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# Incidence of Cereal Cyst Nematodes (*Heterodera avenae* type B and *H. filipjevi*) in southwestern Iran

**Ali Reza Ahmadi[1*] and Zahra Tanha Maafi[2]**

1. Agricultural and Natural Resources Research Centre of Khuzestan, Ahvaz 61335-3341, Iran.
2. Iranian Research Institute of Plant Protection, Tehran 19395-1454, Iran.

**Abstract**: A survey of cereal fields of Khuzestan province during 2008-2011 revealed that cereal cyst nematodes (CCNs) are widely distributed in this region. The CCNs were present in 37 and 35% of the 200 samples collected from wheat and barley fields respectively. The species were identified as *Heterodera avenae* type B and *H. filipjevi* the morphological and morphometric identifications of which were confirmed by rRNA-ITS RFLP. Population density of CCNs ranged from 2 to 103 cysts (mean 18)/100 g of dried soil with an average of 395 (0-3400) J2 and eggs in wheat samples. Whilst the number of cysts in barley samples were counted 3-71 cysts (mean 11) /100 g soil, the J2 and eggs averaged 166 (0-900). The lowest and the highest rates of infestation (8 and 83%) were observed in the regions of Ahvaz and Behbahan respectively. The number of J2 and eggs of CCNs in some regions were greater than the damage threshold level considered for CCNs and it is likely they could cause economic yield loss in these regions.

**Keywords:** Cereal cyst nematodes, distribution, Iran, Khuzestan.

## Introduction

Cereals are the most important food source in the world and 58 percent of the annual cultivation has been allocated to wheat, corn and rice. By the year 2030 the world population will reach about 8 billion people and grain consumption will increase (Fischer *et al*., 2009). Wheat is cultivated in all parts of Iran over an area of 7 million ha with an annual production of 15 million tones, making it the 12th wheat producer country in the world during 2010-2011 (Anon, 2012). Khuzestan province is located in the southwest of Iran, bordering the Persian Gulf and covering an area of 64,000 km$^2$. The province enjoys long and warm summers and short mild winters. The average temperature is 31 °C in summer and 15 °C in winter with average annual precipitation of 266 mm. The area under wheat cultivation is 0.68 million ha with total production of 1.23 million tones, contributing 9% of annual national production in 2009-2010 (Anon, 2011). In Khuzestan province, spring wheat is planted in early November and harvested in mid to late May. The cereal cyst nematodes (CCNs) are considereded as one of the major disease agents of cereals throughout the world. The *Heterodera avenae* group consists of 12 valid and several undescribed species that infect cereals and grasses. The main CCNs attacking cereals are *H. avenae* Wollenweber, 1924, *H. filipjevi* (Madzhidov) Steler, 1984 and *H. latipons* Franklin, 1968 (Rivoal and Cook, 1993). *Heterodera avenae* has been reported in Europe (Rivoal and Cook, 1993) Austrlia (Brown, 1984) India (Khan *et al*., 1990) North America (Miller, 1986) and in several countries of North Africa and West Asia (Sikora, 1988; Al-Yahya *et al*., 1998). *H. filipjevi* occurs in Iran (Sturhan, 1996) Turkey

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* **Corresponding author**, e-mail: alirahmadi2000@gmail.com
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(Rumpenhorst *et al*., 1996), and in several European countries (Subbotin *et al*.,1996; Bekal *et al*., 1997; Rivoal *et al*.,2003; subbotin *et al*.,2003; Holgado *et al*., 2004). *H. latipons* has been detected in the Mediterranean region (Franklin, 1969; Tacconi, 1976; Romero, 1980; Sikora and Oostendorp, 1986; Philis, 1988; Greco, 1994), Southern Russia, Ukraine, Central Asian Republics (Subbotin *et al*., 2003), Iran (Talachian *et al*., 1976), Europe (Stoyanov, 1982; Sabova *et al*., 1988) and Canada (Sewell, 1973). *H. avenae*, *H. filipjevi*, *H. hordecalis* and *H. latipons* have been reported from cereal fields and grasslands in Iran (Tanha Maafi *et al*., 2007). The two first species are the most dominant cyst nematodes in cereal fields, *H. avenae* was found in wheat fields in only one region in the west of the country. *Heterodera hordecalis* was recovered from a few wheat fields and around grasses in western Iran (Tanha Maafi *et al*., 2007, 2009).

A survey of the CCNs in Syria and Turkey showed that 69.9 and 80% of cereal fields respectively were infested to *H. avenae*, *H. filipjevi* and *H. latipons* in Syria and Turkey (Abidou *et al*., 2005). According to a survey on CCNs in the Slovak republic during 2003-2004, *H. avenae* was detected in 56.4% of 188 soil samples, at an incidence of 2-81 cysts in 100 g soil (Renco, 2005). In Iran during a survey on CCNs of Markazy province with 88 root and soil samples of wheat and barley it was shown that 40 % of the sampled fields were infested with *H. filipjevi* and *H. latipons* at population density of 20-40 cysts/300 g soil (Hajihasani *et al*., 2008). The frequency of the CCNs was determined from the relationship between the numbers of samples in which the CCNs were observed divided by the total number of sample taken from that area, multiplied by 100 to express as a percentage (Sawadogo *et al*., 2009). The population density of the CCNs was expressed as the population of cyst, eggs and second stage juveniles in a ginven volume of soil (100 gram of dried soil) (Norton, 1978).

Yield losses due to *H. avenae* were estimated in Australia 20-40 percent on wheat with population density of 2-16 eggs and juveniles/g of soil (Meagher and Brown, 1974); in Tunisia it was 26-96 percent for 10-45 eggs and juveniles/g of soil (Namouchi- Kachouri *et al*., 2009) and 40-92%, 17-77% on wheat and barley with population densities of 15-40 and 16-34 eggs and juveniles/g of soil respectively in Saudi Arabia (Ibrahim *et al*., 1999).

Prior to this research there was not enough information on the status of the CCNs in cereal fields of Khuzestan province. The aim of this study was to determine the occurrence, distribution and population density of CCNs in wheat and barley fields of Khuzestan province, Iran.

## Materials and Methods

### Soil sampling and nematode extraction

The survey was performed in the cereal growing areas in Khuzestan province of Iran for 3 years (2008-2011). One hundred and sixty nine wheat and 31 barley fields were inspected and sampled in 22 regions during the grain filling period to harvest time (from mid-February to late May). About two kilograms of soil were collected from each field at the depth of 30 cm which consisted of 10 subsamples taken in a zigzag pattern across the field. 200 g of soil subsamples were dried at room temperature, the cysts were extracted from 100 g of soil by Fenwick can method (Fenwick, 1940). The number of cyst was counted in each sample and the eggs and second stage juveniles inside the cysts were released by crushing the cysts in a glass crusher. The root tissues of 10 wheat and barley plants from each sample were examined under stereomicroscope for observing mature females and disease symptoms.

### Species identification and population density determination

Nematode population density of CCNs including number of cysts, eggs and second stage juveniles inside the cyst was determined in all of the collected samples (Abido *et al*., 2005). The population densities of CCNs were evaluated as: zero, for no infestation; low, for less than 200; medium, 201 to 500; high, 501 to 1000 and very high for more than 1001 eggs and juveniles / 100 g soil. The nematode population density index in different regions of the province was estimated by





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the authors. It is based on various references of CCNs crop loss (Meagher and Brown, 1974; Ibrahim *et al*., 1999; Hajihasani *et al*., 2010). Distribution maps of CCNs were plotted using ArcGIS 9.3 software. To identify the species of nematodes, vulval cones of several cysts were mounted in glycerin jelly. Second stage juveniles from each cyst were separately fixed in TAF, transferred to glycerin and permanent slides were made (De Grisse, 1969). The species were identified based on morphological and morphometric features (Wouts and Baldwin, 1998; Handoo, 2002) and molecular characters (Subbotin *et al*., 2000). For DNA extraction, one cyst from each species was crushed in 8 µl double distilled water, then transferred to a 0.2 µl micro tube containing 12 µl worm lysis buffer (500 mM KCL, 100 mM Tris-HCL pH 8, 15 mM $MgCl_2$, 10 mM Dithioteritol, 4.5% Tween 20) and homogenised with a micro-homogeniser. The ITS rRNA gene was amplified with the forward TW 81 and reverse AB28 primers (Joyce *et al*., 1994), the PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's instruction. Restriction Fragment Length Polymorphism (RFLP) of amplified ITS product was carried out for *H. avenae and H. filipjevi*. Three to six µl of PCR product was digested by three restriction enzymes according to the manufacture's protocol. The digested DNA was run on a 1.5% TAE buffered agarose gel, stained with ethidium bromide, visualised on gel documentation and photographed.

## Results and Discussion

### Field infestation and distribution maps

CCNs occurred in 47 (32%) and 13 (54%) irrigated and rain-fed wheat fields respectively (Table 1), the percentage of infestation with CCN in irrigated and rain-fed barley fields were 9 (50%) and 6 (67%) respectively in Khuzestan province during 2008-2011 (Table 2). The results showed that out of 200 soil and root samples, 75 samples (37.5%) were infested with an average population of 280 eggs and juveniles per 100 g of soil. CCNs were widely spread in important cereal growing areas in the province i.e. Ahvaz, Andika, Andimeshk, Baghmalek, Behbahan, Dezful, Gotvand, Haftgel, Hendijan, Hoveize, Izeh, Lali, Masjed Soleiman, Omidiyeh, Ramhormoz, Ramshir, Shadegan, Shushtar and Susa. The lowest (8%) and the highest (83%) incidences were found in Ahvaz and Behbahan respectively whereas infestation was not found in some areas i.e. Dasht-e-Azadeghan, Korramshar and Mahshar.

Our results showed that 37.5% of the surveyed fields were infested with CCNs that was in agreement with our previous report (Ahmadi and Tanha Maafi, 2009). Disease incidence in our study was slightly more than that reported from other areas of the country (34%) (Ahmadi and Tanha Maafi, 2008). *H. filipjevi* is the dominant species of CCNs in most cereal growing areas (Tanha Maafi *et al*., 2010) whereas *H. avenae* was confined to the west and southwest of Iran (Tanha Maafi *et al*., 2012; Tanha Maafi *et al*., 2010). Disease incidence on rain-fed wheat and barley fields was greater compared to irrigated wheat and barley fields (59 and 67 versus 32 and 50 percent respectively). CCNs have more devastating impact on rain-fed crops than irrigated crops because drought stress greatly reduces yield (Smiley *et al*., 2005). Currently, the use of wheat and barley varieties resistant or tolerant to the nematodes is considered as one of the most appropriate management strategies for controlling the CCNs, and is widely used in some countries such as Australia, England, Denmark, Sweden and France (Rivoal and Nicol, 2009). Although in our research the CCNs were observed in different wheat cultivars (Chamran, Virinak, Yavarus and Atila), local barley cultivars and weeds (*Lolium prenne*, *Hordeum spontaneum* and *Avena ludovicians*) under field conditions, it would be helpful to examine reaction of a broader range of bread and durum wheat cultivars and weeds to the CCNs under controlled conditions.

The average numbers of cysts, eggs and juveniles per 100 gram of soil in wheat fields was higher than in barley fields (18 and 395 versus 11 and 166 cysts, eggs and juveniles respectively). The results are in agreement with the study of Andersson (1982), who showed that spring wheat was a more suitable host for *H. avenae* than spring barley.





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**Table 1** Population density of cereal cyst nematodes, *Heterodera avenae* and *H. filipjevi*, in soil samples of irrigated and rain-fed wheat fields in Khuzestan province, Iran.

| District | Number of surveyed fields | | Number of infested samples | | Number of *cysts/100 g soil) | Number of *eggs and J2/100 g soil) |
|---|---|---|---|---|---|---|
| | Irrigated | Rain-fed | Irrigated | Rain-fed | | |
| Ahvaz | 12 | 0 | 1 | 0 | 30 | 500 |
| Andika | 0 | 2 | 0 | 1 | 38 | 400 |
| Andimeshk | 7 | 0 | 2 | 0 | 4 | 200 |
| Baghmalek | 1 | 4 | 0 | 0 | 0 | 0 |
| Behbahan | 12 | 0 | 10 | 0 | 16 (3-52) | 519 (0-2367) |
| Dasht-e- Azadeghan | 19 | 0 | 0 | 0 | 0 | 0 |
| Dezful | 9 | 1 | 4 | 1 | 27 (2-99) | 508 (0-2500) |
| Gotvand | 8 | 0 | 3 | 0 | 6 (4-9) | 511 (10-1100) |
| Haftgel | 0 | 3 | 0 | 1 | 23 | 650 |
| Hendijan | 4 | 0 | 2 | 0 | 15 (7-23) | 575 (50-1100) |
| Hoveizeh | 3 | 0 | 1 | 0 | 25 | 100 |
| Izeh | 0 | 5 | 0 | 3 | 17 (14-23) | 344 (0-733) |
| Korramshar | 5 | 0 | 0 | 0 | 0 | 0 |
| Lali | 0 | 2 | 0 | 2 | 44 (32-56) | 200 (0-400) |
| Mah Shar | 5 | 0 | 0 | 0 | 0 | 0 |
| Masjed Soleiman | 0 | 2 | 0 | 2 | 57 (31-103) | 456 (100-867) |
| Omidiyeh | 4 | 0 | 3 | 0 | 12 (5-19) | 200 (100-500) |
| Ramhormoz | 11 | 0 | 5 | 0 | 33 (7-56) | 628 (600-1534) |
| Ramshir | 7 | 0 | 5 | 0 | 18 (10-32) | 1200 (0-3400) |
| Shadegan | 5 | 0 | 1 | 0 | 10 | 500 |
| Shushtar | 17 | 5 | 4 | 3 | 15 (3-37) | 224 (0-600) |
| Susa | 16 | 0 | 6 | 0 | 12 (5-28) | 483 (0-1500) |
| Overall and Mean | 145 | 24 | 47 | 13 | 18 (2-103) | 395 (0-3400) |

* includes both species of *H. avenae* and *H. filipjevi*

**Table 2** Samples characteristics and population density of cereal cyst nematodes, *Heterodera avenae* and *H. filipjevi* in barley fields in Khuzestan province, Iran.

| District | Number of surveyed fields | | Number of infested samples | | Number of cysts/100 g soil | Number of eggs and J2/100 g Soil |
|---|---|---|---|---|---|---|
| | Irrigated | Rain-fed | Irrigated | Rain-fed | | |
| Ahvaz | 1 | 0 | 0 | 0 | 0 | 0 |
| Andika | 0 | 3 | 0 | 1 | 16 (13-19) | 183 (10-267) |
| Baghmalek | 6 | 5 | 6 | 1 | 29 (3-54) | 428 (0-734) |
| Haftgel | 0 | 2 | 0 | 0 | 0 | 0 |
| Hendijan | 1 | 0 | 0 | 0 | 0 | 0 |
| Izeh | 0 | 2 | 0 | 2 | 16 (15-17) | 848 (797-900) |
| Korramshahr | 2 | 0 | 0 | 0 | 0 | 0 |
| Lali | 1 | 1 | 1 | 1 | 41 (11-71) | 333 (0-667) |
| Omidiyeh | 1 | 0 | 1 | 0 | 9 | 200 |
| Ramhormoz | 3 | 0 | 1 | 0 | 20 | 167 |
| Shadegan | 1 | 0 | 0 | 0 | 0 | 0 |
| Shushtar | 1 | 0 | 0 | 0 | 0 | 0 |
| Susa | 1 | 0 | 0 | 0 | 0 | 0 |
| Overall and Mean | 18 | 13 | 9 | 6 | 11 (3-71) | 166 (0-900) |





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In some areas of the province with high nematode population i.e, Ramshir and Behbahan regions, the damage of the disease was very obvious. In Khuzestan province, *H. filipjevi* with an initial population of 9 eggs and J2/ g of soil reduced grain yield, dry biomass, shoot dry weight, plant height and tillering by 40-52, 14-53, 6-69, 8-21 and 10-39 percent respectively during 2008-2009 on wheat (Ahmadi *et al*., 2010). This species reduced wheat grain yield by 11 percent even at the lowest population density of 2.5 eggs and J2/g soil in a microplot trial (Hajihasani *et al*., 2010). *H. avenae* type B with an initial population of 62 eggs and J2/ g of soil reduced grain yield, shoot dry weight and shoot height by 11-21, 5-39 and 6-14 % respectively during 2009-2010 on wheat (Ahmadi *et al*., 2012).

Infested fields showed patches of stunted plants that varied in size (Fig. 1B). The symptoms produced on the roots were proliferation of the roots showing a bushy knotted appearance with several females visible at each knot (Fig. 1A). Above-ground symptoms appear early in the season as pale green patches of plants with fewer tillers.

The population densities of CCNs in soil samples of wheat ranged from 2 to 103 cysts (mean 18) /100 g of soil and 0 to 3400 (mean 395) eggs and J2s /100 g of soil. The highest levels of infestation were found in Masjed Soleiman and Dezful regions, where the total number of cysts per 100 g of soil reached 103 and 99 cysts /100 g of soil respectively. The population densities of CCNs in soil samples of barley were 11-71 cysts (mean 36) /100 g of soil and 0-734 (mean 419) eggs and J2s/100 g of soil. The highest and lowest incidences were observed in Lali and Ramhormoz regions with 100 and 33.3 % respectively.

Distribution map of the CCNs in wheat and barley fields of Khuzestan province is shown in Fig. 3. The occurrence of CCNs in highland regions *i.e.* northern and eastern parts was relatively high compared to the other parts, in lowland areas. The nematode population density index of CCNs in Khuzestan province indicates that about half of the wheat fields showed moderate to high infestation (Fig. 3), and severe infestation was observed in Ramshir region (Fig. 4). Data for barley fields showed that half of the surveyed barley fields were infested by CCNs (Fig. 4). The number of J2 and eggs of CCNs in some regions were greater than that considered as damage threshold level for this nematode (Gill and Swarup, 1971; Meagher and Brown, 1974) and it is likely that these populations could cause economic yield loss. Crop rotation with non-host crops is one of the most effective methods to control the CCNs (Nicol, 2002). The other controlling methods include clean fallow and deep ploughing 2-5 times during May to June in India (Swarup, and Sosa-Moss, 1990), early planting of wheat in order to increase the plant tolerance vigor against nematode attack and application of nematicides in planting (Brown and Kerry, 1987).

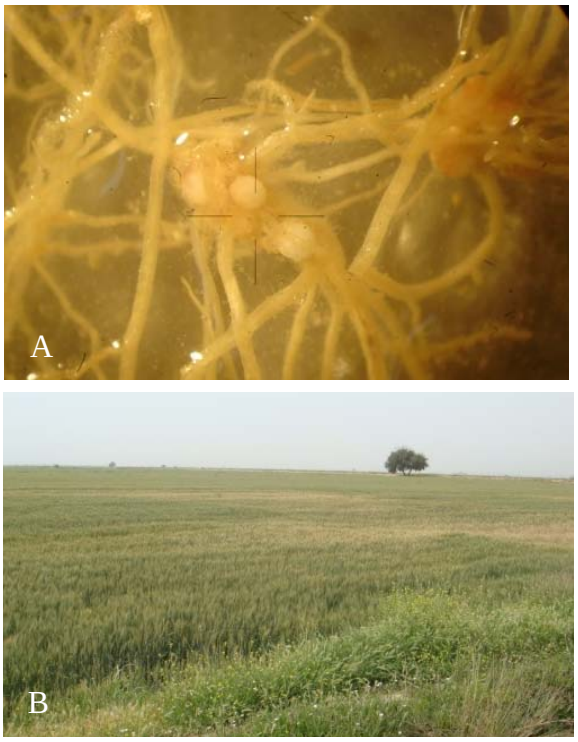


**Figure 1** A, knotted wheat roots attacked by CCNs, with visible white females; B, Showing patches of stunted plants in a wheat field infested with CCNs.





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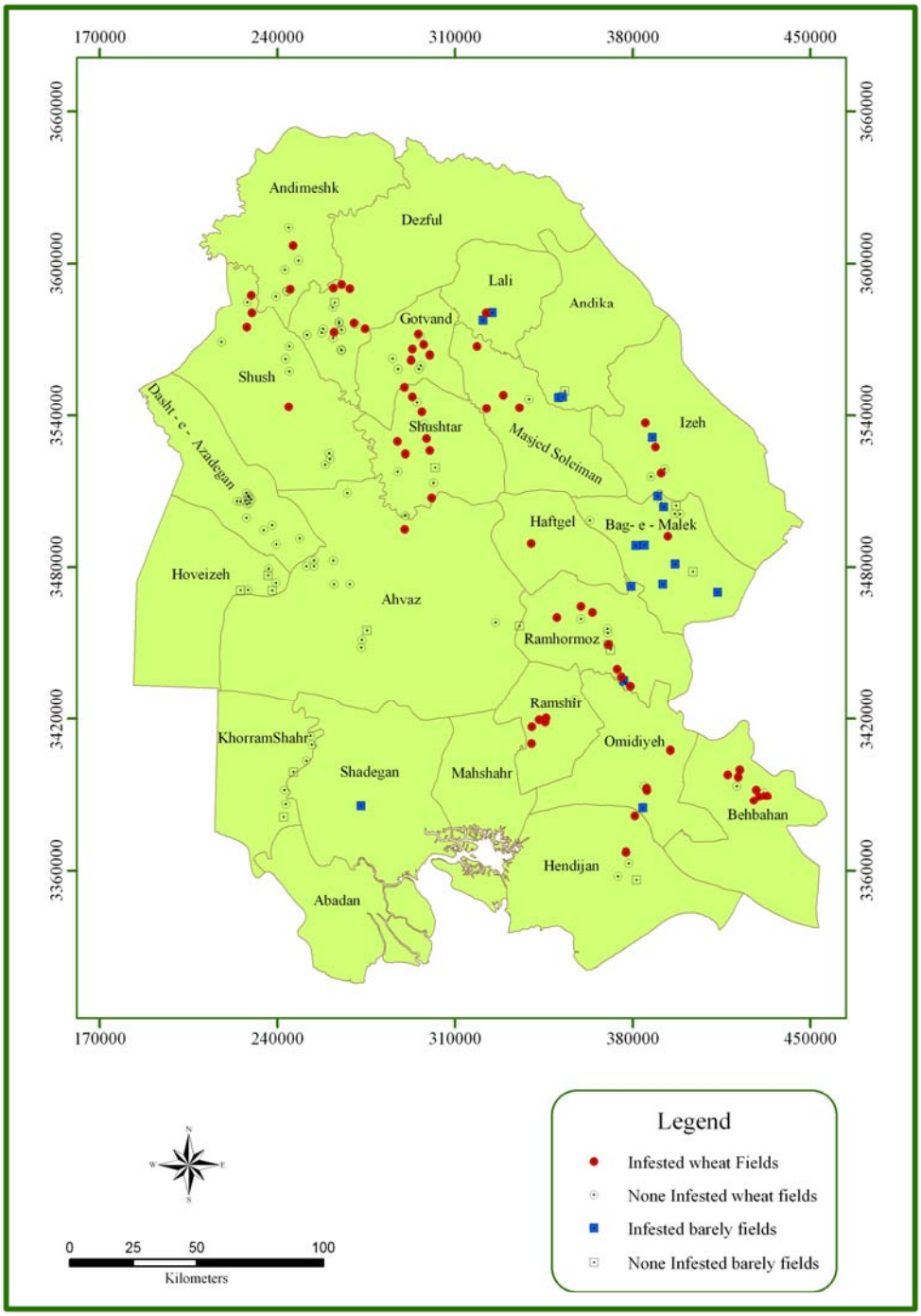


**Figure 2** GIS distribution map of cereal cyst nematodes, *Heterodera avenae* and *H. filipjevi*, in Khuzestan province, Iran.





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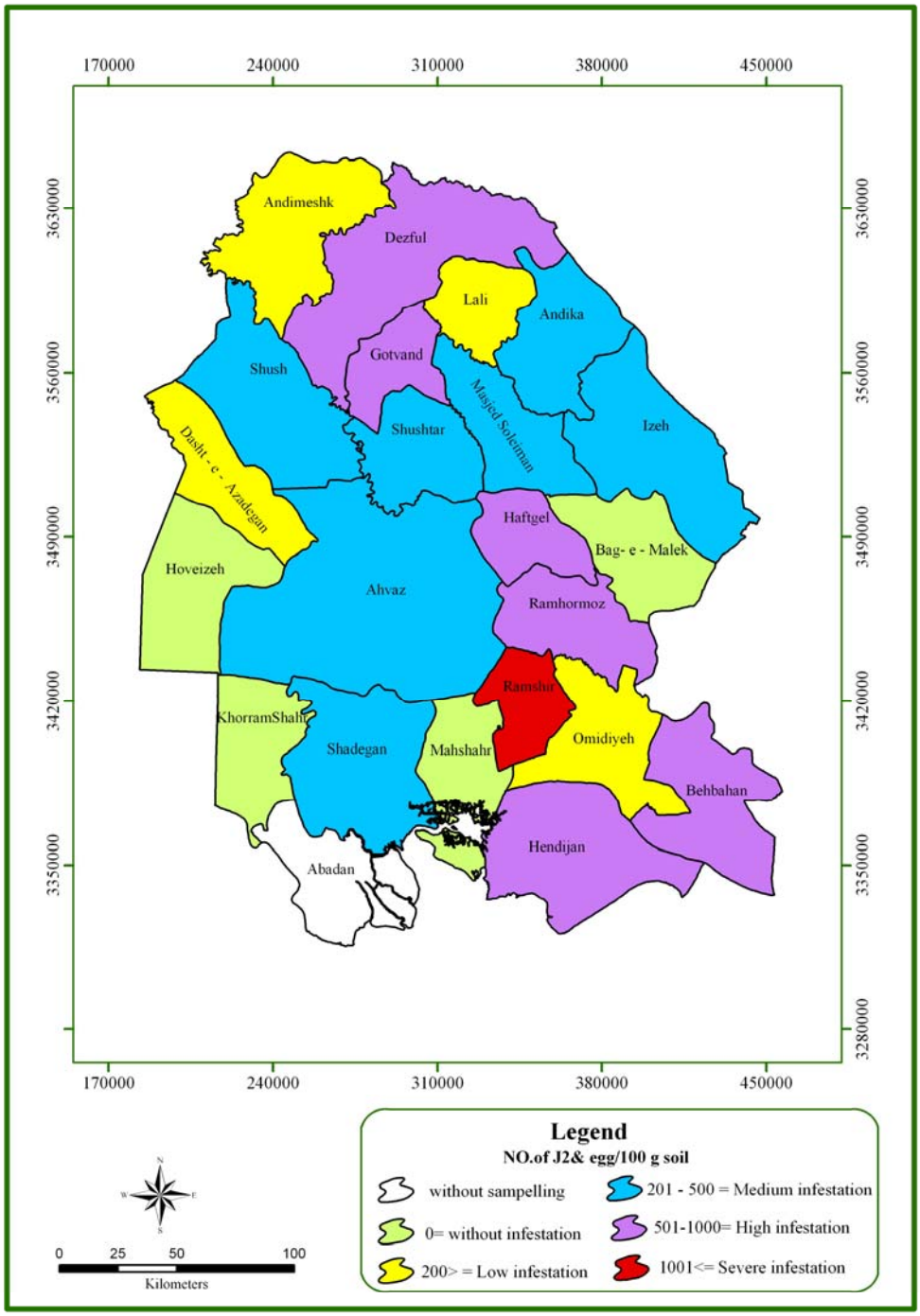


**Figure 3** GIS map of population density of cereal cyst nematodes, *Heterodera avenae* and *H. filipjevi*, in wheat fields of Khuzestan province, Iran.





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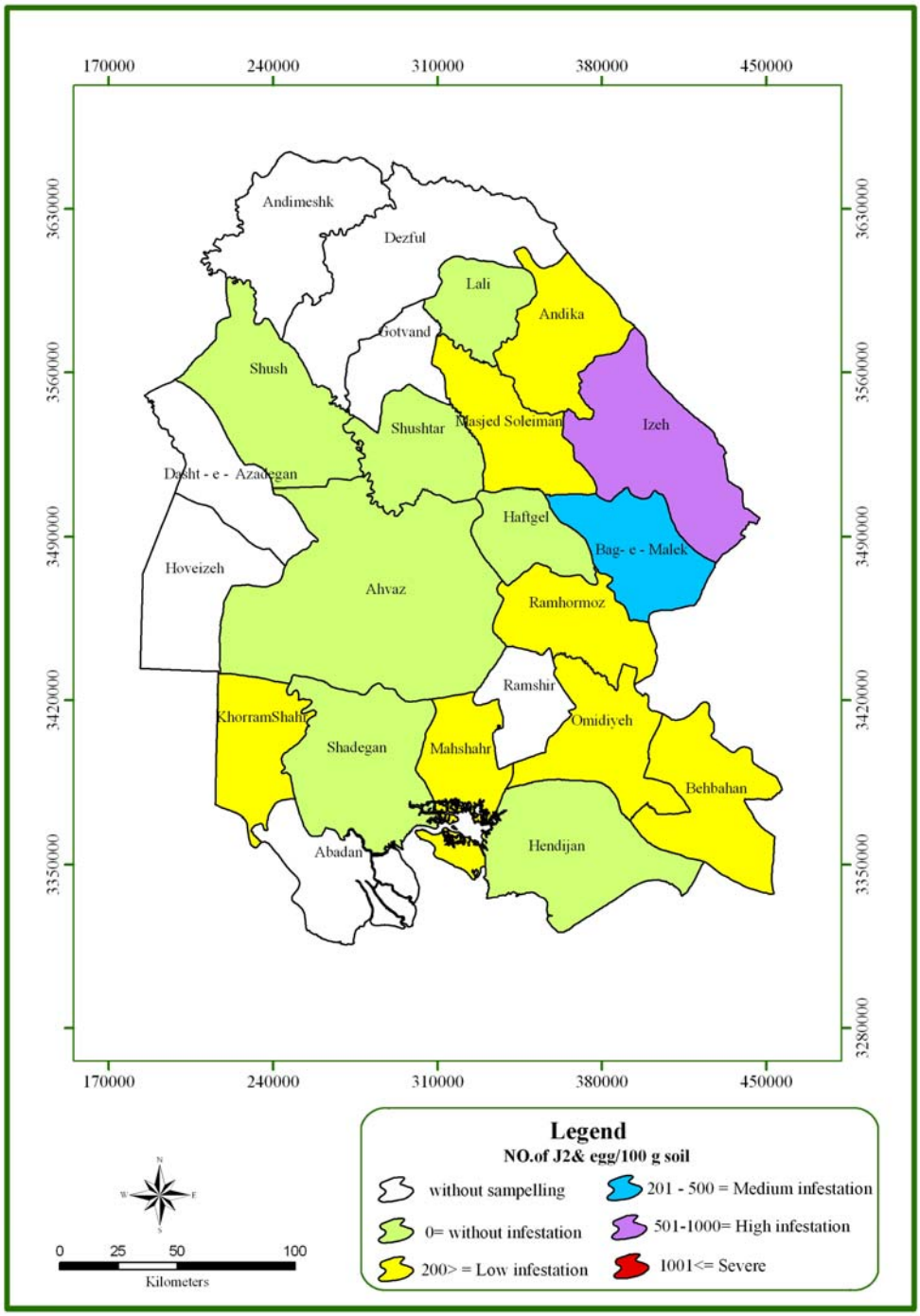


**Figure 4** GIS map of population density of cereal cyst nematodes, *Heterodera avenae* and *H.filipjevi*, in barley fields of Khuzestan province, Iran.





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**Identification of species**

The collected cysts were typically ovoid to lemon-shaped. The main morphological characteristics of the cyst vulval cone that separate the two species, *H. avenae* and *H. filipjevi*, are shown in Fig. 5. *H. avenae* cysts are large, dark-brown to black with, heavy prominent bullae and no underbridge in the vulval cone (Fig. 5A,B). Cysts of *H. filipjevi* are smaller, yellow to light-brown in color. They possess light bullae and a distinct underbridge close to the vulval bridge, thick in the middle and rather weak at the ends (Fig. 5 C, D). The morphological and morpohometric features were in agreement with those published for each species (Table 3) (Subbotin *et al*., 1999). PCR of the rRNA_ITS produced a single fragment of *ca* 1030bp for *H. filipjevi* and *H. avenae*. *Hinf*I and *Pst*I differentiated two morphologically closely related species, *H. filipjevi* and *H. avenae* (Fig. 6). The patterns yielded by using of the restriction enzyme *Rsa*I clearly distinguished *H. avenae* type B from type A which are in agreement with those patterns reported for type B of *H. avenae* (Subbotin *et al*., 2003). The restriction enzyme *Rsa*I does not form any bands in type A. *H. avenae* type B was previously reported from the western provinces of Iran (Tanha Maafi *et al*., 2007; 2009), in this research it was also detected from Khuzestan province.

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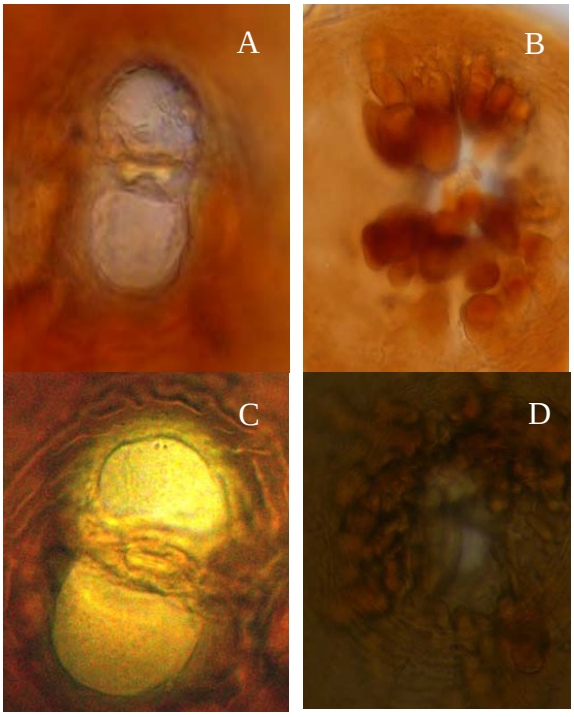


**Figure 5** Terminal region of cereal cyst nematodes. A and B *H. avenae*, showing fenestration, vulval slit and heavy bulae; C, D Vulval cone of *H. filipjevi* showing fenestration, vulval slit, underbridge and light bulae.

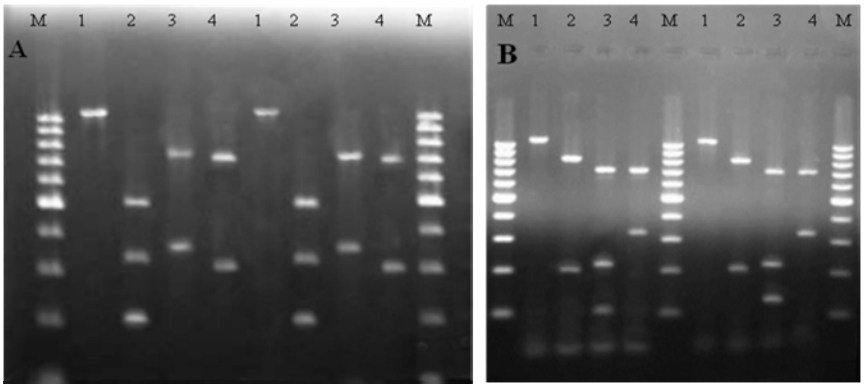


**Figure 6** rRNA-RFLP of *Heterodera filipjevi* and *Heterodera avenae* Type *B*: A: *Heterodera avenae*: M: DNA Ladder 100bp, 1: Unrestricted PCR products, 2: *PstI*, 3: *HinfI*, 4: *RsaI*, B: *Heterodera filipjevi*: M: DNA Ladder 100bp, 1: Unrestricted PCR product, 2: *Hinf*I, 3: *Pst*I, 4: *Rsa*I.





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**Table 3** Morphopmetrics of cysts, con-top and $J_2$ of *Heterodera avenae* and *H. filipjevi* from Khuzestan province, Iran (Measurements in µm, with mean standard deviation, range).

| Character | *H. avenae* | *H. filipjevi* |
|---|---|---|
| Cyst (n) | 10 | 10 |
| Length | 624 ± 83 (524-804) | 567 ± 92 (461-724) |
| Width | 517 ± 67 (385-631) | 394 ± 64 (261-488) |
| Length/ Width | 1.2 ± 0.1 (1.02-1.38) | 1.3 ± 0.1 (1.48-1.76) |
| Con-Top(n) | 10 | 10 |
| Vulval slit length | 9 ± 1.4 (7-11) | 9.9 ± 1.5 (8-13) |
| Fenestral length | 47 ± 1.4 (43-50) | 47.6 ± 4 (47-60) |
| Fenestral width | 20.3 ± 1.8 (17-22) | 24 ± 1.7 (23-29) |
| Vulval bridge width | 9.4 ± 1.8 (7-13) | 7.2 ± 0.7 (6-9) |
| Underbridge lentgth | Absent | 74.2 ± 4.7 (75-90) |
| Second-stage juvenile (n) | 10 | 10 |
| L | 559 ± 18 (520-590) | 647 ± 32.4 (684-782) |
| a | 24.3 ± 1.7 (20-24) | 23 ± 1.2 (24-27) |
| b | 5.3 ± 0.2 (8-10) | 4.2 ± 0.8 (3-7) |
| b' | 4.7 ± 0.3 (4-6) | 4.3 ± 0.8 (3-6) |
| Stylet length | 27.5 ± 0.7 (26-29) | 23.3 ± 1.4 (23-27) |
| Tail length | 67.3 ± 5.2 (58-70) | 52.4 ± 1.3 (57-60) |
| Hyaline tail part | 38.7 ± 1.4 (35-40) | 32.3 ± 3.6 (29-40) |

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# وقوع نماتودهای سیستی غلات (*Heterodera avenae* type B و *H. filipjevi*) در جنوب غربی ایران

**علیرضا احمدی[1*] و زهرا تنها معافی[2]**

۱- مرکز تحقیقات کشاورزی و منابع طبیعی خوزستان، صندوق پستی ۳۳۴۱- ۶۱۳۳۵، اهواز، ایران.
۲- مؤسسه تحقیقات گیاهپزشکی کشور، صندوق پستی ۱۴۵۴-۱۹۳۹۵، تهران، ایران.
* پست الکترونیکی نویسنده مسئول مکاتبه: alirahmadi2000@gmail.com
دریافت: ۱۶ خرداد ۱۳۹۲؛ پذیرش: ۱۷ مهر ۱۳۹۲

**چکیده:** طی یک بررسی در مزارع غلات استان خوزستان در سال‌های ۱۳۹۰-۱۳۸۷ مشخص گردید که نماتودهای سیستی غلات به‌طور وسیعی در این منطقه پراکنده هستند. نماتودهای سیستی غلات به‌ترتیب در ۳۷ و ۳۵ درصد از ۲۰۰ نمونه جمع‌آوری شده مزارع گندم و جو وجود داشتند. گونه‌های *Heterodera avenae* تیپ B و *H. filipjevi* براساس مشخصات ریخت‌شناسی، ریخت‌سنجی و همچنین مولکولی با روش rRNA-ITS RFLP شناسایی گردیدند. تراکم جمعیت نماتودهای سیستی غلات در نمونه‌های گندم بین ۱۰۳-۲ (میانگین ۱۸) سیست و ۳۴۰۰-۰ (میانگین ۳۹۵) تخم و پوره سن دو نماتود در ۱۰۰ گرم خاک خشک و برای نمونه‌های جو بین ۷۱-۳ (میانگین ۱۱) سیست و ۹۰۰-۰ (میانگین ۱۶۶) تخم و پورن سن دو در ۱۰۰ گرم خاک خشک شمارش گردیدند. کمترین و بیشترین میزان وقوع این دو گونه مربوط به مناطق اهواز (۸ درصد) و بهبهان (با میزان ۸۳ درصد) بود. میزان جمعیت تخم و پوره سن دو نماتودهای فوق در بعضی از مناطق نمونه‌برداری شده، از سطح آستانه خسارت درنظر گرفته شده برای این گروه از نماتودها بالاتر است و احتمال خسارت اقتصادی به محصول گندم و جو در این مناطق وجود دارد.

**واژگان کلیدی:** ایران، پراکنش، خوزستان، نماتدهای سیستی غلات.





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