In vitro effectiveness of some plant species as green manure for the control of stem and bulb nematode (*Ditylenchus dipsaci*)

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Abstract: Stem and bulb nematode, *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936, is a plant parasitic nematode that causes significant losses in plant production in the world. One of the promising methods for control of soil-borne diseases and pests that do not accumulate in nature is green manure application. Studies on the effectiveness of green manure on stem and bulb nematode are limited. In this study, the activity of 4 plant species from Brassicaceae family and one *Tagetes patula* L. variety on stem and bulb nematode was investigated in vitro. In the study, rates of motionless nematodes were recorded in the water and sand medium in 4 days and 11 days in five plant species. The efficiency of the plant species was increased over time. In all treatments in the study, the highest rate of motionless nematodes was obtained with arugula. On the 4th and 11th days in the water medium, 84.1% and 95.7% of motionless nematodes were obtained, respectively, while in the sand medium, the rate of motionless nematodes was 60.2% and 86.1%. Following the arugula, *Tagetes patula* showed activity at the rates of 72.9% and 98.3% in the water medium and 40.9% and 81.9% in the sand medium on the 4th and 11th days, respectively. Radish was also found promising with 62.5% and 94.2% in water medium, 59.2% and 80.9% in the sand medium, on the 4th and 11th days, respectively. The data obtained in the study provided preliminary data for green manure applications under field conditions.

Key words: Biofumigation, Brassicaceae, cultural management, nematode suppression, *Tagetes* spp.

Özet: Soğan sak nematodu, *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936, dünya çapında buharla çiçeklerin önemli kayıplarına ve bitki parazitleri olarak nitelendirilen bir bitki parazitidir. Doğada bitki Bộ qualify up and topok kökenli hastalıkları ve zararlıların kontrolünde umit veren yöntemlerden biri yeşil gübremedir. Yeşil gübrelemenin soğan sak nematodu üzerinde etkinliği ile ilgili çalışmalar sınırlıdır. Bu çalışmada, Brassicaceae familyasından 4 bitki türü ve bir *Tagetes patula* L. çeşidinin soğan sak nematodu üzerindeki etkinliği in vitro olarak araştırılmıştır. Çalışmada su ve kum ortamında 4. ve 11. günde 5 bitki türünde nematodlardaki hareketsizlik oranları kaydedildi. Bütün türlerin etkinliğini zanaat kılması araştırılmıştır. Çalışmada bütün uygulamalarda en yüksek hareketsiz nematod oranı 4. ve 95.7 oranlarında hareketsiz nematod elde edilmiştir. Su ortamında 4. ve 11. günlerde sırasıyla %84.1 ve %95.7 oranlarında hareketsiz nematod elde edilmiştir. Kumu sırasıyla *Tagetes patula* 4. ve 11. günlerde sırasıyla %72.9 ve %98.3 oranlarında, kum ortamında ise %40.9 ve %81.9 oranlarında hareketsiz nematod oranları ile etkinlik göstermiştir. *Tagetes patula* 4. ve 11. günlerde sırasıyla %62.5 ve %94.2, kum ortamında %59.2 ve %80.9 hareketsiz nematod oranları ileumin vermiştir. Çalışmada elde edilen verilerle tarla köşelerinde sosyal buharlayıcı olarak kullanılan yeşil gübre uygulamaları için örnek oluşturmuştur.

Anahtar Kelimeler: Biyofumigasyon, Brassicaceae, kültür mücadele, nematod baskılanması, *Tagetes* spp.

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1. Introduction

Nematoda filum of the animal kingdom include plant parasitic microscopic round worms. The plant parasitic nematode species that causes the most economic losses in the 4th place in the world is stem and bulb nematode (*Ditylenchus dipsaci*) (Kühn, 1857) Filipjev, 1936 (Abd-Elgawad and Askary, 2015). Stem and bulb nematode is distributed all over the world, parasites more than 500 plant species and causes yield losses economically (Sturhan and Brzeski, 1991).

The economic damage threshold of the stem and bulb nematode is very low. It has been reported that serious damages occur on plant production when 10 nematodes are found in 500 g soil (Seinhorst, 1956). It is necessary to control the nematode by applying an integrated control strategy in order to continue plant production economically. It is very important for soil sustainability to prioritize cultural and biological control practices such as rotation practices with non-host plant species, growing resistant varieties, use of nematode suppressive plant species and green manure practices in integrated control practices.

Stem and bulb nematode lives in the intercellular parts on the above-ground organs of the plant, damaging the plant. Systemically effective chemicals which are necessary for killing the nematode in plant tissue are very toxic to humans and other organisms in the environment. Their use under field conditions is not economical. For this reason, it is recommended to use of chemicals as soil fumigation for chemical control of nematodes. However, since the synthetic chemicals used are not natural, it is possible that they cause biological accumulation in biological organisms and chronic poisoning. For this reason, plant species with biofumigant effect that naturally decompose and do not leave residues in the soil constitute an important alternative source for the control of nematodes. Effectiveness of plant species such as radish (*Raphanus sativus* L.), arugula (*Eruca sativa* Mill.), broccoli (*Brassica oleracea* L.), canola (*Brassica napus* L.) and...
mustard (Brassica juncea Czern.), from the Brassicaceae family, on nematode control have been found to be high, mostly for Meloidogyne spp. (Johnson et al., 1992; Mojtahedi et al., 1993; Al-Rehiayani and Hafez, 1998; Potter et al., 1998; Stapleton and Duncan, 1998; McLeod and Steel, 1999; Melakeberhan et al., 2006; Kaşkavalcı et al., 2009; Youssef and Lashein, 2013; Kruger et al., 2015).

Out of the Brassicaceae family, Tagetes spp., has become a highly emphasized plant as green manure to plant parasitic nematodes, especially for the suppressive effects on Meloidogyne spp. (Kimpinski et al., 2000; Evenhuis et al., 2004; Seigies and Pritts, 2006; Kaşkavalcı et al., 2009).

Among the plant parasitic nematodes, the most studied nematode species are root knot nematodes (Meloidogyne spp.), which are the most economically important nematodes in the world. There are limited studies on the control of stem and bulb nematode using biofumigant plant species in the world. No study was found in Turkey. With this purpose, it is aimed to determine the in vitro activities of different plant species for the usage as green manure in plant production areas where stem and bulb nematode is infested.

2. Materials and method

2.1. Plant materials

Totally 4 plant species of broccoli (Brassica oleracea var. botrytis L. cv. Standard, Intfa Seed, Konya), cabbage (B. oleracea var. capitata L. cv. Standard, Intfa Seed, Konya), radish (Raphanus sativus L. cv. Standard, Intfa Seed, Konya) and arugula (Eruca sativa Mill. cv. Standard, Intfa Seed, Konya) from Brassicaceae family and a marigold species (Tagetes patula L. cv. Bonanza, Harmony, Tasaco Agriculture, Antalya) were investigated for in vitro effectiveness as green manure against D. dipsaci.

2.2. Nematode inoculums

Pure culture of nematode was used in the study. Nematodes were originally isolated from garlic samples in Karaman Province, Central District in Central Anatolian Plateau (N: 37.10351 E: 33.117). Nematode culture was maintained on sterile carrot discs at 20 °C in dark (Yavuzaslanoglu and Aksay, 2021). Nematodes for the experiment were extracted from two months old sterile carrot disc cultures by washing the surface of the carrot discs with sterile tap water. The nematode suspension was then concentrated to 1 ml. The nematode numbers were counted in whole sample under light microscope at 10x magnification. Living nematodes were obtained with extraction technique (Hooper et al., 2005). The number of motionless nematodes was determined by subtracting the number of motile nematodes obtained in each petri dish from the initial number of 400 nematodes. The motionless nematodes per treatment was calculated and presented as percent (%) (Aydınlı et al., 2019).

2.4. Statistical analysis

Data of percent motionless nematodes per petri dish was analyzed according to fit model of completely randomized plot design with four factors. In addition to three factors investigated in the experiment, repetition of the experiment was included as a factor to the statistical analysis. Effect of factors and their interactions were investigated. Analysis of variance (ANOVA), Tukey's HSD test and Student’s t test were used for determination of statistically significant differences among treatments and their interactions. Statistical analyses were performed using JMP® 5.0 software (JMP, 2020).

3. Results

The number of motionless nematodes (%) obtained was not statistically different in the two experiments performed. Therefore, the data obtained from two experiments were combined and evaluated. While the number of nematodes in the two different media examined in the experiment did not differ statistically, the number of motionless nematodes on the 4th and 11th days in the experiment showed a statistically significant difference (P < 0.05).

In the experiment, no statistically significant interaction was found between the experiment medium and the incubation time. The rates of motionless nematodes at plant species treatments were higher than the negative control (P < 0.05). The interaction of plant species with experiment medium and the incubation time was also found to be statistically significant (P < 0.05).

On 4th day in the water medium, the lowest number of immobile nematodes was obtained in negative control (9.6%). The rate of motionless nematodes obtained in the all plant species was found to be statistically higher than the negative control (P < 0.05). Among the plant treatments, the lowest rates of motionless nematodes were recorded in cabbage and broccoli plant species at 38.5% and 46.9%, respectively. These plant species were found to have statistically significantly lower motionless nematode rates than of the radish, arugula and marigold in the experiment. The highest rate of motionless nematodes
was obtained with arugula (84.1%). Subsequently, 72.9% in marigold and 62.5% in radish were obtained (Table 1).

The rate of motionless nematodes on the 11th day in the water medium did not show a statistically significant difference between plant species, while the control treatment was significantly lower (24.9%) than all plant species treatments. The highest rates of motionless nematodes on the 11th day in the water medium, as on the 4th day, were obtained in marigold (98.3%), arugula (95.7%) and radish (94.2%) treatments. It was recorded as 90.2% and 92.6% in cabbage and broccoli treatments, respectively (Table 1).

The rate of motionless nematodes obtained on the 11th day in the water medium were significantly higher on the 11th day than on the 4th day in all plant treatments but not negative control treatment.

Table 1. Percentage of motionless nematodes (Ditylenchus dipsaci) in plant treatments at water medium with 4 and 11 days incubation times.

| Plant Treatments                        | 4 days incubation | 11 days incubation |
|-----------------------------------------|-------------------|--------------------|
| Broccoli (Brassica oleracea var. botrytis L. cv. Standard) | 46.9±2.9 cB       | 92.6±3.1 aA        |
| Cabbage (B. oleracea var. capitata L. cv. Standard)       | 38.5±2.9 cB       | 90.2±3.1 aA        |
| Radish (Raphanus sativus L. cv. Standard)                  | 62.5±3.1 bB       | 94.2±3.1 aA        |
| Arugula (Eruca sativa Mill. cv. Standard)                   | 84.1±2.9 aB       | 95.7±3.1 aA        |
| Marigold (Tagetes patula L. cv. Bonanza, Harmony)           | 72.9±2.9 aB       | 98.3±3.4 aA        |
| Negative Control                                         | 9.6±3.1 dA        | 24.9±3.9 bA        |

Values are means ± standard error of 10 replicates. Means within a column followed by different lower case letters for each plant treatment are significantly different according to Tukey HSD test at P < 0.05. Means within a row followed by different capital letters for each incubation time are significantly different according to Student’s t test at P < 0.05.

No statistically significant difference was found among the plant and negative control treatments on the 4th day in sand medium. While the rate of motionless nematodes in negative control was 39.1%, it was recorded as 40.9, 52.0, 59.2, 60.2 and 64.4% in marigold, broccoli, radish, arugula and cabbage plant species, respectively (Table 2).

On the 11th day of the experiment in the sand medium, the lowest statistically significant rate of motionless nematodes was obtained in negative control treatment (62.1%). Although there is no statistical difference among plant treatments, the highest rate of motionless nematodes was obtained in arugula plant species (86.1%), followed by marigold and radish plant species at 81.9% and 80.9%, respectively. On the other hand, 80.6% and 77.3% motionless nematodes were obtained in cabbage and broccoli treatments, respectively (Table 2).

The rate of motionless nematodes in all plant treatments and negative control in the sand medium was found to be statistically significantly higher on the 11th day than on the 4th day (Table 2).

4. Discussions

In vitro suppression efficacy of potential green manure plants, which is a promising tool for controlling soil-borne pathogens and nematodes without accumulation in nature, for the control of stem and bulb nematode, which is common in onion and garlic growing areas in our country was investigated. The study provided preliminary data useful for determining the plant species for field experiments.

Table 2. Percentage of motionless nematodes (Ditylenchus dipsaci) in plant treatments at sand medium with 4 and 11 days incubation times.

| Plant Treatments                        | 4 days incubation | 11 days incubation |
|-----------------------------------------|-------------------|--------------------|
| Broccoli (Brassica oleracea var. botrytis L. cv. Standard) | 52.0±6.1 aB       | 77.3±3.4 aB        |
| Cabbage (B. oleracea var. capitata L. cv. Standard)       | 64.4±6.1 aB       | 80.6±3.4 aA        |
| Radish (Raphanus sativus L. cv. Standard)                  | 59.2±6.1 aB       | 80.9±3.4 aA        |
| Arugula (Eruca sativa Mill. cv. Standard)                   | 60.2±6.1 aB       | 86.1±3.6 aA        |
| Marigold (Tagetes patula L. cv. Bonanza, Harmony)           | 40.9±6.1 aB       | 81.9±3.4 aA        |
| Negative Control                                         | 39.1±7.2 aB       | 62.1±3.8B          |

Values are means ± standard error of 10 replicates. Means within a column followed by different lower case letters for each plant treatment are significantly different according to Tukey HSD test at P < 0.05. Means within a row followed by different capital letters for each incubation time are significantly different according to Student's t test at P < 0.05.

It is stated that one of the most important environmental factors for the high suppression efficiency of plants under field conditions is the moisture content in the soil during application. Water plays a critical role in the activity of myrosinase enzyme, which is necessary for the formation of suppressive isothiocyanate compounds (Matthiessen and Kirkegaard, 2006). In the study carried out, obtaining suppression efficiency higher in water medium, even it was not statistically significant, once again revealed that the water is important factor for myrosinase enzyme activity and biofumigation action.

In the experiment, arugula was the plant species with the highest efficiency in both water and sand mediums. In a study conducted under greenhouse conditions, arugula as applied green manure in soil reduced the populations of Meloidogyne hapla. Citwood 1949 and M. chitwoodi. Golden et al. 1980 and Paratrichodorus allius. Jensen, 1963 up to 99% (Riga, 2011). Similar effects have been recorded against root knot nematodes by Kruger et al. (2015).

Radish, which caused a high rate of motionless nematodes in the current experiment, has also been shown to be highly effective on other plant parasitic nematodes. It has been determined by different studies that the potato cyst nematode Globodera pallida Stone, 1973 second-stage larvae and cyst populations had been decreased by radish green manure application (Lord et al., 2011; Ngala et al., 2015). Similarly, higher effects of this plant as green manure has been found on M. citwoodi nematode population (Hafez and Sundararaj, 2000).

Other than the Brassicaceae family, one of the most promising plant species with the suppression effect is the Tagetes species that was shown in our study as well. Seigies and Pritts (2006) reported that after rotation with T. erecta in strawberry plantations, the number of nematodes decreased, the roots of the plants became
stronger and fruit yield increased. In a study conducted in Canada, it was determined that tuber yield increased and *P. penetrans* population density decreased in potato cultivation after rotation using *Tagetes* species (Kimpinski et al., 2000). In organic tomato cultivation, it was determined that there is a decrease in galls formed on the roots in infested soil with *M. incognita* during the autumn period in the co-cultivation with *T. erecta* (Kaşkavalcı et al., 2009). It is recommended to wait 7-10 days until the cultivation after the incorporation of suppressive plant species under field conditions in order to fully realize the biofumigation activity (Youssef, 2015). In our study, high efficiency rates with plant species in water and sand medium were obtained on the 11th day of the experiment and it was found that it increased depending on time.

A significant contribution has been made to the literature on the control of stem and bulb nematode using green manure application. In the next stage of the study, we will focus on the efficacy of promising plant species identified in the current study under field conditions.

**Conflict of Interest**

Authors have declared no conflict of interest.

**Authors’ Contributions**

The authors contributed equally.

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