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OBSERVATIONS ON THE LIFE CYCLE OF *HETERODERACRUCIFERAE* ON ITS MAIN HOSTS UNDER THREE DIFFERENT CONDITIONS IN IRAN

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ABSTRACT

The cabbage cyst nematode, *Heterodera cruciferae* has been reported as one of the pests infecting kohlrabi (*Brassica oleracea* L. var. *gongylodes*) and white cabbage (*Brassica oleracea* L. var. *captita alba*) in Tabriz vegetable farmlands. In this project, its life cycle on these two hosts in the region was studied. Two cultivars of cabbage commonly grown in the region were cultivated under three different conditions: in naturally infested field, in greenhouse, and in micro plots. During the growing season, the nematode life cycle on the crops was monitored. Samples were collected periodically from host plant roots and soil. The nematode completed two generations and initiated the third on the cabbage. These findings can be used to determine the critical harvesting date of the different cabbage crops to avoid nematode reproduction.
Keywords: *Hererodera cruciferae*, life cycle, greenhouse, microplot, naturally infected fields.

INTRODUCTION

Compared to other members of the genus *Heterodera*, the cabbage cyst nematode appears less and insufficient. Much information is still lacking on its biology, population dynamics, crop losses, host range and nematode population structure. Limited economic importance of the nematode as a minor plant pathogen may have contributed to this aspect. Lewis (1971) studied details of the nematode life cycle under field conditions, and indicated that in South Wales two complete generations of brassica cyst eelworm occurred between April and early September and the third generation development proceeds as far as the formation of the egg sac between September and November. In other reports, the number of the nematode generations in a season was found dependent on the nematode hosts growing period and up to three complete generations were possible in Europe (Stone and Rowe, 1976; Subbotinet al., 2010). *Heterodera cruciferae* has three generations per year on late cultivars of cabbage under the conditions of the central regions of Russia (Chizhovet al., 2009). The nematode has been reported from different countries including Armenia, Azarbaijan, Iran, Pakistan and Turkey (Subbotinet al., 2010), but there is no comprehensive and detailed information on the nematode life cycle. In Iran, *H. cruciferae* has already been reported from its main hosts namely kohlrabi (*Brassica oleracea* L. var. *gongylodes*) and white cabbage (*Brassica oleracea* L.var.*captita alba*) (Jabbari and Niknam, 2008). The nematode density in naturally infested fields of the region was found to be 83-280 cysts per 100 gram of soil and 151 cyst per gram of infected host roots (Niknamet al., 2004). Despite such relatively high population densities of the nematode in soil and host plants, the farmers in the region grow the nematode main hosts in infested fields every year or with a short time crop rotation. There is no systematic evaluation on yield loss due to the nematode infection. In addition to the cabbage cultivars, there are other plants such as coriander (*Coriandrum sativum* L., Umbelliferae), sonchus (*Sonchus asper* (L.) Hill., Asteraceae), *Sisymbrium loeselii* L. (Brassicaceae), chenopodium (*Chenopodium album*), Radish (*Raphanus sativus*) and *Lepidium sativum* that are naturally infected with the cabbage cyst nematode in the region.(According to our information, there is no previous documentation on the Coriander, Sonchus and *Sisymbrium loeselii* infection by the nematode and we consider the plants as new hosts for the nematode.

Despite observed yield loss, the establishment of high population densities of the nematode and growing of its main hosts in the region, no regional investigation on the nematode life cycle is available. So, the nematode life cycle on two commonly grown cabbage cultivars (*B. oleracea* L. var. *gongylodes* and *B. oleracea* L.var.*captita alba* both of them with two

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different growing periods, six and three months) was monitored under three different growing conditions in this study.

MATERIALS AND METHODS

1. Nematode

Samples of the cabbage cyst nematode, *Heterodera cruciferae* were collected from naturally infested farms with cabbage cultivation for a long time. Collected nematodes were identified using morphological and molecular means (unpublished data).

2. Hosts and growing conditions

In a naturally infested area in the suburbs of Tabriz city, East Azarbaijan province, northwest Iran crucifers are cultivated in varying periods (Table 1). At the end of March, seeds were direct sown for a growing season to November. A similar period was followed in the greenhouse and micro plots.

2.1. Field*:* In naturally infested fields at Hokmabad, Tabriz vegetables growing area, (38° N 06´ 11.07" and 46° E 16´ 00 32"), East Azarbaijan province, Iran, three out of four cabbage cultivars, K6, K3 and C6 were cultivated. Cultivation of the crops followed the traditional agricultural practices. After planting in late March, samplings commenced on 17 April, and continued until 6 October in seven day intervals. At each sampling, two plants were collected, wrapped in a paper bags, and transferred to the laboratory. The roots of all plants were stained following the lactoglycerin-acid fuchsin staining method (Southey, 1985). Under a stereomicroscope, the samples were checked for penetration by second-stage juveniles (J2) and establishment of other life cycle stages. Every other day, soil temperature was recorded. Soil samples were collected every three weeks with an auger of 7×30 cm. Cysts and J2 were extracted from soil using a modified combined sieving and centrifugation flotation (Jenkins, 1964) and Fenwick (1940) methods.

2.2. Micro plots: The microplots were placed out of field in (38° N 04´ 48.30" and 46° E 14´ 17 72" geographical coordinates) under the same condition of what in fields. Each microplot was a plastic pot with 15×50×35 cm dimensions which were filled with naturally infested field soil. All the cabbage cultivars were sown in separate pots on 7 April, 2012 and sampling of the plants started on 17 April, 2012 with seven days intervals. In each sampling, two plants were collected, put in separate paper bags and transferred to the Lab. Root staining was done following the methods as mentioned above. Soil temperature was also recorded using thermometer, daily. Microplots were kept in the region till January, 2013.

2.3. Greenhouse: A section of greenhouse at the Department of Plant Protection, University of Tabriz (38° N 03´ 46.13" and 46° E 19´ 48 95" geographical coordinates) was allocated for this purpose. In this part of experiment, sterilized soil was used consisting of field soil and sand with 3:1 ratio. Plastic pots having 15×15 cm dimensions were filled with 1 Kg sterilized soil. All the cabbage cultivars were separately sown in the pots. Cysts which were sterilized in 0.1% NaClO for 10-15 seconds, used for inoculation of the plants. Four to six cysts were placed in vicinity of each cultivars of cabbage seeds in sowing time in the pots on 9 April, 2012. During the experiment time, the temperature of the section was set on 25±2°Cand 8:16 hour dark and light photoperiod and with 60±10% relative humidity. Sampling of plants began on 17 April, 2012 and continued until end of October, 2012.

Irrigation and fertilizers application were carried out in consistent with the local farmers agronomic operational schedule.

3. Calculation of Degree-days

In this study following formula was used to calculate the nematode degree-days requirements per generation under field conditions (Caicedo et al, 2012).

$$\Sigma \left[\frac{\text{Daily maximum temperature - Daily minimum temperature}}{2} \right] - 4$$

Since *H. cruciferae* infective juveniles did not invade roots of the host plant when soil temperature is below 4°C (Lewis, 1971), this temperature considered the least temperature for nematode activity. For this purpose, soil temperature was recorded in field conditions.

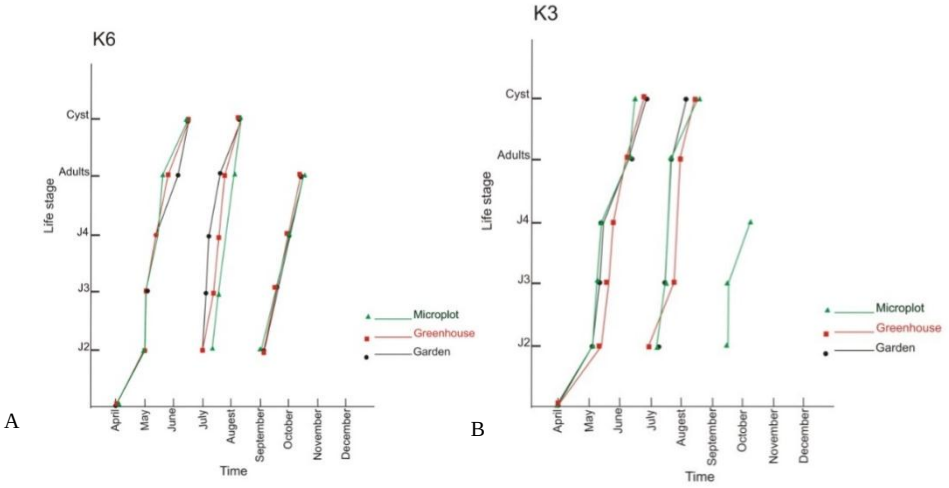

Figure 1. Life cycles of *Heterodera cruciferae* on *Brassica oleracea* L. var. *gongylodes* cultivars (A: K6 and B: K3) in field, greenhouse and micro plot conditions.

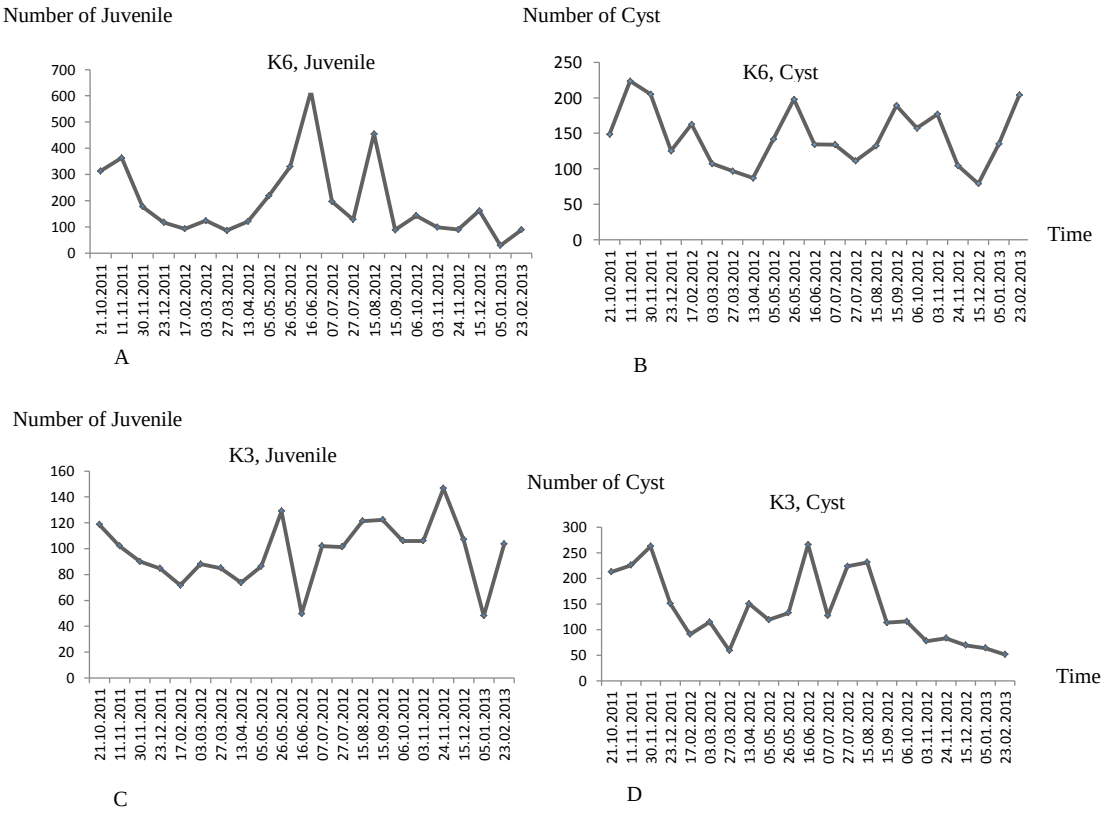

Figure 2. B and D: Cyst and A and C: J2 population changes in rhizosphere of *Brassica oleracea* L. var. *gongylodes* cultivars (A and B: K6 and C and D: K3).

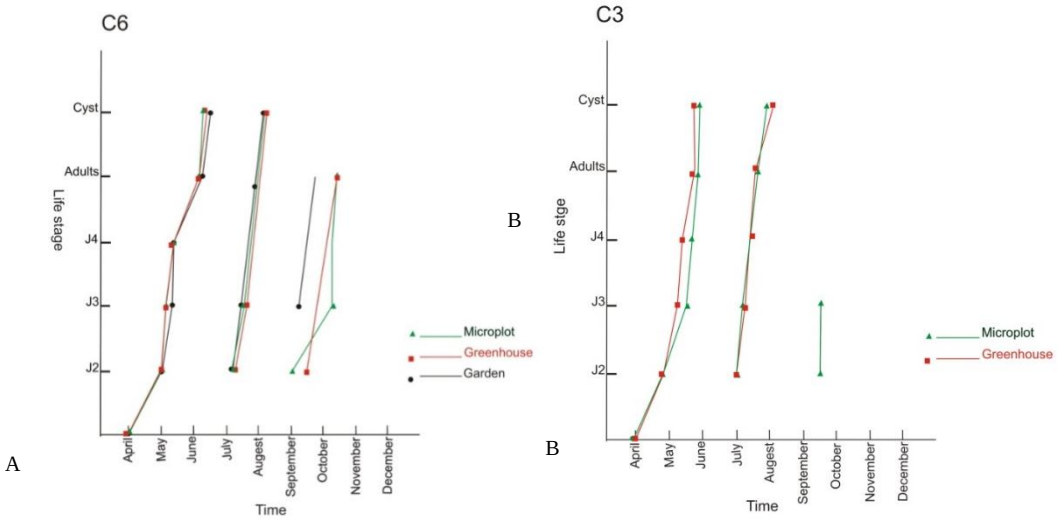

Figure 3. Life cycles of *Heterodera crucferae* on *Brassica oleracea* L.var.*captita alba* cultivars (A: C6 and B: C3) in field, greenhouse and micro plot conditions

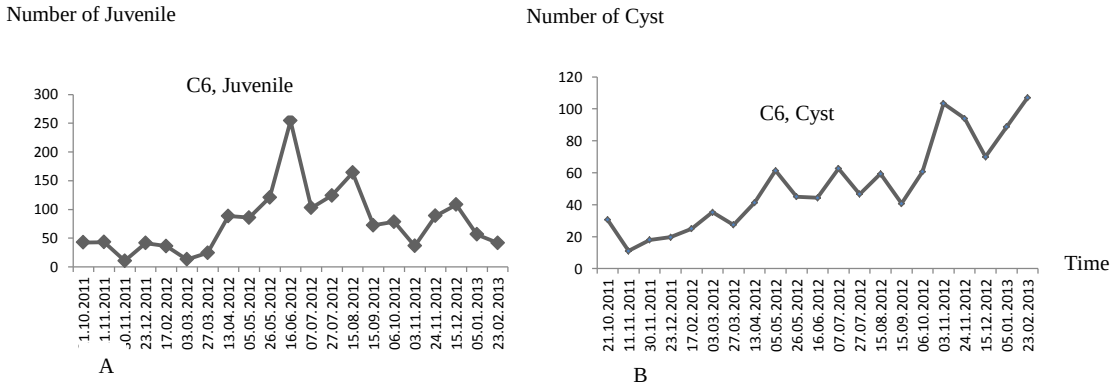

Figure 4. A: Cyst and B: J2 population changes in rhizosphere of *Brassica oleracea* L.var.*captita alba* cultivar with six months growing time.

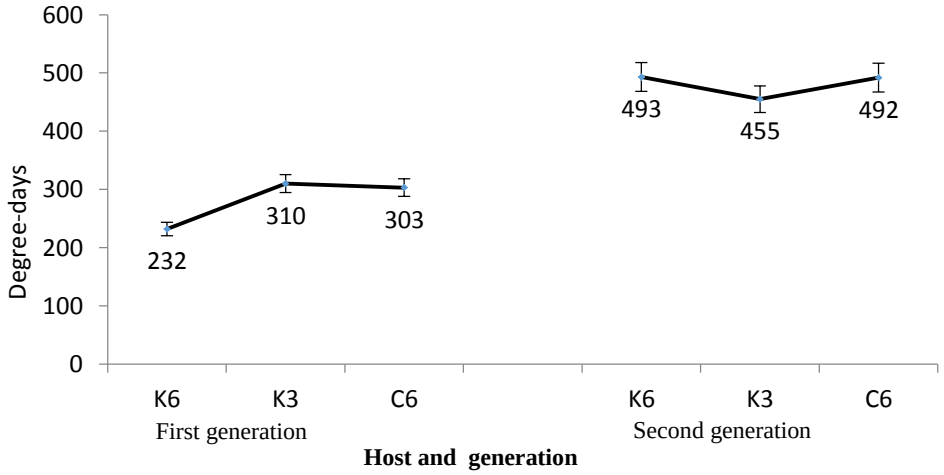

Figure 5. Degree-days required for *H. cruciferae* life cycles under field conditions.

Table 1.The used cabbage cultivars and their codes in the present study.

	Cultivars	Growing period	Code used for cultivars
1	*Brassica oleracea* L. var. *gongylodes*	Six months	K6
2	*Brassica oleracea* L. var. *gongylodes*	Three months	K3
3	*Brassica oleracea* L. var. *captita alba*	Six months	C6
4	*Brassica oleracea* L. var. *captita alba*	Three months	C3

Table 2: Details of data of *Heterodera crucifera* life cycles on cabbage cultivars *Brassica oleracea* L. var. *captita alba* and *Brassica oleracea* L. var. *gongylodes*) grown under three different conditions.

Sowing date	J2	J3	J4	Adult	Cyst	Host	Place	Generation	Duration (Days)
30-Mar. 2012	01 May	05 May.	12 May	09 June	16 June	K6	field	1	45
	30 June	07 July	07 July	21 July	04 Aug.	K6	field	2	35
	03 Sep.	20 Sep.	30 Sep.	07 Oct.	-	K6	field	3- incomplete	
09-Apr. 2012	01 May	05 May	12 May	26 May	19 June	K6	Greenhouse	1	48
	30 June	14 July	-	21 July	11 Aug.	K6	Greenhouse	2	42
	03Sep.	20 Sep.	30 Sep.	07 Oct.	-	K6	Greenhouse	3- incomplete	
07-Apr. 2012	01 May	05 May	12 May	06 June	16 June	K6	Microplot	1	45
	07 July	07 July	-	-	04 Aug.	K6	Microplot	2	28
	01 Sep.	20 Sep.	30 Sep.	7 Oct.	-	K6	Microplot	3- incomplete	
30-Mar. 2012	08 May	05 May	12 May	09 June	16 June	K3	field	1	39
	07 July	14 July	14 July	04 Aug.	04 Aug.	K3	field	2	28
09-Apr. 2012	01 May	05 May	12 May	02 June	13 June	K3	Greenhouse	1	42
	26 June	14 July	-	21 July	11 Aug.	K3	Greenhouse	2	49
07-Apr. 2012	01 May	05 May	12 May	26 May	9 June	K3	Microplot	1	38
	07 July	14 July	-	-	18 Aug.	K3	Microplot	2	42
	15 Sep.	23 Sep.	7 Oct.	-	-	K3	Microplot	3- incomplete	
30-Mar. 2012	01 May	12 May	12 May	09 June	16 June	C6	field	1	39
	07 July	14 July	-	21 July	04 Aug.	C6	field	2	31
	-	6 Sep.	-	20 Sep.	-	C6	field	3- incomplete	
09-Apr. 2012	01 May	05 May	12 May	-	13 June	C6	Greenhouse	1	42
	07 July	14 July	-	26 July	11 Aug.	C6	Greenhouse	2	35
	04 sep.	-	-	06 Oct.	-	C6	Greenhouse	3- incomplete	
07-Apr. 2012	01 May	05 May	12 May	09 June	11 June	C6	Microplot	1	40
	04 July	14 July	-	-	04 Aug.	C6	Microplot	2	31
	O1 sep.	05 Oct.	-	06 Oct.	-	C6	Microplot	3- incomplete	
09-Apr. 2012	24 April	05 May	12 May	19 May	26 May	C3	Greenhouse	1	32
	04 July	09 July	-	23 July	11 Aug.	C3	Greenhouse	2	39
07-Apr. 2012	24 April	05 May	12-May	19 May	22 May	C3	Microplot	1	47
	30 June	07 July	-	21 July	04 Aug.	C3	Microplot	2	35
	14 sep.	15 Sep.	-	20 Oct.	-	C3	Microplot	3- incomplete	

RESULTS AND DISCUSSION

1- Brassica oleracea L. var. *gongylodes*

1.1. Six months growing

1.1.1. Fields

This cultivar was under cultivation from April till October. Twenty-one to 30 days after seed sowing, vermiform infective juveniles (J2) were observed in the roots (Figure 1). At this time, average air and soil temperatures were 10 and 6°C, respectively. Thirty-five days after seed sowing time, third stages (J3) of the nematode were monitored in the host plants root system. The fourth stage juveniles (J4) appeared in root system collected from the fields, on 12 May, when host plants were 42 days old. Virgin or young females without eggs in their bodies were found on stained roots on 9 June. By production of brown, lemon-shape, egg filled cysts, first generation of cabbage cyst nematode, was completed on 16-19 June, 77 days after sowing and 45 days after J2 penetration to root system (Figure 1). At the time of the J2 penetration into cabbage root system, number of infective juveniles in soil were increased and a reduction in cyst numbers were noticeable. On the other hand, an increase in the number of cysts in infected soils was realized when cysts were producing on roots (Figure 2). Fourteen days after detecting brown cyst on host roots, there was another increasing in number of J2 inside root systems which show the commencement of second generation. This event had synchrony with J2 population decrease in soil (Figure 2). This generation started 18 days after production of the first generation cysts on roots. The second generation was completed by appearance of cysts on 4 August, and it took 35 days. Second generation duration was found shorter than the first generation of the nematode. All data of first and second generations are summarized in Table 2. The nematode had third an incomplete generation, which started on 3 September. This generation just continued until the formation of young females which were appeared on early October, but the generation never completed cysts.

1.1.2. Micro plots

In micro plots the same procedure of life cycle was also revealed and the nematode passed two complete generations which were started on 1 May and 7 July and ended on 16 June and 4 August, for first and second generations, respectively (Figures 1 and 2 and Table 2). Duration the generations were 45 and 28 days and they showed just small difference compared with the field condition in which the span of the generations were 45 and 35 days for first and second generations, respectively. The third generation was also incomplete and no cyst was produced on infected roots under this growing system (Figure 1).

1.1.3. Greenhouse

The same scenario was observed in greenhouse, and the nematode completed two generations from 30 April to 11 August, under greenhouse conditions. First generation took 48 days and ended on 19 June. Second generation started on 30 June and ended on 11 August and took 44 days. The third generation which was started on 3September was not completed, too (Figure 1 and Table 2).

1.2. Three months growing

1.2.1. Field

The cultivar was grown in field just till first half of August, although we asked the farmers to keep a number of cabbage plants intact to continue our study on them. The same data were realized for the life cycle of the nematode but with some differences in *Brassica oleracea* L. var. *gongylodes* having three months growing period. First generation lasted from 8 May up to 16 June and took 39 days. Second generation was started at 7 July and after 28 days ended on 4 August (Figure 1). The third generation of the nematode was not traceable under field conditions.

1.2.2. Micro plot

H. cruciferae followed the same process of life cycles in micro plot conditions as observed in the field conditions. First generation of the nematode started on 30 April and ended 38 days later on 9 June and second generation took 42 days, from 7 July until 18 August. Under micro plot conditions and on this host, third incomplete generation of the nematode was detected. Second stage juveniles of the generation were observed in the root on 15 September. Accordingly, the third and fourth stage juveniles were found in stained roots on second half of September and first half of October, respectively (Figure 1).

1.2.3. Greenhouse

Two complete and an incomplete generations of the nematode were detectable during the growing season from 30 April till 6 October on the cultivars in greenhouse (Figure 1 and Table 2). First generation occurred from 30 April until 13 June with 42 days duration. Second

one took 49 days and ended on 11 August which was started on 26 June. The incomplete generation of the nematode observed on the host.

2- Brassica oleracea L. var. *captita alba*

2.1. Six months growing

2.1.1. Field

Like *Brassica oleracea* L. var. *gongylodes* cultivar, cabbage cyst nematode completed two generations on this cultivar. First generation began on 1 May and increased in roots in coincidence with a reduction in number of the nematode infective stage in soil and this generation lasted until 16 June after 39 days of J2 penetration into host roots. Second generation of the nematode started on early July (4 July), took approximately one month (31 days) and the cysts of this generation were produced on infected roots on 4 August. On six months growing cultivar, the third incomplete generation started on 4 September and adults of the nematode appeared on cabbage roots in 20 September (Figure 3). Cysts of the third generation did not produce, so the generation was not completed (Figure 3). All details of the nematode life cycles are shown in Figures 3, 4 and Table 2.

2.1.2. Micro plot

Two complete and one incomplete generations of the nematode were also observed on six months growing *Brassica oleracea* L. var. *captita alba* under this situation. The seeds were sown in micro plots on 7 April, and on 11 June and 4 August, first and second generations of the nematode completed, after 40 and 31 days, respectively. The third incomplete generation of the nematode occurred in this growing condition on 04 September (Figure 3).

2.1.3. Greenhouse

The number of the nematode life cycles is similar to the above mentioned status, so that two complete and one incomplete generation on this cultivar were realized (Figure 3 and Table 2). First generation with 42 days was between 1 May till 13 June and second generation took less time (35 days) which started on 7 July and ended on 11 August. Third generation was incomplete on this host and under greenhouse condition.

2.2. Three months growing

2.2.1. Field

On the time of our study, three months growing *Brassica oleracea* L. var. *captita alba* was not under cultivation in the fields and it was not possible to continue the experiment regularly. Therefore, the data are not available.

2.2.2. Micro plot

On three months growing cabbage, two complete and one incomplete generation of the nematode were also detected as seen in the other experiments (Figure 3 and Table 2). The first generation starting time was 24 April and ended after 28 days on 22 May. Second generation ended on 4 August after 34 days which was started on 30 June. Third generation's J2 were observed on roots on 14 September, but the life cycle was not completed.

2.2.3. Greenhouse

The cultivar under greenhouse conditions could host two complete generations of cabbage cyst nematode (Figure 3 and Table 2). First generation started on 24 April and was ended on 26 May after 32 days. Second generation duration showed one week more than first one and lasted from 4 July until 11 August. The third incomplete generation was not detected in greenhouse on this cultivar.

3. Degree days

The amount of degree days above 4ºC required for the nematode life cycle in both generations under field conditions is shown in Figure 5. The degree days required by *H. cruciferae* to develop from J2 penetration in first generation up to the commencement of the next generation was calculated 232-310 in field conditions on different hosts. In second generation on six months growing cabbage, since this generation is coincident with warmer climate of the season and the duration of this generation is shorter, therefore, it needs 455-493 degree-days that is more than first generation. Koshy and Evans (1986) calculated the amount of degree-days which is required by the nematode in order to complete one generation on oilseed rape plants root under controlled conditions. They estimated that the degree days above 5°C is 680 from egg hatching to producing of egg sac stages by the nematode, of which 210 degree days were required to make the egg sac. In total, 470 degree-days are enough for nematode from hatching to cyst stage. This amount is comparable with that of degree-days obtained (455-498 degree-days) for the Iranian population of *H. cruciferae* second generation on its main host and under field conditions in the current study (Figure 5).

The rates of cyst nematode development are affected by soil and air temperature, food quality and quantity and these are main factors that could explain the differences between the generation spans of the nematodes. The span of life cycle for most *Heterodera* species is reported around 30 days (Lilley et al., 2005). Number of their generations per year varies among species and populations as well from different climatic areas (Subbotin et al., 2010). Time of the sowing, transplanting and host growth duration affects nematode life cycle (Lewis, 1971).

Based on the results, it was observed that cabbage cyst nematode has two complete generations on *B. oleracea* L. var. *gongylodes* and *B. oleracea* L. var. *captita alba* cultivars with six and three months growing time between April and August in vegetables growing area of Tabriz, northwest, Iran. The third generation is just partially completed in the late growing season that will be acting as overwintering phase of life cycle of the nematode for the next cultivated cabbage or other hosts in the next spring season. The same situation was revealed in South Wales in England where the nematode has two complete generations between April and early September and one incomplete generation (Lewis, 1971). Under different climates and conditions cabbage cyst nematode could have up to three complete life cycles (Stone and Rowe, 1976 and Subbotin et al., 2010). In middle regions of Russia three generations of the nematode have been reported (Chizhov et al., 2009).

The nematode develops most rapidly during the summer months in second generation, due to high soil temperatures (Lewis, 1971). In Tabriz conditions, the understudy area, the nematode had also two complete generations on six months growing cabbages; first one lasts nearly 1.5 months (according to the cabbage variety takes 39-48 days), from late April to middle of June. The second generation starts on 30 June to 7 July, is faster, takes almost one month, and ends on early August (28-42 days). On the cabbage cultivars with three months growing period, except field condition, on the cultivar with K3 code, the second generation in all other experiments spent more time compared with the first generation. The cultivar K3 needs 39 and 28 days to complete first and second generations, respectively.

As already mentioned, cabbage cyst nematode genetically acts as a single population in the region of Tabriz (unpublished data). On the other hand, in spite of different species and varieties of cabbages that are cultivated in the region and they were also used in current study and under the three different growing conditions, two complete and one incomplete generations of the nematode were consistently observed. So, the homogeneity of the nematode population, long term cultivation of the host plants in the fields and similar agricultural operations may have allowed the nematode to adapt itself with biological, ecological and environmental conditions of the region. Therefore, the same number and procedure of life cycles shown by the nematode in the vegetable growing area of Tabriz could be justified and are acceptable.

Research on cabbage cyst nematode is limited and majority of them are old and could not explain details of its life cycle and the parameters that influence the number of generations. Therefore, it remains open and needs more investigations on different aspects of the nematode biology including life cycle under different climatic conditions and on its other hosts as well.

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