الجزایر: پیامدهایی برای مدیریت نماتدها

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چکیده: نماتد *Heterodera avenae*، که یکی از نماتدهای سیستی مهم در غلات محسوب می‌شود، تولید گندم و جو را در شمال آفریقا تهدید می‌کند. با این‌حال، اطلاعات دقیقی درباره پراکندگی آن در خاک‌های الجزایر وجود ندارد. در این مطالعه، توزیع مکانی و عمودی *H. avenae* در ۱۸ مزرعه غلات واقع در شش منطقه از استان شلف (الجزایر) و در دو عمق خاک (۰ تا ۱۰ سانتی‌متر و ۱۰ تا ۳۰ سانتی‌متر) بررسی شد. با استفاده از روش شناورسازی «فنویک (Fenwick)» و تحلیل‌های میکروسکوپی، سیست‌ها و لاروهای سن دوم (J2) استخراج و شمارش شدند. نتایج نشان‌دهنده نرخ آلودگی بین ۸ تا ۳۰ درصد بود. تفاوت معناداری بین اعماق مختلف خاک مشاهده نشد ($p > 0.05$) که نشان می‌دهد شخم‌زنی‌های سطحیِ رایج، باعث یکنواخت شدن توزیع نماتدها در خاک می‌شوند. بیشترین تراکم سیست (٤/٤٨ ± ۰/۰۱) سیست در ۱۰۰ گرم خاک) در منطقه «تاجنه (Tadjena)» و کم‌ترین میزان (۰/۰٤ ± ۰/۰۲) در منطقه اولاد فارس (Ouled Fares) ثبت شد. تراکم لاروهای سن دوم در تمام مزارع پایین‌تر از آستانه خسارت اقتصادی (۱۲ لارو در هر گرم خاک-طبق مطالعات پیشین) بود. این امر نشان‌دهنده ریسک فوریِ پایین است، اما بر اهمیت پایش مستمر برای جلوگیری از طغیان‌های احتمالی در آینده تأکید دارد. این یافته‌ها بینش‌های کاربردی برای مدیریت تلفیقی آفات (IPM) فراهم می‌کنند و بر ضرورت به‌کارگیری راهکارهای کنترلی که تمامی اعماق خاک را در نظر می‌گیرند، در سیستم‌های زراعیِ مناطق نیمه‌خشک تأکید می‌ورزند.

واژگان کلیدی: عوامل زراعی-بوم‌شناختی، پایش نماتد سیستی غلات، تعیین کمیت نماتد، عمق نمونه‌برداری