

Disease incidence, identification, and monthly fluctuations in the population density of root-knot nematodes *Meloidogyne javanica* on cucumber plants in Semel District, Duhok, Kurdistan Region, Iraq

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Abstract. A survey was performed in four locations of Semel District – as follows: Sartenk, Qesir yazdin, Sharia, and Grshin (Bawerde) – by selecting 4 greenhouses planted with cucumber plants (*Cucumis sativus* L.) on each location, during both growing seasons (spring and autumn) of the year 2015. The results showed that the incidence of root-knot disease increased to its maximum level (37.48%) in the autumn season and then decreased to its minimum level (34.67%) in the spring season. According to the surveyed locations, the highest disease incidence (73.05%) was recorded in Sartenk location and the lowest (13.54%) in Sharia location. Results of the interaction between the locations and seasons revealed that the highest disease incidence (80.5%) appeared in Sartenk location during the autumn growing season followed by 65.60% in the same location during the spring growing season, whereas the lowest percentage was reported in Sharia during the spring season (3.47%). Depending on the perineal patterns for species identification of *Meloidogyne* spp. on cucumber plants, results showed the presence of *Meloidogyne javanica* in all surveyed locations. Generally, in one of the greenhouses of Sartenk location, the population density of *M. javanica* reached its maximum level (1,762 nematodes/200 gm soil) in September 2015, while the minimum level (337.5 nematodes/200 gm soil) was recorded in May the same year.

Keywords: *Meloidogyne javanica*, disease incidence, identification, population density, cucumber

1. Introduction

Cucumber (*Cucumis sativus* L.) is considered as one of the essential vegetables and one of the most common individuals of the family Cucurbitaceae [20], and it grows quickly, within a very short time from the date of planting as compared to other crops [39]. Cucumber is affected by nematodes, fungal, viral, bacterial, and six genera of plant-parasitic nematodes as follows: *Meloidogyne*, *Helicotylenchus*, *Pratylenchus*, *Scutellonema*, *Rotylenchulus*, and *Aphelenchoides* [15]. The *Meloidogyne* genus has the ability to affect 5,500 species of plants [5]. The *Meloidogyne* species are economically very important soil-borne pests that cause diseases to the root systems of many crops [33].

Generally, the symptoms of plants caused by root-knot nematodes can manifest above and under the ground. The symptoms above the ground may include spare growth, patchiness, chlorosis, stunting, defoliation, loss of yield, and wilting [34]. Underground symptoms consist of gall formation on roots, and the infection of plants tends to have few secondary roots. The number of egg masses and galls on the roots differs by the population density of nematodes in the soil, environmental factors, and host susceptibility [28]. At the end of 2012, nearly 100 nominal species of *Meloidogyne* were identified [25]. Thus, this study aims to determine root-knot disease incidences and carry out species identification of root-knot nematodes *Meloidogyne* on cucumber plants, while it also aims to calculate the monthly fluctuations in nematode population density during the growing season of cucumber plants in Semel District, Duhok, Kurdistan Region, Iraq.

2. Materials and methods

A. Survey of greenhouses planted with cucumber for the detection of root-knot disease

Survey was performed by selecting 4 greenhouses where cucumber was planted in 4 locations within Semel District as follows: Sertank, Grshin (Bawerdie), Sharia, and Qesiryazdin, during 2015 in both spring (May, June, and July) and autumn (August, September, and October). Roots and soil samples were taken using a systemic pattern (zigzag method), as described by Coyne et al. [11], where soil from the rhizosphere area and root samples of infected cucumber plants were collected at a depth of 20–30 cm from each greenhouse, taking into account that the samples represented an area of each greenhouse. Samples were placed separately in polyethylene bags, moist, with little amounts of distilled water, and for each sample the sampling date, planting date, and location name were recorded. The samples kept in cool boxes (insulated container) with pieces of ice and brought

to the laboratory where preserved at 4 °C for a period not exceeding 3 days. Each house was considered as a single unit and sampling sites deposited on alternations of planting lines to assess disease incidence depending on the presence of galls on the examined roots and according to the following equation used by Kayani et al. [18]:

$$\% \text{ of disease incidence} = \frac{\text{No of infected plants}}{\text{Total no. of examined plants}} \times 100$$

This experiment consists of 8 treatments (4 locations \times 2 lugs) with 4 replications and was carried out as factorial experiment in Completely Randomized Design (CRD). Data were analysed using SAS program, and the means of disease incidence in the surveyed locations were compared based on Duncan's multiple range test, $p \leq 0.05$ [32].

B. Identification of root-knot nematodes Meloidogyne spp. by perineal pattern

Perineal patterns of *Meloidogyne* species were prepared according to a method mentioned by Hussain et al. [14], through which perineal patterns of 10 females were prepared for each infected cucumber in the greenhouses. The perineal patterns were compared with standard diagrams, and then *Meloidogyne* species was identified.

C. Monthly fluctuations in the population density of root-knot nematode M. javanica in a greenhouse located in Sertank village, planted with cucumber plants

One of the cultivated greenhouses in the Sertank village location was selected to study the fluctuation of nematode population density during 2015 for spring lug (May, June, and July) and autumn lug (August, September, and October). Soil samples were gathered as mentioned previously, placed on a piece of polyethylene in laboratory, and mixed well by hand for homogeneity. Four random samples (200 gm/sample) were taken for nematode extraction using tray method as used by Kago et al. [16]. The population density of *M. javanica* was calculated using a method described by Van Benzooijen [36], with some modifications. The number of J2s (second-stage juveniles) was counted in the suspension of each sample, which was determined to a particular volume in each beaker, the nematode suspension was carefully mixed by shaking it with a sterilized glass rod for homogeneity, and 1 ml of suspension was directly transferred to a counting dish with the help of a sterilized glass pipette. Nematodes were counted under stereomicroscope with 5 replications for each sample.

This experiment consists of 6 treatments (6 months) with 4 replications performed in CRD. Data were analysed as mentioned previously to compare the means of nematode population density/month.

D. Soil analysis for some physical and chemical properties

- i. Soil texture:** Soil texture was determined by the hydrometer method according to Bowles [8] to estimate soil contents of clay, silt, and sand.
- ii. Soil pH:** It was determined by pH-meter model Hans Herbert Mennerich (geotechnical), Hanover, as described by Van Reeuwijk [37].
- iii. Electrical conductivity (E.C.):** The electrical conductivity was estimated by using E.C.-meter, model D8120, and adjusted to 25 °C, as stated by Van Reeuwijk [37].
- iv. Organic matter:** Oxidizable organic matter was determined by the Walkley and Black, wet dichromate oxidation procedure, as mentioned by Nelson and Sommers [23].
- v. Total Nitrogen:** Total nitrogen in soil was estimated according to the Kjeldhal method, as described by Rowell [31], using concentrated H₂SO₄ and H₂O₂ with heating for soil digestion.

3. Results and discussion

3.1 Survey of greenhouses planted by cucumber plants in Semel District to detect the presence of root-knot disease

The symptoms associated with root-knot nematode were clear in four surveyed locations, where stunting, wilting, chlorosis, loss of yield, death of plant, patchiness, and formation of galls on roots were detected (*Fig. 1*).



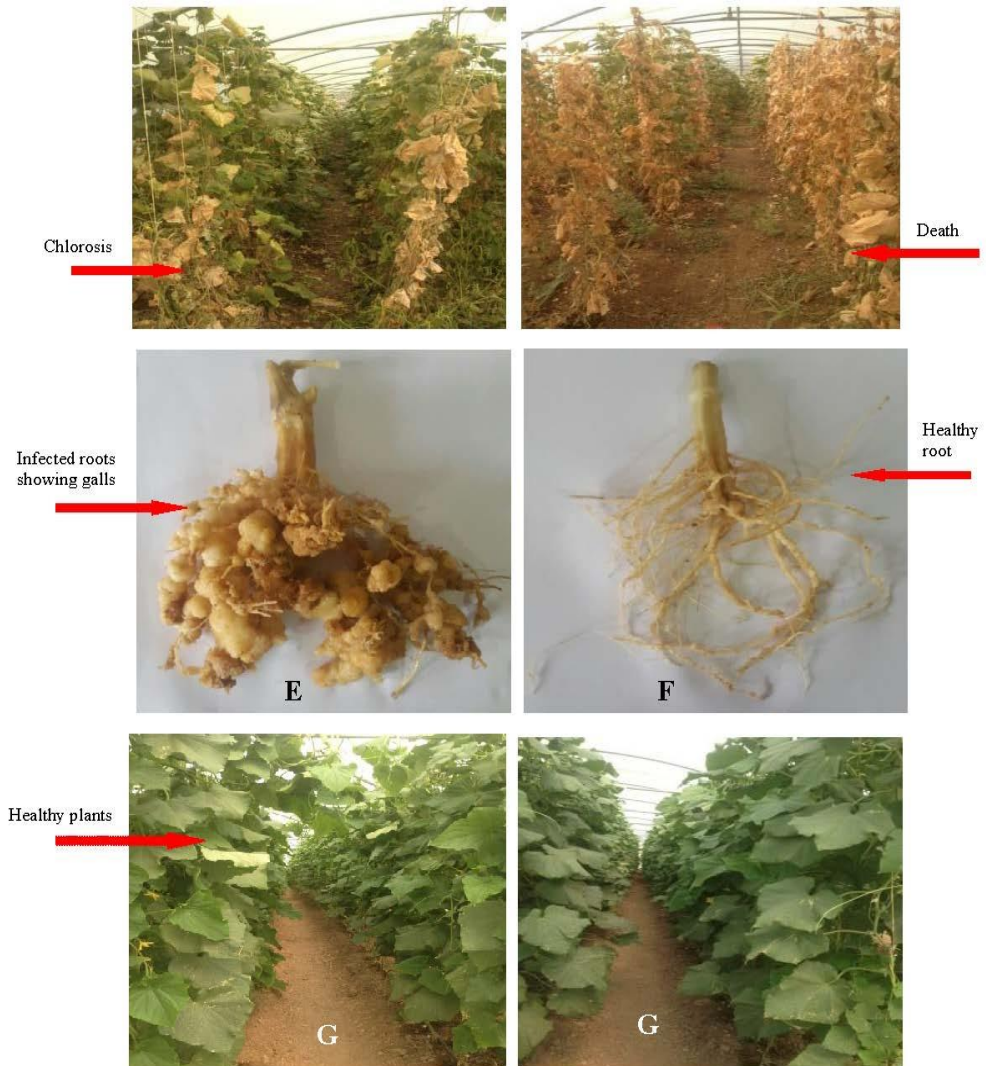


Figure 1. Patchiness (A), stunting (B), chlorosis (C), death (D), and root galling (E) as symptoms of root-knot nematode *M. javanica* compared to healthy plants (F and G) in the surveyed locations

3.2 Disease incidence of root-knot disease on cucumber plants caused by *M. javanica*

Results in Fig. 2 showed that the highest disease incidence (37.48%) was recorded in the autumn season, while the lowest one (34.67%) in the spring season though the difference was not significant, as revealed by the results of the statistical analysis. Regarding the surveyed locations, disease incidence reached its maximum level (73.05%) in Sartenk with a significant difference from the disease incidence rates in the other locations, and then it decreased to its minimum level (13.54%) in Sharia location, which did not differ significantly from the disease incidence rate in Qesir yazdin (Fig. 3). On the other hand, the interaction between seasons and locations (Fig. 4) was significant in its effect on disease incidence, and in general the highest disease incidence rate (80.5%) was reported in Sartenk location, in the autumn season, which did not differ significantly from the disease incidence in the same location in the spring season; however, the lowest disease incidence (3.47%) was found in Sharia location in the spring season, without significant differences from the disease incidence of Qesir yazdin in both seasons and from that of Grshin in the spring season.

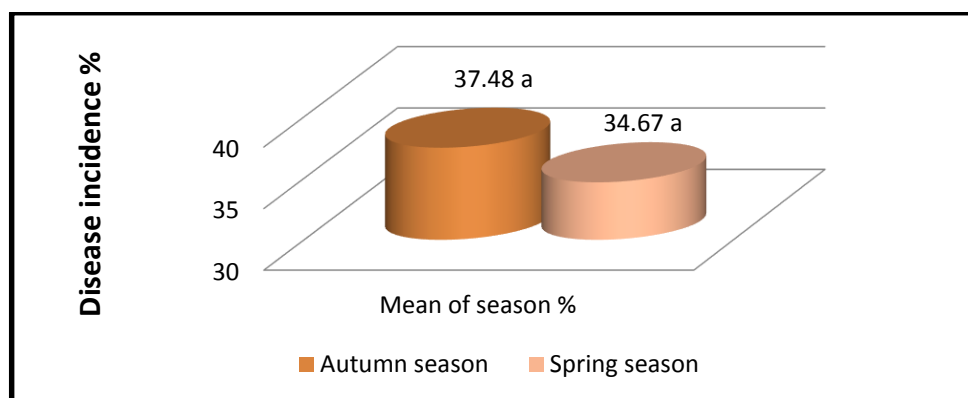


Figure 2. Disease incidence of root-knot in both growing seasons: spring and autumn. Means do not differ significantly based on Duncan's Multiple Range test ($P \leq 0.05$). Each number is a mean of 16 values (4 locations \times 4 replications).

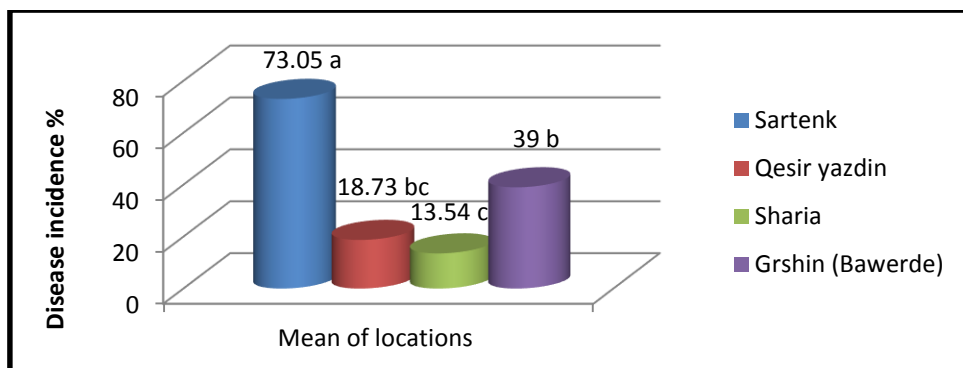


Figure 3. Disease incidence as affected by different locations.
Means followed by different letter(s) differ significantly based on Duncan's Multiple Range test ($P \leq 0.05$).
Each number is a mean of 8 values (2 seasons \times 4 replications).

The physical and chemical properties of the surveyed soil showed that the soil of most tested greenhouses was sandy clay loam, the maximum percentage of sand (73.92%) was found in Sartenk and the minimum percentage (35.72%) in Qesir yazdin. The maximum percentage of organic matter (5.1%) was recorded in the soil of Qesir yazdin and the minimum (1.67%) in the soil of Sartenk.

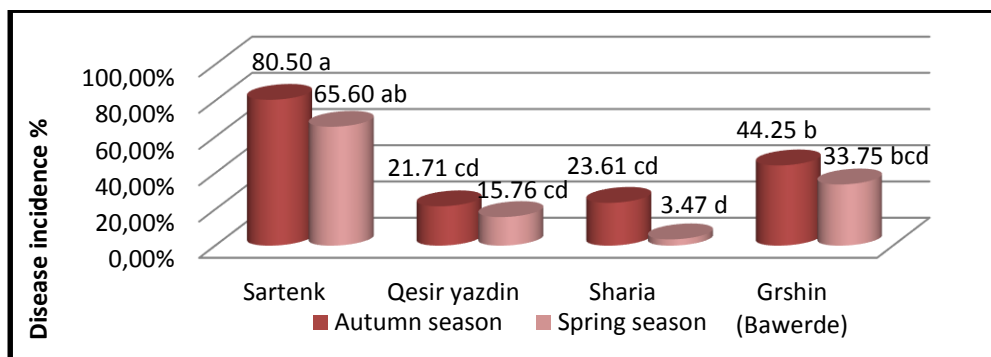


Figure 4. Disease incidence during both seasons (spring and autumn) in 2015 in the surveyed locations.
Means followed by different letter(s) differ significantly based on Duncan's Multiple Range test ($P \leq 0.05$).
Each value is a mean of 4 replications.

Results of pH were almost similar in all locations, ranging from 7.2 to 8. The E.C. increased in the soil of Sharia (6.31) and then declined in the soil of the other locations, the minimum (0.61) being reported in the soil of Sartenk. The C/N ratio was found to be high (14.7) in Grshin followed by Qesir yazdin (13.2), and a low value (3.2) was recorded in the Sartenk soil (*Table 1*).

The increase in the disease incidence of root-knot nematode is due to a number of reasons such as appropriate temperature for nematode activity, including egg hatching and juvenile development, which is consistent with Madulu and Trudgill [21], Trudgill [35], Park et al. [27], and Ismail et al. [15], as well as planting of susceptible cucumber varieties during the growing season, as found by Agrios [2], Chaudhary et al. [10], and Kayani et al. [18]. In addition to that, the soil may be planted with the same suitable plant hosts during the period preceding the autumn season and the remaining of infected plant debris roots in the soil.

Table 1. Physical and chemical analysis of the soil in the surveyed locations

Locations	Green houses	Clay %	Silt %	Sand %	Soil texture	Organic matter %	pH	E.c. dSm ⁻¹	C/N ratio
Grshin	1	29.77	15.44	54.79	Sandy clay loam	3.41	7.26	6.26	5.5
	2	25.01	19.88	55.11	Sandy clay loam	2.52	7.3	5.35	9.1
	3	28.27	23.64	48.09	Sandy clay loam	2.36	7.63	5.25	6.1
	4	22.03	27.16	50.81	Sandy clay loam	2.65	7.76	1.46	14.7
Qesir yazdin	1	15.98	28.08	55.99	Sandy loam	5.10	7.35	5.60	13.2
	2	22.56	36.19	41.25	Loam	1.74	7.52	3.41	6.01
	3	25.35	38.49	35.72	Loam	2.86	7.14	5.10	7.4
	4	20.15	17.60	62.28	Sandy clay loam	3.13	7.45	4.82	6.5
Sharia	1	22.08	31.90	40.02	Loam	3.21	7.35	6.31	7.07
	2	26.08	16.23	57.70	Sandy clay loam	4.47	7.65	4.9	13.03
	3	25.43	28.51	46.07	Sandy clay loam	3.55	7.46	5.12	8.1
	4	26.21	35.26	38.52	Loam	4.26	7.41	5.35	6.8
Sertank	1	12.55	13.53	73.92	Sandy loam	1.67	7.43	1.43	3.4
	2	27.54	22.86	49.60	Clay loam	1.88	7.70	0.62	3.2
	3	16.77	31.51	51.72	Loam	3.48	7.63	0.61	7.2
	4	18.91	29.17	51.92	Loam	3.07	8.00	1.47	5.2

E.c. = electric conductivity, dSm⁻¹ = decimals, C= carbon, N = nitrogen

However, the decrease of disease incidence in the spring season largely attributed to low temperature in the winter in addition to the absence of the host, which led to the death of numerous nematode eggs and J2s, which was confirmed by Ami [6] and Ami and Al-Sabie [7]. The variability of disease incidence among surveyed locations can be traced back to some chemical and physical properties of the soil. Thus, the highest disease incidence appeared in the Sartenk greenhouse, where the soil contains a high percentage of sand and a low percentage of clay particles, where the pores of such soil are suitable for nematode movement [13], [19] toward the roots of its hosts [12]; moreover, the low value of electrical conductivity (E.C.) in the soil of Sartenk compared to the soil of other locations played a role in nematode activity [17]. Therefore, the nematode population increased in sandy soil compared to clay soil [38], [30], while the presence of clay in low percentage in the soil also attracts nematode juveniles from long distances towards the roots of plants through adsorption of root exudates on their surface. This view is consistent with what has been reported by Prot and Van Gundy [29], which means that soil texture has influence on the movement of nematodes toward the plant roots and then on their reproduction [22], [14].

Furthermore, increasing organic matter percentage in the soil reduced nematode population [24], whereas in this study the absence of the effect of organic matter in showing differences in disease incidence between locations may be due to the convergence of its value in the majority of soils in the study locations. Also, the impact of pH on disease incidence is almost identical in the soil of all locations due to the convergence of its values, where the pH of soil was within the appropriate range for nematode survival and reproduction [26]. The ratio of C/N was less than 20 (have enough nitrogen), which caused a decrease in the number of nematodes as reported by Agbenin [1] and Agu [3]. Therefore, in our study, the role of C/N ratio may be the same because its value was less than 20 in all studied locations. The decrease in the disease incidence in Sharia and Qesir yazdin may also be due to the application of some methods for nematode control such as the use of nematicides, animal manure, planting of garlic among cucumber plants, and fallowing.

3.3 Identification of root-knot nematodes by perineal pattern

Results of recognition of root-knot nematodes depending on the morphological characters of perineal patterns of mature females extracted from infected roots of cucumber plants in 4 surveyed locations, including Sartenk, Qesir yazdin, Sharia, and Grshin (Bawerde), during both seasons in 2015 revealed that the extracted nematodes belong to *M. javanica* (Fig. 5) in all surveyed locations, and according to certain characters emphasized that the perineal patterns were for root-knot nematode (*M. javanica*). This result is consistent with

those of many nematologist researchers (4, 9, and 17) who found the same species in the infected cucumber roots.

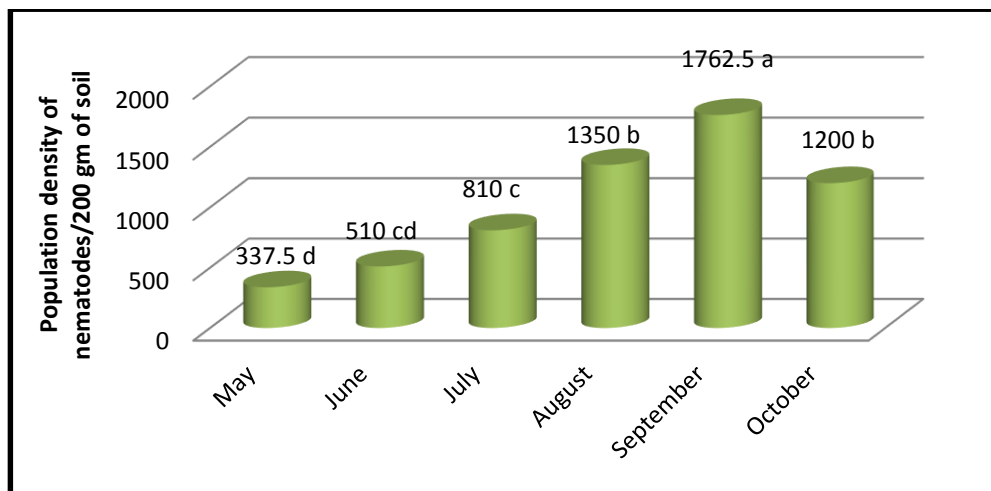


Figure 5. Monthly fluctuation of population density of *M. javanica* in one of the greenhouses in Sartenk location during 2015.

Means with different letters differ significantly based on Duncan's Multiple Range test ($P \leq 0.05$).

Each value is a mean of 4 replications.

4. Monthly fluctuations of nematode population density of root-knot nematodes in the greenhouse of Sartenk village in Semel District

The results revealed that the period of soil sampling has a significant effect on nematode population density in the soil, where it reached the highest level (1,762.5 nematodes/200 gm of soil) in September 2015 with significant differences as compared to the other months, followed by August (1,350 nematodes/200 gm of soil), whereas the lowest level of nematode population density (337.5 nematodes/200 gm of soil) was found in May during the same year (Fig. 6). The variation in nematode population density in the soil of Sartenk location during the study months was due to temperature values having a direct impact on nematode activity and reproduction and on nematode population density in the soil.

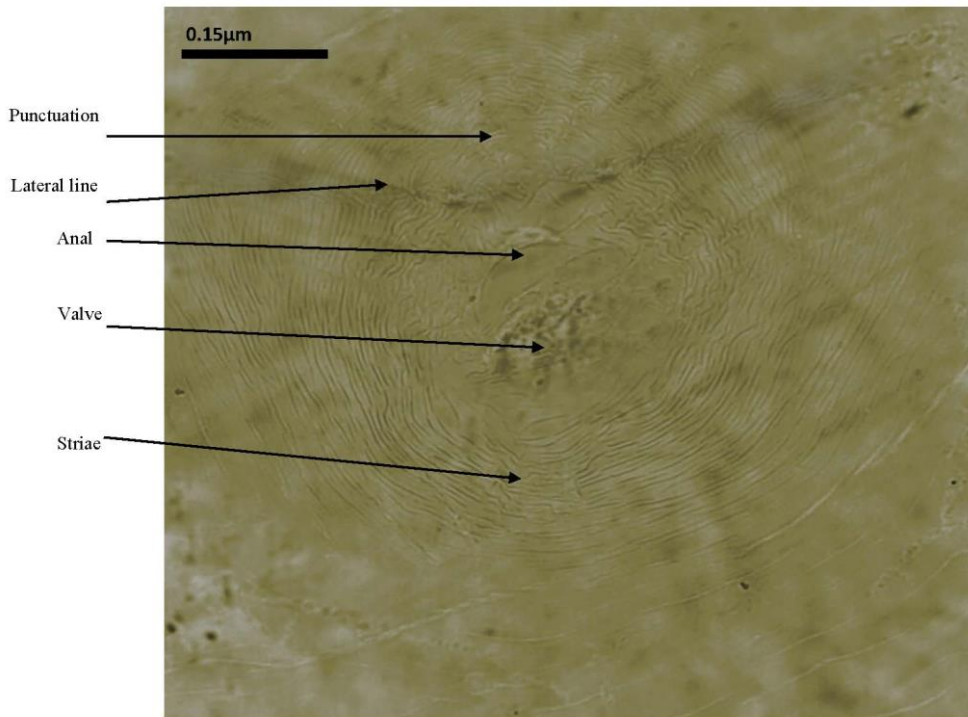


Figure 6. General morphology of perineal pattern of mature adult females of *M. javanica*

Thus, the increasing nematode numbers in September can be traced back to the suitability of soil temperature for nematode activity and egg hatching as well as the existence of susceptible hosts for root-knot nematodes, which caused an increase in nematode population density in the soil from summer until September. This is consistent with what have been recorded by Hussain et al. [14] and Ismail et al. [15]. The decrease in nematode population density in October was due to the death of infected cucumber plants as a result of severe damage of the root system at the end of the growing season in addition to the gradual decrease of soil temperature. This observation was supported by Ami and Al-Sabie [7]. On the other hand, the increase of nematode population density after May was attributed to the further development and reproduction of root-knot nematodes and the production of multiple generations during the growing season as a result of favourable soil temperatures for this nematode species, which is likely to have adapted to the soil temperatures for the greenhouse during the summer period besides the suitability of soil texture and the existence of susceptible hosts

(cucumber plants), which also indicates that the root system of cucumber plants did not deteriorate during this period of nematode parasitism.

4. Conclusions

1. Survey results showed the existence of root-knot disease in all greenhouses of summer plants by cucumber and the highest disease incidence was observed in the autumn growing season while the lowest in spring growing season.

2. Results of root-knot nematode associated with the identification of cucumber plants either by perineal pattern or molecular identification revealed that the widespread species of nematode was *M. javanica*.

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