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Original article (Orijinal araştırma)

Effect of green manure on nematode populations in onion production[1]

Soğan yetiştiriciliğinde yeşil gübrelemenin nematod popülasyonları üzerindeki etkisi

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Abstract

Allium cepa L. (Asparagales: Amaryllidaceae) is an essential crop for human nutrition. Besides its nutritional properties, it holds an important place due to its immune-boosting effects. The effect of green manure treatments with three biofumigant plant species for controlling plant-parasitic nematodes, which cause economic losses in onion production, has been investigated. Forage turnip, *Brassica rapa* L. (Brassicales: Brassicaceae), brown mustard, *Brassica juncea* (L.) Hook.f. & Thomson (Brassicales: Brassicaceae) and common vetch, *Vicia sativa* L. (Fabales: Fabaceae) were grown before onion production and incorporated into the soil in May-June in the study undertaken in a grower field in Karaman Province in 2021-2024. Total plant-parasitic nematode numbers were between 0-270.9 nematodes/100 g dry soil in green manure treatments. *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 (Rhabditida: Anguinidae) (35%), *Pratylenchus* spp. Filipjev, 1936 (Rhabditida: Pratylenchidae) (29%), *Heterodera* spp. Schmidt, 1871 (Rhabditida: Heteroderidae) (3%), *Paratylenchus* spp. Micoletzky, 1922 (Rhabditida: Tylenchulidae) (22%), *Geocenamus* spp. Thorne & Malek, 1968 (Rhabditida: Dolicodoridae) (8%) and *Helicotylenchus* spp. Steiner, 1945 (Rhabditida: Hoplolaimidae) (3%) were the plant parasitic nematodes. The most abundant plant parasitic nematodes were *Ditylenchus dipsaci* and *Pratylenchus* spp. Nematode populations did not differ by green manure treatments while they were affected by precipitation and varied among growing years.

Keywords: Biofumigation, cultural management, green manure, plant parasitic nematodes, population development

Öz

Allium cepa L. (Asparagales: Amaryllidaceae) insan beslenmesinde vazgeçilmez ürünlerden birdir. Besleyici özelliklerinin yanında bağışıklığı destekleyici etkileri nedeniyle önemli bir yere sahiptir. Soğan üretiminde ekonomik kayıplara neden olan bitki paraziti nematodların kontrolü için biyofumigasyon etkinliği bulunan üç bitki türü ile yeşil gübreleme uygulamalarının etkinliği araştırılmıştır. Karaman İli'nde üretici tarlasında 2021-2024 yıllarında yapılan çalışmada soğan üretimi öncesi yem şalgamı, *Brassica rapa* L. (Brassicales: Brassicaceae), kahverengi hardal, *Brassica juncea* (L.) Hook.f. & Thomson (Brassicales: Brassicaceae) ve adi fiğ, *Vicia sativa* L. (Fabales: Fabaceae) bitkileri yetiştirilerek Mayıs-Haziran ayında toprağa karıştırılmıştır. Yapılan çalışmalarda toplam bitki paraziti nematod sayıları 0-270.9 nematod/ 100 g kuru toprak olarak elde edilmiştir. Tespit edilen bitki paraziti nematodlar; *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 (Rhabditida: Anguinidae) (%35), *Pratylenchus* spp. Filipjev, 1936 (Rhabditida: Pratylenchidae) (%29), *Heterodera* spp. Schmidt, 1871 (Rhabditida: Heteroderidae) (%3), *Paratylenchus* spp. Micoletzky, 1922 (Rhabditida: Tylenchulidae) (%19), *Geocenamus* spp. Thorne & Malek, 1968 (Rhabditida: Dolicodoridae) (%8) ve *Helicotylenchus* spp. Steiner, 1945 (Rhabditida: Hoplolaimidae) (%3) olup; en baskın nematod türleri ise *Ditylenchus dipsaci* ve *Pratylenchus* spp. olarak belirlenmiştir. Nematod popülasyonları yeşil gübre uygulamalarına göre farklılık göstermezken, yağış miktarından önemli oranda etkilenmiş ve yıllara göre değişiklik göstermiştir.

Anahtar sözcükler: Biyofumigasyon, kültürel mücadele, yeşil gübre, bitki paraziti nematodlar, popülasyon gelişimi

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Introduction

Onion, belonging to the Alliaceae family, has nutritional and medicinal importance. Rich in minerals such as phosphorus and calcium, carbohydrates and protein and vitamin C. The onion plant has sweet taste, aromatic, thermogenic, antiperiodic, antibacterial, stimulant, appetite-stimulating, diuretic and tonic properties (Sitapara et al., 2024). Onions have high economic value and play a vital role in human nutrition, with 111 million tons production worldwide. India, China and Egypt are the top three producers. Türkiye is 5th globally, after United States with a production value of 2.6 million tons in 2023 (FAO, 2025).

Biofumigation via green manure application provided promising results for sustainability of the soil (Jian et al., 2020; Fan et al., 2021; Rasa et al., 2021). Green manure affects soil microbial diversity and abundance by improving soil physicochemical properties (Salahin et al., 2013; Yang et al., 2016; Nivelle et al., 2016; Iderawumi & Kamal, 2022). It positively contributes to soil microbial community structure by enhancing soil organic material (Xie et al., 2017; Asghar & Kataoka, 2022; Prajapati et al., 2023). Green manure significantly increases the abundance and diversity of beneficial organisms in the ecosystem (Ga´mez-Virue et al., 2010; Moreira et al., 2016). Green manure application increases the tolerance of the plants to biotic stress as it causes well development of the crops (Liu et al., 2021; Pott et al., 2021).

There are over 20,000 identified species of nematodes belonging to the phylum Nematoda, 4,100 of which are plant-parasitic nematodes (Abd-Elgawad & Askary 2015; Mitiku, 2018). Plant-parasitic nematodes are in the fourth place among the plant growth constraints which cause annual economic damage of about 200 billion dollars worldwide (Huang et al., 2020). Chemical applications provide economical management in high value plant species, while mostly cultural management practices are used for field crops (Sasanelli et al., 2021; Khan et al., 2022). Besides plant based cultural management practices such as planting cover crops, intercropping, green manure applications and traps crops are increasing (Monfort et al., 2007; Lopez-Perez et al., 2010). Riga (2011) investigated the effectiveness of the Brassicaceae plants as green manure in combination with organochloride content nematicide; 1,3-Dikloropropen under greenhouse and field conditions. The population levels of *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, 1980 (Rhabditida: Meloidogynidae), *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941 (Rhabditida: Pratylenchidae) and *Paratrichodorus allius* Jensen, 1963 (Triplonchida: Trichodoridae) populations were reduced, while free living nematodes and nonpathogenic *Pseudomonas* spp. Migula, 1894 (Pseudomonadales: Pseudomonadaceae) populations were not affected. Economic analysis of this study revealed; 35% economic benefits compared to chemical management. Dutta et al. (2019) suggested the biofumigation for integrated management of plant parasitic nematodes based on current studies on different species of plant parasitic nematodes. Green manure applications of 15 different Brassicaceae plant species significantly reduced the development of *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Rhabditida: Meloidogynidae) (McLeod & Steel, 1999). Recently Musa (2022) reported variable effects of different Brassicaceae species and hairy vetch green manure applications on *Ditylenchus* spp. Filipjev, 1936 (Rhabditida: Anguinidae) populations on faba bean production. Based on promising results of biofumigant plants on plant parasitic nematodes in vitro and in vivo conditions, therefore the objective of the study is to evaluate the effect of the forage turnip, *Brassica rapa* L., brown mustard, *Brassica juncea* (L.) Hook.f. & Thomson (Brassicales: Brassicaceae) and common vetch, *Vicia sativa* L. (Fabales: Fabaceae) as green manure treatments in field conditions for onion production for nematode population growth.

Materials and Methods

Experimental area

The experiment was set to investigate the effect of green manure treatments as a plant protection method against stem and bulb nematode, *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 (Rhabditida:

Anguinidae). The experiments were carried out between 2021 and 2024 in a grower field infested with stem and bulb nematode in Karaman Province (37°05'43.8"N 33°06'18.7"E). *Ditylenchus dipsaci* infested in the experiment area was identified by PCR technique using species specific primers according to Yavuzaslanoglu et al. (2018).

Experimental design

Forage turnip, *Brassica rapa* cv. Polybra (Ulusoy Agriculture, Ankara), brown mustard, *Brassica juncea* cv. ISCI99 (Farmi Türkiye, İzmir) and common vetch, *Vicia sativa* Selçuk99 (Akdanoğlu Agriculture, Karaman) were used in the experiment as green manure plants based on their biofumigation potential (Kruger et al., 2013).

Green manure seeds were planted on 10.03.2021 and 30.09.2022 in the first and second repetitions of the experiment, respectively. The green manure plants were incorporated into the soil at flowering stage in 06.06.2021 and 13.05.2023. The optimum onion planting time in Central Anatolian Region was mid-March-beginning of April, it would be late for onion planting after green manure incorporation, therefore onion planting was carried out in the following years of green manure application. The experiment carried out between the growing seasons of 2021 and 2024.

The experiment was carried out in randomized block design with four replications. According to the standard pesticide experimental instructions, the plot sizes were arranged as 28 m^2 (7X4 m). A 140 cm safety strip has been left between the blocks to avoid the effect of soil drift during plowing, and a 100 cm safety strip has been left between the plots within the block (Anonymous, 2019).

The inter-row and on-row distances of green manure plants were set as 35x10 cm for forage turnip and 17.5x5 cm for mustard and common vetch plants. Plants were irrigated to ensure their emergence.

In the experiment, negative control plots did not receive any treatment. Only weed control has been carried out in the plots. When green manure treatments were incorporated into the soil, a registered chemical fumigant for nematodes (Metam Fluid®, Safa Agriculture, Konya) with the active ingredient of metam sodium (500 g/ L), was applied to the positive control plots at the dose of 100 L/ decare (2.8 L/ plot), which was recommended by the manufacturer. Positive control plots were covered with transparent plastic for 15 days after chemical application.

The incorporation of green manure plants into the soil was carried out by plowing with a rotary machine. After incorporation, it was waited for 15 days for deterioration of green manure plants. After that, onion variety Betapanko (Beta Tohum, Konya), susceptible to stem and bulb nematode (Yavuzaslanoglu, 2019) was planted to all the experiment plots at a planting density of 35x10 cm.

The amount of green manure incorporated in a plot was calculated by weighing the amount of green manure plants grown in 1 m^2 area in the plot and multiplying by 28 m^2 plot area. The nutrient requirements of the plants were met with synthetic fertilizer. All the phosphorus and nitrogenous (Di Ammonium Phosphate; 18% N, 46% P) were given at planting. The amount of fertilizer was calculated as 10 kg of pure phosphorus per decare, according to the requirements of the plants. In the experimental plots, weed control was carried out physically and irrigation was carried out when necessary. No other plant protection application has been carried out.

Nematode population monitoring

Sampling for nematode populations from plant and soil was carried out monthly throughout the experiment, in April, May and June when the nematodes were most active in their habitats. Modification of the "Baermann Funnel Method" was used to extract nematodes from plant and soil samples (Hallmann & Subbotin, 2018). To extract motile nematodes from soil samples, 100 g of soil sample was placed on a filter paper covered sieve, in a 15 cm diameter petri dish that was filled with water and allowed for extraction for 48 hours to collect nematodes in the bottom of the petri dish. At the end of the period, petri dishes were transferred into cylindrical measures to sink to the bottom of the measures for at least 8 hours, then the water volume was reduced to fit into 15 ml tubes, allowing the nematodes to settle to the bottom and water volume was reduced to 1 ml and nematode suspension and condensed in a 15 ml test tube (Hallmann & Subbotin, 2018). Nematodes were counted in 100 µl of the prepared samples under the microscope at 10x magnification. Nematode numbers were multiplied by 10 to determine the total number of nematodes in 1 ml of sample and therefore in 100 g of fresh soil. Dry weight of 10 g soil was determined by drying soil at 110ºC for 2 nights. Nematode numbers were converted as per 100 g of dry soil by multiplying the nematode numbers counted in 100 g fresh soil with multiplication factor (100/dry weight of 100 g fresh soil for each sample). Nematodes were identified morphologically at genus level. Nematodes were then separated into feeding groups according to Yeates et al. (1993) and population changes were evaluated. Taxonomic classification of the nematodes was made according to GBIF (2026).

Statistical analysis

Data of biomass and nematode numbers per 100 of dry soil provided normality by Shapiro Wilk test. Data for biomass and nematode populations were analyzed using two-way Analysis of Variance (ANOVA), considering treatment, year/sampling time, and their interactions as fixed effects. Mean separations were performed using Tukey’s Honestly Significant Difference (HSD) test at a significance level of $p<0.05$. Multivariate correlation analysis was conducted between variables of nematode numbers and temperature and precipitation data during the experiment in 2021-2024. Significant relationships were determined by pairwise correlations. Scatterplot of correlations were presented and correlation coefficient (r) for each relationship was indicated on the scatterplot with a circle color map. Statistical analysis was performed using JMP© Pro16.0.0 software (JMP, 2025).

Results and Discussion

Soil temperatures and precipitation in the experimental area

Monthly average soil temperature at a depth of 20 cm and monthly total precipitation in Karaman from 2021 to 2024, during the experiment, was obtained from the Karaman Provincial Directorate of Meteorology and is presented in Figure 1.

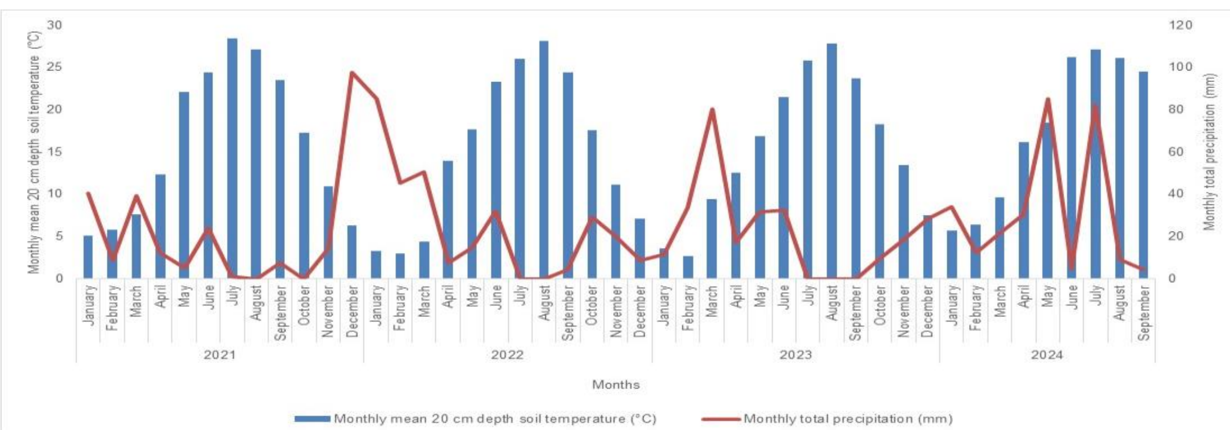

Figure 1. Monthly mean soil temperatures at 20 cm soil depth (ºC) and monthly total precipitation (mm) in Karaman in 2021-2024.

Between April and June when the plants were actively growing, soil temperatures varied between 12.3-26.2ºC, during the experiments. Temperatures were between 25.8-28.4ºC from July to August. From September to April, the monthly mean lowest temperature was 2.7ºC and the highest 24.5ºC. Precipitation was higher in December-June and lowered in July-November in all years of the experiment. Total precipitation was between 251-297 mm per year during the experiment. The highest precipitations recorded in each year were in December 2021 (97.8 mm), January 2022 (85 mm), March 2023 (80.4 mm) and May and July 2024 (85 and 82.2 mm).

Biomass weight of green manure plants

The biomass weights of green manure plants differed across years, and green manure treatments and their interactions were also found significantly different (Table 1).

Table 1. Effect of year, treatment, replication and year*treatment interaction on green manure biomass in the experiment.

Source	DF	Sum of Squares	F Ratio	Prob > F
Year	1	2552,7563	74,0095	<,0001
Treatment	2	753,5073	10,9228	0,0012
Replication	3	59,1789	0,5719	0,6421
Year*treatment	2	2129,5122	30,8694	<,0001

* The significance level is $p \leq 0.05$.

Biomass weight of brown mustard was a mean of 22.0 and 26.6 kg/28 m^2 plot area in the two years of the experiment. It did not differ between the years. However, the biomass of forage turnip and common vetch was higher in the second year of the experiment. Biomass of forage turnip was the highest in the first year of the experiment (23.8 kg/plot), while common vetch gave the highest biomass in the second year of the experiment (61.3 kg/plot) (Table 2).

Table 2. Biomass weights (kg/plot) obtained from the treatments (Data were presented in the form of mean±standard error of mean).

Treatments	2021	2023
Brown mustard	22.0±0.9bc	26.6±4.8bc
Forage turnip	23.8±1.2bc	34.0±2.8b
Common vetch	14.3±0.8c	61.3±3.6a
Positive control	-	-
Negative control	-	-

Significant differences were indicated by different lowercase letters, -: not applicable.

Nematode populations

Nematode species belonging to 4 feeding groups were determined in the monthly samplings during 2021-2024 from the experimental plots. The results revealed that plant parasitic nematodes, fungivores nematodes, bacterivorous nematodes and omnivorous nematodes were distributed in the experimental area.

Plant parasitic nematode genera detected in the experimental area were *Ditylenchus* spp. (35%), *Pratylenchus* spp. Filipjev, 1936 (Rhabditida: Pratylenchidae) (29%), *Heterodera* spp. Schmidt, 1871 (Rhabditida: Heteroderidae) (3%), *Paratylenchus* spp. Micoletzky, 1922 (Rhabditida: Tylenchulidae) (22%), *Geocenamus* spp. Thorne & Malek, 1968 (Rhabditida: Dolicodoridae) (8%) and *Helicotylenchus* spp. Steiner, 1945 (Rhabditida: Hoplolaimidae) (3%). The most abundant plant parasitic nematode genera in the experiment area were *Ditylenchus dipsaci* and *Pratylenchus* spp. species.

In the experiment total plant parasitic nematode population level varied between 0-270.9 nematodes/ 100 g dry soil. Nematode populations differed significantly only among sampling times. Green manure treatments and their interactions with sampling times did not show any significant differences (Table 3).

Table 3. Effect of treatment, replication, sampling time and treatment*sampling time interactions on total number of plant parasitic nematodes in the experiment

Source	DF	Sum of Squares	F Ratio	Prob > F
Treatment	4	33214,65	2,1601	0,0754
Replication	3	1747,68	0,1515	0,9286
Sampling time	11	942493,8	22,2888	<,0001*
Treatment*sampling time	44	157106,2	0,9288	0,6021

* The significance level is $p \leq 0.05$.

The highest number of plant parasitic nematodes was recorded in April and May 2021 and May 2024 in positive control treatment (mean 145.6-175.5 nematodes/ 100 g dry soil). The highest number of nematodes was obtained in May 2024 for other treatments. The numbers of nematodes in all treatments were low in 2023 during the experiment (Table 4).

Table 4. Total number of plant parasitic nematodes/ 100 g dry soil at sampling dates in the green manure treatments (Data were presented in the form of mean±standard error of mean)

Sampling time and date	Green manure treatments				
	Brown mustard	Forage turnip	Common vetch	Positive control	Negative control
1. 8.04.2021	121.6±35.6ab	215.7±40.6ab	82.8±22.9ab	145.6±42.1a	145.1±42.2ab
2. 5.05.2021	40.2±23.1bc	172.7±40.5abc	78.5±9.9ab	175.5±12.1a	161.5±30.9ab
3. 8.06.2021	64.0±11.7bc	64.8±25.2bcd	81.1±40.4ab	102.4±30.3ab	146.8±40.3ab
4. 20.04.2022	36.3±18.7c	30.4±13.6cd	38.1±14.1b	27.1±2.4b	36.7±11.5bc
5. 10.05.2022	33.4±7.2c	51.2±4.9cd	49.8±6.8ab	29.1±7.3b	48.1±16.8bc
6. 9.06.2022	18.9±8.1c	79.9±53.4bcd	18.9±6.6b	21.7±9.9b	20.9±14.2bc
7. 6.04.2023	2.0±0.0c	2.0±0.0d	2.0±0.0b	2.0±0.0b	2.0±0.0c
8. 13.05.2023	5.6±3.2c	0.0±0.0d	0.0±0.0b	0.0±0.0b	0.0±0.0c
9. 17.06.2023	5.6±5.6c	0.0±0.0d	5.7±3.3b	0.0±0.0b	0.0±0.0c
10. 29.04.2024	85.9±16.3bc	99.8±33.9abcd	87.2±25.0ab	24.7±7.1b	54.1±26.8bc
11. 27.05.2024	179.1±25.8a	239.1±35.6a	270.9±141.4a	167.1±49.5a	215.9±65.9a
12. 30.06.2024	35.4±11.8c	103.3±54.1abcd	61.5±35.3ab	89.4±41.7ab	42.0±4.2bc

Significant differences in the columns were indicated by different lowercase letters.

The most abundant plant parasitic nematodes of *D. dipsaci* and *Pratylenchus* spp. were examined separately. The population of *D. dipsaci* changed 0-248.2 nematodes/ 100 g dry soil and 0-13.3 /plant in the experiment. The only sampling time was significantly different in the soil population of *D. dipsaci* (Table 5). The plant populations were not significantly different among treatments and sampling times (Data were not presented).

Table 5. Effect of treatment, replication, sampling time and treatment*sampling time interactions on total number of *D. dipsaci* / 100 g dry soil in the experiment

Source	DF	Sum of Squares	F Ratio	Prob > F
Treatment	4	16503,44	1,5124	0,2005
Replication	3	2086,72	0,255	0,8577
Sampling time	11	632910,5	21,0919	<,0001*
Treatment*sampling time	44	64848,07	0,5403	0,9911

* The significance level is $p \leq 0.05$.

The population of stem and bulb nematode increased significantly in the last year of the experiment. The highest nematode numbers were observed in May 2024. In the first two years of the experiment, the nematode population varied between 2.9-86 nematodes/100 g soil in all treatments. No nematodes were found during the third year of the experiment. In the fourth year, 12.7-248.2 nematodes/ 100 g dry soil were recorded in all treatments (Table 6).

Table 6. Number of *D. dipsaci* / 100 g dry soil at sampling dates in the green manure treatments (Data were presented in the form of mean±standard error of mean).

Sampling time and date	Green manure treatments				
	Brown mustard	Forage turnip	Common vetch	Positive control	Negative control
1. 8.04.2021	24.8±8.9c	86.0±22.2b	30.0±11.2b	44.6±4.3b	33.0±11.6b
2. 5.05.2021	10.6±7.5c	28.2±3.2b	48.0±7.9ab	55.1±23.3b	46.5±20.2b
3. 8.06.2021	23.9±6.7c	36.2±12.1b	29.0±16.9b	45.8±10.5b	58.1±25.1b
4. 20.04.2022	17.8±7.5c	14.9±5.4b	20.3±6.9b	9.0±5.0b	25.3±10.5b
5. 10.05.2022	9.4±6.2c	27.2±11.2b	23.1±12.5b	2.9±2.9b	14.1±7.1b
6. 9.06.2022	13.5±5.1c	54.7±40.6b	16.2±6.7b	5.4±3.1b	7.8±7.8b
7. 6.04.2023	1.0±0.0c	1.0±0.0b	1.0±0.0b	1.0±0.0b	1.0±0.0b
8. 13.05.2023	0.0±0.0c	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0b
9. 17.06.2023	2.8±2.8c	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0b
10. 29.04.2024	81.6±14.8b	90.5±30.9b	73.1±24.7ab	12.7±7.6b	49.1±27.7b
11. 27.05.2024	157.5±16.9a	226.1±40.5a	248.2±138.4a	151.5±44.2a	204.3±63.3a
12. 30.06.2024	24.1±8.6c	81.5±48.1b	45.9±29.5ab	74.3±42.4ab	31.5±3.7b

Significant differences in the columns were indicated by different lowercase letters.

The number of *Pratylenchus* spp. in 100 g dry soil significantly varied among the sampling dates in the experiment (Table 7).

Table 7. Effect of treatment, replication, sampling time and treatment*sampling time interactions on total number of *Pratylenchus* spp./ 100 g dry soil in the experiment

Source	DF	Sum of Squares	F Ratio	Prob > F
Treatment	4	484,1024	1,111	0,3529
Replication	3	514,1284	1,5732	0,1975
Sampling time	11	6060,278	5,0576	<,0001*
Treatment*sampling time	44	6316,958	1,318	0,1084

* The significance level is $p \leq 0.05$.

The number of *Pratylenchus* spp./ 100 g dry soil was significantly different across sampling dates in forage turnip and negative control treatments. The highest population was 36.8 nematodes/ 100 g dry soil in forage turnip in May 2021. In the negative control treatment, the highest population was recorded in May 2022 being 19.8 nematodes/ 100 g dry soil (Table 8).

Table 8. Number of *Pratylenchus* spp./ 100 g dry soil at sampling dates in the green manure treatments (Data were presented in the form of mean±standard error of mean).

Sampling time and date	Green manure treatments				
	Brown mustard	Forage turnip	Common vetch	Positive control	Negative control
1. 8.04.2021	6.1±5.1a	25.1±14.1ab	1.0±0.0a	6.2±3.0a	16.4±6.6ab
2. 5.05.2021	2.6±2.6a	36.8±8.5a	8.2±2.7a	16.6±9.7a	19.5±5.4ab
3. 8.06.2021	8.1±5.1a	7.7±4.8b	23.3±20.0a	10.7±4.4a	15.6±5.2ab
4. 20.04.2022	3.7±2.7a	9.5±5.4ab	9.1±5.3a	3.4±2.4a	8.6±4.7ab
5. 10.05.2022	9.3±5.8a	3.1±3.1b	8.5±5.5a	0.0±0.0a	19.8±7.2a
6. 9.06.2022	2.5±2.5a	8.3±5.1ab	0.0±0.0a	5.3±2.4a	2.6±2.6ab
7. 6.04.2023	1.0±0.0a	1.0±0.0b	1.0±0.0a	1.0±0.0a	1.0±0.0ab
8. 13.05.2023	0.0±0.0a	0.0±0.0b	0.0±0.0a	0.0±0.0a	0.0±0.0b
9. 17.06.2023	2.8±2.8a	0.0±0.0b	5.7±3.3a	0.0±0.0a	0.0±0.0b
10. 29.04.2024	3.1±1.1a	5.5±3.3b	6.5±2.9a	4.5±3.5a	1.0±0.0ab
11. 27.05.2024	17.3±13.8a	5.7±4.1b	12.8±5.3a	12.7±6.2a	4.3±2.8ab
12. 30.06.2024	3.7±2.4a	3.8±3.8b	3.9±2.5a	5.1±2.0a	3.9±1.3ab

Significant differences in the columns were indicated by different lowercase letters.

The plant populations of *Pratylenchus* spp. were between 0-3.3 nematodes/ plant in the experiment. The highest number of nematodes was recorded in May 2023 in common vetch and brown mustard treatments being 3.3 and 1.6 nematodes/ plant, respectively.

Tylenchus spp. Bastian, 1865 (Rhabditida: Tylenchidae) (28%), *Filenchus* spp. Andrássy, 1954 (Rhabditida: Tylenchidae) (1%), *Aphelenchus* spp. Bastian, 1865 (Rhabditida: Aphelenchidae) (29%) and *Aphelenchoides* spp. Fischer, 1894 (Rhabditida: Aphelenchoididae) (42%) were the fungivores nematodes found in the green manure experimental plots. The occurrence frequencies are in parentheses.

The populations of soil fungivores nematodes ranged between 0-490.8 nematodes/ 100 g of dry soil in the experiment. The total number of fungivores nematodes significantly differed across sampling times in this experiment while there was not any significant difference among green manure treatments (Table 9).

Table 9. Effect of treatment, replication, sampling time and treatment*sampling time interactions on total number of fungivores nematodes/ 100 g dry soil in the experiment

Source	DF	Sum of Squares	F Ratio	Prob > F
Treatment	4	42270,3	1,4513	0,2191
Replication	3	50982,9	2,3339	0,0756
Sampling time	11	2364595	29,522	<,0001*
Treatment*sampling time	44	274165,8	0,8557	0,7237

* The significance level is $p \leq 0.05$.

Total number of fungivores nematodes was the highest in 2021, with an average of 253.1 nematodes/ 100 g dry soil recorded in all treatments. The fungivores nematode populations decreased in 2022 and 2023 similarly to plant parasitic nematodes; the average number of fungivores nematodes was 47.3 nematodes/ 100 g dry soil in all treatments. The populations were recorded higher in 2024 being on average 143.6 nematodes/ 100 g dry soil in all treatments (Table 10).

Table 10. Total number of fungivores nematodes/ 100 g dry soil at sampling dates in the green manure treatments (Data were presented in the form of mean±standard error of mean).

Sampling time and date	Green manure treatments				
	Brown mustard	Forage turnip	Common vetch	Positive control	Negative control
1. 8.04.2021	185.8±54.1ab	240.8±28.3b	164.1±29.9ab	273.8±95.0ab	342.0±91.7a
2. 5.05.2021	114.5±8.1bc	163.6±32.3bc	89.2±19.2b	224.4±46.3abc	159.9±18.1abc
3. 8.06.2021	88.8±43.1bc	100.9±18.8bc	63.8±28.2b	99.8±21.3bcd	94.1±27.8bc
4. 20.04.2022	92.9±18.3bc	68.4±25.7bc	81.2±31.9b	79.2±42.5bcd	68.2±13.6c
5. 10.05.2022	79.1±23.7bc	94.4±20.8bc	33.3±16.8b	87.9±21.0bcd	44.9±9.9c
6. 9.06.2022	14.0±8.4bc	5.7±5.7bc	24.6±20.7b	19.6±5.5cd	23.2±7.1c
7. 6.04.2023	0.0±0.0c	0.0±0.0c	8.6±8.6b	13.2±13.2d	49.1±45.3c
8. 13.05.2023	16.7±5.5bc	19.1±11.2bc	8.4±5.4b	5.7±5.7d	33.7±4.5c
9. 17.06.2023	150.8±36.1abc	149.7±51.9bc	171.4±24.6ab	80±11.9bcd	89.7±21.6bc
10. 29.04.2024	149.4±27.8abc	221.2±29.7bc	168.0±34.7ab	127.8±37.9bcd	228.8±79.6abc
11. 27.05.2024	107.7±37.8bc	171.5±65.9bc	136.1±22.9b	100.3±13.9bcd	102.3±16.1bc
12. 30.06.2024	3.7±2.4a	3.8±3.8b	3.9±2.5a	5.1±2.0a	3.9±1.3ab

Significant differences in the columns were indicated by different lowercase letters.

Cephalobus spp. Bastian, 1865 (Rhabditida: Cephalobidae) (16%), *Eucephalobus* spp. Steiner, 1936 (Rhabditida: Cephalobidae) (26%), *Acrobeles* spp. von Linstow, 1877 (Rhabditida: Cephalobidae) (5%), *Acrobeloides* spp. (Cobb, 1924) Thorne, 1937 (Rhabditida: Cephalobidae) (35%), *Rhabditus* spp. Dujardin, 1844 (Rhabditida: Rhabditidae) (7%), *Panagrolaimus* spp. Fuchs, 1930 (Rhabditida: Rhabditidae) (3%), *Wilsonema* spp. Cobb, 1913 (Plectida: Plectidae) (4%) and *Monhystera* spp. Bastian, 1865 (Monhisterida: Monhisteridae) (4%) were the bacterivorous nematodes in the experimental area. The abundance of genera of *Acrobeloides*, *Eucephalobus* and *Cephalobus* was higher.

The number of bacterivorous nematodes differed across experiment replications. The number of bacterivorous nematodes differed significantly among sampling times. Green manure treatments have no significant effect on bacterivorous nematodes (Table 11).

Table 11. Effect of treatment, replication, sampling time and treatment*sampling time interactions on total number of bacterivorous nematodes/ 100 g dry soil in the experiment

Source	DF	Sum of Squares	F Ratio	Prob > F
Treatment	4	429106,1	1,7923	0,1324
Replication	3	1001623	5,5782	0,0011*
Sampling time	11	6165381	9,3643	<,0001*
Treatment*sampling time	44	2068614	0,7855	0,8259

* The significance level is $p \leq 0.05$.

Total number of bacterivorous nematodes in the soil from the experiment ranged between 2.8-989.7 nematodes/ 100 g of dry soil. The highest populations belonged to bacterivorous nematodes among the feeding groups of nematodes detected in the experimental area. The highest nematode populations were obtained in 2021 in all green manure treatments. It decreased in 2022 and 2023 and increased again in 2024 as in other nematode feeding groups. The average number of nematodes from all treatments was 141.2 nematodes/ 100 g dry soil (Table 12).

Table 12. Total number of bacterivorous nematodes/ 100 g dry soil at sampling dates in the green manure treatments (Data were presented in the form of mean±standard error of mean).

Sampling time and date	Green manure treatments				
	Brown mustard	Forage turnip	Common vetch	Positive control	Negative control
1. 8.04.2021	274.7±60.1a	464.6±102.8a	447.7±190.8a	989.7±615.7a	864.6±607.4a
2. 5.05.2021	124.5±31.9abc	246.3±50.8abc	181.64.1ab	410.244.1ab	623.5±177.0a
3. 8.06.2021	79.4±31.1bc	174.1±25.5bc	104.7±19.8b	270.8±106.2ab	341.8±88.0a
4. 20.04.2022	212.1±66.1ab	213.3±84.5abc	125.9±39.8b	95.6±14.1ab	128.9±45.5a
5. 10.05.2022	129.4±53.2abc	71.2±29.0bc	53.2±14.6b	82.1±43.4ab	73.9±11.5a
6. 9.06.2022	77.1±22.3bc	113.2±66.5bc	38.7±16.2b	90.7±17.8ab	51.8±21.3a
7. 6.04.2023	11.6±6.7c	11.3±4.6c	11.6±4.9b	20.1±11.8b	18.1±7.2a
8. 13.05.2023	25.9±14.9bc	8.4±5.3c	2.8±2.8b	26.8±16.4ab	22.0±10.7a
9. 17.06.2023	11.0±6.3c	10.9±6.3c	14.2±7.1b	8.6±5.4b	19.8±7.1a
10. 29.04.2024	102.7±30.9abc	103.1±21.3bc	85.2±27.1b	138.7±50.5ab	62.8±16.7a
11. 27.05.2024	175.2±38.1abc	272.2±37.2ab	125.7±17.1b	111.6±32.2ab	239.8±49.7a
12. 30.06.2024	163.4±42.8abc	142.0±56.1bc	130.3±13.0b	148.7±50.2ab	116.7±13.2a

Significant differences in the columns were indicated by different lowercase letters.

Omnivorous nematodes belonging to the order Dorylaimida were recorded at low population density (0-99.2 nematodes/100 g dry soil) in the experiment.

Multivariate correlation analysis revealed that the numbers of *Ditylenchus* spp. (r=0.72), *Aphelenchus* spp. (r=0.68), *Acrobeles* spp. (r=0.87) and *Panagrolaimus* spp. (r=0.87) were significantly correlated with monthly total precipitation in Karaman Province in 2021-2024 (Figure 2). Distribution of significantly related nematode populations and precipitation in the sampling times was presented on Figure 3.

Direct effect of green manure plants on soil nematodes is based on the formation of toxic compounds from bioactive compounds released from green manure plant tissues. In addition, green manure has indirect effects on nematode populations such as improving soil structure and increasing the amount of organic matter, thereby increasing the populations of non-pathogenic microorganisms, development and stress tolerance of plant (Thoden et al., 2011; Lopes et al., 2019; Genç Kesimci, 2022). In Türkiye, the use of brown mustard plant as green manure in tomatoes production has improved yield and quality parameters under field conditions (Fıçıcı, 2018). It has also been shown that the use of legume plants as intercrops in cereal production systems has positive effects on subsequent crop yields (Özel & Acar, 2023). However, the selection of green manure plant species is of high importance for plant protection purposes; Brassicaceae plants are preferred for plant protection purposes as green manure due to their high glucosinolate contents. Toxic decomposition products of glucosinolates such as isothiocyanates and nitriles are responsible biofumigation activity under soil conditions (Bohinc et al., 2012). In addition to Brassicaceae, other cyanogenic pant species such as *Sorghum* spp. and *Vicia* spp. have biofumigation potential for biocontrol of soilborne pathogens (Panasiuk & Bills, 1984; Vetter, 2000).

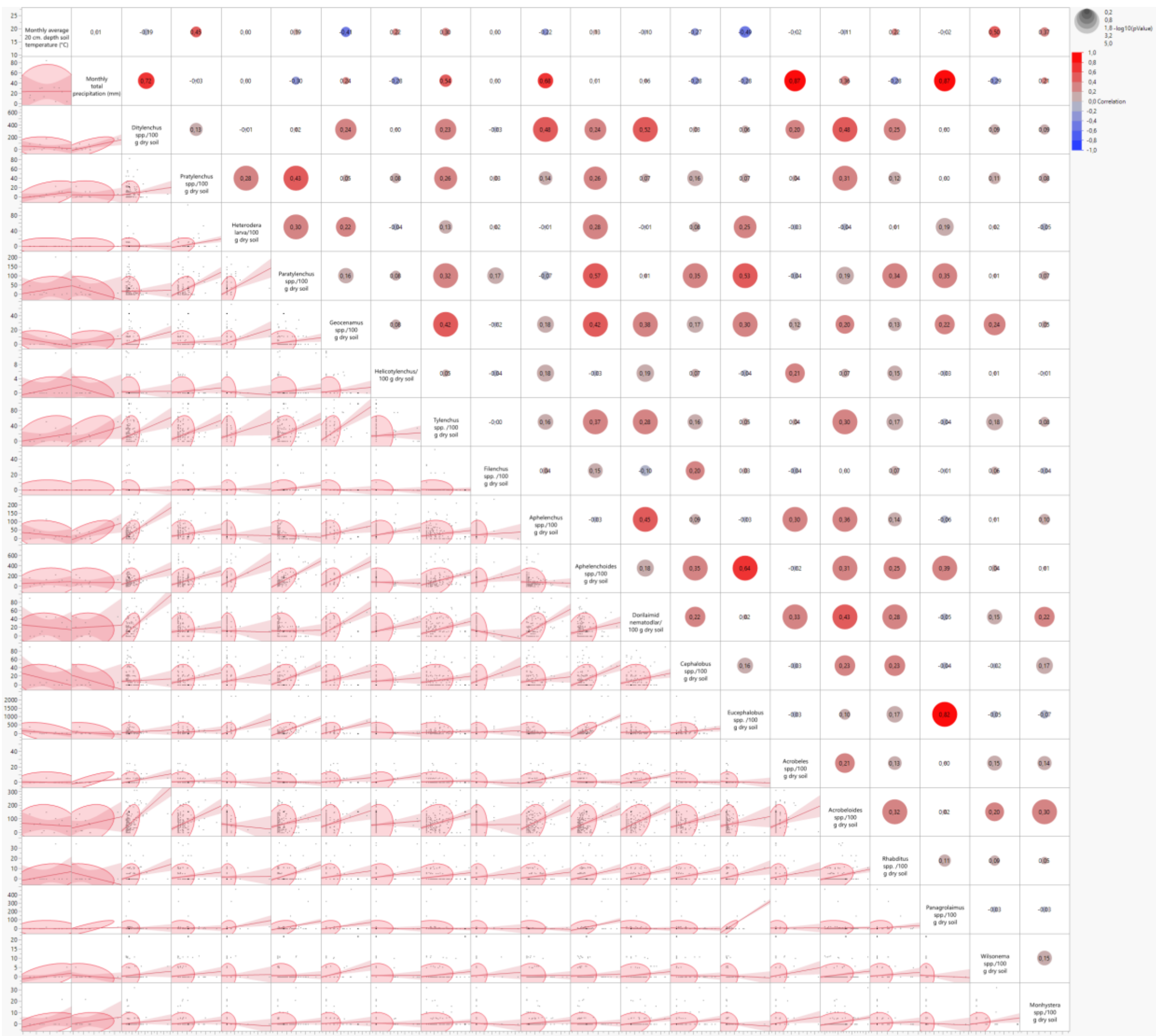

Figure 2. Scatterplot of multiple correlations between nematode numbers, monthly average 20 cm depth soil temperature and monthly total precipitation in Karaman in 2021-2024.

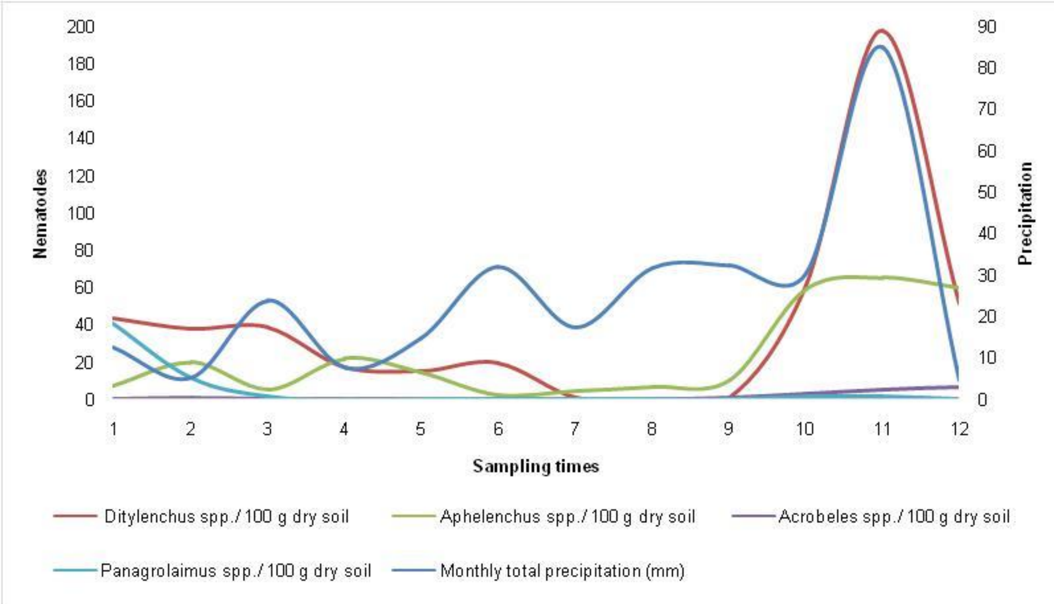

Figure 3. Numbers of nematodes and significantly correlated monthly total precipitation in Karaman in 2021-2024.

Based on the biofumigation potential of Brassica and *Vicia sativa* was investigated in the current study. There was not any significant difference among treatments on nematode populations in current study, the nematode populations were significantly higher than damage threshold for *D. dipsaci* which are 0.2-1 nematodes/ 100 g soil (Seinhorst, 1960). In the experiment, nematode population was recorded up to 248 nematodes/ 100 g of dry soil. Although there was not any significant effect of the green manure treatments on the nematode populations in the current experiment, considerable effects had been recorded on *Meloidogyne* spp. and *Rotylenchulus reniformis* Linford & Oliveira, 1940 (Rhabditida: Rotylenchulidae) populations using *Brassica* spp. on cucumber production (Waisen et al., 2020). Adverse effects of *Brassica* spp. were shown on *Globodera rostochiensis* as well (Fatemy & Sepideh, 2016). Similarly, in strawberry production, a decline in the number of *Pratylenchus* spp. was recorded with green manure applications (Belair & Coulombe, 2009). Compatible with our results; Musa (2022) reported no significant effect of the green manure applications on *D. dipsaci* on faba bean production. Only *D. gigas* populations were reduced significantly compared to fallow control treatment in green manure experiment conducted with different species of Brassicaceae and hairy vetch (Musa, 2022). *Ditylenchus dipsaci* completes its life cycle approximately in 10-15 days at 20-25ºC (Behmand et al., 2022). Relatively higher reproduction capacity of the nematode causes rapid increase of the population in favorable field conditions. However, nematode population decreases were recorded after incorporation of the green manures during the experiment.

In the current study, the nematode populations varied among sampling times through the experiment. The number of the nematodes increased significantly in the last year of the experiment. Effects of the climatic conditions on nematode populations were seen clearly in this study. The precipitation was low in the first three years of the experiment and then it increased in 2024. The watering capacity was limited during the experiment. Higher soil moisture caused an increase in nematode populations in the soil in the corresponding periods of the experiment (Figures 2&3). The increasing effect of precipitation on the soil nematode community population growth was also shown clearly by Landesman et al. (2011), Bristol et al. (2023), Hu et al. (2025), Li et al. (2025). Therefore, it should be considered in planning management practices for plant parasitic nematodes.

The green manure applications will contribute to the control of the plant parasitic nematode populations in field conditions. Further studies are needed for improving the effectiveness of the green manure applications for the control of plant parasitic nematodes.

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