PAPER • **OPEN ACCESS**

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To cite this article: Mohammed H. Aldarraji and Dhia S. Alwaily 2024 *IOP Conf. Ser.: Earth Environ. Sci.* **1371** 032041

IOP Conf. Series: Earth and Environmental Science **1371** (2024) 032041 doi:10.1088/1755-1315/1371/3/032041

Morphological Diagnosis of *Heterodera avenae* in Iraq and Their Pathogenicity on Three Wheat Varieties

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Abstract. Wheat is one of the dominant crops in many countries being used by human and livestock. Cereal Cyst nematodes one of very important pest that reduce yield up to 50% worldwide. In this study, the survey was conducted across seven Governorate the Heterodera type appeared in three of them (Misan, Baghdad, Al-Najaf) chosen based from their wide area of small grain production and geographical position in 2020. Cyst were identified by morphological methods. Species identification has been validated by molecular method. As a result of the study, that the wheat variety Buhooth 22 was less affected by the infection and the infection rate was 40% and a significant difference was recorded between it and the other two varieties, Ibaa 99 and Rashid. The infection rate was high and reached 53.3 and 60% respectively. This is first study in Iraq in terms of morphometric nematode identification in case of Cyst nematodes. These results can be of signifigance for improving strategies to control Cereal Cyst nematode affecting wheat production in Iraq.

Keywords. Morphological, Nematodes, Wheat varieties, *Heterodera avenae*.

1. Introduction

Cereal cyst nematodes *Heterodera avenae* is the principal nematode species on temperate cereals, and in Europe more than 50% of the yields in major cereal growing areas are infected by this nematode [1]. A morphologically sound identification method can be used to detect an increasing number of species belonging to the H. avenae group. According to research, there are twelve species of cyst nematode in the genus Heterodera. Of these, three—H. avenae, H. filipjevi, and H. latipons—cause substantial economic losses in small grain crops worldwide [2].

The original test array did not capture the full range of this species' polymorphism, new path types have been identified, and the patho type approach, which was initially developed to distinguish between H. avenae populations in northern Europe, is insufficient for describing virulence phenotypes in other regions. Thirty virulence traits have been found from 69 H. avenae populations studied globally, and many additional nations still need to do their own virulence phenotypic assessments [3]. In addition, nine other genera of plant parasitic nematodes were detected by the survey: Aphelenchus,

Haplolaimus, Helicotylenchus, Heterodera, Paratylenchus, Pratylenchus, Tylenchorhynchus, Tylenchus, and Xiphinema [4,5].
There are eleven known species of Heterodera avenae and many more that have not yet been named or described [6,7]. Of these, three—H. avenae Wollenweber, H. latipons Franklin, and H. filipjevi (Madzhidov)—are known to be the most damaging cyst nematode pests to cereals. While Stone Heterodera avenae is found all over the globe, H. latipons is mostly found in the Mediterranean, and H. filipjevi is primarily found in Eastern Europe and West Asia [8].
Three species of H. avenae group nematodes were found in recent surveys of cereal fields in Syria and the Central Anatolian Plateau of Turkey. These locations are major wheat and barley farming regions in Western Asia. Although H. avenae's distribution was small, it was reported in Turkey and detected in Syria [9]. Several biochemical techniques have shown useful for nematode identification, since successful identification based on morphology becomes more challenging. The distinct species of Heterodera were identified by the use of isozyme analysis. Polymorphism was found in populations of Heterodera avenae, and RAPD analysis was able to discriminate between Heterodera species [10]. The first investigation to find Heterodera avenae in three governorates in Iraq (Al-Najaf, Baghdad, and Maysan) was carried out by [11].

2. Materials and Methods

2.1. Nematode Extraction from Soil Samples
Plant parasitic nematodes were extracted by Whitehead-Hemming tray method Whitehead-Hemming Heretofore referred to as the Whitehead method by rough sieve (4.76 mm) [12].

2.2. Morphological Identification
Using a stereomicroscope, we examined the collected nematodes, selected the ones we were interested in, killed them, preserved them, and then transferred them to anhydrous glycerin, following the procedure outlined in [13]. Using a drawing tube connected to an Olympus BH-2 light microscope, permanent slides were created and morphometric data was gathered. A digital camera was attached to the same microscope in order to capture photomicrographs [14]. A comprehensive literature review and collection formed the basis for morphometric and morphological investigations of all known species of Helicosae [15].

2.3. Phenotypic Diagnosis of Perineal Pattern Section of Vesicular Nematode
The cyst nematodes, widespread worldwide, were diagnosed based on the morphological characteristics of the Perineal pattern of adult females according to [16].

2.4. Pathogenicity of Heterodera sp on Three Wheat Cultivars
The experiment was conducted on 1/11/2020, Soil sterilized with formalin was prepared, then the soil was filled into plastic 1 Kg pots. Wheat seeds of three varieties (Ibaa 99, Buhooth 22, and Rashid) were obtained from the Department of Inspection and Certification of Seeds, Saad River Branch. 3 seeds were grown for each pot with the addition of 200 Cyst nematodes eggs/pot each with three replications [11].
Pathogenesity of cyst nematodes *H. avenae* was evaluated in the three wheat varieties according a 5 degree scale [17] it consists of five degrees. The percentage of severity of nematode infection was calculated by using McKinney equation [18].

$$\text{Injury severity} = \left(\text{Sum}\left(\text{Number of infected plants} \times \text{degree of infestation}\right) / \left(\text{Total number of plants} \times \text{highest score}\right)\right) \times 100$$

3. Results and Discussion
The cyst nematodes *Heterodera avenae* was morphometric diagrammed (Figure1) and microscopic characterized (Figure2). The results indicated in Table (1) btained when taking biometric measurements of the vesicular nematodes of the studied model Paratype and comparing them with the

original Holotype, where all measurements were taken in mμ and according to the measurements of de man 1884 equation and the number of nematodes that were measured was 10, the total body length of the nematode was 457.5 (L) and the total body length divided by the largest body width 23.2 (a), the length of the body divided by the length of the esophagus 3.7 (b) , the total body length divided by the distance between the beginning of the body and the posterior end of the esophageal glands in the case of overlap of the esophagus with the intestine 3.4 (b‘) and the total body length divided by the length of the tail (the distance between the anus and the end of the body C), 7.7 the length of the tail divided by the width of the body in the anal area 4.9 (c‘), the width and height of the head were (5.1) and (8.8) respectively, length of the spear (Style) 26.0, conical front part of the spear (conus) 11.3, length of the pointed front section of the spear x 100 divided by the length of the spear 43.2 (m), as for dorsal glandular duct opening (DGO) 5.5, the distance between the nodes of the spear and the opening of the dorsal esophageal gland divided by the length of the spear × 100 21.2(O), middle esophagus (median bulb) 71.8.

MB% = % distance from anterior to median bulb relative to length of esophagus =57.1 . As for excretory pore = 101.8, oesophagus length=125.8, end of gland=136.0, the width of the body was=19.8, anal body width =12.3, the tail was 60.0 in length, length of the transparent area of the tail (Hyalin) 35.3. The results agreed with the findings [15] . In the taxonomic key, the length of the tail is less than 80, ranging from(68-70) and tail end area (hyalin) 41(35-45), length of spear 27(24-28), DGO was (5-6), as for the width of the body BW (20-24). It was confirmed by [19] there are some important characteristics in diagnosing nematodes *H. avena* from the rest of the other species the length of the tail ranges from (60-70), and the length of the spear is (26-28).

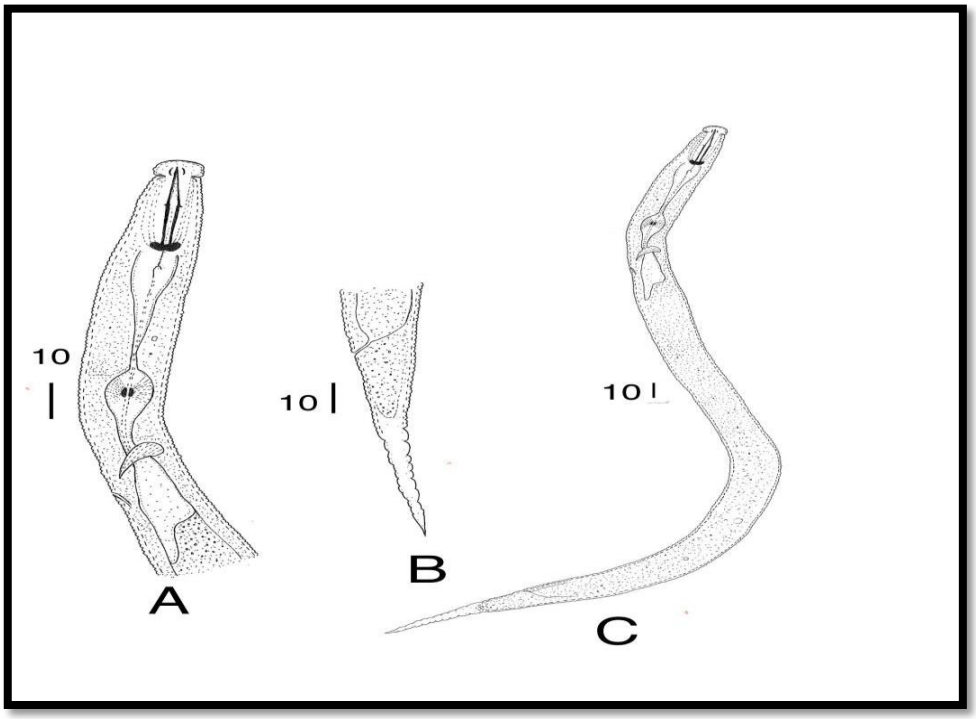

Figure 1. Morphometric real drawing of the cyst nematode, A) showing the front (anterior body) part revealing the well diviloped spear (Stylet), Bulboid Esophagus and nurve ring and esophagus postrioer ended with the intestain, B) the postrior tail region, and C) the entire body of the vermiform J2 cyst nematode.

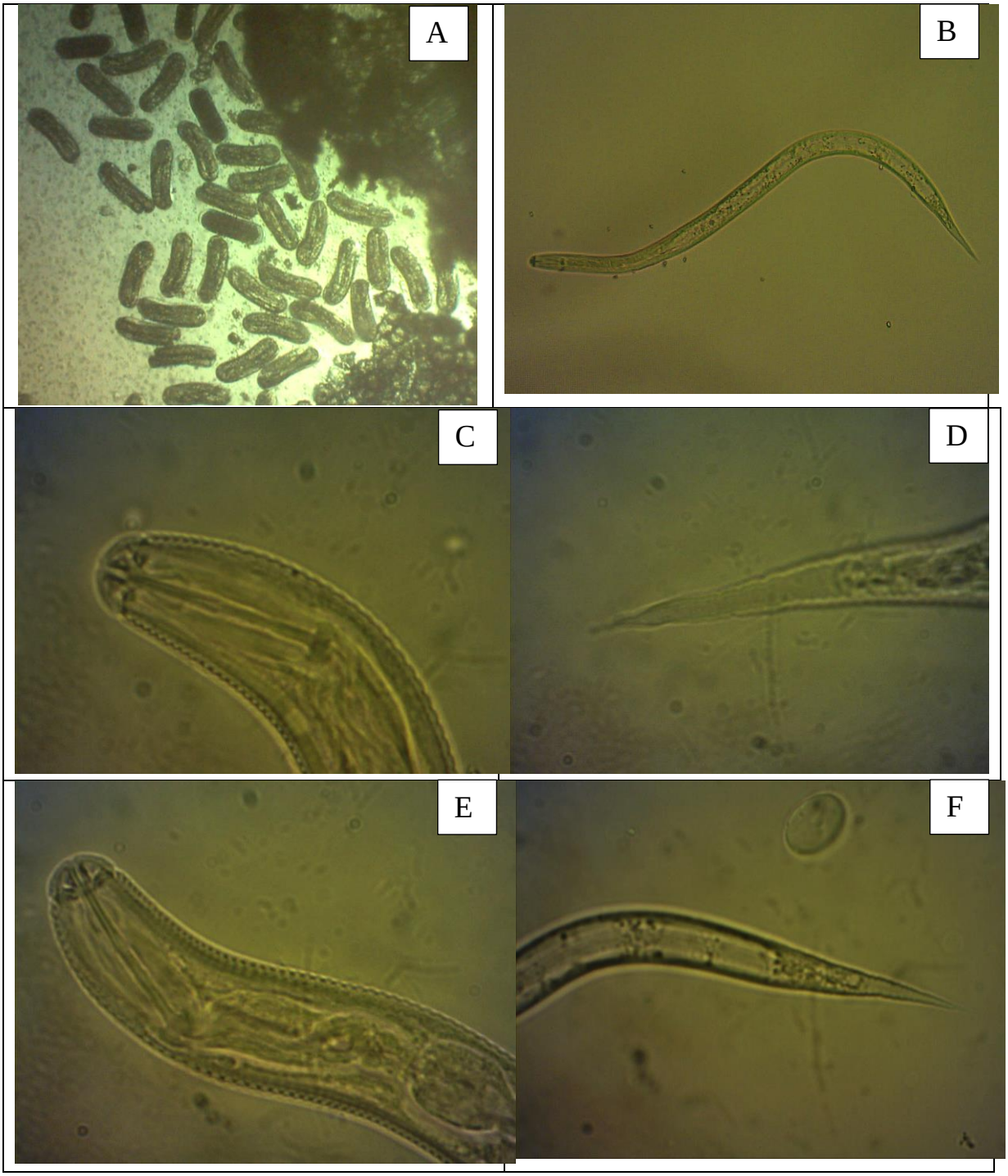

Figure 2. Microscopic characterization of cyst nematodes *H. avenae* showing nematode eggs (A), the vermiform Js (B), Anterio part of lips and stylet head framework (C), tail hilgh (D), Anterior body part and overlapping esophus (E), and thye tail region showing tail terminus (F).

Table 1. Morphometric measurements of the Cyst nematode *H. avenae.*

Characters	Holotype	Para type
Slide No.	N	10.0
L	493.0	457.5 ± 25.2(439.0-493.0)
L'	433.0	398.3 ± 25.0(380.0-433.0)
A	24.1	23.2 ± 1.4(22.0-24.7)
B	3.7	3.7 ± 0.2(3.4-4.0)
b'	3.4	3.4 ± 0.2(3.2-3.7)
C	7.9	7.7 ± 0.4(7.3-8.2)
c'	4.5	4.9 ± 0.4(4.5-5.4)
Head Height	5.0	5.1 ± 0.3(5.0-5.5)
Head Width	9.0	8.8 ± 0.5(8.0-9.0)
Stylet	26.0	26.0 ± 0.8(25.0-27.0)
Conus	10.0	11.3 ± 1.0(10.0-12.0)
M	40.0	43.2 ± 2.7(40.0-46.2)
DGO	6.0	5.5 ± 0.6(5.0-6.0)
O	24.0	21.2 ± 2.7(18.5-24.0)
Median bulb	75.0	71.8 ± 7.2(61.0-76.0)
MB	60.0	57.1 ± 3.1(53.6-60.0)
Excretory Pore	103.0	101.8± 8.4(90.0-110.0)
Oesophagus	125.0	125.8 ± 12.3(110.0-140.0)
End Of Glands	135.0	136.0 ± 12.4(120.0-150.0)
Overlapping	10.0	10.3± 0.5(10.0 -11.0)
Body Width(BW)	20.0	19.8 ± 0.5(19.0-20.0)
Anal Bady Width	13.0	12.3± 1.0(11.0 -13.0)
Tail	60.0	59.3 ± 1.0(58.0-60.0)
Hyalin	38.0	36.3± 1.7(36.3-38.0)

The study that was conducted to know the characteristics of the perineal pattern (Vulval cone) of the *H. avenae* nematode showed, where the lemon shape adult female was detected, the wall color of the cyst is dark brown and has a zigzag pattern, the wall color of the bags is dark brown and has a zigzag pattern and these characteristics we notice in figure (3) (A, B), the head is slightly skewed.

The study agreed with the findings of the researchers [19], the cyst shape of *H. avenae* and *H. ustralis* is lemon shaped while the rest of the species such as *H. pratensis* and H. mini are round. The researcher also stated that the color of the cyst is important in distinguishing between the types of nematodes, as *H. avenae*, *H. ustralis, pratensis and H. mini* are characterized by a brown to dark brown color, while the rest take the bags other colors.

The researcher also explained that the vulva is conical in all species except the type *H. flipjevi* is longer than the rest and the bubbles are white on the edges, and there is a distinct lower bridge in all species [20].

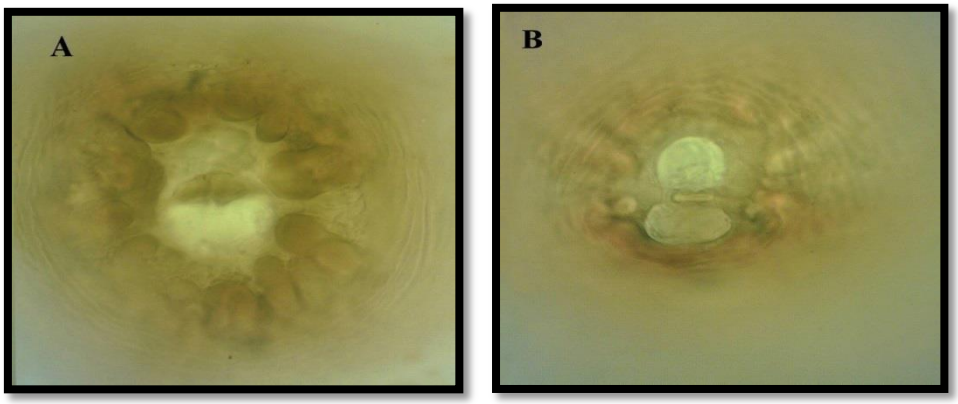

Figure 3. A-B: Represents a section of the perineal pattern (vulval cone) of cyst nematode *H. avenae* adult female, showing the vulva bridge, semifenstration and bullae

As for the pathogenicity test, the results of the statistical analysis shown in figure (4) indicated that the variety Buhooth 22 was less affected by the infection and the infection rate was 40% and a significant difference was recorded between it and the two cultivars. As for the two cultivars Ibaa 99 and Rashid, the infection rate was high and reached 53.3 and 60% respectively as in the figure (5).

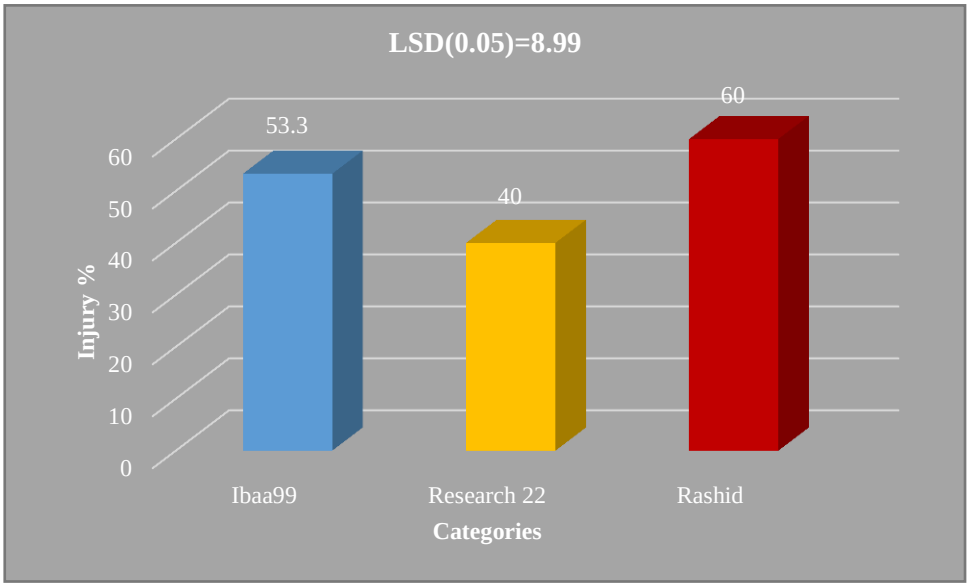

Figure 4. Represents the pathogenicity of cys nematodes *H. avenae* on three wheat varieties.

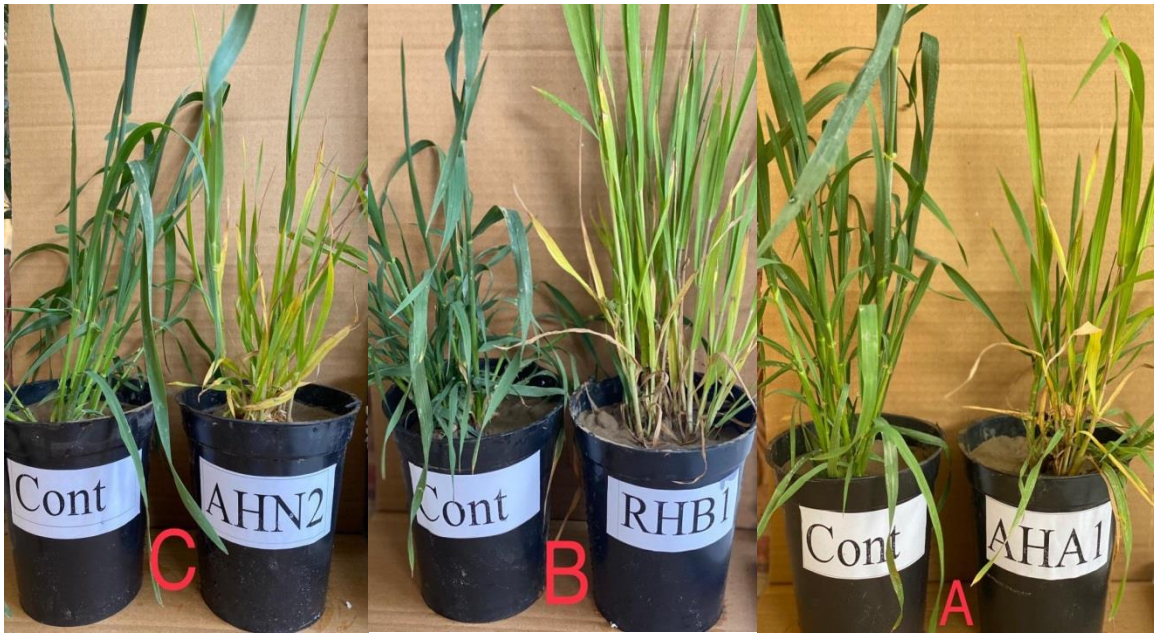

Figure 5. Growth reduction at different levels caused by cyst nematodes *H. avenae* on the three wheat varieties (A.Ibaa 99, B. Buhooth 22 and C. Rashid) under study.

In order to understand the true impact of these pests on cereal yield losses, it is crucial to establish their economic importance. Nematode surveys in Syria and Turkey revealed that H. latipons and H. filipjevi are widely distributed in major cereal-cultivating areas, with high population densities in some regions [21]. Because of the diversity in this category of nematodes, which might occur in mixed populations, effective integrated control methods require studies that use reliable methodologies for the precise identification of species [6].

Incorporating variations observed in certain populations into the morphometric compendium, the measurements of most specimens closely matched the original and subsequent redescriptions of

species. This key is significant because it provides an all-inclusive guide to identifications and works well with all described populations of the 12 valid Heterodera spp. in the H. avenae group, including the specimens of this group saved in the Nematode Collection [22]. In order to consider a broader view of the fundamental challenges that arise when dealing with diverse populations within the avenae group, the author acknowledges the value of molecular data and implies that, for populations where morphological data is uncertain, molecular data could be beneficial [15].
There is an extensive variation in morphological characters within the *H. avenae* group. Table 1, a compilation of characters commonly used for species identification, further emphasizes the heterogeneity of the group and supports the view that the H. avenae group is not monophyletic [23]. In this study, we used both technique morphological techniques to detect the *Heterodera avenae* from Iraq. Although the effect of *Heterodera avenae* on the crops in Iraq have been reported previously by Stephan [24], our study can be considered as the first study on *Heterodera avenae* diagnosis in Iraq.

Conclusion

The study found that Buhooth 22 had a 40% infection rate, significantly lower than Ibaa 99 and Rashid. Infection rates were high (53.3 and 60%). This is Iraq's first Cyst nematode morphometric identification study. These findings may improve Iraqi wheat output Cereal Cyst nematode control techniques.

References

[1] Qing, X., Peng, H., Ma, J., Zhang, Y. M., Li, H., Peng, D., ... & Long, T. (2022). Phylogeography of Chinese cereal cyst nematodes sheds lights on their origin and dispersal. Evolutionary applications, 15(8), 1236-1248.
[2] Keçici, A. İ., Bozbuğa, R., Öcal, A., Yüksel, E., Özer, G., Yildiz, Ş., ... & İmren, M. (2022). Diversity and identification of plant-parasitic nematodes in wheat-growing ecosystems. Microorganisms, 10(8), 1534.
[3] Al-Khafaji, R. T. (2018). An assessment of nematodes affecting wheat in Montana (Doctoral dissertation, Montana State University).
[4] Smiley, R. W., & Yan, G. (2015). Discovery of Heterodera filipjevi in Washington and comparative virulence with H. avenae on wheat. Plant Disease, 99(3), 376-386.
[5] Smiley, R.W. (2016). Cereal Cyst Nematodes. A Pacific Northwest ExtensionPublication. https://catalog.extension.oregonstate.edu/sites/catalog/files/project/pdf/ pnw620_2.pdf
[6] Abidou, H., Valette, S., Gauthier, J. P., Rivoal, R., El-Ahmed, A., & Yahyaoui, A. (2005). Molecular polymorphism and morphometrics of species of the Heterodera avenae group in Syria and Turkey. Journal of Nematology, 37(2), 146.
[7] Ahmadi, A. R., & Tanha Maafi, Z. (2014). Incidence of Cereal Cyst Nematodes (Heterodera avenae type B and H. filipjevi) in southwestern Iran. Journal of Crop Protection, 3(1), 75-88.
[8] Dababat, A. A., Imren, M., Erginbas-Orakci, G., Ashrafi, S., Yavuzaslanoglu, E., Toktay, H., ... & Mekete, T. (2015). The importance and management strategies of cereal cyst nematodes, Heterodera spp., in Turkey. Euphytica, 202(2), 173-188.
[9] Toumi, F., Hassan, G., Waeyenberge, L., Viaene, N., Dababat, A. A., Nicol, J., ... & Moens, M. (2015). Distribution of the cereal cyst nematodes (Heterodera spp.) in wheat and barley fields in north-eastern regions of Syria. Journal of Plant Diseases and Protection, 122(5), 255-263.
[10] Bogale, M., Baniya, A., & DiGennaro, P. (2020). Nematode identification techniques and recent advances. Plants, 9(10), 1260.
[11] Aldarraji, M. H., & Alwaily, D. S. (2021). DETECTION OF CYST NEMATODE ON WHEAT PLANTS IN MIDDLE AND SOUTH OF IRAQ. Int. J. Agricult. Stat. Sci. Vol, 17(1), 2333-2337.
[12] May, D. B., Johnson, W. A., Zuck, P. C., Chen, C. C., & Dyer, A. T. (2016). Assessment and management of root lesion nematodes in Montana wheat production. Plant disease, 100(10), 2069-2079.
[13] De Grisse, A. T. 1969. Redescription ou modification de quelques techniques dans L'etude des nematodes phytoparasitaires. Mede. Rijks. fak. LandbWet Gent, 34: 351-369
[14] Golhasan, B., Heydari, R., Alvarez-Ortega, S., Esmaeili, M.,
[15] Handoo, Z. A. (2002). A key and compendium to species of the Heterodera avenae group (Nematoda: Heteroderidae). Journal of Nematology, 34(3), 250.
[16] Perry, R. N., Moens, M., & Jones, J. T. (Eds.). (2018). Cyst nematodes. CABI.

[17] Kerry, B. R., Irving, F., & Hornsey, J. C. (1987). Variation between strains of the nematophagous fungus, Verticillium chlamydosporium Goddard. I. Factors affecting growth in vitro.
[18] Matrood, A. A., & Rhouma, A. (2021). Evaluation of the efficiency of Paecilomyces lilacinus and Trichoderma harzianum as biological control agents against Alternaria solani causing early blight disease of eggplant. Pakistan Journal of Phytopathology, 33(1), 171-176.
[19] Subbotin, S., Sturhan, D., Rumpenhorst, H. J., & Moens, M. (2003). Molecular and morphological characterisation of the Heterodera avenae species complex (Tylenchida: Heteroderidae). Nematology, 5(4), 515-538.
[20] (MCLEOD, R.W. & KHAIR, G.T. (1977). Observations on the morphology of Heterodera avenae in Australia. Nematologica 23, 382- 389
[21] Mokrini, F. (2016). Root-lesion and cyst nematodes in cereal fields in Morocco: species identification, population diversity and crop resistance (Doctoral dissertation, Ghent University).
[22] Stec, D., Morek, W., Gąsiorek, P., & Michalczyk, Ł. (2018). Unmasking hidden species diversity within the Ramazzottius oberhaeuseri complex, with an integrative redescription of the nominal species for the family Ramazzottiidae (Tardigrada: Eutardigrada: Parachela). Systematics and Biodiversity, 16(4), 357-376.
[23] Dababat, A., İmren, M., Pridannikov, M., Özer, G., Zhapayev, R., Mokrini, F., ... & Morgounov, A. (2020). Plant-parasitic nematodes on cereals in northern Kazakhstan. Journal of Plant Diseases and Protection, 127(5), 641-649.
[24] Stephan, Z.A. (1986). Host Status of Cyst Nematodes in Iraq.In : Cyst Nematodes. Boston, MA: Springer US