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### A Field Study on Determination of Nematode Diversity in Canola Fields in Tekirdağ

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## Tekirdağ İli Canola Tarlalarında Nematod Çeşitliliğinin Belirlenmesi Üzerine Bir Arazi Çalışması

Öz: Tekirdağ'da Malkara, Süleymanpaşa, Muratlı, Hayrabolu ve Ergene ilçelerinde 28 kanola Anahtar Kelimeler tarlasından alınan toprak örnekleri nematod faunasının belirlenmesi için incelenmiştir. Her Nematod çeşitliliği, tarlada 0-60 cm derinlikten toprak örneği alınmış ve bu örneklerden modifiye Baermann Funnel Kanola, Tekirdağ, metoduyla 28 cinse ait nematod türleri toplanmıştır. Bunlardan 8 tanesi bakteri ile beslenen, 3 Türkiye tanesi fungus ile beslenen, 5 tanesi omnivor, 3 tanesi predatör ve kalanı bitki paraziti türlerdir. Sürvey alanlarındaki nematodların colonizer-persister değerleri 1 ile 5 arasında değişmiştir. Maturity ve kommunite indeksleri de hesaplanmış ve 4 tanesi şu değerleri almıştır: Enrichment (E): 34.6±15.2; Maturity (MI): 2.68±0.05; Plant parasite (PPI): 2.69±0.39; Shannon's diversity (H'): 1.80±0.26. En yaygin cinsler Filenchus (100%), Mesodorylaimus (92%), ve Ditylenchus (85.1 %) olmuştur. 100 cm<sup>3</sup> toprakta yoğunluk bakımından en yüksek değer Acrobeloides (192 birey/100 cm<sup>3</sup> toprak)'te bulunmuş, bazı topraklarda ise Ditylenchus (57 birey/100 cm<sup>3</sup> toprak), Filenchus (180 birey/ 100 cm<sup>3</sup> toprak) ve Mesodorylaimus (165 birey/100 cm<sup>3</sup> toprak) birey sayısıda yüksek bulunmuştur. Kanola tarlalarında teşhis edilen bitki paraziti nematodlar ise Boleodorus tylactus, Filenchus cylindricus, F. sheri, F. thornei, Geocenamus brevidens, Helicotylenchus digonicus, Malenchus fusiformis, Paratylenchus variabilis, Pratylenchoides alkani, Rotylenchus robustus, Tylenchorhynchus annulatus, ve T. cylindricus'tur.

### **1. INTRODUCTION**

Canola (Brassica napus L. var. napus ) is an annual herbaceous plant with winter and summer-grown

varieties. It is classified in the Brassicaceae family and Brassica (Cruciferae) genus. Canola is among the plants grown as a source of vegetable oil from 240 plant species in this genus. Canola plant has been produced with the

breeding studies of rapeseed, which is the third largest vegetable oil source and the second largest protein meal source in the world with the seeds it produces [1]. Plant seeds contain 38-50% oil and 16-24% protein [32]. Prohibited from cultivation in the past because of containing erucic acid, which is harmful to human health, and glucosinolate, toxic to animal health, canola is now grown in many countries [27]. The pulp of this plant, whose seeds are used in oil production for human consumption, is also used to make animal feed and biofuel.

Canola is grown on 73.776.943 hectares of land in the world, and Canada, China, India, Germany, France, Australia, and Poland are world leaders in terms of production [34]. Türkiye has a 37.601 ha cultivation area, and, Tekirdağ ranks first with a 12.261 ha area in terms of production [4]. Although it is grown in all districts in Tekirdağ province, the production is more intensive in Çorlu, Hayrabolu, Marmara Ereğlisi, Malkara, Muratlı, and Süleymanpaşa.

The soil micro and macrofauna include several pests, insects, and microorganisms. Members from phylum Nematoda constitute almost the majority of the total organisms. The phylum includes animal-plant parasites and marine and free-living non-parasitic nematodes. Dozens of species with different feeding habitats, such as plant parasites (e.g. Meloidogyne spp.), bacterivores (e.g. Acrobeles spp.), omnivores (e.g. Dorylaimus spp.), or predatory (e.g. Seinura spp.), and fungivores (e.g. Mononchus sp.) live in agricultural areas [20].

There are limited studies on nematode diversity associated with canola in a limited number of countries and Türkiye as well [29, 9]. Therefore a study in canola fields in Tekirdağ is planned. The study aimed at the determination of free-living and plant parasitic nematodes. Nematode genera in the surveyed fields were determined. Nematodes were grouped based on feeding style and feeding habitat. The maturity and community indices were calculated. Plant parasitic nematode species were described based on morphometric characteristics.

#### 2. MATERIAL AND METHOD

#### 2.1. Study Area Information

The study was conducted in the province of Tekirdag, located northwest of Turkey, on the coast of the Marmara Sea. Viticulture, sunflower, and wheat cultivation are the most common agricultural activity in the region. Canola is also grown in all districts in Table 1 and is cultivated in rotation with sunflower and wheat. Table 1. Canola production area, districts, total production in 2021 [4]

DISTRICTS	TOTAL	TOTAL
	PRODUCTION	PRODUCTION
	AREA (ha)	(ton)
ÇERKEZKÖY	2.3	9
ÇORLU	2.200	7.900
ERGENE	700	2.800
HAYRABOLU	1325	4.630
KAPAKLI	9.6	24
MALKARA	1800	7.010
MARMARA	1.550	5.950
EREĞLİSİ		
MURATLI	1750	6.970
SARAY	250	1.000
SÜLEYMANPAŞA	2.250	8.300
ŞARKÖY	425	4.250

# 2.2. The Survey, Soil Collection, and Nematode Recovery

Within the scope of the study, canola fields in Malkara, Muratlı, Hayrabolu, Marmara ereğlisi, Çorlu, Süleymanpaşa, and Şarköy districts were surveyed in May, and soil samples were collected from 28 fields (Figure 1). Soils taken from 0-60 cm depth in the root zone of 5 randomly selected plants in each field were collected, and approximately 1 kg of soil was arranged per field. While sampling, attention was paid to ensure at least a 1 km distance between each field.



92

Figure 1. Map of the study area with the number of samples

Nematodes were isolated from the collected soils by the sugar centrifugation method of Jenkins [10]. In this method, 200 g of each sample was taken into a bowl, and water was added. This thoroughly mixed mixture was first passed through a 200, then 400 mesh sieve and the nematodes remaining at the bottom of the 400 mesh sieve were collected into the tube. The collected nematode suspension was first centrifuged at 1750 rpm for 5 minutes, then a solution containing 475 g/l sugar was added instead of water and centrifuged again for 1 minute. In the last step, the suspension was passed through a 400 mesh sieve, and the remaining nematodes were washed with tap water and collected.

# **2.3.** Nematode identifications, the diversity, and ecologic indices of nematodes

Species identifications were carried out from female nematodes. Slides of females were prepared by heatkilling (at 60°C) and fixing in a double-strengthened TAF (Trietmanolamine-formalin) solution [22]. The classifications were conducted based on [24]. Plant parasitic nematodes were identified on a species level, and the morphometric parameters were determined based on de Man [7].

The feeding habitat and colonizer-persister values (c-p value 1-5) of each nematode species were determined [6, 33]. The frequency of occurrence (f %) of species was calculated by dividing the number of samples in which the total number of samples recorded the species/genera. Shannon's diversity index (H'), Evenness index (J'), Enrichment (EI), Structure index (SI), Channel index (CI), Basal index (BI), Maturity index (MI), Maturity index 2-5, and Plant parasitic index (PPI) were calculated [23, 26, 8].

### 3. RESULTS AND DISCUSSION

In this study, a total of 28 taxa of nematodes were found in canola fields. Identified nematodes were divided into two groups free-living non-parasites and plant parasites. All identified taxa were classified based on feeding habitat as bacterivores, fungivores, herbivores, omnivores, and predators. Free-living nematodes included bacterivores, fungivores, omnivores, and predator species. Their c-p values ranged between 1 to 5 (Figure 2).



**Figure 2.** A. The proportion of nematode taxa in terms of feeding habitat B. The proportion of nematode taxa in terms of colonizer-persister (c-p) values

Diversity indices were determined for 28 fields, and mean values were in Table 1. Significant variations in indices were observed in 28 fields. The Channel indices (CI) that reach 100 in some locations indicate the dominance of fungivore nematodes and a higher rate of organic decomposition. Due to the higher presence of bacterivore c-p1 and fungivore c-p2 species, an Enrichment (E) value of 59.65 is calculated in one field. The Maturity index (MI) values in canola fields in Tekirdağ were between 2-3.48. Values above three were obtained from soils with good soil structure, and an MI index higher than three indicates high organic matter.

Table 1. Indices of diversity for canola fields in Tekirdağ

Indices	Mean	Minimum- maximum	
Basal (BI)	30.4±17.3	5.31-53.73	
Channel (CI)	93.03±12.6	64.7-100	
Evenness (J')	$0.8 \pm 0.12$	0.6-0.92	
Enrichment (E)	34.6±15.2	12.5-59.65	
Maturity (MI)	$2.68 \pm 0.05$	2-3.48	
Maturity 2-5	$2.69 \pm 0.51$	2-3.55	
Plant parasite (PPI)	$2.69 \pm 0.39$	2-3	
Structure (SI)	67.4±21.7	40-94.24	
Shannon's diversity	$1.8 \pm 0.26$	1.44-2.21	
(H')			

The lowest nematode population in 100 cm<sup>3</sup> soil was counted as 23, and the highest as 476. The number of nematodes was determined to be the lowest in arid soils. However, only some nematodes were isolated from soils taken from heavy, stony fields.

The most common nematode taxa in Tekirdağ were bacterivore species. In some soils, the rate of bacterivore species reached 57.3%, while in some, it remained at the level of 3%. The percentages of herbivores, bacterivores, omnivores, fungivores, and predators varied between 8.1-45.6%, 3-56.1%, 7.6-65.9%, 4.3-46.8, and 1.9-38.6% respectively. Predators were the least common, and their incidence was below 25%. *Acrobeloides, Mesodorylaimus,* and *Ditylenchus* were leading taxa in frequency and density (Table 2).

Twelve species belonging to nine genera in the Tylenchida order were determined as plant parasites. (Table 3). Migratory ectoparasites were predominant, and endoparasite *Pratylenchoides alkani* was present only in eight fields.

When species density at  $100 \text{ cm}^3$  was considered, individuals were generally found below the economic loss threshold in species like *Helicotylenchus digonicus* and *P. alkani*. Species' morphometrics and morphologic parameters also fit published descriptions (Table 3-5). The classification and frequency of occurrence are given in Table 6 and Table 7.

	Helicotylenchus		Rotylenchus		Tylenchorhynchus		Filenchus	
	digonicus	[35]	robustus	[37]	cylindricus	[2]	sheri	[13]
n	7	18	7	1	5	13	6	5
L	0.71±0.04 (0.69-0.77)	0.64-0.76	0.93±0.01 (0.90-0.95)	0.91-1.10	0.93±0.01 (0.91-0.93)	0.65-0.99	0.45±0.02 (0.41-0.47)	0.46-0.51
а	27.6±0.93 (26.3-28.4)	20-25	31.15±2.33 (28.5-34.9)	24-29	33.5±1.50 (32.1-35.65)	28-35	30.4±0.94 (29.1-31.2)	31-38
b	4.93±0.12 (4.8-5.1)	5.3-6.1	7.3±0.13 (7.3-7.58)	6.3-7.9	5.73±0.12 (5.6-5.91)	4.2-6	6.53±0.12 (6.4-6.7)	5-6
c	42.1±4.59 (36.8-48)	38-51	65.6±3.39 (61.4-69.7)	50-82	20.5±0.70 (19.6-21.3)	13-20	6.11±0.02 (6-6.2)	-
<b>c'</b>	1.04±0.23 (0.85-1.37)	-	0.84±0.02 (0.81-0.90)	-	2.56±0.12 (2.4-2.7)	-	6.9±0.34 (6.51-7.35)	6.7-7.6
Stylet	24±0.85 (24-25.1)	26-29	30.8±0.48 (29.2-30.4)	33-40	22.3±0.2 (22.1-22.6)	24-27	10	7-8
Tail	15.9±2.12 (14.3-18.9)	-	14.2±0.71 (13.6-15.2)	20-28	47.1±1.87 (44.4-49.4)	-	71.2±3.43 (67.2-75.6)	-
Vulva %	62.5±0.5 (62-63)	60-64	59.3±0.94 (58-60)	55-58	54±1.63 (52-56)	54-64	65	61-63

 $\label{eq:table 3. Morphometrics of Helicotylenchus digonicus, Rotylenchus robustus, Tylenchorhynchus cylindricus, Filenchus sheri. Measurements except for L in \mum. mean \pm (SD) range.$ 

Table 4. Morphometrics of Boleodorus tylactus, Filenchus thornei, Filenchus cylindricauda, Pratylenchoides alkani, Measurements except for L in

	Boleodorus tylactus	[30]	Filenchus thornei	[13]	Filenchus cylindricauda	[13]	Pratylenchoides alkani	[36]
n	9	-	9	1	5	1	8	-
L	0.54±0.02 (0.52-0.59)	0.5	0.72±0.09 (0.66-0.79)	0.73	0.88±0.02 (0.88-0.91)	1	0.75±0.02 (0.72-0.75)	0.82-1.21
a	36.4±0.78 (35.2-37)	21	36.4±0.78 (35.2-37)	36.7	35.5±1.2 (33.19-37)	40	28.8±0.6 (28-29.44)	29-35
b	6.5±0.37 (5.9-6.9)	5	6.5±0.37 (5.9-6.9)	7.8	5.92±0.17 (5.68-6.10)	6.5	5.17±0.2 (4.97-5.45)	4-4.9
c	4.6±0.04 (4.58-4.68)	10	4.2±0.42 (3.9-5)	3.95	6.46±0.28 (6.23-6.86)	6.5	18.6±0.94 (17.9-20)	14-17
c'	6.1±0.28 (5.68-6.48)	-	12.3±0.94 (11.3-13.6)	-	9.32±0.02 (9.21-9.43)	-	2.94±0.2 (2.69-3.26)	-
Stylet	11.4±1.16 (10-13)	12-14	10.7±0.76 (9.5-11.6)	10.5	11.6	13	17.2±0.84 (16-18.5)	22-25
Tail	45.3±4.3 (41.2-51.6)	63.5-71.5	176±4.6 (169-181)	187	136±3.74 (132-141)	-	46.9±2.24 (43.9-49.8)	-
Vulva %	60	69	60	58.1	69±0.81 (68-70)	64	55.2±0.74 (55-56)	55

 $\mu$ m. mean $\pm$  (SD) range.

94

 $\label{eq:table 5. Morphometrics of Malenchus fusiformis, Paratylenchus variabilis, Geocenamus brevidens, Tylenchorhynchus annulatus, Measurements except for L in \mum. mean \pm (SD) range$ 

	Malenchus fusiformis	[31]	Paratylenchus variabilis	[21]	Geocenamus brevidens	[2]	Tylenchorhynchus annulatus	[25]
n	2	1	2	3	6	11	5	-
L	0.33-0.36	0.35	277-295	296.1±23.2 (247.7-336.2)	0.67±0.01 (0.65-0.67)	0.54-0.69	0.93±0.03 (0.89-0.96)	0.75-0.91
а	27.2-30	17	17.7-24.6	22.8±1.4 (20.5-25.0)	23.9±1.96 (21.3-25.6)	23-27	32.6±1.61 (30.5-34.4)	26.8-35.9
b	4.8-5	5	3.95-4.21	4.2±0.4 (3.8-4.9)	3.66±1.24 (3.5-3.8)	4.2-5.2	5.26±0.24 (5-5.58)	5.3-6.8
c	5	5.1	10.22-14.14	12.9±1.6 (9.1-15.0)	12±0.65 (11.3-12.9)	11-13	16.6±0.69 (16.3-17.6)	15.6-19.1
c'	6.4-6.5	-	2.46-2.54	2.6±0.4 (2.0–3.4)	2.74 (2.73-2.75)	-	2.64±0.06 (2.55-2.7)	2.2-3.4
Stylet	10	10	17.9-21.3	17.6±0.8 (16.3-19.0)	13.2±0.9 (12.2-14.9)	14-16	18.4±0.95 (17.6-19.8)	17.7-21.8
Tail	67-72	-	27.1-27.9	23.3±3.3 (16.5-31.7)	55±4.4 (49-59.2)	-	55±3.26 (51-59)	41.4-53.6
Vulva %	65	64	85	83.9±1.0 (82.4-85.4)	55±2.44 (55-58)	-	55±1.24 (55-57)	52.2-57.5

Table 6. Free-living nematode taxa identified from canola fields in Tekirdağ

Species/genus	Order	Family	c-p class	Feeding type	Frequency of occurence	Abundance in 100 cm <sup>3</sup>
Achromadora Cobb, 1913	Chromadorida	Achromadoridae	3	Bacterivore	23	1-4
Acrobeles Cobb, 1924	Rhabditida	Cephalobidae	2	Bacterivore	26.9	3-21
Acrobeloides von Linstow, 1877	Rhabditida	Cephalobidae	2	Bacterivore	85.7	5-192
Aporcelaimellus Heyns, 1965	Dorylaimida	Aporcelaimidae	5	Omnivore	7.1	2-9
Aphelenchoides sacchari Hooper, 1958	Aphelenchida	Aphelenchoididae	2	Fungivore	21.4	2-57
Aphelenchoides obtusus Thorne & Malek, 1968	Aphelenchida	Aphelenchoididae	2	Fungivore	7.1	2-4
Aphelenchus avenae Bastian,1865	Aphelenchida	Aphelenchoididae	2	Fungivore	25	1-5
Cephalobus Bastian, 1865	Rhabditida	Cephalobidae	2	Bacterivore	57.7	5-8
Clarkus Jairajpuri, 1970	Mononchida	Mononchidae	4	Predator	25	3-5
Ditylenchus myceliophagus Goodey, 1958	Tylenchida	Anguinidae	2	Fungivore	85.1	3-57
Dorylaimus Dujardin, 1845	Dorylaimida	Dorylaimidae	4	Omnivore	25	3
Eudorylaimus Andrássy, 1959	Dorylaimida	Dorylaimidae	4	Omnivore	38.4	6-7
Mesodorylaimus Andrassy 1959	Dorylaimida	Dorylaimidae	4	Omnivore	92	4-170
Mesorhabditis Osche, 1952	Rhabditida	Rhabditidae	1	Bacterivore	21.4	3-11
Monhystera Bastian, 1865	Monhysteria	Monhysteridae	2	Bacterivore	13.3	2
Prodorylaimus Fuchs, 1930	Dorylaimida	Dorylaimidae	4	Omnivore	7.1	4-8
Rhabditis Dujardin, 1845	Rhabditida	Rhabditidae	1	Bacterivore	15.4	6-10
Seinura Fuchs, 1931	Aphelenchida	Aphelenchoididae	2	Predator	10.7	7-28
Tripyla Bastian, 1865	Triplonchida	Tripylidae	3	Predator	17.9	1-4
Wilsonema Cobb, 1913	Plectida	Plectidae	3	Bacterivore	3.6	2

Family	Genus	Species	c-p	Feeding strategy	Frequency of occurrence	Abundance in 100 cm <sup>3</sup>
Boleodorinae	Boleodorus	Boleodorus tylactus Thorne, 1941	2	M2	17.8	4-7
Belanolaimidae	Tylenchorhynchus	<i>Tylenchorhynchus annulatus</i> (Cassidy, 1930) Golden, 1971		M2	32.1	2-7
	2 2	Tylenchorhynchus cylindricus Cobb, 1913	3	M2	21.4	2-4
Hoplolaimidae	Helicotylenchus	Helicotylenchus digonicus Perry, Darling Thorne, 1959	3	M2	46.4	4-8
1	Rotylenchus	Rotylenchus robustus deMan, 1876	3	M2	21.4	2-70
Paratylenchidae	Paratylenchus	Paratylenchus variabilis, Raski, 1975	2	M2	7.1	18
Pratylenchidae	Pratylechoides	Pratylenchoides alkani Yüksel, 1977	3	M1	28.5	6-8
Telotylenchidae	Geocenamus	Geocenamus brevidens (Allen, 1955) Siddiqi	3	M2	53.7	20-48
		Filenchus sheri Siddiqi, 1986	2	M2	50	2-77
Tylenchidae	Filenchus	Filenchus thornei Andrassy, 1963	2	M2	25	3-24
		<i>Filenchus cylindricus</i> Thorne & Malek, 1968) Niblack & Bernard, 1985	2	M2	35.7	2-40
	Malenchus	<i>Malenchus fusiformis</i> (Thorne and Malek, 1968) Siddiqi, 1979	2	M2	3.6	75

Table 7. Herbivore nematode species identified from canola fields in Tekirdag, c-p values, feeding strategies, occurrence, and abundance rates

M1: Migratory endoparasite M2: Migratory ectoparasite

The results obtained from this study indicate the presence of different free-living nematode species in canola fields. Free-living nematodes play an essential role in the nutrient cycle in the soil and contribute to the improvement of soil permeability and texture. Some species can also feed on pathogens or other pests that are parasitic on plants [28]. In this study, herbivore plantparasitic nematodes, which do not cause economic yield loss in canola, were detected and were therefore called weak parasites. Virus vector species such as *Xiphinema*, *Longidorus*, and *Trichodorus* were also not found.

Our results were compatible with other researchers' studies. In rapeseed fields in Italy, nematodes belonging to 24 genera were identified. Free-living bacterivores (50.18%) and fungivores (42.90%) took the first two places regarding the occurrence rate. The most abundant genera were Aphelenchus (23.71%), Acrobeloides (20.49%).and Aphelencoides (19.18%) [14]. Additionally, nematodes such as Aphelenchoides limberi, A. daubichaensis, A. delhiensis, A. confusus, A. rutgersi, D. dipsaci, P. thornei, and P. neglectus have been detected in canola fields in several countries [5, 9]. Growth anomalies and damage have been reported in canola seedlings due to D. dipsaci feeding [29]. On the contrary, symptoms like plant damage caused by nematodes were not observed in the canola fields surveyed in this study in Tekirdağ.

On the contrary, in our study, species belonging to *Boleodorus, Filenchus, Geocenamus, Helicotylenchus, Malenchus, Paratylenchus, Pratylenchoides*, and *Rotylenchus* genera were determined in canola fields in Tekirdağ. *Boleodorus* and *Filenchus* species are known as one of the most common root hair-feeding, highly proliferative nematodes on earth with a short life cycle [15, 16]. Similarly, in our study in Tekirdağ, *Filenchus* was the most common (100% occurrence) and abundant (in highly infested fields 98 individuals/ 100 cm<sup>3</sup>). Additionally, the ability of *Filenchus* species to feed with fungal species such as *Rhizoctonia solani, Fusarium oxysporum*, and *Pythium ultimum* has been clarified in

several studies [17, 18]. Ditylenchus myceliophagus, Aphelenchus avenae, Aphelenchoides sacchari, and A. obtusus, extracted from canola fields in Tekirdağ, were the other nematodes of which fungal feeding behavior was determined in laboratory studies. Meanwhile, Pratylenchoides Geocenamus brevidens, alkani, Tylenchorhynchus annulatus, and T. cylindricus species in this study found in canola fields have previously been extracted from orchards and vegetable growing areas [12].

The number of plant-parasitic species identified in canolagrowing areas in the world was low. The glucosinolate component secreted from canola was reported to have a nematode suppressive effect on many plant-parasitic species [11]. For instance, it has been reported that the population of *Pratylenchus thornei*, a significant pest in wheat, is considerably reduced when canola is planted in the field [19]. As in other studies in the world, in this study in Tekirdağ, only a few species of plant parasitic nematodes were detected. The low number of plantparasitic species in canola fields in Tekirdağ may be attributed to the result of some nematicidal components secreted from the plant.

This study was the most recent pioneer study conducted on nematode biodiversity one of the provinces where canola is grown most extensively in Türkiye. As a result of the study, it was determined that there were no harmful nematode species such as *Meloidogyne* in the canola fields, and the populations of the detected species were not at a level that would cause harm.

### 4. CONCLUSION

In a study covering canola fields in Tekirdağ, Turkey, nematodes from different trophic groups were identified. They were classified as free-living non-plant parasitics and plant parasitics. Free-living nematodes belong to 19 genera, while plant parasitics belong to 9 genera. Most free-living were bacterivores, and plant parasitics were ectoparasite species. None of the plant-parasitic species was quarantined pests. Except for *Pratylenchoides alkani*  and *Rotylenchulus robustus*; the rest of the species did not cause significant plant damage, even under higher populations. It is unknown whether the role of the plant's allelochemical secretions is responsible for the low nematode richness. More studies must be conducted to reveal the interaction between the canola plant and nematode population growth.

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