

Cereal Cyst Nematode, *Heterodera filipjevi* (Madzhidov, 1981) in Wheat Fields of Chaharmahal and Bakhtiari Province, Iran and Its Distribution Based on Interpolation by Geographic Information System

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ABSTRACT

To identify the Cereal Cyst Nematodes (CCN) and their distribution and population density in wheat fields in Chaharmahal and Bakhtiari Province based on interpolation by geographic information system, a total of 212 composite soil and root samples were randomly collected from different wheat fields of nine districts of the province during 2013-2014. The soil samples were explored for number of cysts, second-stage Juveniles (J2) and eggs. The species were identified based on morphological, morphometric and molecular characteristics. Analysis was performed by Arc GIS software using interpolation technique for determination of raster map. The results showed that 42% of soil samples were infested with *Heterodera filipjevi* containing an average of 994 eggs and J2 per 200 g of soil. The maximum infestation was observed in Dehsahra region in Lordegan district with an average of 4720 eggs and J2 per 200 g soil. Wheat fields of Kohrang, Kiyar and Farsan districts showed low population density while moderate infestation was observed in Ardal, Ben, Saman and Borujen districts. Instead high population density of *H. filipjevi* was found in Shahrekord and Lordegan. Based on interpolation of population density and interpolation maps, the eastern part of Shahrekord and southern and western parts of Lordegan districts were predicted as regions with high infestation to *H. filipjevi* and considered as hotspots for this disease.

Keywords: Cereal cyst nematodes, Chaharmahal and Bakhtiari, Distribution, GIS, Interpolation, Population density.

INTRODUCTION

Wheat is the main staple crop in all countries in Central Asia. The consumption rate of wheat is higher than in the rest of the world, at over 200 kg year⁻¹ capita⁻¹. However, production of wheat per capita is slightly declining and wheat yield is remaining very low (Muminjanov, 2015).

Wheat is the most important agricultural crop in Iran and is cultivated under both irrigated and rain-fed conditions comprising of 38 and 62%, respectively, of the area as growing wheat. The total area under wheat cultivation in Iran was estimated at 6.4 million ha with an annual production of 9.3 million tons in 2012-2013. Wheat in Chaharmahal and Bakhtiari Province was

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produced on 72219 ha with an annual production of 93507 tons (Anon, 2015).

Chaharmahal and Bakhtiari Province is located in the southwestern part of Iran. The annual average of temperature differs in different areas. In the center of the province, Boroujen and Shahrekord, the temperature is in the range of 10.5-11.5°C, whilst in cooler regions viz. Kohrang it is 9.2°C and rises to 15.2°C in Lordegan. The average rainfall is measured about 560 mm annually. Cereal Cyst Nematodes (CCNs) are known as the most important group of plant parasitic nematodes on wheat and barley as a worldwide basis (Dababat et al., 2014). The "*Heterodera avenae* group" is a complex of 12 species and many intraspecific pathotypes that invade roots of cereals and grasses. The three main species which are the most economically important, are *Heterodera avenae* Wollenweber, 1924, *H. filipjevi* (Madzhidov, 1981) Stelter, 1984, and *H. latipons* Franklin, 1969. These species cause economic yield losses, especially in non-irrigated wheat production systems in North Africa, West Asia, China, India, Australia, the United States and parts of Europe (Rivoal and Cook, 1993; Smiley and Nicol, 2009; Dababat et al., 2014).

Heterodera avenae is the most common species of CCNs in that it has a global distribution and is the most damaging and widely distributed species on temperate cereals. This species occurs in most European countries, Australia, Canada, South Africa, North Africa, Japan, Saudi Arabia and Turkey (Nicol et al., 2003; Dababat et al., 2015). The distribution of *H. filipjevi* was recently demonstrated to be wider than previously documented and losses may be high especially in drought conditions (Rivoal and Nicol, 2009). The economic importance of *H. filipjevi* was investigated in Turkey and the results showed average yield losses up to 36% (Elekcioglu et al., 2009). Five species of CCNs, namely *H. avenae* type B, *H.*

filipjevi, *H. hordecalis*, *H. latipons* and *Heterodera* sp. 1 were reported from cereal fields and grasslands in Iran. Among these species, *H. filipjevi* was most widely distributed followed by *H. latipons* and *H. avenae* type B (TanhaMaafi et al., 2007). Preliminary studies of distribution and population density of *H. filipjevi* in wheat fields of Isfahan Province in Iran showed 52% of soil samples infested with *H. filipjevi* (Karimipour Fard and Tanha Maafi, 2010). In a survey on CCNs in Markazi Province in Iran, it was shown that 40% of the sampled fields were infested with *H. filipjevi* and *H. latipons* (Hajihasani et al., 2008).

Another survey of the CCNs on cereal fields of Khuzestan Province revealed that CCNs were widely distributed, that *H. avenae* type B and *H. filipjevi* were present in 37 and 35% of the 200 samples collected from wheat and barley fields respectively, and that the number of Juveniles (J2) and eggs of CCNs in some regions were greater than the damage threshold level (Ahmadi and Tanha Maafi, 2014).

Field microplot studies in Iran on the effect of different initial population levels of *H. filipjevi* on wheat cultivar Sardari, indicated that grain yield was reduced even at the lowest population density (500 eggs and J2 200 g⁻¹ of soil) and reached a maximum loss of 48% at initial population density of 4,000 eggs and J2 200 g⁻¹ of soil (Hajihasani et al., 2010).

The assessment of crop loss caused by *H. filipjevi* to some cultivars of spring wheat in Khuzestan Province, showed that an initial population of 2,000 eggs and J2 200 g⁻¹ of soil was reduced of grain yield, dry biomass, shoot dry weight, plant height and tillering by 52 (40-73), 40 (14-53), 38 (6-67), 15 (8-21) and 24 (10-39)% respectively (Ahmadi et al., 2013). Recent field studies in Isfahan Province have also indicated the economic importance of *H. filipjevi* in both irrigation and drought stress conditions. The grain

yield reduction in three cultivars varied from 24 to 28% under drought stress conditions and from 20 to 23% under normal irrigation conditions (Karimipour Fard *et al.*, 2015).

There was no sufficient and reliable information on the status of the CCNs in wheat fields of Chaharmahal and Bakhtiari Province. Therefore, the objective of this study was to determine the occurrence, distribution and population density of CCNs in wheat fields of this province based on interpolation by Geographic Information System (GIS).

MATERIALS AND METHODS

Soil Sampling, Nematode Extraction and Population Density Determination

In total, 212 composite soils as well as root samples were randomly taken from different wheat fields of nine districts of Chaharmahal and Bakhtiari Province including Ardal, Ben, Borujen, Farsan, Kohrang, Kiyar, Lordegan, Saman and Shahrekord during 2013-2014. The sampling was done during the grain filling period until harvesting time. About 3 kg of soil were collected from each field consisting of 10 subsamples taken in a zigzag pattern across the field and then 200 g of each soil sample were processed by the Fenwick can method (Fenwick, 1940) for cysts extraction.

To determine the population density in each soil sample, the intact cysts were counted, then three cysts from each sample were randomly selected for excising the vulval cone region for species identification and other cysts crushed in a glass crusher and the number of J2 and eggs were determined. The roots of 10 wheat plants from each sample were examined under a stereomicroscope to observe the presence of mature females and cysts. The population densities of CCNs and the mean of population densities of samples belonging to

each district were evaluated based on the ranking presented by Ahmadi and Tanha Maafi (2014) as: Zero, for no infestation; low, for less than 400; medium, 401-1,000; high, 1,001 to 2,000; and very high for more than 2,001 eggs and J2 200 g⁻¹ soil.

Infestation and Population Density Interpolation Based on GIS

Spatial interpolation is the process of using points with known values to estimate values at other unknown points. Distribution and population density maps were prepared based on spatial interpolation using Geographic Information System (GIS). To achieve this aim by using points with known values (sampled points), values at other unknown points were estimated based on interpolations using Arc GIS, 9 software (Sutton *et al.*, 2009).

Because, the variation of counted eggs and J2s was very high, interpolation process was carried out by using Inverse Distance Weighted (IDW) and raster map of eggs and J2s population were prepared. In the IDW interpolation method, the sample points are weighted during interpolation such that the influence of one point relative to another, declines with distance from the unknown points (Sutton *et al.*, 2009).

Soil sampling was taken again from some regions predicted with high population of identified species through interpolation in 2016.

Species Identification

To identify the specimens, permanent slides were made by excising the vulval cone region of cysts and mounting them in glycerin jelly, and by fixing J2 from each cyst in TAF and mounting them in glycerin (De Grisse, 1969). The species were identified based on morphological and



morphometric features (Wouts and Baldwin, 1998; Handoo, 2002) and molecular characteristics (Subbotin *et al.*, 2000). Therefore DNA of eight J2s and eggs was extracted according to Tanha Maafi *et al.* (2003). 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 QIA quick Gel Extraction Kit (Qiagen) according to the manufacturer's instruction. Restriction Fragment Length Polymorphism (RFLP) of amplified ITS product was carried out for *H. filipjevi*. Three to six μ l 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, visualized on gel documentation and photographed.

RESULTS AND DISCUSSION

Identification of Species

Morphological and morphometric studies of cyst vulval cones and J2s revealed the presence of *H. filipjevi* in Chaharmahal and Bakhtiari Province. The cysts were typically ovoid to lemon-shaped with small protuberance of the vulval cone, light-brown to dark in color. The Vulval cone, bifenestrated, vulval bridge narrow, vulval slit short, bullae numerous and the underbridge was thick in the middle and rather weak at the ends with bifurcated ends (Figure 2).

The J2s share the following morphological characters: robust stylet, stylet knobs large and slightly concave, tail gradually tapering, terminating in a rounded tip. The morphological and morphometric features were in agreement with the typical characters published for *H. filipjevi* (Table 1) (Subbotin *et al.*, 1999).

Morphometric characteristics of cyst, vulval areas and J2s of three populations

from districts of Chaharmahal and Bakhtiari were compared with the population of Khuzestan and Isfahan Provinces, but no difference was observed (Table 1).

PCR of the *rRNA-ITS* gene produced a single fragment of 1,030 bp. The patterns yielded by using the restriction enzymes clearly distinguished by *H. filipjevi* (Figure 3). It can be distinguished from those of the RFLP pattern of *H. avenae* by different fragments of length generated by *Hinf*I (498, 318, 187, 41 bp) and *Pst*I (708, 341 bp) (Subbotin *et al.*, 1999; 2003).

Field Infestation, Distribution and Interpolation Maps

Out of 106 soil samples, collected from wheat fields of different districts of Chaharmahal and Bakhtiari Province, 45 soil samples (42%) were infested by *H. filipjevi* averaging 994 eggs and juveniles per 200 g of soil. The mean number of cyst in infested soil samples was 15 cyst 200 g⁻¹ soil.

Heterodera filipjevi was found in all districts of Chaharmahal and Bakhtiari and was the only identified species of CCNs in this province. This result was in agreement with the previous studies which reported that *H. filipjevi* was the dominant species of CCNs in most cereal growing areas of Iran (Tanha Maafi *et al.*, 2007; 2010), including Khuzestan Province (Ahmadi and Tanha Maafi, 2014). In some areas of the province i.e. Shahrekord, Lordegan and Borujen districts with high population of eggs and J2 (1,410, 1,075 and 850 eggs and J2 200 g⁻¹ soil; Table 2), cereals showed clear symptoms of cereal cyst nematode attacks including pale green patches with fewer tillers and roots showing bushy knotted appearance.

The maximum infestation occurred in the Dehsahra region in Lordegan district with 64 cysts 200 g⁻¹ soil and 4,720 eggs and J2 200 g⁻¹ soil. The minimum infestation was found

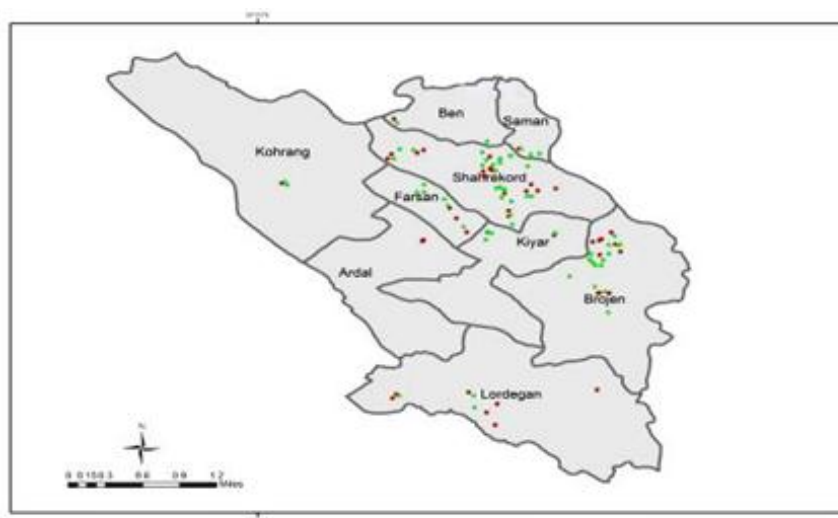


Figure 1. Distribution map of cereal cyst nematode *Heterodera filipjevi*, in Chaharmahal and Bakhtiari Province. Red and green points indicate the infested and non-infested fields, respectively.

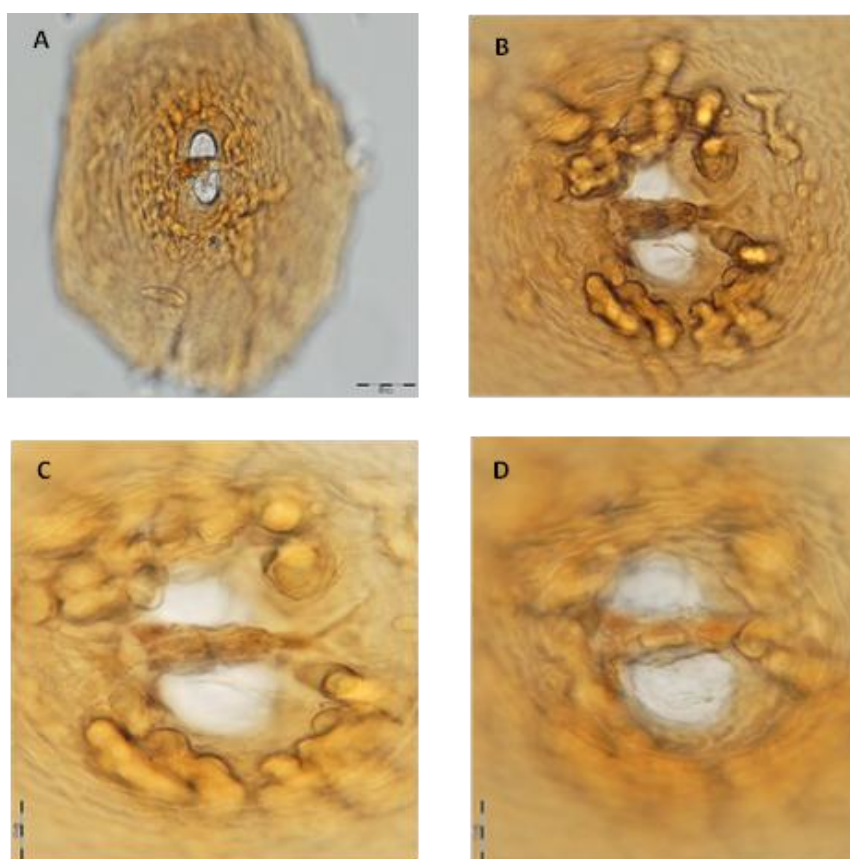


Figure 2. Vulval cone region of *Heterodera filipjevi*: (A) Fenestration; (B) Bullae; (C) Underbridge, (D) Vulval slit.



Table 1. Morphometric characteristics of cysts, vulval cone and second stage juveniles of *Heterodera filipjevi* from Chaharmahal and Bakhtiari, Khuzestan and Isfahan Provinces (measurements in μm).

Character	Location			
	Lordegan	Ardal	Saman	Isfahan Province (Tanha Maafi et al., 2007)
Cyst (n)	10	10	10	10
Length	695±20.13(670-730)	650±23.09(620-680)	696±32.38(660-750)	594±58(470-690)
Width	533±24(510-580)	441±48.6(370-500)	484±34.7(410-520)	464± 47(350-520)
Length/Width	1.3±0.04(1.24-1.35)	1.47±0.11(1.36-1.67)	1.44±0.12(1.3-1.73)	1.3±0.07 (1.15-1.44)
Vulvalcone (n)	5	5	5	3
Vulval slit length	10.2±0.83(9-11)	9.4±1.14(8-11)	9.8±1.3(8-11)	9.8±1.7 (8-11)
Fenstral length	51.6±6.1(44-60)	46.6±2.4(44-50)	47±1.58(45-49)	46.4±8.4 (37-53)
Fenstral width	26.2±2.58(23-29)	21.8±1.3(20-23)	28.8±1.3(27-30)	22± 6.6(15-28.5)
Vulval bridge width	8.2±1.3(7-10)	7.8±1.3(6-9)	7.4±1.14(6-9)	8.8±2.3 (7-11)
Under bridge length	80.2±5.4(72-86)	81.6±2.07(79-84)	76.8±2.58 (74-80)	83±5 (78-88)
Second-stage juvenile (n)	10	10	10	20
L	540±22.11(520-580)	533.7±10(520-550)	537.8±18.84(520-580)	524± 26(458-591)
a	22.64±1.11(21-24)	22.8±1.19(21.2-25.1)	22.8±0.88(21.2-24)	24± 1.4(21.4-26.6)
b	4.72±0.61(4.1-5.9)	4.93±0.21 (4.6-5.3)	4.57±0.45 (4.1-5.7)	4.6± 0.3(4.2-5.6)
b'	3.98±0.6(3.4-5.1)	3.89±0.17(3.6-4.2)	3.89±0.42 (3.4-4.9)	4.3± 0.8(3-6)
c	9.12±0.45(8.52-9.7)	8.97±0.6(8.25-10.1)	8.89±0.65(8-9.7)	9.3±0.7(8-11.5)
c'	3.34±0.31(2.9-3.85)	3.34±0.24(3-3.8)	3.43±0.22(3.1-3.8)	3.6±0.26(3.1-4.1)
Stylet length	24.45±1.49(22-26)	23.89±1.1(22.4-25.6)	24±1.33(22-26)	24.3± 0.8(23-26)
Tail length	56.85±3.87(50-61)	61.35±1.85(59-64)	59.45±3.32(52-64)	56.7± 3.8(44-61)
Hyaline tail part	33.77±1.61(32-36)	33. 94±1.63(32-36)	33.97±1.49(32-36)	32.9± 2.6(29-38)

Table 2. Mean population density of cereal cyst nematode, *Heterodera filipjevi*, in soil samples collected from wheat fields in different districts in Chaharmahal and Bakhtiari Province, Iran.

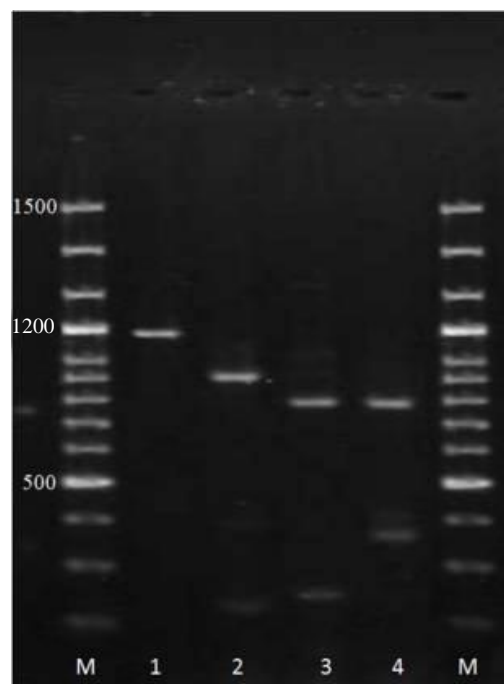
District	Number of collected soil samples	Number of infested soil samples	Number of cysts 200 g ⁻¹ soil	Population of eggs and J2 200 g ⁻¹ soil
Ardal	3	3	4(2-6)	652(240-956)
Borujen	25	10	13.4(1-29)	852(24-2060)
Ben	3	1	5	679
Saman	5	2	17.5(10-25)	851(360-1341)
Shahrekord	40	17	18.4(3-36)	1409(24-2915)
Farsan	9	3	10.4(4-19)	84(42-130)
Kohrang	3	1	6	56
Kiyar	8	1	2	110
Lordegan	10	7	17.9(3-64)	1075(120-4720)

in Gandoman region in Borujen district with 1 cyst 200 g⁻¹ soil and 24 eggs and J2 200 g⁻¹ soil.

As stated before, Lordegan district was one of the most infested areas, here most of the area under wheat cultivation is under rain-fed conditions. CCNs have more devastating impact on rain-fed crops than irrigated crops because drought stress greatly reduces yield (Smiley *et al.*, 2005). *H. filipjevi* is also widely distributed in Turkey and the average yield loss was 40% on wheat, with the highest losses occurring under drought conditions (Rivoal and Nicol, 2009). In Iran, grain yield reduction percentages under drought stress conditions are also significantly greater than under irrigation conditions, indicating that water stress increases the severity of injuries in infected plants (Karimipour Fard *et al.*, 2015).

Distribution map and GIS map of mean population density of *H. filipjevi* in wheat fields of Chaharmahal and Bakhtiari Province is shown in Figures 1 and 4 respectively. According to the results, *H. filipjevi* was found in almost half (42%) of the wheat fields of Chaharmahal and Bakhtiari Province. The nematode population density indices of *H. filipjevi* based the ranking presented by Ahmadi and Tanha Maafi (2014) indicates that the wheat

fields of Kohrang, Kiyar and Farsan districts had low infestation, while moderate population density was observed in Ardal, Ben, Saman and Borujen districts. The maximum population density of *H. filipjevi* was found in Shahrekord and Lordegan (Figure 4). Therefore more than half of the

**Figure 3.** rDNA-RFLP of *Heterodera filipjevi* (Lordegan population). M: DNA ladder (100 bp); 1: Unrestricted PCR product; 2: *Hinf* I; 3: *Pst* I; 4: *Rsa* I.



districts of the province showed moderate to high infestation of *H. filipjevi*.

Based on GIS map (Figure 4), the population density of *H. filipjevi* in infested wheat fields of eastern and southern parts was higher than in other parts of the province. Based on interpolation of infestation and interpolation map (Figure 5) the eastern part of Shahrekord and western parts of Lordegan districts where the number of collected samples was low, were predicted as regions with high infestation and considered as a hotspot for this disease.

The results of prediction of population density through interpolation were validated by the presence of *H. filipjevi* with high population density in the predicted regions of province after soil sampling for verification (Figure 6). Based on validation results, high percentage of infested soils with high population of *H. filipjevi*, were confirmed in the predicted regions including, southern and western parts of Lordegan and eastern parts of Shahrekord (Table 3).

The nematode population density in 58%

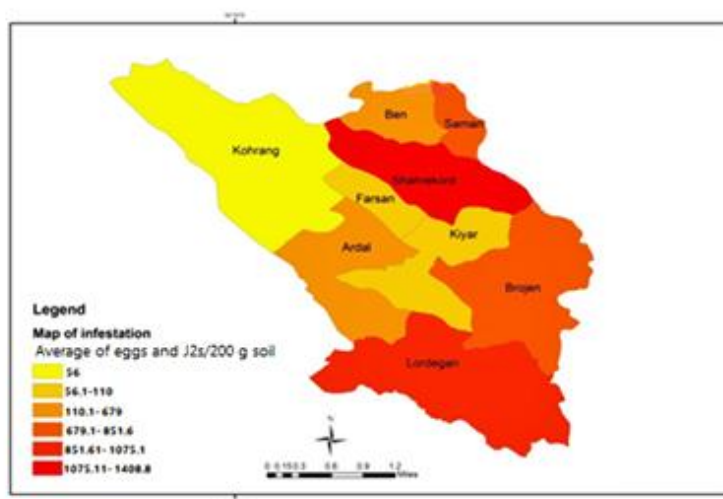


Figure 4. GIS map of mean population density of *Heterodera filipjevi*, in wheat fields of districts of Chaharmahal and Bakhtiari Province.

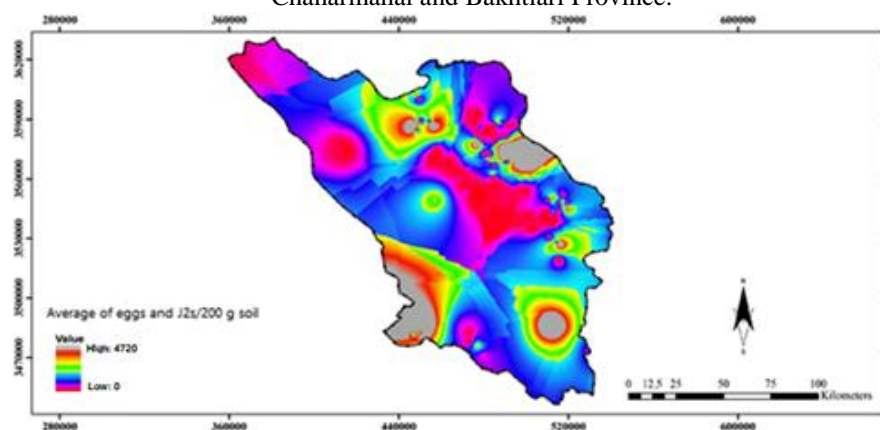


Figure 5. Interpolation map of population density of *Heterodera filipjevi* in Chaharmahal and Bakhtiari Province.

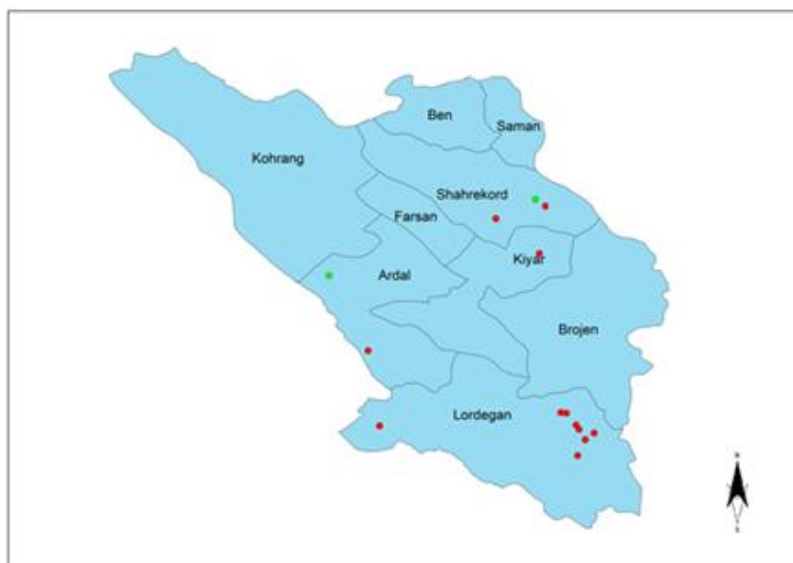


Figure 6. Infested (Red dots) and non-infested (Green dots) samples to *Heterodera filipjevi*, in soil sampling for validation in Chaharmahal and Bakhtiari Province.

Table 3. Comparison between results of soil sampling and interpolation prediction for validation of infestation to *Heterodera filipjevi*, in some regions of Chaharmahal and Bakhtiari Province.

Region	Number of collected soil samples	Number of infested soil samples	Infested soil samples (%)	Maximum of population	Average of eggs and J2 200 g ⁻¹ soil	Prediction of population density based on interpolation
Western parts of Lordegan	7	7	100	3604	2310	High population density
Southern parts of Lordegan	3	2	66.7	1500	1220	High population density
Eastern parts of Shahrekord	4	3	75	1400	1078	High population density

percent of infested soil samples was more than 500 eggs and J2 200 g⁻¹ soil and this population was greater than that which caused a reduction of grain yield by 11% (Hajihassani *et al.*, 2010). Due to consecutive droughts in recent years in Chaharmahal and Bakhtiari Province and the fact that more than half of the area under wheat cultivation (56%) of the province is allocated to rain-fed (Anon., 2015), *H. filipjevi* may be causing economic yield loss especially in rain-fed wheat fields.

Many attempts have been made to control CCNs around the world, including cultural practices, chemical control, biological

control and development of resistant wheat varieties. The use of resistant crop cultivars and crop rotation with non-host crops are considered the most effective and economical methods for managing these nematodes (Nicol, 2002; Dababat *et al.*, 2014).

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وقوع نماتد سیستمی غلات (*Heterodera filipjevi* (Madzhidov, 1981) در مزارع
گندم استان چهارمحال و بختیاری و تعیین پراکنش آن بر اساس درون یابی با سامانه
اطلاعات جغرافیایی

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چکیده

به منظور شناسایی، تعیین پراکندگی و تراکم جمعیت نماتدهای سیستمی غلات در مزارع گندم استان چهارمحال و بختیاری بر اساس درون یابی با سامانه اطلاعات جغرافیایی، مجموعاً ۲۱۲ نمونه مرکب خاک و ریشه از مزارع گندم ۹ شهرستان استان طی سالهای ۱۳۹۲-۱۳۹۳ به صورت تصادفی جمع آوری گردید. سیستم‌ها از نمونه‌های خاک استخراج و تعداد سیستم‌ها و تخم و لارو سن دوم درون هر نمونه شمارش گردید. شناسایی گونه بر اساس مطالعات ریخت شناسی و ریخت سنجی و همچنین مشخصات مولکولی انجام شد. با بکارگیری تکنیک درون یابی با استفاده از نرم افزار Arc GIS نقشه پیوسته تراکم جمعیت تخم و لارو سن دوم تعیین شد. نتایج نشان داد ۴۲ درصد از نمونه‌های خاک با میانگین جمعیت ۹۹۴ تخم و لارو سن دوم به *H. filipjevi* آلودگی دارند. منطقه ده صحرا در شهرستان لردگان با متوسط جمعیت ۴۷۲۰ تخم و لارو در ۲۰۰ گرم خاک حداکثر آلودگی را به نماتد مذکور داشت. مزارع گندم شهرستانهای کوهرنگ، کیار و فارسان دارای آلودگی کم و مزارع شهرستانهای اردل، بن، سامان و بروجن از مناطق با آلودگی متوسط به نماتد *H. filipjevi* بودند. شهرستانهای شهرکرد و لردگان آلودگی بالایی به نماتد مذکور داشتند. بر اساس درون یابی تراکم جمعیت و نقشه درون یابی، شرق شهرستان شهرکرد و بخش‌های غربی شهرستان لردگان مناطقی با آلودگی بالا پیش بینی شدند.