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Full Length Research Paper

Distribution, frequency of occurrence and population density of root knot nematode in Hamelmalo – Eritrea

K. K. Chaudhary*, Daniel Brhane, Habtom Okube, Temesgen Zaid and Eyob Dagnew

Department of Plant Protection, Hamelmalo Agricultural College, P. O. Box 397, Hamemmalo, Keren, Eritrea.

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A survey was conducted during January to May 2011, to assess the occurrence and population density of root-knot nematode on different crops in 13 different localities of Hamelmalo subzone (Eritrea). A total 449 samples were collected from the study area and nematodes in their rhizosphere soil and root extracted by Cobb's decanting and sieving technique, Baermann funnel technique and root maceration methods. Out of the 449 collected samples, 344 samples were found to be infested with root knot nematode as soil population was visible. Awatle (97.8%), Shewwa (90%) and Engdaw (88.2%) are the provinces of the Hamelmalo that had very high population of root knot nematode. The least infection was reported from the Stur (52.3%) province. At individual crop level, the highest frequency of occurrence was reported from tomato (86.5%) and least was from rocket (50%) crop. Root knot nematode population in 100 cc soil (density) was reported as maximum (73.5%) for the chilli crop and minimum (12.24%) for the rocket vegetable. Measurement of disease incidence was based on the gall index and egg mass index, and this varied from 2 to 5 on 5 point scale. Highest disease incidence (5 GI and EMI) was reported from tomato, okra and potato crops, followed by the spinach, chilli and molukhyia crop, while the least disease incidence (2 GI and EMI) was reported from the rocket crop.

Key words: Root knot nematode, vegetable crops, frequency, density, disease incidence, Hamelmalo.

INTRODUCTION

Eritrea, situated in the horn of Africa (East Africa), has a total area of 124,320 square kilometers and a population of about 5.0 million. The country attained independence in 1991 after 30 years struggle, and so still country in infancy stage. Eritrea has six administrative provinces/regions (zobas); Anseba, Debub, Gash Barka, Maekel, North Red Sea and South Red Sea. Out of the six regions, Anseba region is situated in the northwestern part of Eritrea. The Zoba covers an area of 22,834 km^2, ranging in altitude from 450 to 2,000 m above sea level. The capital of this region is Keren. Zoba Anseba is divided into 11 sub-Zobas, and these sub-zobas are in turn made up of villages or kebabi. Sub-zone Hamelmalo is the 11th and recently created sub-zone of the Anseba region located in the southern part of the region. This sub-zone consists of 8 kebabis and 47 villages, with 452.2 Km^2 total surface areas. Hamelmalo is the modern centre of vegetable cultivation of Eritrea.

Agriculture is the backbone of Eritrean economy; it is the livelihood for the vast majority of the Eritrean people. About 70% of the population depends on agriculture and its allied fields such as crop production, livestock, forestry and traditional fishing (Rena, 2004). Although, the country economy is based on the agriculture, the agriculture of the country is still facing many challenges like very small agricultural area (only 3.6% of total land), draught, erratic rain fall, lack of irrigation, seasonal migration of farmers, pest, disease and weeds. According to the Ministry of Agriculture's Report for 2003, annually there is 25% yield loss because of insect pest, although sometime this loss can reach up to 70 to 80%. Information about the plant parasitic nematodes damage is not yet clear, but the presence of these nematodes

*Corresponding author. E-mail: kamal22055@yahoo.co.in. Tel: 002917348344. Fax: 00291-1-401589

are reported by the Ministry of Agriculture (Mo, 2003). According to Sasser and Freckman (1987) the average annual yield loss on a worldwide scale due to damage caused by plant parasitic nematode is 12.3%. In plant parasitic nematodes, root knot nematode, *Meloidogyne* species are more damaging one. This genus is considered the most important among plant-parasitic nematodes (Sasser and Freckman, 1987), mainly due to the wide host range known to exceed 3000 wild and cultivated plant species (Hussey and Janssen, 2002).

In Eritrea, information about the root knot nematode is limited, only Hadish (1997) reported the presence of this nematode. Moreover, the country's climatic conditions are suitable for the nematode activity and favor the development of nematode throughout the year. In addition, the inadequate investment in agricultural research, traditional method of farming, unavailability of suitable nematicides, continuous cultivation of susceptible crops in the same field and use of traditional seed or planting material are also some common situations that favor the rapid development of nematode population in the country. However, the information on the distribution and damage of field crops by root knot nematodes is meager in Eritrea, especially in the province (sub-zoba) of the Hamelmalo. Hence, a survey study was conducted to find out the geographical distribution, frequency of occurrence and disease incidence due to root knot nematode in the Hamelmalo sub-zoba.

MATERIALS AND METHODS

Nematode survey

Nematological surveys were carried out in Hamelmalo sub-zone of the Anseba region (Eritrea) from January 2011 to May 2011 (Figure 1). A total of 449 soil and root samples were collected from the rhizosphere region up to 15 cm from the base of the plants and at a depth of 15 cm in vegetable and field crops. Soil samples were collected using a standard (2.5 cm diameter and 30 cm depth) soil probe. The root and soil samples were collected from both the agricultural fields only. The sampling sites were located on the bank of Anseba River and this is the only source of irrigation for the cultivated crops. The altitude of the study site was 1286 m above sea level and geographical location is 15° 53' North latitude and 38° 66' East longitude. Environmental conditions during sampling time were clear sky and no rain, but annual rain fall was as reported 430 mm; temperature varies from 20 to 40°C; soil was irrigated and sandy loamy type. Mixed cropping was usually practiced, and on most of the location vegetable was grown under the traditional farming system.

Processing of soil and root sample

Separate samples were collected from both the poor growth area and an area of relative good growth; this was obvious for comparison and getting actual frequency and relative density of the nematode. In each sample, 500 cc soils was been collected. After mixing soil properly in the laboratory, it was divided in subsample and the nematodes from a composite subsample of 100 cc soil were extracted by means of modified Cobb's decanting and sieving technique (Flegg, 1967) and Baermann funnel technique (Hooper, 1990). For root population, root sample was processed by a modified maceration method (McSorley et al., 1999). Sieves used in nematode extraction were U. S. standard sieve series of 100, 200 and 325 meshes with openings of 149, 74 and 44 µ, respectively. Nematode suspension was collected in a 250 ml beaker for the process of counting and identification. Prior to counting, solution containing nematodes were agitated thoroughly and nematode populations were counted in 1 ml distilled water suspension in a counting dish and a mean of 3 counts was taken in each case.

Nematodes were counted at 400 X magnifications using a compound microscope. Counting of root-knot nematodes was based on second stage juveniles (J2) only. Isolated nematodes were killed at 70°C and fixed in 5% formaldehyde solution, processed to anhydrous glycerin (Seinhorst, 1959), and then examined under a compound microscope to identified the root knot nematode. Identification of the Genus *Meloidogyne* was based on the head shape and morphology of males and morphology of second stage juveniles according to Eisenback (1985). The mature females were also isolated from the galled roots and identified by the examination of perineal pattern. For assessment of second stage juvenile population in the infested root, the root were washed free of soil and chopped in 1 cm length by scissor. This chopped root were immersed in sufficient amount of water and macerated by home mixer and grinder for 1 to 2 min. Water was added to this suspension and 1 ml aliquot was taken by micropipette for nematode counting. The number of juveniles were determined from mean of three replicated.

In addition, for getting the information about the egg masses and gall numbers, the roots were immersed in an aqueous solution of phloxine B 15 mgL^{-1} (Southey, 1986) for 15 min to make the egg masses visible by giving red color.

Data analysis

The data were analyzed according to the prevalence of nematode population based on three factors; frequency, relative density and disease incidence. The frequency (F) of the root knot nematode genus was determined from the relationship between the numbers of samples in which the root knot nematode was observed divided by the total number of sample taken from that area or plants, multiplied by 100 to express as a percentage (Sawadogo et al., 2009). The relative density (D) of the root knot nematode was express as the population of RKN in fixed volume of soil, and the calculation was based on that of Norton (1978). Also, relative density of the genus root knot was determined by the total number of root knot nematode from particular area or plant divided by the total number of plant parasitic nematode, multiplied by 100 to express as percentage.

The determination of disease incidence was based on the nematode population per root system and it was expressed by the number of egg masses and gall index. The numbers of egg masses were counted and gall index (GI) and egg mass index (EMI) were determined on the following scale: 0 = 0; 1 = 1 – 2; 2 = 3 – 10; 3 = 11 – 30; 4 = 31 - 100 and 5 = greater than 100 galls or egg masses per root system (Taylor and Sasser, 1978).

RESULTS

Geographical distribution of root knot nematode in Hamelmalo sub-zone

The survey was conducted to assess the incidence of

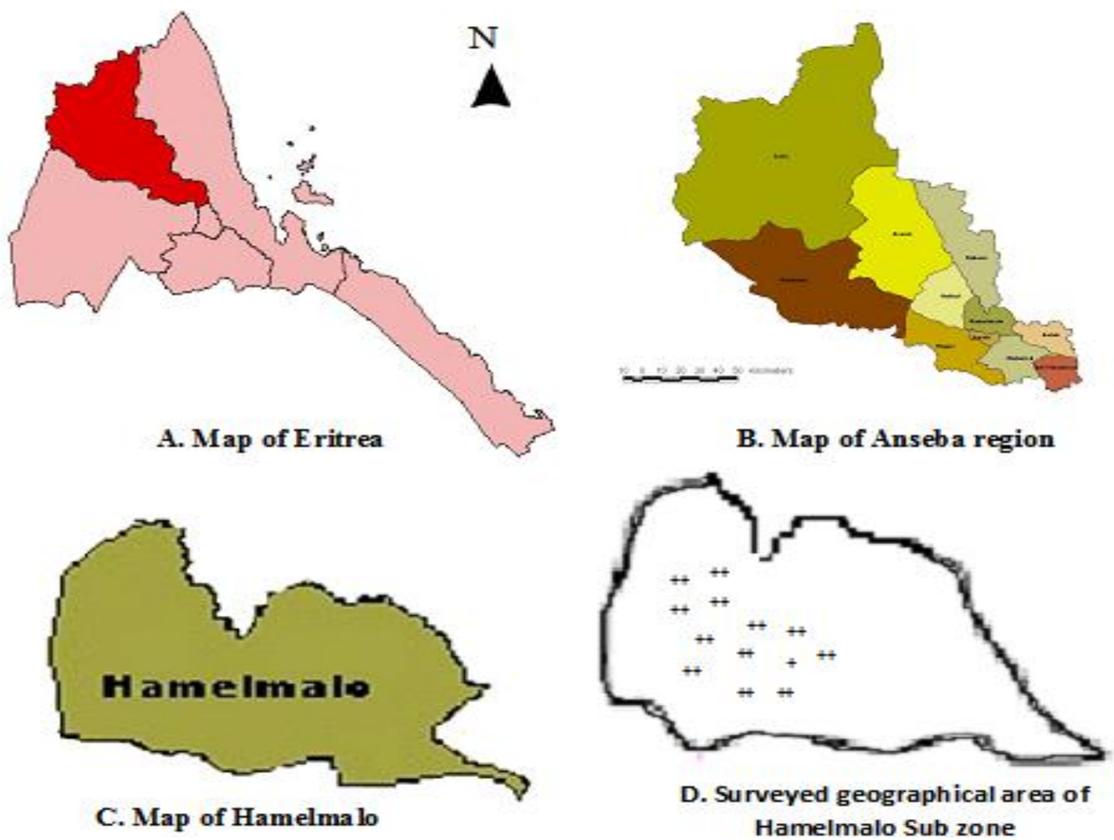

Figure 1. Geographical location of Anseba region, Hamelmalo sub-zone and the surveyed area.

Table 1. Number of root knot nematode present in 100 cc soil and root samples from the different localities of the Hamelmalo sub-zone.

S/N	Name of the area	Samples analyzed	Sample with RKN	Frequency (F %)**
1	Embadirho	30	22	73.33
2	Bietkelb	7	5	71.43
3	Engilae	58	46	79.31
4	Shewwa	20	18	90
5	HAC (mekelasi)	75	61	81.33
6	Stur	42	22	52.38
7	Gebenahmed	16	9	56.25
8	Awatle	94	92	97.87
9	Engdaw	17	15	88.24
10	Tsebab	16	10	62.5
11	Encodo	43	26	60.47
12	Genfelom	16	9	56.25
13	Nigsti	15	9	60

** Percentage of samples in which the genus was found.

root-knot disease on 9 different crops and 13 geographical area of the Hamelmalo sub-zone. The frequencies of the nematode genus in all the surveyed areas highly varied from one area to another (Table 1). Out of the 449 analyzed samples, 344 were found infected with root knot nematode in all the 13 areas, therefore the overall frequency occurrence of this genus was 76.61%. The frequency of occurrence was maximum

Table 2. Frequency of occurrence and Relative Density of root-knot nematodes on different crops of Hamelmalo subzone.

SN	Name of Crop	Total sample analyzed	Sample with RKN	Frequency (F %)**	Mean Density (D %)*	GI[1]/ EMI[2]
1	Tomato (*Lycopersicon esculentum*)	186	161	86.56	73.34±1.84	5/5
2	Spinach (*Spinacia oleracea*)	37	22	59.46	51.56±12.94	4/4
3	Chilli (*Capsicum annuum*)	41	33	80.49	73.57±1.88	4/4
4	Potato (*Solarium tuberosum*)	31	23	74.19	62.77±4.99	4/4
5	Okra (*Abelmoschus esculenta*)	41	34	82.93	61.80±5.25	5/5
6	Carrot (*Daucus carota*)	25	15	60	29.39±5.71	3/3
7	Molukhyia(*Corchorus olitorius*)	37	26	70.27	59.97±8.06	4/5
8	Cucumber (*Cucumis sativus*)	25	17	68	45.82±13.49	3/3
9	Rocket (*Eruca sativa*)	26	13	50	13.51±0.41	2/2

* Mean density ± standard error of nematodes per 100 cc soil.**Percentage of samples in which the genus was found. [1]Gall index as par 0 to 5 scale (Taylor and Sasser, 1978); [2]Egg mass Index as par 0 to 5 scale (Taylor and Sasser, 1978).

(97.87%) in Awatle and minimum (52.38%) in Stur geographical region. From Awatle area, a total of 94 samples were collected from the different crops of which 92 samples showed the presence of this genus. In frequency of occurrence, Awatle region was immediately followed by the Shewwa (90%) and Engdaw (88.24%). Stur (52.38), Gebenahmed (56.25) and Genfelom (56.25) are the geographical areas that showed less frequency of the occurrence for this genus. From Stur, out of 42 samples, 22 showed the presence of *Meloidogyne* genus.

Incidence of root knot nematode on different agricultural crops

The frequency and density of root knot nematode on all surveyed crops highly varied from area to area and within the area, from crop to crop. During survey, samples were collected from the 9 different crops in all 13 geographical locations, and all the crops showed the presence of the root knot nematode. Hence, overall incidence of the nematode genus was 100%. Table 2 shows the frequency and density of root knot nematode on different crops. Maximum frequency and density was reported from the tomato crop. For this crop, 186 samples were collected from the 13 region and 161 samples showed the presence of this nematode genus, hence the frequency of the occurrence was 86.55%. Okra (82.93%) and chilli (80.49%) crops were almost same with the tomato, while least frequency of occurrence was reported on the rocket (50%). For the rocket vegetable, only half of the observed samples showed the presence of the root knot nematode.

In case of density, almost similar types of trends were reported on all the crops in all the geographical areas. Population densities of the nematode ranging from 13 to 73% /100 cc soil were reported from the various host plants. Maximum relative density of the genus was reported from the chilli crop (73.57%) and this was immediately followed by the tomato crop (73.34%). The remaining crops like spinach, potato, okra, molukhiya and cucumber also had very good density of this genus, but it is at par to each other. Least density was reported from the carrot (29.39) and rocket vegetable (13.51%).

The susceptibility of the crops was determined by the multiplication of the nematode inside the plant roots. The expression of the multiplication was based on the Taylor and Sasser (1978) galls and egg mass indexes. These 0 to 5 scoring scales are most useful for the estimation of disease caused by the root-knot nematode. Both gall and egg mass indexes (average) ranged from 2 to 5. The highest egg mass and gall index (5 each) was reported on tomato and okra. This was followed by the chilli, potato, spinach and molukhiya; these crops showed 4 gall and egg mass indexes each (Table 2). The lowest galls and egg mass indexes was reported from rocket vegetable; for this crop both indexes are at 2.

DISCUSSION

Overall, 77% of the sampled had root knot populations and this was high enough to indicate a potential for damage and contributing the largest portion of risk. The survey results identified nine host plant associated with 13 geographical area of the Hamelmalo sub-zone. During survey, it was observed that plant growth was highly variable in most of the surveyed fields. The survey provides information on occurrence, frequency, density and disease incidence of root knot nematode associated with vegetable crops in Hamelmalo sub-zone. A study conducted under the project of IMP has shown worldwide pattern of distribution of root knot nematode genus. So these results were in accordance with the observations made in different parts of the world (Sasser, 1980).

Plant parasitic nematodes cause annual yield losses of about $ 100 billion worldwide, with 70% of the damage attributed to root-knot nematodes (*Meloidogyne* spp.) It infects a wide range of crops, particularly vegetable, and causes losses up to 80% in heavily infested field. According to the Feldmesser et al. (1971), vegetables are the most susceptible host for this genus. The study area is famous for the center of modern vegetable production in all over Eritrea (Rena, 2004), all the surveyed 13 locations are having maximum amount of vegetable crops only. The status of vegetable crops as a good host for *Meloidogyne* spp. has been previously reported (Feldmesser et al., 1971; Sasser and Freckman, 1987; Siddiqi, 2000; Sikora and Fernandez, 2005; Kaskavalci, 2007) and this was also supported by this survey.

Root knot nematode (*Meloidogyne* sp.) infestations on tomato are common in Africa and worldwide (Netscher and Sikora, 1990; Sikora and Fernandez, 2005), with an estimated yield losses ranging from 28 to 68% (Adesiyan et al., 1990). Maximum frequency, density and disease incidence from these crops are in conformity with the previous study related to this crop. Infection of root knot nematode on the spinach and molukhiya crops were reported during this survey and result are similar with the findings of Di vito et al. (2004), Anamika et al. (2010) and Olubunmi et al. (2011) on the infection on these crops. The members of the Brassicaceae family are used as an alternative control measure for root knot nematode, *Meloidogyne* sps. The biocidal efficacy of the rocket vegetable (*Eruca sativa*) have been well established against the plant parasitic nematode (Kaul, 1985; Curto et al., 2005). During the survey, minimum frequency, density and incidence of root knot nematode was reported on rocket vegetable, thus indicating the biocidal potential of this crop.

The results of this survey provide not only the information of root knot nematode associated with vegetable crops grown in Hamelmalo subzone but also indication of their frequency of occurrence, geographical distribution, and possible potential for crop damage and economic impact. Surveyed plant species were selected either because they were common to the area and of economic importance or because they showed some disease symptoms (poor growth, yellowing, etc.) It also provides a basis for estimating how widespread and severe this problem was. Moreover, the high density of the root knot nematode species and the frequent occurrence of this nematode population in this survey were expected because in the study areas, farmers are not using any control measure for reducing the population of this nematode. These observations suggest a lack of success in nematode management programs in Eritrea. Surveys are useful for determining the relative importance of various nematode problems on a regional basis, and yield loss estimates can be improved by a clear understanding of nematode distribution. In general, growers with high nematode densities tend to apply nematicides regularly, but the nematicides are apparently not adequately reducing the nematodes' densities. Nematicide treatments would therefore have a greater chance of success if applied as preventative measures rather than after crop mortality rates increase.

Further research is needed to determine the significance of this nematode on other crops and to determine its possible interaction with other soil pathogens and the environment. This survey provides important background information for planning and administering nematode management strategies in Eritrea.

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REFERENCES

Adesiyan SO, Caveness FE, Adeniji MO, Fawole B (1990). Nematode Pest of Tropical Crops. Heinemann Educational Books, Nigeria.

Anamika SS, Devi J (2010). Survey report of incidence and intensity of root knot nematode (*Meloidogyne incognita*) on Spinach (*Spinacea oleracea*) in U.P. and Bihar. (web: http://www.idosi.org/ejas/ejas2(2)10.htm assess on 23 April, 2011). Eurpean J. Appl. Sci., 2: 51-54.

Curto G, Dallavalle E, Lazzeri L (2005). Life cycle duration of *Meloidogyne incognita* and host status of Brassicaceae and Capparaceae selected for glucosinolate content. Nematology, 7: 203-212.

Di Vito M, Vovlas N, Castillo P (2004). Host–parasite relationships of *Meloidogyne incognita* on spinach. Plant Pathol., 53: 508-514. Doi: 10.1046/j.0032-0862.2004.01053.x

Eisenback JD (1985). Diagnostic characters useful in the identification of the four most common species of root-knot nematodes (*Meloidogyne* spp.). In: Sasser JN, Carter CC (eds) An Advanced Treatise on *Meloidogyne*. Vol. I, Biology and control. North Carolina State University Graphics, Raleigh, NC, USA, pp. 95-112.

Feldmesser J, Edwards DI, Epps JM, Heald CM, Jenkins WR, Jensen HJ, Lear B, McBeth CW, Nigh EL, Perry VG (1971). Estimated crop losses from plant-parasitic nematodes in the United States. Comm. Crop Losses. Special Publication No. 1. Society of Nematologists, Hyattsville, Maryland.

Flegg JJM (1967). Extraction of *Xiphinema* and *Longidorus* species from soil by a modified Cobb's decanting and sieving technique. Ann. Appl. Biol., 60: 439-437.

Hadish M (1997). A report on the status of plant nematology, Asmara, Eritrea

Hooper DJ (1990). Extraction and processing of plant and soil nematodes. In: Luc et al. (eds) Plant Parasitic Nematodes in Subtropical and Tropical Agriculture, CAB International, Wallingford, UK, pp. 45–68.

Hussey RS, Janssen GJW (2002). Root-knot nematodes: *Meloidogyne* species. In: Starr et al. (eds) Plant Resistance to Parasitic Nematodes. CAB International, Wallingford, UK, pp. 43-70.

Kaskavalci G (2007). Effect of soil solarization and organic amendment treatment for controlling *Meloidogyne incognita* in tomato cultivars in Western Anatolia. Turk. Agric. For., 31: 159-167.

Kaul V (1985). Studies on the plant parasitic nematodes of Kashmir. PhD thesis, University of Kashmir, Srinagar, India.

McSorley R, Frederick JJ, McGovern RJ (1999). Isolation of *Meloidogyne incognita* from caladium corms. Nematropica, 29: 245-248.

Mo A (2003). Report on the crop protection activities. In: Basic agricultural facts of Anseba Region, Keren. (web: http://www.eritreaembassy-japan.org/data/AgronomyinSpateIrrigatedAreasofEritrea.pdf assess on 23 April, 2011).

Netscher C, Sikora RA (1990). Nematode parasites of vegetables. In: Luc et al. (eds) Plant parasitic nematodes in subtropical and tropical agricultural, CAB international, Wallingford, UK, pp. 237-283.

Norton DC (1978). Ecology of plant parasitic nematodes. Wiley, New York.

Olubunmi A, Adenike FO (2011). *Lawsonia inermis* in the control of *Meloidogyne spp.* on *Corchorus olitorus.* EJEAFChe. 10: 2000-2006. (web: http://ejeache.uvigo.es/component/option,com_docman/task,cat_view/gid,117/temid,33 , assess on 23 April, 2011).

Rena R (2004). Emerging Eritrean Agriculture in Accordance with Global Competition: A Case Study on Elabered Estate. Osmania J. Social Sci., 4: 34-44.

Sasser JN (1980). Root-knot nematode: A global menace to crop production. Plant Dis., 64: 36-41.

Sasser JN, Freckman DW (1987). A world perspective on nematology: the role of the society. In: Veech JA, Dickson DW (eds) Vitas on Nematology. Society of Nematologists, Hyattsville, Maryland, pp. 7-14.

Sawadogo A, Thio B, Kiemde S, Drabo I, Dabire C, Ouedraogo J, Mullens TR, Ehlers JD, Roberts PA (2009). Distribution and Prevalence of Parasitic Nematodes of Cowpea (*Vigna unguiculata*) in Burkina Faso. J. Nematol., 41: 120-127.

Seinhorst JW (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica, 4: 67-69.

Siddiqi MR (2000). Tylenchida Parasites of Plants and Insects. Second Ed. CAB International, Wallingford, UK, p. 848.

Sikora RA, Fernandez E (2005). Nematode parasites of vegetables. In: Luc et al. (eds) Plant parasitic nematodes in subtropical and tropical agriculture. 2ed edition, CAB International, Wallingford, UK, pp. 319-392.

Southey JF (1986). Laboratory methods for work in plant and soil nematodes. Ministry of Agriculture, Fisheries and Food, London, pp. 202.

Taylor AL, Sasser JN (1978). Biology, Identification and control of root knot nematodes (*Meloidogyne spp*.). Coop. Pub. Deptt. Plant Pathol., North Carolina State University and United States Agency for International Development, Graphics, Raleigh, pp. 111.