Pakistan J. Zool., vol. 47(3), pp. 847-855, 2015.

Incidence of Plant-Parasitic Nematodes Associated with Okra in District Layyah of the Punjab, Pakistan

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Abstract. - A systemic survey was conducted to assess the reliable estimate of nematodes and their infestation level with okra plants from twelve grower's fields located within three major vegetable production localities of District Layyah. From each locality, four sampling sites were selected randomly and twenty samples of root and soil were taken. Nematode population in 20g of roots and 100cm^3 of soil samples was determined by the sieving-cum-modified Baermann funnel techniques. *Meloidogyne incognita* were the predominant species in all surveyed localities. Other plant parasitic nematode genera identified were *Pratylenchus*, *Aphelenchus*, *Criconema*, *Helicotylenchus*, *Hoplolaimus*, *Longidorus* and *Xiphinema*. The incidence of infestation of root knot nematode 86, 85 and 87% in the okra production areas of Layyah, Karor and Chobara, respectively. The incidence ranged from 70 to 95% with an average of 82.5%. The gall index ranged from 4 to 5 with a mean of 4.5 with maximum gall size. Both incidence and gall index varied from field to field and within the fields from each locality. This survey yielded the first report of *Meloidogyne* spp. infestation and other nematodes in vegetable production area of Layyah.

Key words: *Meloidogyne incognita*, plant parasitic nematodes, okra (*Hibiscus esculentus*), *Pratylenchus* spp.

INTRODUCTION

Okra (*Abelmoschus esculentus* Moench.) is one of the warm season crops that is grown in the tropical and sub-tropical regions of the world (Rashid *et al*., 2002). The crop is a leading Asian vegetable in Pakistan cultivated on 13919 ha. All parts of okra plant including fresh or immature okra fruits are consumed as vegetable and roots and stem are expended in cane juice clearing (Chauhan, 1972). Its leaves and stems are used in the manufacture of paper, coffee, fiber and ropes (Jideani and Adetula, 1993). The fruit mucilage is usually used to replace blood plasma (Benjamin *et al*., 1951), to reduce fluid friction in turbulent flow (Castro and Neuwirth, 1971), and to stabilize foams (Woolfe *et al*., 1977) as well as suspensions (Wahi *et al*., 1985). More interestingly, it has also been used for medicinal purposes as laxative and expectorant (Muresan and Popescu, 1993). Young leaves are cooked like 'spinach' by the Africans (Busson) may also act as a diuretic and have gastric ulcer and wound healing properties (Weniger and Robineau, 1988).

Seeds of okra having high quality of edible oil and high level of proteins are consumed to complement other protein sources (Bryant *et al*., 1988). Pods of okra comprise of mucilage, which holds a mixture of pectin and carbohydrates and is used as thickener in food industries (Woolfe *et al*., 1977; Nilufar *et al*., 1993). Okra flour is an efficient source of food additive in wheat flour for baking bread with excellent technological and sensory characteristics (Acquistucci and Francisci, 2002). In Pakistan, people usually fry the tender parts of okra and cook them in curries. It is considered as rich source of protein. The yield is 7.8 mt/ ha, which is less than Saudi Arabia (13.5) and India (11.6), major okra growing regions of the world (FAO, 2011). The reason for low yield are probably sandy

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0030-9923/2015/0003-0847 $ 8.00/0

soils and unawareness of growers to pest infestations particularly plant parasitic nematodes, the hidden enemies. As more intensive and continuous cultivation increased, soil-borne diseases and nematodes have become an important constraint in vegetable production (Anwar *et al*., 1992, 2007). Furthermore, farmers are continuously growing same vegetables in the same fields which enhanced infestation level in soil (Hussain *et al*., 2012). Nematodes are the most successful and abundant metazoans (Boucher and Lambshead, 1994) and they occupy a wide range of ecological niches. They are parasites of animals and plants (Blaxter and Bird, 1997) and cause serious diseases in both. The impact of nematodes on humans is evaluated through yield reductions in food and fiber crops, through feebleness of livestock, and by direct infection; nematodes such as hookworm and Ascaris. More than billion people are effected world-wide. Plant-parasitic nematodes are considered a single major unmanageable biotic cause of plant stress and crop loss (Bird and Kaloshian, 2003).

Many species of plant-parasitic nematodes including *Meloidogyne* spp. (*M. incognita* and *M. javanica)*, *Belonolaimus longicaudatus*, *Helicotylenchus* spp. (*H. dihystera* and *H. pseudorobustus*), *Hoplolaimus seinhorsti* and *Tylenchorhynchus indicus* are serious pests of okra (Rathour *et al*., 2006.). Nematode parasitism may result in secondary infection by soil-borne fungal and bacterial pathogens (Abawi and Chen, 1998; Sikora and Carter, 1987) or transmission of plant viruses (Brown *et al*., 1995), which can negatively influence yield (Orr and Robison, 1984). Yield reductions among vegetables have reached as high as 30% for susceptible genotypes in the presence of plant parasitic nematodes in some production regions in the Punjab (Anwar *et al*., 2007; Anwar and McKenry, 2012). Vegetable crops usually are among the most susceptible and worst affected by nematodes (Sharma *et al*., 2006; Anwar *et al*., 2007). Infection of roots by nematodes alter uptake of water and nutrients and interferes with the translocation of minerals and photosynthates (Anwar, 1995; Williamson and Hussey, 1996). Such alterations can change the shoot: root ratio (Anwar and Van Gundy, 1989) leading to poor plant growth.

Okra is known to be highly susceptible to root-knot nematodes and infected plants are stunted, exhibiting signs of nutrient deficiency and characteristic large swellings on both primary and secondary roots (Thies *et al*., 2004; Sikora and Fernandez, 2005). Although, recent reports in Pakistan have been published on association, distribution, and density of plant parasitic nematodes on various crops planted in field settings (Anwar and Akhtar, 1992; Anwar and McKenry, 2012; Hussain *et al.*, 2012; Anwar *et al*., 2013; Kamran *et al*., 2013). Our recent survey revealed that vegetable production area of Layyah is highly infested with plant parasitic nematodes particularly with *Meloidogyne* spp (Anwar *et al*., 2007). In some localities of District Layyah, nematode infestation was recorded low which was due to cropping pattern or fallowing of land employed by farmers. But in most of localities, farmers are unaware about this hidden pathogen and no management is being done by themselves. Most of farmers use pesticides to control this disease which is not reliable and fruitful to use. Usage of high amount of pesticides could be drastic for human and animals as they have residual effects on plants. Local yield losses were recorded as 35-40% during our survey. This promoted this study with the objectives to quantify and document the occurrence, distribution, density and prevalence of nematode populations found associated with okra, a major vegetable crop of Layyah region.

MATERIALS AND METHODS

Field sampling

During 2009-2010, a survey was conducted in 12 major okra growing regions of Layyah (Fig. 1). From each tehsil, four sampling sites were selected randomly from grower's fields. Twenty samples of root and soil were taken from each sampling site located at Bhagal, Lalazar, Chowk Azam, Hira Minor, Fetehpur, Kazmi chowk, Qaziabad, Rajan Shah, Nawan Kot, Kapoori, Shergarh, and Rafiqabad. A total of two hundred and forty root and soil samples were carefully collected with Oak field tube of 2.5-cm diameter. Samples were placed in labeled plastic bags, sealed, and brought back to the nematology laboratory where they were stored at 4°C until processed for nematode presence. The climatic conditions of two years (temperature, humidity, rain fall) from all localities were noted down. The survey was repeated in following year in same localities.

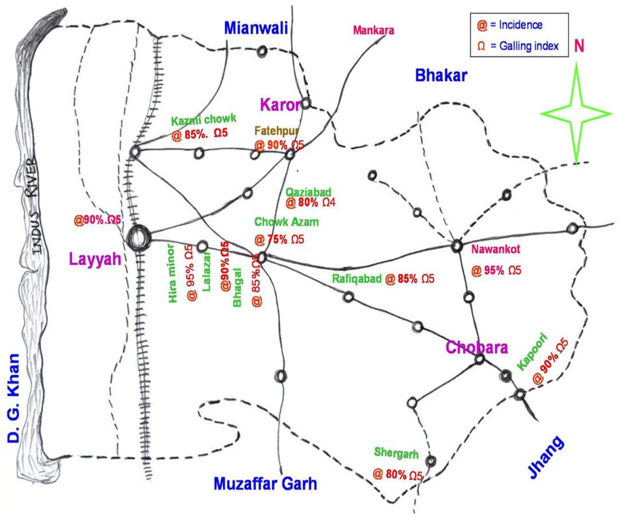

Fig.1. Map representing field infestation of *Meloidogyne* spp. on okra in sampling sites in three localities of Layyah.

Root sampling and extraction of nematodes

The roots were separated from the soil, washed, dried and weighed. The whole root systems of each individual plant were stained with Phloxin B (Holbrook *et al*., 1983) and assessed for the presence of egg masses. Severity of root knot nematodes was determined in terms of individual plants gall and egg mass indices on a 0 to 5 scale (Quesenberry *et al*., 1989), where 0 = no galls or egg masses, 1 = 1 or 2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 > 100 galls or egg masses per root system. The entire root system was diced, chopped and a 20 g composite root sample was processed for the extraction of nematodes by placing in a mist-chamber for 5-days to hatch the eggs (McKenry and Roberts, 1985). After 5-days the nematodes were harvested and identified under stereo-binocular microscope.

Soil sampling and extraction of nematodes

Each soil sample was thoroughly mixed by shaking the plastic bags then a 100-cm^3 sub-sample was extracted using a combined sieving and Baermann funnel method that allowed nematode extraction in mist over a three-day period (McKenry and Roberts, 1985). Collected nematodes population in Petri plates were identified and counted under stereo-binocular microscope.

Perennial patterns

In addition, mature females of root knot nematodes were dissected out from the infected roots and perennial patterns were prepared as described by Taylor and Netschler (1974). *Meloidogyne* species were identified on the basis of female perineal patterns, morphological characters of males and second stage juveniles (Eisenback, 1985; Jepson, 1987).

Collected nematodes were killed at 70˚C, and fixed in 4% formalin and placed in vials. Prior to counting, solutions containing nematodes were agitated thoroughly then 3-ml poured into a counting dish. Nematode populations were quantified under stereo microscope 60 × magnification. Counting of root-knot nematodes was based on second stage juveniles only. Identification of other species of plant parasitic nematodes was based on the morphology and measurements of adults (Handoo and Golden, 1989; Handoo, 2000; Choi, 2001).

The incidence of root knot nematodes of individual plant okra fields was assessed as follows (Hussain *et. al.,* 2012)

$$\text{Incidence (\%)} = \frac{\text{Total number of infected plants}}{\text{Total number of observed plants}} \times 100$$

The prevalence of root knot nematodes in okra fields of district Layyah was determined as follows (Hussain *et. al.,* 2012)

$$\text{Prevalence (\%)} = \frac{\text{Number of fields infested with root knot nematodes}}{\text{Total number of fields surveyed in district Layyah}} \times 100$$

Assessment of yield losses

The criteria used to assess yield losses comprised grower interviews, visual assessment based on foliage growth of okra standing crop in the fields (necrosis, chlorosis, stunting, wilting and pod

Table I.- **Population, roots response to infection, and incidence of *Meliodogyne* spp. at 12 okra field.**

Localities	Okra field / Sampling sites	Nematode population and root response[1]			Incidence %	Soil type
		Roots (20g)	Soil (100cm^3)	Gall Index[2]		
Layyah	Bhagal	345f	201e	5	85	Sandy loam
	Lalazar	234j	175f	5	90	Sandy loam
	Chowk Azam	350e	209d	5	75	Sandy loam
	Hira Minor	320h	234b	5	95	Sandy loam
Karor	Fetehpur	370c	178f	5	90	Sandy loam
	Kazmi chowk	401a	256a	5	85	Sandy loam
	Qaziabad	327g	123h	5	80	Sandy loam
	Rajan Shah	375b	202e	4	70	Sandy loam
Chobara	Nawan Kot	289i	133g	5	95	Sandy
	Kapoori	324gh	231b	5	90	Sandy
	Shergarh	356d	211d	4	80	Sandy
	Rafiqabad	346ef	224c	5	85	Sandy

[1]Means with in a column sharing the same letter are not significantly different from each other at P = 0.05 according to Duncan Multiple Range Test
[2]Gall indices: 0-5 scale; where 0 = no galls, 1 = 1-2 galls; 2 = 3-10 galls; 3 = 11-30 galls; 4 = 31-100 galls, and 5 = > 100 galls per root system (Quesenberry *et al*., 1989).

Table II.- **Occurrence frequency plant parasitic nematodes other than root-knot nematodes and their population in three localities.**

Nematode genera	Layyah		Karor		Chobara	
	Root (20 g)	Soil (100 cm)	Root (20 g)	Soil (100 cm)	Root (20 g)	Soil (100 cm)
Aphelenchus	-	-	-	-	0	5
Criconema	0	21	-	-	-	-
Helicotylenchus	0	12	0	6	-	-
Hoplolaimus	-	-	0	25	0	7
Longidorus	-	-	0	35	0	4
Pratylenchus	-	-	9	13	7	18
Xiphinema	0	19	-	-	0	11

-, Not detected

damaging), root symptoms and expert opinion from local Agriculture Department of District Layyah. The interviews of growers were based on soil condition, cultural practices, organic manipulation by animals, chemical treatment of soil and crops, fertilization and crop conditions during whole crop season, market based quantitative and qualitative yield losses, insect damage and density on crops and finally cultivars grown. These losses were expressed as percentage of yield losses.

Data analysis

Data on nematodes was subjected to analysis of variance using SAS (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

During this survey we observed variable level of infestation in region of District Layyah, Punjab Pakistan. Eight genera of plant-parasitic nematodes were commonly detected from twelve sampling sites of three localities associated with the okra growing region (Tables I, II). Magnitude of population, root response to infection, and incidence of *Meliodogyne* spp. in 12 okra fields and frequency of other seven plant parasitic nematode genera varied greatly among the sampling sites. This variation might be due to variation in soil types, cultivars used and other soil edaphic factors (Fig. 2, Table I) (Anwar

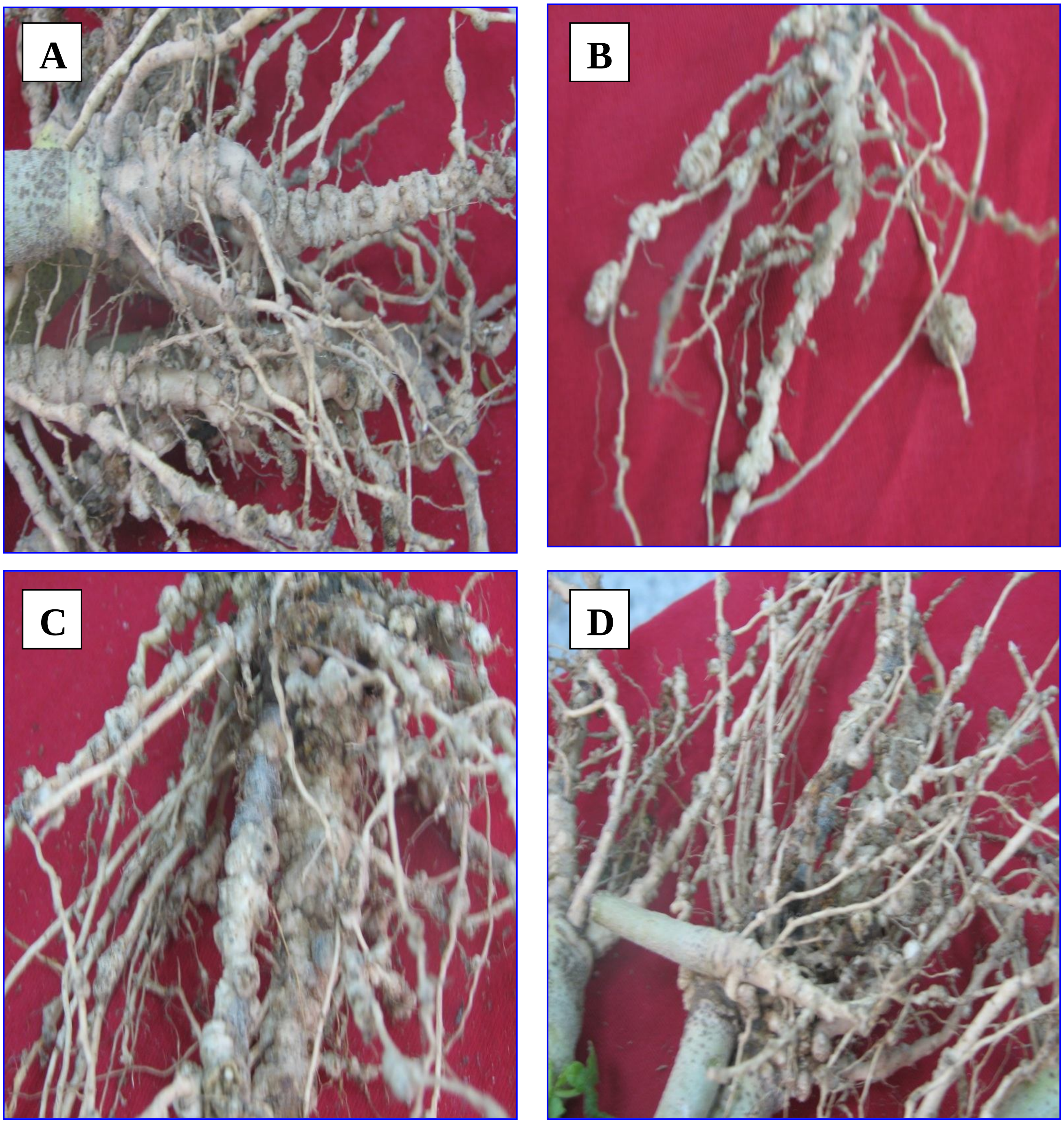

Fig. 2. Variation in root galling and gall size of four okra genotypes; **A**, Pusa swami; **B,** 019236, **C**. Sabzpari China red; **D**, Perbhani kranti, induced by *Meloidogyne* spp. detected during survey of Layyah.

and Din, 1987; Starr *et al.*, 1993).

Two species of root knot nematode, *M. arenaria* (Neal) Chitwood and *M. incognita* (Kofoid and White) Chitwood were common. These sedentary endoparasites of vascular tissues were recovered from all the sampling sites with a population frequency, gall and egg mass indices, and incidence that were highly variable. These nematodes induced unpredictable numbers of root galls and egg masses on roots (Fig. 2, Table I). Galled roots exhibited arrested root systems with few feeder roots. *Meloidogyne* spp., were the predominant species in all surveyed localities.

Second on the list were root lesion nematode, *Pratylenchus* spp. the migratory endoparasite and cortical feeder. Six ectoparasitic nematodes (*Aphelenchus, Criconema*, *Helicotylenchus*, *Hoplolaimus*, *Longidorus,* and *Xiphinema),* were also recovered from rhizosphere soil of okra fields though not at each of the field localities (Table II).

Most nematodes identified in this survey can be predicted to be a major menace to vegetable production and should be considered as serious pests (Anwar *et al*., 2007; Anwar and Mckenry, 2012). Initial pathogenicity tests should include, *M. incognita,* and *M. arenaria* which taken alone can be of serious economic importance in the tropics and sub tropics (Anwar *et al*., 2007; Davide, 1988). *Meloidogyne* spp. are common in vegetable soils world-wide where they parasitize vascular root tissues and induce their familiar root galls. Root knot nematode, *M. incognita*, is among the most common (Anwar and Mckenry, 2010; Abawi and Widmer, 2000, Davis *et al*., 2003; Sasser, 1979; Barker and Olthof, 1976). In addition to extensive root galling leading to arrested root systems and its presence is often been associated with increased incidence and severity of *Fusarium* wilts of several field crops (Anwar and Khan, 1973; Martin *et al*., 1994). The result is reduced yield of vegetable crops due to nematode feeding that can range up to more than 40% (Anwar and Mckenry, 2012), depending on soil texture and prevailing weather conditions (Starr *et al*., 1993).

Root lesion nematode, *Pratylencus* spp. is migratory endoparasites of roots feeding among cortical tissues. Their infections can result in necrotic brown lesions and tunneling within rootlets. This can interfere with water and nutrient movement within plant tissues as well as increased leakage of harvested, stored food due to lesions (Dorhout *et al*., 1991). *Pratylenchus penetrans* is known to enhance the severity of *Verticillium* wilt of vegetables (Vrain, 1987). Presence of these serious plant parasitic nematodes in abundance on okra produced within warmed fields should be taken seriously by growers. The association of these nematodes with vegetable crops has been reported to limit yields worldwide and there are now reports of yield losses from Punjab, Pakistan (Anwar and McKenry, 2012), India (Sehgal and Gaur, 1999), and USA (McSorley *et al*., 1987).

Other nematode genera identified during this study included *Aphelenchus, Criconema*, *Helicotylenchus*, *Hoplolaimus*, *Longidorus*, and *Xiphinema*. These are ectoparasites of epidermal root tissues and have not been documented as dangerous pests of vegetables. Their feeding leads to pruning of root hairs and damage to epidermal tissues, which reduce the ability of roots to absorb water and nutrients from soil leading to poor foliage growth (Endo, 1975). The occurrence of these ectoparasitic nematodes genera has frequently been found in commercially grown vegetable crops (Anwar and McKenry, 2010; Anwar and Akhtar, 1992; Barker *et al*., 1998). Species of *Xiphinema* in addition to the direct root damage caused by their feeding also are known to transmit viral diseases like tomato ring spot nepovirus (Tom RSV), tobacco ringspot nepovirus (Tob RSV) that infects tomato, tobacco, and soybean and has an economically important impact on cucurbits (Fulton, 1962; Imle and Samson, 1937; Brown *et al*., 1993, 1995). As virus vectors they can be damaging at very low population levels. Least damaging of the listed nematodes to vegetables is *A. avenae*, a nematode that derives its food from fungi and bacteria and is more associated with damage to mushroom culturing (Khanna and Kumar, 2005). A variation in occurrence frequency and density of each nematode species surveyed from these vegetable crops appears to be influenced by cropping pattern. It is reported that plant-parasitic nematodes in cultivated soil may be affected by the planting of cover crops, the use of alternate crop sequences, soil types and length of fallow (Brodie and Murphy, 1975; Brodie *et al*., 1970).

The interviews of growers were based on edaphic factors, climatic conditions and cultural practices from soil preparation to crop maturity. It was also noted that fields manipulated with animal manure were of less infested as compared to fields with no manure or Urea fertilizer. We hypothesized that animal dung has bacteria which actively produce antagonistic mechanism with nematodes or help fungus *Arthrobotrys oligospora* present in dung to produce three dimentional adhesive network to trap nematodes. (Wang *et al.,* 2014). In recent study it was noted that urea in cow dung send signal to fungus *Arthrobotrys oligospora* to produce trap for nematode. (Wang *et al*., 2014). Furthermore, temperature recorded during crop season was much higher (40-45°C) which facilitated *Meloidogyne incognita* to produce more population. Sandy soil with high pore size is also another factor for nematode penetration and movements through the soil.

The results of this study indicate that plant parasitic nematodes are widely distributed on okra crop cultivated in Layyah production region of the Punjab.

CONCLUSION

This information on nematode occurrence on vegetable crops will be helpful for growers for planning and administering nematode management strategies to reduce the nematode populations below their threshold levels. This study further suggests that magnitude of nematode problem needs serious consideration to tackle by the use of useful nematode management strategies.

ACKNOWLEDGMENT

We are grateful to higher education commission for providing us funds to conduct survey in District Layyah. We are also thankful to Czech University of Life Sciences, Prague for providing funds and facilities to conduct identification of nematode species in laboratory under project number PROJ201500056.

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(Received 18 September 2013, revised 7 January 2015)