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Effect of Plant Age and Nematode Inoculation Density on Final Population of *Heterodera schachtii* on Chinese Cabbage

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배추 묘령과 선충접종밀도가 사탕무씨스트선충의 증식에 미치는 영향

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ABSTRACT: Sugarbeet cyst nematode (*Heterodera schachtii*) has recently been detected as a severe pest of Chinese cabbage fields in Korea. Two studies were performed to evaluate the effect of inoculation density of *H. schachtii* and plant age of Chinese cabbage on the final population of the nematodes. Chinese cabbage inoculated with high inoculation density (4 juveniles or eggs/g soil) showed significant differences in the number of cysts, females and eggs. The 40 day- old plants inoculated with high nematode densities yielded the highest nematode populations after 30 and 60 days of inoculation, compared with other plant age groups (0, 20, 30 day-old)used in the experiment. We, thus, summarize that, maximum nematode multiplication rates are made on comparatively older plants at the high nematode densities. Therefore, taking plant age into consideration at the transplanting of Chinese cabbage was recommended for the management of *H. schachtii*.

Key words: Hatching, Juveniles, Population density, Management, Sugarbeet cyst nematode

초 록: 사탕무씨스트선충(*Heterodera schachtii*)은 최근 우리나라의 배추 재배지의 문제선충이다. 본 연구는 사탕무씨스트선충의 밀도와 배추의 묘령이 사탕무씨스트선충의 증식에 미치는 영향을 알아보기 위하여 수행하였다. 접종농도가 높은(4마리 유충 또는 알/g 토양) 처리에서 씨스트 수나 암컷 또는 알 수가 유의하게 높았다. 40일 묘령 배추에 고밀도 선충 접종 시 30일이나 60일 후 다른 어린 묘령 배추(파종처리, 20일묘, 30일묘)에 비하여 증식 선충 밀도가 높았다. 높은 선충 밀도 조건에 묘령이 오래 된 모종 식재 시 선충 증식율이 높기 때문에 사탕무씨스트선충 관리를 위해서는 정식 모종의 묘령 고려가 필요하다.

검색어: 부화, 유충, 증식밀도, 관리, 사탕무씨스트선충

Chinese cabbage, *Brassica rapa chinensis* L. is a significantly important crop cultivated in spring, ranked as third crop produced in Korea (KREI, 2012). Chinese cabbage has a high market demand because of its multipurpose use, particularly for the production of Kimchi (KREI, 2012), but suffers severe yield loss when infected by sugar beet cyst nematode (SBCN), *Heterodera schachtii*. The average temperature for growing Chinese cabbage ranges from 10-15℃ (KMA, 2011). Chinese cabbage growers at the highland area of Korea have been suffering a lot for the economic loss caused by sugar beet cyst nematode, SBCN since 2011, the first reported year (Lee et al., 2013).

SBCN is one of the most serious pests of sugar beet, Chinese cabbage, broccoli, cauliflower, radish, spinach and most of the Brassicaceae crops of the world (Whitehead, 1998) and causes severe economic damage in Chinese cabbage field in Korea. Hatching starts when the adult female dies and its body form a

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cyst of dark brownish-color in average field temperature 10-15℃. Successful hatching ensures a high number of juveniles that could lead the severe field damage of a single cabbage field. Therefore, population of SBCN is a burning issue to control management.

A group of European nematologists, firstly stated the importance of population density of SBCN in sugar beet production. Helinga (1942) first stated a linear correlation between the logarithm of the initial population density and yields of sugar beet. Later in 1956, Jones modified the relation. After that, Seinhorst (1965) initiated the first expression of population density by inoculating eggs/g soil with yield. Heijbroek (1973) showed a clear relationship between population densities of cysts, eggs and larvae on the yields of sugar beet. Most of the papers addressed the information on either population density or tolerance limit of SBCN on sugar beet. However, only a few papers have been reported on the age of plants and population density of SBCN (Griffin, 1981a, b). Though, SBCN mainly named after sugar beet as first introduced and identified from sugar beet but most of the cyst nematode has a relatively narrow host range (Franklin, 1972; Sharma, 1998) and it is somewhat difficult to complete eradication from the fields. Usually, farmers chose the young plantlets (2-3weeks old) for transplanting (1st week of April) in the field during the low field temperature (12℃) (KMA web source). In addition, there is huge variability in the number of eggs/cysts in the field depending on the previous cropping history (Griffin, 1974). A previous field data surveyed showed different population density of SCBN depending on surveyed sites (Kwon et al., 2016). In addition, planting period was different depending on the elevation of highlands cultivated vegetables in Korea. Generally, farmers planted about 20-day old plants in highlands, but some farmers planted much older plants because of unsuitable planting condition such as drought soil condition. Thus, we hypothesized that comparatively older aged plants are more sensitive to nematode infection.

To reduce the damage caused by SBCN several researches have been made, but none of them were actually designed for Chinese cabbage. We, therefore, wanted to see the effect of inoculation density and plant age on the final population of SBCN. The reason in our trials was to relate the plant age in contrast with population density with the previous study results, which showed that 20 days' extract of Chinese cabbage gave the highest hatching of SBCN (Kabir et al., 2015). Their results showed that there is a relationship between the plant age and nematode density while planting in the field. There could also be a question regarding the chemical properties of the extract but before going through the chemical properties, we wanted to prove whether there is any relationship between the plant age or not?

This could be an ample opportunity to find out the effect of population density of SCBN and plant age of Chinese cabbage. The outcomes of this study could suggest planting adaptive Chinese cabbage seedlings for the local growers which could minimize the nematode population and less yield loss. It could make a significant change for the local farmers as well on the national economy of Korea by suggesting the adaptive Chinese cabbage seedlings age in the field.

Materials and Methods

Effect of plant age on the final population of *H. schachtii*

Chinese cabbage in pot

Four stages of different plant age e.g. 0 days' seedlings, 20, 30 and 40 days old Chinese cabbage (cultivar: Chungwang) were used for the experiment. Each plant age condition had 5 replications. Each pot (size 10.5 × 13.5 cm) was filled with 800 g autoclaved soil to ensure no contamination of other soil nematodes. All the plants were inoculated with juvenile of *H. schachtii* at the rate of 1 (800 J2/pot), 2 (1600 J2/pot) and 4 (3200 J2/pot) second juvenile (J2) per gram soil, respectively on the following day after planting. Inoculation was done using a pipette. For 0-day old (seedling) plants, seeds were directly sown in the pot. However, 20, 30 and 40 days old seedlings were kept in 25℃ growth chamber (GC-1000TLH, GEIO Tech, Korea) with 11h light and 13h dark condition.

Eggs and juveniles of *H. schachtii*

Cysts were extracted from the soil collected from Chinese cabbage field a highly infested with sugar beet cyst nematode in Taebaek, Korea. Cysts of *H. schachtii* were isolated by sieving method (Barker 1985). Collected SBCN infested soils from the field were sieved using 20, 60 and 400 mesh sieves. Only 60 sieves particles were filtered with Whatman no. 100 and then healthy (not broken/damaged) cysts were isolated by hand picking with forceps. Isolated cysts were then kept for 24h in a Baermann funnel with water until successful hatching occurred. Hatched juveniles were collected from the funnel and diluted to make nematode suspension with a concentration of 150 juveniles/ml of water. Afterward, desired volumes were inoculated into different pots using a pipette for the inoculation of eggs (for 2nd experiment), collected healthy cysts were crushed using a sonicator (Polytron PT1300D, Kinematica AG, Swizerland) and then water containing eggs were diluted to make egg suspension and followed the same procedure as inoculation of J2.

Population density of *H. schachtii* after 30 and 60 days of inoculation

H. schachtii populations were counted after 30 and 60 days of inoculation. At first, the plant shoot was cut above the ground level of the pot and soil with root was washed carefully using tap water and the resultant water was sieved using 20, 60 and 400 mesh sieves. Cysts of 60-mesh sieve were filtered with Whatman no. 100 filter paper to remove the water content. Then, the filter paper in a petri dish was taken and placed under stereomicroscope (SM2 1000, Nikon, Japan) to count cyst, female and total cysts. 5 healthy cysts (undamaged, with egg cysts) were transferred into a small vial in 5 ml water. The cysts were sonicated at 800 rpm using Polytron PT 1300D sonicator (Kinematica AG, Switzerland). To estimate total numbers of eggs, a subsample of 1ml suspension of eggs was taken from the stock solution and numbers of eggs were counted under stereomicroscope. For counting 2nd stage juveniles were extracted using a modified Baermann funnel technique (Barker 1985).

Number of leaves of Chinese cabbage after 30 and 60 days of inoculation

The number of total leaves and the length and width of first 5 leaves were measured by a cm scale. Also, weight of shoot of the plant was measured after detaching from root. Initially, the root weight was measured just after washing and later after drying to find the moisture content of the root.

Effect of inoculation density on the final population of SBCN in Chinese cabbage pot

Only 40 days old plants were transplanted and inoculated with eggs (1, 2, 4 and 8 eggs/ gram) and juveniles (1, 2, 4 and 8 J2/ gram) of soil separately to find out the effect of inoculation density. The final population was counted after 60 days of inoculation. Inoculation and counting final population were followed as described in the earlier experiment.

Statistical analysis

All the data obtained from our experiments were analyzed two-way ANOVA using SAS 9.4 Software (SAS 9.3 institute Inc., NC, USA). Level of significance tests were done using fisher's least significance difference among the factors at $P \leq 0.005$ and $P \leq 0.001$.

Results

Effect of inoculation density on final population of SBCN in Chinese cabbage pot

Inoculation with eggs and juveniles were done to justify the effect of plant age at different inoculation densities. Plants inoculated with eggs resulted in various number of cyst, female and eggs. Though the number of female and eggs/cyst were not statistically different, the number of cyst was highly significant (Fig. 1A). Whereas, the plants inoculated with juveniles showed different results. Increased number of cyst, female and number of eggs were observed but the results were statistically non-significant (Fig. 1B).

Percent correlated weight reduction was calculated to see the correlated weight loss occurred while inoculation with eggs and juveniles.

In the set of both inoculum test (eggs and juvenile's inoculation), juveniles caused more plant weight loss (Fig. 2).

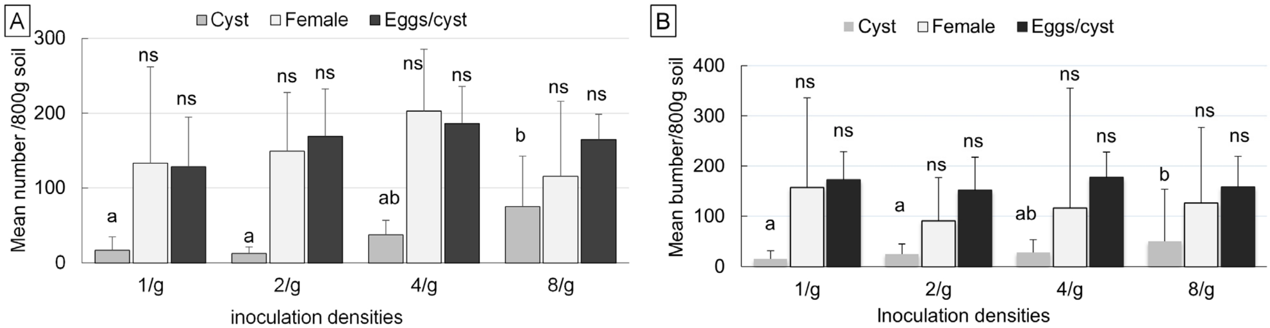

Fig. 1. Number of final population after inoculating with different densities of eggs (A) and juveniles (B) in potted Chinese cabbage at 25℃ incubator. Bar on the graph was standard deviation. Only the cyst data in (Fig. A) showed a statistically significant at $P < 0.05$ based on Tukey's Studentized Range Test.

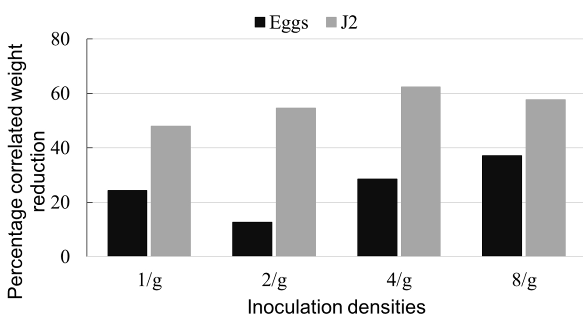

Fig. 2. Percentage of correlated weight reduction of Chinese cabbage after inoculating with different inoculation densities of eggs and juveniles in potted Chinese cabbage at 25℃ incubator.

Effect of plant age and inoculum density on final population of SBCN in Chinese cabbage pot

SBCN population after 30 days of inoculation

Numbers of cysts were increased in the higher plant age and higher inoculation, numbers were not significantly different (Table 1). Initially, the number of female was very low (in seedlings) but numbers were increased in the higher pant age (20, 30 or 40 days old plants). The highest number of females was counted in 40 days old plants at 4/g inoculation density. Although the number of total cysts was also found as lower number in younger plants (seeds), there was not significant. In

Table 1. Mean number of cysts, female, total cysts and eggs of different plant age at different inoculation densities after 30 days of inoculation

Plant age	Inoculation density/g soil	Number of			
		Cyst	Female	Total cyst	Total eggs
0 (seed)	1	0.0 ± 0.0a*	3.4 ± 3.8b	3.4 ± 3.8b	317.4 ± 315.5b
	2	0.0 ± 0.0a	15.6 ± 8.4b	15.6 ± 8.4b	1333.3 ± 586.3b
	4	0.0 ± 0.0a	8.2 ± 4.7b	8.2 ± 4.7b	432.5 ± 248.7b
20 days	1	0.6 ± 0.9a	35.2 ± 14.4b	35.8 ± 14.2b	3100.1 ± 1171.3b
	2	2.8 ± 4.1a	50.0 ± 32.8ab	52.8 ± 36.6ab	8098.2 ± 7081.9ab
	4	0.4 ± 0.5a	25.6 ± 21.8b	26.0 ± 21.9b	3601.5 ± 3424.5b
30 days	1	0.4 ± 0.9a	32.4 ± 22.8b	32.8 ± 23.2b	4856.0 ± 3391.1b
	2	4.8 ± 6.6a	36.2 ± 18.2b	41.0 ± 20.5b	6306.7 ± 3437.9ab
	4	0.6 ± 1.3a	21.0 ± 23.1b	21.6 ± 22.5b	3923.0 ± 6240.6b
40 days	1	1.4 ± 3.1a	12.8 ± 13.6b	14.2 ± 13.9b	2120.1 ± 2081.9b
	2	0.4 ± 0.9a	42.6 ± 22.1ab	43.0 ± 21.9ab	6267.6 ± 3880.2ab
	4	4.0 ± 4.6a	91.4 ± 54.8a	95.4 ± 56.1ab	15332.0 ± 8236.5a

*Mean numbers followed by the same letters in each row are not significantly different at the 5% level by Tukeys Studentized Range Test.

older plant age, the number of total cysts was also recorded very high comparatively with the younger plant age. The maximum numbers were counted in 40 days' older plants at 4/g inoculation density.

Number of total leaf and weight of Chinese cabbage

The number of leaves of each plant age did not show any noticeable changes in the graph (Fig. 3A). Interestingly, the total number of leaves in seedlings showed statistically highly significant. However, in 40 days old plant, at all inoculation densities gave the maximum number of leaves. The shoot weight gave a better understanding of the effect of plant age. The seedlings showed very low shoot weight (Fig. 3B). Nonetheless, the 20-day old plant weight was increased twice than the direct sowing plants (seeds). However, the data set were not statistically significant. In 40-day old plant at 0/g inoculation density ranked the highest weight and 4/g gained the lowest one. 30 and 40-day old plants showed a significant result.

SBCN population after 60 days of inoculation

To predict the population density over time, number of cysts, females, total cysts and the number of eggs were counted

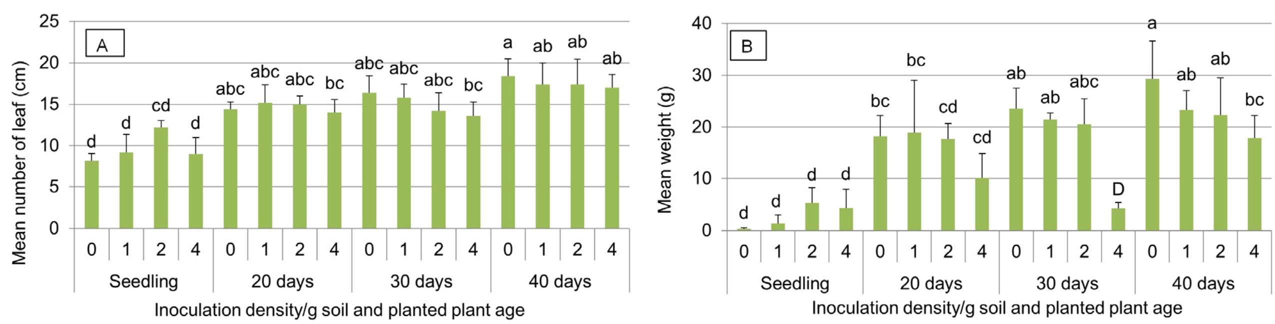

Fig. 3. Number of leaf (A) and weight (B) of Chinese cabbage 30 days after sugarbeet nematode inoculation on potted Chinese cabbage depending on inoculation densities and plant age at 25°C incubator. Bars on the graph were standard deviation. The same uppercase letter on the bars indicated that there is no significant difference among means (Tukey's Studentized Range Test, $P < 0.05$).

Table 2. Mean number of cysts, female, total cysts and eggs of different plant age at different inoculation densities after 60 days of inoculation

Plant age	Inoculation density/g soil	Number of			
		Cyst	Female	Total cyst	Total eggs
0 (seed)	1/g	2.2 ± 2.3c*	106.8 ± 97.1bc	109.0 ± 97.3c	14682.8 ± 11119.5bc
	2/g	3.0 ± 0.7c	72.8 ± 50.3bc	75.8 ± 50.7c	12764.7 ± 8481.5bc
	4/g	0.8 ± 1.3c	16.8 ± 12.5c	17.6 ± 13.5c	2811.1 ± 2172.7c
20 days	1/g	7.6 ± 6.0bc	83.6 ± 26.0bc	91.2 ± 31.6c	11092.6 ± 5313.4bc
	2/g	1.4 ± 2.2c	17.8 ± 6.9c	19.2 ± 9.0c	2183.2 ± 1138.6c
	4/g	15.6 ± 21.0bc	72.4 ± 24.1bc	88.0 ± 40.3c	11576.2 ± 6829.8bc
30 days	1/g	9.4 ± 8.2bc	68.8 ± 53.0bc	78.2 ± 60.4c	8785.9 ± 7158.7bc
	2/g	57.8 ± 39.4bc	193.8 ± 249.2bc	251.6 ± 287.8bc	20177.9 ± 24077.1bc
	4/g	31.6 ± 19.2bc	21.8 ± 9.1c	53.4 ± 21.0c	5288.4 ± 3095.8bc
40 days	1/g	81.2 ± 27.7b	703.8 ± 240.3a	785.0 ± 263.5a	87035.8 ± 21931.4a
	2/g	60.6 ± 39.0bc	264.2 ± 166.7bc	324.8 ± 205.2bc	41828.2 ± 36146.7b
	4/g	255.6 ± 101.8a	337.6 ± 250.0b	593.2 ± 342.1ab	43487.6 ± 33009.8b

*Mean numbers followed by the same letters in each row are not significantly different at the 5% level by Tukey's Studentized Range Test.

after 60 days of inoculation at different inoculation densities. Like previous (after 30 days of inoculation), the number of cysts showed higher number at higher plant age and at higher inoculation densities (Table 2). In higher plant age, the number of total cysts was also recorded very high compared with the younger plant age. The maximum number was counted in 40-day old plants at 4/g inoculation density. All the results were statistically highly significant.

Number of leaves of Chinese cabbage

At all inoculation, densities the resulted data were very close to each other in the number of leaf (Fig. 4A). However, 40-days old plant at 0 to 2/g inoculation density gave the maximum number of leaves and the data were highly significant.

Discussion

There were a few evidences found about the effect of inoculation density and plant age on the final population of SBCN. However, no evidence of inoculation densities of *H. schachtii* on Chinese cabbage has been reported. Plant inoculated with eggs showed a highly significant increasing rate of cyst. At higher inoculation density, the number of eggs and juveniles was reduced. Crowding is the possible effect for this case as we assume. The females might have a tendency to go for a short dormancy because of the crowding effect that was explained by Zheng and Ferr (1991) as obligate diapause.

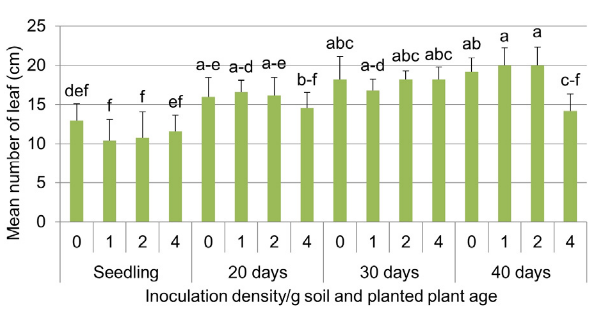

Fig. 4. Number of leaves of Chinese cabbage plants counted 60 days after sugarbeet nematode inoculation on potted Chinese cabbage depending on inoculation densities and planted plant age at 25°C incubator. Bars on the graph were standard deviation. The same uppercase letter on the bars indicated that there is no significant difference among means (Tukey's Studentized Range Test, $P < 0.05$).

However, Steele (1975) reported that, if there are minimal environmental changes that are sufficient to alter either plant root growth or number of invading larvae, will affect the density of larvae. These changes could lead in large shifts in population s of females and males.

Our assumption was, therefore, the higher population density could cause high damage percentage and affected in higher plants than the younger, which is similar what Seinhorst (1986) found. But in other way, looking at the number of cysts found after 30 days of inoculation does not fit. Seinhorst (1965) also mentioned that, the average nematode is the same at all densities (size or activity of the nematode are not influenced by the density of the population) which contradicts our result. Temperature could be another issue here as mentioned by Griffin (1981a). He mentioned that soil temperature has a double influence on the relationship of SBCN and sugar beet. Also, Nemazi (1961) reported that larger secondary roots with a thicker epidermis trapped more female nematodes than did the smaller primary roots. Percent reduction rate was higher in case of juvenile inoculation which means it has a clear effect on the foliar root and total growth if the inoculation density is high. As only roots are affected by nematodes thereby, leaves are sensitive to their effect. However, light and relative humidity could have an issue regarding the individual weight of Chinese cabbage while they kept in incubator. Moreover, in the middle of-experiment, some plants in the incubator were affected by the attack of aphids, which could partially affect the weight loss result. However, we did not go through the root analysis issue. Nevertheless, it is clear that SBCN are more likely to attack older aged plants than the younger one but still the population density is a big mystery.

Our results coincided with, after 30 days of inoculation, older plants (40 days aged) resulted with high number of total cyst, females and eggs while inoculated with high (4/g) inoculation densities. However, the scenario was completely different after 60 days of inoculation. The number of total cysts, female and total eggs reached maximum at 1/g inoculation density in 40-day old plants. In question to final population, significant change has been observed at different inoculation density in 40-day old plants after 30 and 60 days of inoculation. Therefore, we can conclude that higher aged plants are more sensitive to nematode infection, which partially proved our hypothesis. However, overall final population density was higher in 40-day old plants after 30 and 60 days of inoculation. This result is completely different from what Griffin (1981b) said in his paper. He found that older plants are less susceptible to nematode pathogenicity than younger one. Captivatingly in this study, after 30 and 60 days of inoculation, the final population density did not show the similar pattern to results of Kabir et al. (2015). They reported that 20-day old plant extract gave the maximum hatching of SBCN and 40-day old plants showed the lowest effect on hatching at room temperature. The change of final population in different aged plants can give a possible explanation of having something interesting, which was started by Selvan et al. (1993) that, density-dependent factors within a host have an important influence on the population dynamics of parasites. The average mean number of total cyst and females were very close which logically support the statement of Weischer and Brown (2000) who found, females were prominent when the inoculation density was small with the proportion of male increasing as the number of parasites invading a host increased. However, we did not take into consideration sex determination of inoculated juveniles.

More interestingly, after 30 days of inoculation, the number of cyst was high at 2/g inoculation density in 30-day old plant, however, after 60 days of inoculation, numbers were found high at 4/g inoculation density in 40-day old plants. Initially, after 30 days of inoculation, the population increased up to a certain level of density but after 60 days, the final population density decreased again, when overpopulation initiates intraspecific competition as explained by Koppenhöfer and Kaya (1995), Selvan et al. (1993), and Hominick and Tingley (1984). They reported that all nematodes during their life cycle experience little or more intraspecific competition if resources are limited or high population. In general, populations can exhibit uniform, random, or patchy distributions but the pattern observed depends upon the scale over which it is measured Dutilleul and Legendre (1993). Also, the dauer stage could have had a considerable effect on the final population density though close scrutiny for dauer juveniles was not taken into account in the study. However, Molz (1920), first reported that the ratio of SBCN is strongly influenced by physiological condition of the host plant. Nevertheless, Seinhorst (1986) reported that, hatching only after stimulation by host root diffusate as in SBCN including some other nematodes. Similar statement is also reported by Wesemael et al. (2006) that hatching of second-stage juveniles (J2) of *M. chitwoodi* on young plants did not require host root diffusate stimulus, whereas at the end of the plant growing season, egg masses contained a percentage of unhatched J2 that require host root diffusate to cause hatch.

In consideration of the physical appearance of the plants, we measured total number of leaves, leaf length, width and as well as weight of plant to express a clear idea how the nematode affects plant growth in different plant age. 30 and 60 days after inoculation, the maximum number of leaves were found at 0/g inoculation density in 40-day old plants and 1/g inoculation density in 40-day old plants, respectively. Plant weight was also found higher both at 0/g inoculation density in 40-day and 30-day old plants, respectively. That means, zero or less inoculation density has no effect on foliar appearance (shoot growth). Moreover, logically, the older plants should have more leaves and weight than the younger ones. Our results showed lower inoculation density at comparatively older plants has less effect on the growth of the plant. However, Griffin (1981b) found that there is a close relationship between root weight, soil temperature and population density. Therefore, we cannot ignore that soil temperature and intraspecific competition of nematode population are also responsible for the actual effect. In Fig. 2A, the number of leaves was prominently increased in all stages but less in 4/g inoculation densities. However, the effect of inoculation is clearer in Fig. 2B. Plant weight showed remarkable changes in the yield. Initially, it was increasing but in 20 days aged plant weight was drastically fallen down. We assume this could be the effect of stress tolerance of the plants. Effect of inoculation density was much more prominent in 30-day and 40-day old plants by decreasing final weight of the plant at different inoculation densities, respectively. Especially, at 4/g inoculation density, the plant lost its weight by a significant level and the data sets were statistically significant.

Therefore, we believe that our results could make a little hope for the local farmers. After analyzing results, we suggest that comparatively younger plants (20-day old plants) are recommended to be transplanted in the field, which have shown less affected by nematodes. However, direct seeding seedling have has also shown even more effective results than 20-day old plants. However, younger plants may be more vulnerable to other insect pest and diseases. In addition, younger seedlings are much more sensitive to other environmental factors. Therefore, we assume that planting 20-day old plants would be the best choice for sugar beet nematode-free plants. However, more research will be needed to understand the effect more accurately.

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