



OCCURRENCE AND IDENTITY OF THE CAUSAL AGENTS OF WILT/ ROOT-ROT DISEASE IN CHICKPEA IN ABU HAMAD AREA, SUDAN

Hanan Ibrahim Mudawi*

The Environment, Natural Resources and Desertification Research Institute,
National Centre for Research, Ministry of High Education,
Khartoum, Sudan.

Email: hananmodawi@gmail.com

Mohamed Osman Idris

Department of Plant Protection, College of Agriculture,
Khartoum University, Khartoum, Sudan.

Hanaa Sedhum Zawam

Plant pathology Research Institute,
Agricultural Research Center, Giza, Egypt.

*Corresponding Author

ABSTRACT

Purpose: A survey was undertaken at Chickpea production areas in Adu Hamad, northern Sudan, for three seasons, 2006/007, 2007/008 and 2008/009, to determine the distribution and population density of plant-parasitic nematodes, their interaction with two *Fusarium* spp., and the microbial flora associated with chickpea.

Design/methodology/approach: Thirteen Hawashas (fields) were divided into three sectors: north, centre and south. Nematode population density/100g of soil, absolute density, prominence values and their frequency occurrence were recorded. The total fungal and bacterial count, and the neutral pH in the three sectors were also recorded.

Findings: Eleven substantial nematode populations were detected: *Aphelenchus avenae*, *Meloidogyne* spp., *Ditylenchus dipsaci*, *Heterodera* spp. *Rotylenchulus reinformis*, *Tylenchulus* spp. and *Criconemoide* spp. recorded the highest population density, whereas *Helicotylenchus* spp., *Tylenchorhynchus* spp., and *Trichodorus* spp recorded the lowest. *Meloidogyne* spp. were the most conspicuous plant-parasitic nematode in the northern and southern sectors, with a high prominence value of 57.14% and 66.70% respectively, and being recovered from 55.38% of soil samples. The total fungal and bacterial count ranged from 7.51×10^5 – 5.13×10^4 and 1.24×10^8 – 3.25×10^7 . The neutral pH of the three sectors explained the affinity of nematodes to highly propagate and the higher number of the bacterial count over the fungal count.

Original/value: for the first time, this survey sheds some light on the presence of some serious plant-parasitic nematodes genera and their distribution in the rhizosphere of chickpea in the Adu Hamad area, and explains its low productivity.

Keywords: survey; *Cicer arietinum*; plant-parasitic nematodes; wilt/root-rot disease; Sudan

Reference to this paper should be made as follows: Mudawi, H. I., Idris, M. O. and Zawam, H. S. (2018) 'Occurrence and Identity of the Causal Agents of Wilt/Root-Rot Disease in Chickpea in Abu Hamad Area, Sudan', *Int. J. Sudan Research*, Vol. 8, No. 1, pp.001–014.

INTRODUCTION

During the years 1999–2004, chickpea was grown in an area of approximately 1701–1935 hectares annually. This was mostly in the northern and southern part of Sudan, which was more suitable for chickpea production compared to the central and southern parts of the country (Sheikh Mohamed, 1995), with a low productivity range of 4.2–11.76Kg ha (El Khedier, 2007). Chickpea production in northern Sudan dropped sharply from 180Kg/feddan to 40Kg/feddan during the years 2000–2009, which compelled farmers to change to other profitable crops. These included the common bean (*Phaseolus vulgaris*), Fenugreek (*Trigonella foenum-graecum*) and cumin (*Cuminum cyminum*) (personal contact with farmers). They also cultivated wilt/root-rot susceptible cultivars which were released during these years (El Khedier, 2007).

More than 330 chickpea genotypes were evaluated for resistance to wilt/root-rot in the sick-plot at Hudeida Research Station Farm (ARC) in northern Sudan (Ali et al., 2002). This resistancy was destroyed due to root infection by soil-borne organisms such as plant parasitic nematodes, fungi, bacteria and viruses. Parasitic nematodes were known to cause an estimated yield loss of 14% in chickpea (Sharma and McDonald, 1990). Because of their microscopic size and the non-specific symptoms of an infection, these organisms live out of sight and, generally, out of mind of the growers and plant protection workers. Otherwise, most farmers and extension staff are not able to identify nematodes and other soil borne diseases (Sharma, 1997). Wall and Virginia (1999) and O'Donnell et al. (2001) reported that soil bacteria and fungi play vital roles in various biochemical cycles and are responsible for the recycling of organic compounds. They influence above-ground ecosystems by contributing to plant nutrition, plant health, soil

structure and soil fertility. Bacteria make up the most abundant group of microorganisms in the soil (3.0×10^6 – 5.0×10^8) per gram of soil, followed by the actinomycetes (1.0×10^6 – 2.0×10^7), fungi (5.0×10^3 – 9.0×10^6), yeast (1.0×10^3 – 1.0×10^6), algae and protozoa (1.0×10^3 – 5.0×10^5) and nematodes (50–200) counts per gram of soil. There are wide differences in the relative proportions of individual genera found in particular soils (Atals and Bartha, 1998).

In addition, interactions commonly occur between nematodes and other soil pathogens, complicating any quick recognition of the problem and assessment of the damage done. Several studies have shown that interactions of *F. oxysporum* f. sp. *ciceris* and *M. incognita*, *M. artiellia*, *M. javanica* or *Pratylenchus* spp. in chickpea can lead to a breakdown of resistance to an unidentified race of the Fusarium wilt pathogen (Uma Maheswari et al., 1997; Castillo et al., 2003).

Castillo et al. (2008), estimated the threshold of tolerance of chickpea of *Meloidogyne* spp. to range between 0.01–4.28 nematode/g of soil (*M. incognita* 1.00–2.00 and *M. javanica* 0.10–4.28), *Heterodera ciceri* 0.40–1.40 nematode/g of soil, and *Rotylenchulus reniformis* 0.50–0.10 nematode/g of soil, from different sources and countries. Di Vito et al. (1992) estimated a threshold level density of *Pratylenchus* spp. to be 0.31–10.28 nematode/g of soil under field conditions in Syria.

In Sudan, nematological research has not received much attention, and there is no record of nematode pests occurring on chickpea in the country. Previous studies have listed plant parasitic nematodes associated with cotton, sorghum, millet, wheat, groundnut, pigeonpea, and ornamental plants, with some species found to be more parasitic and pathogenic causing economic losses to these crops (Yassin, 1974, 1986; Magbool, 1997).

Despite economic importance and strong national and international breeding programmes in chickpea, average yield has not improved considerably over the years. The annual growth rate of chickpea production is low (0.007%) during the last decade (1993–2003), and average yields have been almost static (FAO, 2006). The low yield of chickpea is attributed to its susceptibility to several fungal, bacterial, viral and nematode plant pathogens (Dubey et al., 2007).

Therefore, a survey was undertaken to determine the distribution and population density of plant-parasitic nematodes and their interaction with the two *Fusarium* spp. under investigation. The survey also included the microbial flora associated with chickpea growing areas in three sectors at Adu Hamad area, Northern Sudan.

MATERIALS AND METHODS

Soil Sample Collection

Systematic surveys for plant-parasitic nematodes were established and carried out in three localities during three seasons in May 2006/007, 2007/008 and 2008/009, of the main chickpea growing areas of El Rubatab area (Abu Hamad), Northern Sudan (Figure 1). The total area under survey was 4.81Km. This area is 67.23Km south of Abu Hamad Locality, within a geographical position of latitude 190.62'–180.55'north and a longitude 330.32'–330.70' east. This area was divided into three sectors, North, Centre and South. The soil samples were randomly collected from ten Hawashas (fields), chosen along a predetermined route in each region. One bound of soil samples were randomly collected with a hoe to a depth of 30cm from chickpea rhizosphere soil. Ten soil subsamples from each Hawasha were thoroughly mixed in a plastic bag and fully labelled. They were then brought to the laboratory and stored at 5°C until they were examined. A total of 300 samples belonged to chickpea cultivar Shinde.

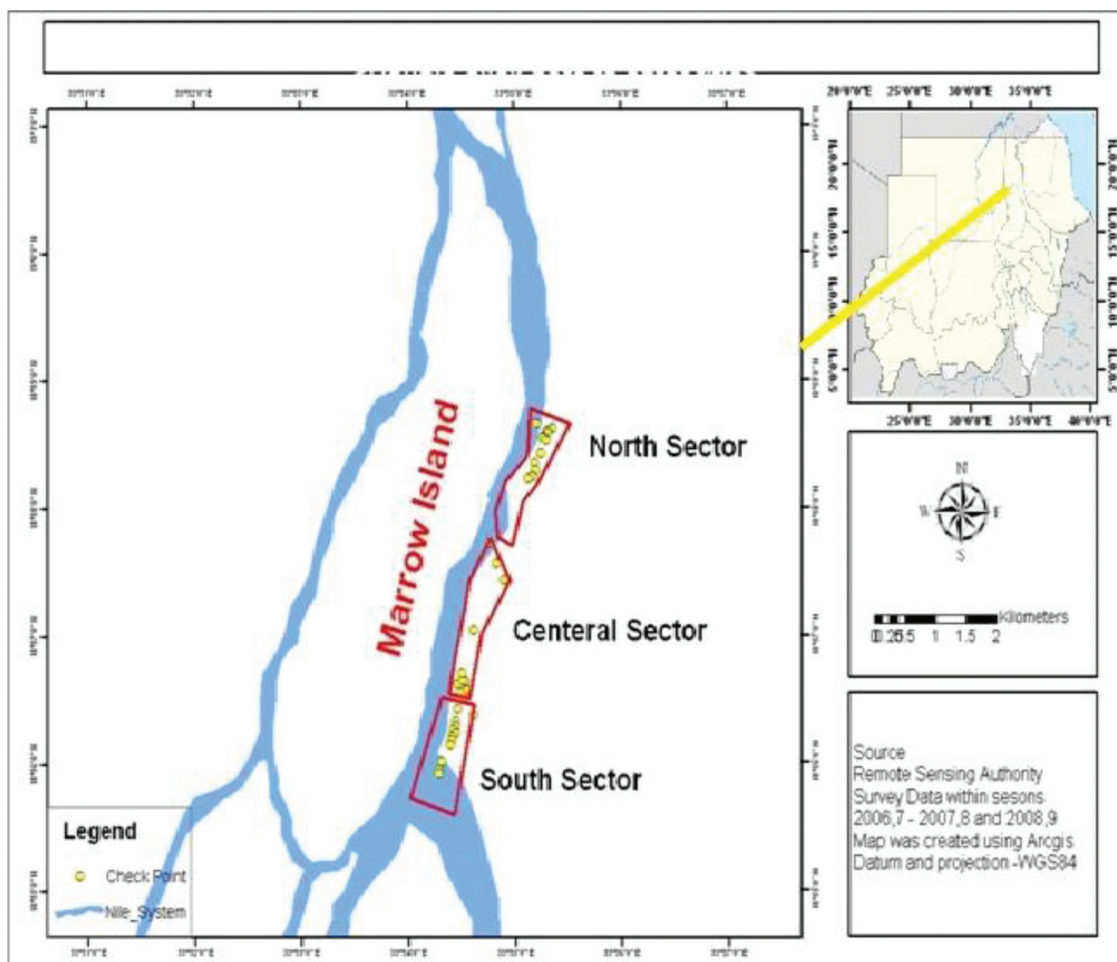


FIGURE I Map of Abu Hamad Locality, River Nile, Northern Sudan, indicating the area surveyed

Source: Remote Sensing Authority. Map was created using Arcgis Datum and Projection – WGS84

Nematode Extraction

Nematodes were extracted and counted from soil samples obtained from the selected sectors using the Modified Baermann tray method (Southey, 1986; Hooper et al., 2005). Simple sieves were used; each sieve was overlaid with double tissue paper and placed in a plastic tray. One hundred grams of soil sample containing roots was evenly spread in a thin layer over the tissue paper. Enough water to saturate the tissue paper was carefully added to the tray then left for 24 hours. The nematode suspension was collected in a 500ml Erlenmeyer flask. The suspension was reduced to 200ml after one hour for the nematode count. The mean number of nematodes per 1ml was determined.

For collection and identification of nematodes, the Baermann funnel technique was used (Whitehead and Hemming, 1965). A rubber tube was placed on the stem of a 9.5cm conical glass funnel, and the tube was secured by a pinch clamp. The funnel was placed in a suitable support (a 50ml Erlenmeyer flask) and almost filled with water. Ten grams of each soil subsample was placed in a double tissue paper; this was flooded to enclose the soil, and then gently submerged in the water in the funnel.

Preservation of Nematodes

The method used for fixation and preservation of nematodes was that described by Kleynhans (1999) and Hooper et al. (2005), which involved the killing, fixation and permanent slide preparation of live nematodes. The prepared slides were double sealed with nail varnish, labelled and identified.

Nematode Population Density Assessment

The importance of each genus was determined by its relative density (nematode number per unit volume of soil), absolute frequency (rate of occurrence), relative frequency; the prominence value of the different nematodes were calculated according to Norton (1978) in which:

$$\text{Absolute frequency occurrence} = \frac{\text{Number of sample containing a species} \times 100}{\text{Number of sample examined}}$$

$$\text{Relative frequency occurrence} = \frac{\text{Absolute frequency occurrence of species} \times 100}{\text{Sum of frequency occurrence of all species}}$$

$$\text{Absolute density} = \frac{\text{Total number of individuals of a species}}{\text{Number of samples containing this species}}$$

$$\text{Prominence value} = \frac{\text{Number of samples containing a species} \times 100}{\text{Number of samples collected}}$$

According to Castillo et al. (2008) the threshold of tolerance of the most important nematodes attacking chickpea ranged from 0.02–4.28 individuals/gram of soil. Thus, an individual was considered abundant when its mean number was greater than 2.00 individuals per 100g of soil.

MICROBIOLOGICAL ANALYSES

Quantification of Fungal and Bacterial Isolates

To quantify the fungal and bacterial isolates, soil subsamples were thoroughly mixed, and a suspension of 1g (dry weight equivalent) in 9ml of sterilized distilled water was prepared from each subsample. A serial dilution of the soil suspensions was prepared (ten-fold) and used in the estimation of bacterial and fungal populations by a standard spread-plate dilution method described by Seeley and Van Demark (1981); this was done in triplicate. Nutrient agar (NA) was used for bacteria isolation and potato dextrose agar (PDA), supplemented with 0.05% (w/v) chloramphenicol to avoid bacterial contamination that was used for fungal isolation. The colonies of microorganisms were counted and the number of viable cells was calculated as the total number of colony forming units (cfu). *Fusarium* species were isolated by soil suspensions, where 1g of soil was suspended in 0.05% water agar media (WA), supplemented with 0.05% (w/v) chloramphenicol. Enumeration, fungal colonies resembling *Fusarium* spp. were obtained and plated on Spezieller Nährstoffarmer Agar (SNA), KH_2PO_4 1.00g, KNO_3 1.00g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.50g, KCl 0.50g, Glucose 0.20g, Sucrose 0.20g, Agar 20.00, Distilled water 1.00L, and the media were allowed to dry for three days. Pure cultures were observed for *Fusarium* species and then maintained on sterilized soil-agar at 4°C. The identification of isolates was based

on cultural, microscopic characteristics with reference to Leslie and Summerell's *Fusarium* laboratory manual (2006), and the soil pH was determined; three replicates were measured for each sector.

Analysis of Data

Data are square root transformed for analysis to avoid zero. A Least Significant Range Test was used to test significant differences between treatments at $p \leq 0.05$ (Gomez and Gomez, 1984).

Results

The total number of nematodes/100g of soil was significantly different ($P \leq 0.05$) between the three selected sectors (Table 1). No significant differences were detected ($P \leq 0.05$) between the central and the southern sectors. The central region showed the highest infestation number of nematodes during the three successive surveyed seasons, 2006/007, 2007/008 and 2008/009, giving a total range of 3270.00–2556.67 nematodes/100g of soil. The soil pH of the Abu Hamad sectors ranged from 7.35–7.62. The soil pH in the north was higher than in the south and centre; this is shown in Table 1. However, differences in the soil pH values of the different sampling locations were observed to be statistically significant at $P \leq 0.05$, the northern sector recorded the highest pH, although the pH of the three sectors is considered neutral.

Table 1 Mean nematode population density/100g of soil, over three seasons 2006/07, 2007/08 and 2008/09 and the soil pH at three chickpea production sectors in the Abu Hamad area of northern Sudan

Sector	Nematode density/100g of soil ^a				Soil pH ^b
	2006/007	2007/008	2008/2009	Total mean	
North	2140.00 (45.73) ^{*B}	2580.00 (49.99) ns	2090.00 (44.92) ns	2270.00	7.62 (2.89) ^A
Center	3340.00 (56.95) ^A	3380.00 (57.63) ns	3090.00 (54.81) ns	3270.00	7.42 (2.90) ^B
South	2540.00 (49.53) ^A	2750.00 (51.54) ns	2380.00 (47.45) ns	2556.67	7.35 (2.94) ^C
LSD($P \leq 0.05$)	7.75	11.84	8.60		0.01

^aFive replications were used for each sample in each sector.

^bThree replications were used for each sample in each sector.

* Data are square root transformed for analysis to avoid zero, data with the same letter are not significantly different ($P \leq 0.05$), according to Least Significant Range Test.

Source: Devised by authors

Free-living and plant-parasitic nematodes were identified in 22 out of the 30 soil samples. The results in Tables 2 and 3 show the differences between nematodes in frequency, density and prominence value percentages. Eleven genera of stylet-bearing nematodes representing eight families were identified from soil samples collected from investigated chickpea fields. By sector, five genera were recovered from the north, nine from the centre and seven from the south sector, with a significant difference at $P \leq 0.05$ between them, both in the mean nematode number/100g of soil and the total absolute density between sectors.

In the collected soil samples from the Abu Hamad area, seven genera were identified and considered most prevalent according to Castillo et al.'s (2008) estimation. In descending order of the total mean density of nematodes/100g of soil these were:

- *Aphelenchus avenae* (236.30);
- *Meloidogyne* spp. (220.00);
- *Ditylenchus dipsaci* (166.67);
- *Heterodera* spp. (133.33);
- *Rotylenchulus reinformis* (133.33);
- *Tylenchulus* spp. (80.00);
- *Criconemoide* spp. (46.67).

The least prevalent were:

- *Helicotylenchus* spp. (26.67);
- *Trichodorus* spp. (20.00); and
- *Tylenchorhynchus* spp. (13.33) (see Table 3).

Table 2 Absolute density of soil and the population range of the nematode genera associated with chickpea in three major producing sectors, from 23 soil samples^N collected from 30 chickpea fields in Abu Hamad at Northern state 2006/007

Genera	North		Abu Hamad sectors Center		South	
	Absolute density	Range*	Absolute density	Range	Absolute density	Range
<i>Rotylenchulus reinformis</i>	90.00	800–1000	20.00	0.00–200	66.67	400–800
<i>Heterodera</i> spp.	100.00	600–800	60.00	200–1200	80.00	0.00–800
<i>Ditylenchus dipsaci</i>	70.00	400–1200	40.00	200–600	100.00	800–1200
<i>Meloidogyne</i> spp.	80.00	400–1200	80.00	400–1200	86.67	600–1000
<i>Tylenchulus</i> spp.	0.00	–	40.00	0.00–400	6.25	800–1000
<i>Trichodorus</i> spp.	0.00	–	00.00	–	60.00	0.00–600
<i>Criconemoide</i> spp.	0.00	–	70.00	600–800	00.00	–
<i>Helicotylenchus</i> spp.	0.00	–	80.00	0.00–800	00.00	–
<i>Tylenchorhynchus</i> spp.	0.00	–	20.00	0.00–200	00.00	–
<i>Aphelenchus avenae</i>	35.00	200–600	96.30	200–1800	93.33	600–1600
<i>Panagerllus</i> spp.	100.00	800–1200	0.00	–	70.00	400–1000

^NNematodes were collected from seven hawashas in the north, ten in the centre, and six in the south.

*Nematode population range from each selected hawashas from each sector.

Source: Devised by authors

The survey revealed that, in the central sector, almost all the nematodes genera were detected. *Meloidogyne* spp. were the most conspicuous plant-parasitic nematodes associated with chickpea and were present in 21.82% of sampled chickpea in the north, sharing the high prominence value of 57.14% with *Aphelenchus avenae*. The highest in the absolute density in

the north region was *Heterodera* spp., and *Panagerllus* spp. reached 100.00 nematode/100g of soil (Table 2). However, *Rotylenchulus reinformis* and *Panagerllus* spp. recorded the highest population range of 800–1000 and 800–1200. In the central sector, the percentage of the relative frequency of occurrence of *Aphelenchus avenae* (27.54%) was the highest, although the most important nematode in this region was *Ditylenchus dipsaci*, giving a high prominence value percentage of 40.00%. *Meloidogyne* spp. was most important nematode in the southern sector, with a percentage prominent value of 66.70% and a percentage frequency of occurrence of 18.31% (Table 3).

Table 3 Frequency of occurrence (%), the prominence value (%) and Mean density/100g soil, of nematode genera associated with chickpea in three major producing sectors, from 23 soil samples^N collected from 30 chickpea fields in Abu Hamad in Northern state 2006/007

Genera	Abu Hamad sectors ^N						
	North		Center		South		Mean density/ 100g of soil
	Relative Frequency %	Prominence value %	Relative Frequency %	Prominence value %	Relative Frequency %	Prominence value %	
<i>Rotylenchulus reinformis</i>	16.36	28.57	1.91	10.00	14.08	50.00	133.33 ^b
<i>Heterodera</i> spp.	18.18	28.57	11.44	20.00	5.63	16.70	133.33 ^b
<i>Ditylenchus dipsaci</i>	12.73	28.57	15.25	40.00	14.08	50.00	166.67 ^{ab}
<i>Meloidogyne</i> spp.	21.82	57.14	15.25	20.00	18.31	66.70	220.00 ^a
<i>Tylenchulus</i> spp.	0.00	0.00	3.81	10.00	14.08	33.30	80.00 ^{cd}
<i>Trichodorus</i> spp.	0.00	0.00	0.00	00.00	4.23	16.70	20.00 ^e
<i>Criconemoide</i> spp.	0.00	0.00	13.35	20.00	0.00	00.00	46.67 ^{de}
<i>Helicotylenchus</i> spp.	0.00	0.00	7.63	10.00	0.00	00.00	26.67 ^e
<i>Tylenchorhynchus</i> spp.	0.00	0.00	3.81	20.00	0.00	00.00	13.33 ^e
<i>Aphelenchus avenae</i>	12.73	57.14	27.54	30.00	19.72	50.00	236.30 ^a
<i>Panagerllus</i> spp.	18.18	28.57	0.00	0.00	9.86	33.30	113.33 ^{bc}

^N Nematodes were collected from (i) seven hawshas at north, (i) ten at centre, and (ii) six at south.

* Total number of nematodes extracted from all selected hawshas at the three sectors.

^a Data are square root transformed for analysis to avoid zero, data with the same letter are not significantly different ($P \leq 0.05$), according to Duncan multiple rang test.

Source: Devised by authors

The mean total fungal counts of each soil sample ranged from 6.47×10^5 cfu/g of soil, 5.13×10^4 cfu/g of soil, 7.51×10^4 cfu/g of soil from the north, centre and south sectors, respectively. The highest counts were observed in the north, while the lowest counts were observed in south, as shown in Table 4. Differences in the average total fungal counts of the sampling locations were not statistically significant ($P \leq 0.05$). Throughout the different sampling sectors several types of fungal genera were observed. Among these are, *F. oxysporum*, *F. solani*, *Aspergillus* spp., *Penicillium* spp., *Heminthosporium* spp., *Verticillum* spp., *Rhizoctonia* spp., and *Ascochyta* spp.

The most prevalent species in the three sectors was *F. oxysporum*, which was significantly high ($P \leq 0.05$) in the south sector with a total colony count of 1.76×10^3 /g of soil (Table 4). The propagules number of *F. solani* was very low. Conversely, *F. solani* was detected in the highest concentration of 1.56×10 cfu/g of soil in the central sector, and it was significantly higher

($P \leq 0.05$) than *F. oxysporum*. *Meloidogyne* spp. and *F. oxysporum* were the most abundant microorganisms in the south sector.

The mean total bacterial counts of each soil sample ranged from 12.45×10^8 cfu/g of soil, 3.25×10^7 cfu/g of soil, and 7.00×10^7 cfu/g of soil from the north, centre, and south sectors, respectively (Table 4). There were differences in the average total bacterial counts of the different sampling locations, and were statistically significant ($P \leq 0.05$). However, the highest counts were observed in the north sector, and the lowest count was observed in the south sector.

Table 4 Means of the occurrence of bacterial, fungal fauna, *F. oxysporum* and *F. solani* (cfu/g of soil), of the collected soil samples from three sectors at the Abu Hamad area of Northern Sudan

Sector	Bacterial count (cfu/g of soil)	Fungal count (cfu/g of soil)	Fusarium oxysporum (cfu/g of soil)	Fusarium solani (cfu/g of soil)
North	1.24×10^8 A	6.47×10^4 B	0.16×10^3 B	0.13×10 C
Center	7.00×10^7 B	5.13×10^4 B	1.33×10^3 B	1.56×10 A
South	3.25×10^7 C	7.51×10^5 A	1.76×10^3 A	1.33×10 B
LSD ($P \leq 0.05$)*	12.26	2.43	0.41	0.12

Three replications were used for each count in each sector.

*Data were square root transformed for analysis; data with the same letter are not significantly different ($P \leq 0.05$), according to Least Significant Rang Test.

Source: Devised by authors

Discussion

The results obtained from the total fungal and bacterial count (Table 3), which ranged from 5.13×10^4 – 7.51×10^5 and 3.25×10^7 – 1.24×10^8 , fell in comparison with the range reported by earlier workers (Atals and Bartha, 1998). Expectedly, the total bacterial count was generally higher than those of fungi, irrespective of the sampling locations.

All the soil pH was in the neutral range (Table 1); this favours microbial growth (Agrios, 2005; Ogunmwonyi et al., 2008). The neutral soil pH of the three sectors could explain the affinity of nematodes to highly propagate during the three surveyed seasons 2006/007, 2007/008 and 2008/009; it also explains the higher total bacterial count over the total fungal count (Table 4). According to Davis (2004), the disease incidence and severity are greater in soils with a pH of 5.5 to 7.5. Soil pH is also known to influence the activity of mineralizing and nitrifying bacteria and mycorrhiza as well as pathogenic organisms (Haverkort et al., 1998); therefore the population density of nematodes and fungi were observed to decrease when the soil pH increased. On the other hand, the bacterial propagules increased when the pH increased, giving an assumption that the abundance of nematodes and fungi is influenced with decreased pH exponentially, and *vice versa* with bacteria propagules. The neutralized soils of the Adu Hamad area are the cause of the reduced density of *Fusarium* spp. shown in the three sectors (Table 4), since *Fusarium* spp. diseases are generally more severe in acid pH soils (Agrios, 2005). Sugha et al. (1994) reported that the maximum Fusarium wilt occurs at pH 5.2, with a slight decline towards neutrality.

With the exception of *Aphelenchus avenae* and *Panagrellus* spp., all the nematodes in this study have been reported as some of the nematodes attacking the chickpea crop. *Meloidogyne* spp., *Ditylenchus dipsaci*, *Heterodera* spp., and *Rotylenchulus reinformis*, are the most

frequent and abundant genera representing the major plant parasitic nematodes in chickpea production areas in northern Sudan (Table 3). In fact, these nematodes are universally parasitic to *Cicer arietinum*. L. as stated by Castillo et al. (2008). The mean densities of these nematodes were higher than the referenced reports in different countries growing chickpea (Di Vito et al., 1992, 1994; Castillo et al., 2008). The high frequencies and population densities found for *Meloidogyne* spp. and *Ditylenchus* spp. are fostered by the neglect options for control measures. Although the stem and bulb nematode *Ditylenchus dipsaci*, which are rarely reported to infect chickpea plants (Castillo et al., 2008), showed a high population density and prominence value in the southern region (Table 3), infection of this nematode has only been reported in South Australia, causing severe yield losses in young chickpea plants: adult plants were resistant to the nematode (Thompson et al., 2000).

The root-knot nematodes, *Meloidogyne* spp., were the most conspicuous plant-parasitic nematodes associated with chickpea with its high population densities in this study, having a high prominent value in the north and south, and the highest mean density/100g of soil. Its importance as a pathogen of chickpea is reported in many countries, Egypt, Ghana, India, North Africa, Syria, Pakistan and Ethiopia (Di Vito et al., 1994; Ali and Sharma, 2003; Sikora et al., 2005; Castillo et al., 2008). Most of the disease complexes studied include *Meloidogyne* spp. and different soil pathogenic fungi (Taylor, 1990; Bertrand et al., 2000; Back et al., 2002), especially *F. oxysporum* f. sp. *ciceris* (Castillo et al., 2003). The presence of *M. incognita*, *M. artiellia* and *M. javanica* in soils infected with *Fusarium* spp. enhances the incidence, rate of disease development, and severity of Fusarium wilt in grain legumes (Nene et al., 1989; Sharma and McDonald, 1990; Sharma et al., 1992). These nematodes are able to attack a wide host range including many weeds, and can complete several generations within a growing season; this leads to a very high post-harvest nematode population density in soils (Sikora et al., 2005; Castillo et al., 2008). Experimental evidence indicated that *Meloidogyne* spp. causes a serious yield decline of chickpea crops. Yield losses of up to 60% were caused by *M. incognita* infections in India, whereas in southern Italy, *M. artiellia* caused a yield reduction of 50% in winter sowing and 80% in spring sowings (Castillo et al., 2008).

Cyst-forming nematodes, mainly *Heterodera* spp., recording an absolute density of 100 nematode/100g of soil in the northern sector (Table 2), are more prone than other plant-parasitic nematodes to dispersion over time and space. This is because eggs within cysts can tolerate long periods of desiccation and persist in soil for several years in the absence of a host plant (Agrios, 2005).

The following parasitic nematode genera were also identified in the chickpea rhizosphere, exhibiting different parasitic behaviour, sedentary endoparasitic (*Meloidogyne* spp., *Heterodera* spp., *Rotylenchulus* spp., and *Tylenchulus* spp.), migratory endoparasitic (*Ditylenchus* spp. and *Tylenchorhynchus* spp.), ectoparasitic, feeding on subsurface tissue (*Helicotylenchus* spp. *Trichodorus* spp. and *Criconemoides* spp.). Most of these genera have been previously found to be associated with chickpea elsewhere (Nene et al., 1989; Sharma and McDonald, 1990; Sharma et al., 1992; Castillo et al., 2008). The presence of mixed populations of plant parasitic nematodes is likely to accelerate root damage; this is because lesions can develop at feeding sites throughout the root tissue, leading to a good broad explanation of the yearly reduction in yield in the Adu Hamad area recently.

Unexpectedly, there were no reports of the occurrence of *Pratylenchus* spp. (a migratory endoparasitic) in the surveyed area of Abu Hamad, the nematode that ranked second after the root-knot nematodes in terms of their global economic impact on this crop (Castillo and Vovlas, 2007). This could be due to the high abundance of *Meloidogyne* spp.; knowing that both

nematodes have the same habitat, and according to the principle of competitive exclusion of Agrios (2005), no two species occupy exactly the same niche or perform exactly the same function in an ecosystem. Thus *Meloidogyne* spp. may be suppressing *Pratylenchus* spp. Also, the neutralized soils of the Adu Hamad area played a part in the absence of *Pratylenchus* spp. Willis (1972) reported that the population of *P. penetrance* (Cobb.) colonizing alfalfa roots was greater at pH 5.2 and 6.4, and *P. allenii* Ferris populations in soybean roots were significantly greater at pH 6.0 than at 4.0 or 8.0.

The presence of the fungivorous nematode, *Aphelenchus avenae*, resulted in a reduction in the number of fungi in the central sector, 5.13×10^4 cfu/g of soil (Table 4). This nematode has a high potential to propagate itself by feeding on more than 92 species of soil fungi (Ishibashi, 2005). Hence, reporting a high density (96.30 nematode/100g of soil) and frequency percentage (27.54%) in this sector, attributed to low fungal propagation. The distribution of one group of fungal-feeding nematode suggests that they feed on fungi associated with this rhizosphere, and since *Fusarium oxysporum* are moderately present in these soils, it could be the food web of the nematode, hence reducing its population density. However, Ishibashi (2005) reported that *A. avenae* has less affinity to *F. oxysporum* and *Verticillium dahliae* than for *Rhizctonia solani* and *Botrytis cinerea*, and explains it to be due to the thinner width of their hyphae than the length of the nematode stylet.

Surprisingly, the free-living nematode *Panagrellus* spp. that has been found abundantly in both the north and south regions, are considered a suitable alternative live food for the rearing of many larval fish species (Ricci et al., 2003).

As cultural practices within the surveyed sectors have remained basically unchanged for the last ten years, we believe that this survey provides a general and realistic representation of the nematode distribution and explains the low productivity of chickpea in the Adu Hamad area. Although this is a preliminary survey, it sheds some light, for the first time, on the presence of some serious plant-parasitic nematodes genera in the rhizosphere of chickpea in northern Sudan, Adu Hamad area. The consecutive cultivation of chickpea in Abu Hamad area year after year, using uncertified varieties that are susceptible to wilt/root-rot disease complex, and the negligence of using fertilizers, has led to the accumulation of inoculum and hence the development of an epidemic.

For all these reasons, chickpea production in the Abu Hamad area decreased over the past ten years and eventually stopped. El Hawata and Al Gezira farmers in central Sudan have started to grow chickpea and are facing the same problems as those in the Abu Hamad area. Further studies are needed to identify the species of these genera, their pathogenicity and a feasible management approach.

REFERENCES

- Agrios, G. N. (2005), *Plant Pathology*, 5th edn, Elsevier Academic Press, UK, USA, pp. 295–872.
- Ali, M. E. K., Inangn, S. and Sugimoto, Y. (2002), 'Sources of resistance to Fusarium wilt of Chickpea in Sudan', *Phytopathologia Mediterranea*, Vol. 41, No. 3, pp. 163–169.
- Ali, S. S. and Sharma, S. B. (2003), 'Nematode survey of chickpea production areas in Rajasthan, India', *Nematol. Mediterr.*, Vol. 31, No. 2, pp. 147–149.
- Atals, R. M. and Bartha, R. (1998), *Microbial Ecology: Fundamentals and Applications*, 4th edn, Benjamin Cummings Publishing Company, pp. 300–350.
- Back, M. A., Haydock, P. P. J., and Jenkinson, P. (2002), 'Disease complexes involving plant parasitic nematodes and soilborne pathogens', *Plant Pathology*, Vol. 51, No. 6, pp. 683–697.

- Bertrand, B., Nunez, C. and Sarah, J. L. (2000), 'Disease complex in coffee involving *Meloidogyne arabicida* and *Fusarium oxysporum*', *Plant Pathology*, Vol. 49, No. 3, pp. 383–388.
- Castillo, P., Navas-Cortés, J. A., Blanca B., Landa, B. B., Jiménez-Díaz, R. M. and Vovlas, N. (2008), 'Plant-parasitic nematodes attacking chickpea and their in planta interactions with rhizobia and phytopathogenic fungi', *Plant Disease*, Vol. 92, No. 6, pp. 840–853.
- Castillo, P., Navas-Cortés, J. A., Gomar-Tinoco, D., Di Vito, M. and Jiménez-Díaz, R. M. (2003), 'Interactions between *Meloidogyne artiellia*, the Cereal and Legume Root-Knot Nematode, and *Fusarium oxysporum* f. sp. *ciceris* Race 5 in Chickpea', *Phytopathology*, Vol. 93, No. 12, pp. 1514–1523.
- Castillo, P. and Vovlas, N. (2007), *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, Biology, Pathogenicity and Management. In: Hunt, D. J. and Perry, R. N. (Series Eds): *Nematology, Monographs & Perspectives*, Vol. 6, Brill Academic Publishers, Martinus Nijhoff Publishers and VSP, Leiden. The Netherlands, pp. 331–354.
- Davis, R. M. (2004), 'Carrot Diseases and their Management', In Naqvi, S. A. M. H. (Ed.): *Diseases of Fruits and Vegetables Diagnosis and Management*, Vol. I, Dordrecht: Kluwer Academic Publishers, 400pp.
- Di Vito, M., Greco, N. and Saxena, M. C. (1992), 'Pathogenicity of *Pratylenchus thornei* and chickpea in Syria', *Nematologia Mediterranea*, Vol. 20, No. 1, pp. 71–73.
- Di Vito, M., Greco, N., Halila, H. M., Mabsoute, L., Labdi, M., Beniwal, S. P. S., Saxena, M. C., Singh, K. B. and Solh, M. B. (1994), 'Nematodes of cool-season food legumes in North Africa', *Nematol. Mediterr.*, Vol. 22, No. 1, pp. 3–10.
- Dubey, S. C., Suresh, M. and Singh, B. (2007), 'Evaluation of *Trichoderma* species against *Fusarium oxysporum* f.sp. *ciceris* for integrated management of chickpea wilt', *Biological Control*. Vol. 40, No. 1, pp. 118–127.
- El Khedier, M. A. (2007), *Principles of Field Crops Production (1st Edn)*. Khartoum, Sudan: El Shareef academic Library for Production and Distribution, pp. 20–25.
- FAO (2006), Food and Agriculture Organization of the United Nations statistical database (FAOSTAT). Faostat, production statistics of crops. Online database. Retrieved on 2nd November 2015 from: <http://faostat.fao.org>
- Gomez, K. A. and Gomez, A. A. (1984), *Statistical Procedures for Agriculture Research* (2nd edn), Wiley, New York, NY, 680pp.
- Haverkort, A. K., Mulder, A. and Van Der Waart, M. (1998), 'The effect of soil pH on yield losses caused by the potato cyst nematode *Globodera pallid*', *Potato Research*, Vol. 36, No. 3, pp. 216–226.
- Hooper, D. J., Hallmann, J. and Subbotin, S. A. (2005), 'Methods for extraction, processing and detection of plant and soil nematodes', In Luc, M., Sikora, R. A and Bridge, J. (Eds): *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. CAB International, Wallingford (2nd Edn), pp. 53–86.
- Ishibashi, N., Takayama, S. and Kondo, E. (2005), 'Propagation and feeding behavior of the mycetophagous nematode, *Aphelenchus avenae*, on four species of soil fungi', *Japanese Journal of Nematology*, Vol. 35, No. 1, pp. 13–19.
- Kleynhans, K. P. N. (1999), *Preserving nematodes. A manual for Nematology*. ARC- Plant Protection Research Institute, Pretoria, South Africa, pp. 43–47.
- Leslie, J. F. and Summerell, B.A. (2006), *Fusarium laboratory manual* (1st Edn), Blackwell Publishing, Asia, 387pp.
- Magbool, M. A. (1997), Plant nematode problems and their control in the Near East region, *FAO Plant Production and Protection Paper No.144*, Proceedings of the Expert Consultation on Plant Nematode Problems and their Control in the Near East Region Karachi, Pakistan 22–26 November 1992. Retrieved on 2nd November 2015 from: <http://www.fao.org/docrep/v9978e/v9978e00.HTM>
- Nene, Y. L., Haware, M. P., Reddy, N. M. V., Philips, J. P., Castro, E. L., Kotasthane, S. R., Gupta, O., Singh, G., Shukia, P.; and Sah, R. P. (1989), 'Identification of broad based and stable resistance to wilt and root-rots in chickpea', *Indian Phytopathol.* Vol. 42, No. 4, pp. 499–505.
- Norton, D. C. (1978), *Ecology of plant-parasitic nematodes*. New York: Wiley, 268pp.
- O'Donnell, A. G., Seasman, M., Macrae, A., Waite, I. and Davie, J. T. (2001), 'Plants and Fertilizers as drivers of change in microbial community structure and function in soil', *Plant Soil*, Vol. 232, No. 1–2, pp. 135–145.
- Ogunmwonyi, I. N, Igbinosa, O. E, Aiyegoro, O. A and Odjadjare, E. E.(2008), 'Microbial analysis of different top soil samples of selected site in Obafemi Awolowo University', *Nigeria Scientific Research and Essay*, Vol. 3, No. 3, pp. 120–124,
- Ricci, M., Fifi, A. P., Ragni, A., Schlechtriem, C. and Focken, U. (2003), 'Development of a low cost technology for mass production of the free living nematode *Panagrellus redivivus* as an alternative live food for first feeding fish larvae', *Applied Microbiology and Biotechnology*, Vol. 60, No. 5, pp. 556–559.

- Seeley, H. W. and Van Demark, P. J. (1981), *Microbes in action. A laboratory manual of Microbiology* (3rd edition), W. H. Freeman and Company USA, p. 350.
- Sharma, S. B. (1997), Diagnosis of key nematode pests of chickpea and pigeonpea and their management. Proceedings of a Regional Training Course, ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), Andhra Pradesh, India, pp 74–98.
- Sharma, S. B. and McDonald, D. (1990), 'Global status of nematode problems of groundnut, pigeonpea, chickpea, sorghum and pearl millet, and suggestions for future work', *Crop Prot.*, Vol. 9, No. 6, pp. 453–458.
- Sharma, S. B., Smith, D. H. and McDonald, D. (1992), 'Nematode constraints of chickpea and pigeonpea production in the semi- arid tropics', *Plant Diseases*, Vol. 76, No. 9, pp. 868–874.
- Sheikh Mohamed, A. I. (1995), Chickpea Improvement. Production and improvement of cool-season Food legumes in the Sudan. In the Proceedings of the National Research Review Workshop, 27–30 August 1995, Agricultural Research Corporation, Wad Medani, Sudan. Nile Valley Regional Program, ICARDA, Cairo, Egypt.
- Sikora, R. A., Greco, N., and Veloso Silva, J. F. (2005), Nematode parasites of food legumes. In Luc, M. R. Sikora, A. and Bridge, J. (Eds): *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. CABI Publishing, Wallingford, UK, pp. 259–318.
- Southey, J. F. (1986), *Laboratory Methods for Work with Plant and Soil Nematodes*. Ministry of Agriculture, Fisheries and Food, London, 202pp.
- Sugha, S. K., Kapoor, S. K. and Singh, M. B. (1994), 'Soil characteristics and their relation to *Fusarium* wilt of chickpea (*Cicer arietinum* L.)', *Tropical Science*, Vol. 34, No. 3, pp. 282–288.
- Taylor, C. E. (1990), 'Nematode interaction with other pathogens', *Ann. Applied Biol.*, Vol. 116, No. 3, pp. 405–416.
- Thompson, J. P., Greco, N., Eastwood, R., Sharma, S. B., and Scurrah, M. (2000), 'Integrated control of nematodes of cool legumes', In Knight, R. (Ed.), *Linking Research and Marketing Opportunities for Pulses in the 21st Century*. (Proc. Int. Food Legumes Res. Conf. 3rd.). Kluwer Academic Publishers, Dordrecht. The Netherlands, pp. 491–506.
- Uma Maheswari, T., Sharna, S. B., Reddy, D. D. R. and Haware, M. P. (1997), Interaction of *Fusarium oxysporum* f.sp. *ciceris* and *Meloidogyne javanica* on *Cicer arietinum*. *J. Nematol.*, Vol. 29, No. 1, pp.117–126.
- Wall, D. H. and Virginia, R. A. (1999), 'Controls on soil biodiversity insights from extreme environments', *Appl. Soil Ecol.*, Vol. 13, No. 2, pp. 137–150.
- Whitehead, A. G. and Hemming, J. R. (1965), 'A comparison of some quantitative methods of extracting small vermiform nematodes from soil', *Annals of Applied Biology*, Vol. 55, No. 1, pp. 25–38, Version of Record online: 28 June 2008.
- Willis, C. B. (1972), 'Effect of soil pH on reproduction of *Pratylenchus penetrans* and forage yield of alfalfa', *Journal of Nematology*, Vol. 4, No. 4, pp. 292–295.
- Yassin, A. M. (1974), 'Role of *Pratylenchus sudanensis* in the syndrome cotton wilt reference to its vertical distribution', *Sudan Agric. J.*, Vol. 2, No. 1, pp. 48–52.
- Yassin, A. M. (1986), 'Nematode parasites of crop plants in the Sudan', *Tech. Bull.*, 4. ARC.

BIOGRAPHIES

Dr Hanan Ibrahim Mudawi is a Researcher Professor Assistant in Bio-pesticides and Plant Pathology, currently working at the Department of Bio-pesticides and Bio-fertilizers, Environment and Natural Resources and Desertification Research Institute, National Center of Research, Ministry of High Education, Khartoum, Sudan. She was awarded a PhD in Bio-pesticides by the Sudan Academy of Sciences, achieved her MSc in plant protection at the University of Khartoum, and BSc (Honours) in Agricultural Sciences at the University of Gazera. She has held, organized and attended many training courses in different fields, and has supervised both undergraduate and postgraduate students. She has also participated in many projects dealing with organic agriculture, is a Member of several scientific societies, and has published papers in peer-reviewed journals. She is a Consultant for private companies in the field of plant pathology and nematodes. Dr Hanan Ibrahim Mudawi is the corresponding author and can be contacted at: hananmodawi@gmail.com.

Dr Mohamed Osman Idris is an Associate Professor in Plant Pathology, currently working at the College of Agriculture, Khartoum University, Khartoum, Sudan. He has been a Lecturer on plant pathology since 1975. He was awarded his PhD at Reading University, UK, an MSc at the University of Khartoum, and BSc (Honours) at the University of Khartoum. He is an internal and external examiner for postgraduate programmes at many universities in Khartoum. To date he has supervised studies for 55 MSc Degrees and 5 PhD Degrees. He has published 22 papers in national and international reputable peer-reviewed journals and conferences. His main research theme is fungal diseases and mycotoxins in Sudan agricultural products. He is the Chairman of the Mycotoxins Committee Sudanese Standards, and a Member of the Metrology Organization. He is currently a Consultant to many government and private organizations in Sudan.

Dr Hanaa Sedhum Zoam is a Researcher Professor Assistant and a plant pathologist and a nematologist. She is currently working at the Department of Nematological Research Diseases, Plant Pathology Research Institute (PPATHRI), Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Cairo, Egypt. She graduated from the Faculty of Agriculture of Cairo University in 1988, and obtained her MSc at Cairo University in 1994. Her PhD in Nematology was awarded by the Faculty of Agriculture at the El Zagazig University in 1999. She has published a number of papers in peer-reviewed journals. Her current research is in plant parasitic nematodes and their management. She can be contacted at zawamhanaa@yahoo.com.