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# Distribution of the entomopathogenic nematodes in apple growing areas of Karaman, Turkey

E. Yavuzaslanoglu[1†], U. Gozel[2], C. Gozel[2] and M. Aydogdu[3]

[1]Karamanoglu *Mehmetbey University, Technical Sciences Vocational School, Plant and Animal Production Department, Karaman, Turkey*
[2]*Çanakkale Onsekiz Mart University, Agricultural Faculty, Plant Protection Department, Çanakkale, Turkey*
[3]*Ministry of Food Agriculture and Animal Production, Central Research Institute for Field Crops, Department of Geographic Information Systems, Ankara, Turkey*

[†]Corresponding author: eyavuzaslanoglu@kmu.edu.tr

## Abstract

Study was conducted to determine the distribution of the entomopathogenic nematodes in apple growing areas in Karaman province, Turkey and their efficacy on *Galleria mellonella* (Lepidoptera: Pyralidae). Soil samples were collected from 130 apple orchards in April 2012 and 2013. Entomopathogenic nematodes were found in 25 samples (19.23%). Entomopathogenic nematodes species isolated: *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) (16.92%) and *Steinernema feltiae* (Filipjev, 1934) (Rhabditida: Steinernematidae) (2.3%). Entomopathogenic nematodes distributed in low sand content and low-fertilized soils in Karaman province. The efficacy of *H. bacteriophora* and *S. feltiae* on *G. mellonella* was 100% after 72 h with 50 infective juveniles at 25 °C.

**Keywords:** Karaman, apple, entomopathogenic nematodes, biological control.

Apple is the most important product among the soft seed fruits in Turkey. In 2014, 2.480.444 tonnes in 171.417 ha were produced in Turkey (Anonymous, 2013). Karaman is the second-largest producer, providing 12.23% of the total apple production in Turkey. Total apple production was 349.793 tonnes from 20.962 ha in Karaman (Anonymous, 2013). One of the constraints of apple growing in Karaman province is pests (Eren *et al*., 2013). The pesticides are used extensively by apple producers for control of pests. The damage of the pesticides has been recognised. Therefore, other alternative ecologically friendly control methods are encouraged. Integrated control methods are developed for a specific plant considering the geographical area and soil properties. Entomopathogenic nematodes (EPNs) have an active role in the integrated control of pests (Gaugler, 2002; Grewal *et al*., 2005).

There are three genera of EPNs *Steinernema*, *Neosteinernema* and *Heterorhabditis* in Steinernematidae and Heterorhabditidae families. In total, 35 species have been identified, with 25 in the *Steinernema* genera, one in the *Neosteinernema* genera and nine in the *Heterorhabditis* genera (Burnell & Stock, 2000). The first identification of EPN strain in Turkey was *Steinernema feltiae* (Filipjev 1934) Wouts, Mracek, Gerdin & Bedding, 1982 (Rhabditida: Steinernematidae) in the Black sea region (Ozer, 1995). After this, Kepenekci *et al*., (1999) isolated *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) in Aksaray province. *H. marelatus* (Liu & Berry 1996) was

isolated in addition to *S. feltiae* and *H. bacteriophora* in Ankara (Kepenekci & Susurluk, 2000; Susurluk *et al*., 2001).

Other species of EPNs found in Turkey were *S. carpocapsae* (Weiser, 1955) in the Mediterranean and Marmara regions (Kepenekci, 2002; Güneş & Gözel, 2011), *S. anatoliense* (Hazir, Stock & Keskin, 2003) in the East Anatolian region (Hazır *et al*., 2003), *S. affine* (Bovien, 1937) (Hazır *et al*., 2003a) and *S. weiseri* (Mrácek, Sturhan & Reid, 2003) in the Marmara region (Aydın, 2007).

*Steinernema feltiae* (found in six regions) and *H. bacteriophora* (found in five regions) species are the most widespread species in Turkey (Hazır *et al*., 2003a). Entomopathogenic nematodes are found in sandy to clay soils at pH 5.6-7.9 (Rosa *et al*., 2000; Hazır *et al*., 2003a). In the Marmara region, EPNs (*S. affine, S. carpocapsae, S. feltiae* and *H. bacteriophora*) were recovered from sandy loam, sandy clay loam and loamy sand soil. Organic matter ranged from 0.43-7.05% and pH varied from 5.13-8.09 (Güneş & Gözel, 2011). The isolation frequencies of EPNs from different ecological areas vary between 2.03% and 12.1% in Turkey (Hazir *et al*., 2003a; Aydın, 2007; Armagan, 2010; Günes & Gözel, 2011). The aim of the study was to investigate the distribution of EPNs in apple-growing areas in Karaman province in relation to the physicochemical characteristics of the soil and their efficacy on *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae.

## Material and Methods

**Soil sampling:** The apple-growing area in Karaman province was surveyed in April 2012 and 2013. The apple-growing area in Karaman province was divided into two parts. In the first year, the central district and the Kazimkarabekir district of Karaman 62 soil samples were collected. In the second year, Ayrancı, Ermenek, Sariveliler and Basyayla districts 68 samples were collected. In total, 130 soil samples were used for isolation of EPNs. Soil sampling locations were identified using GPS (Global Positioning System) on the Karaman province map (Fig. 1).

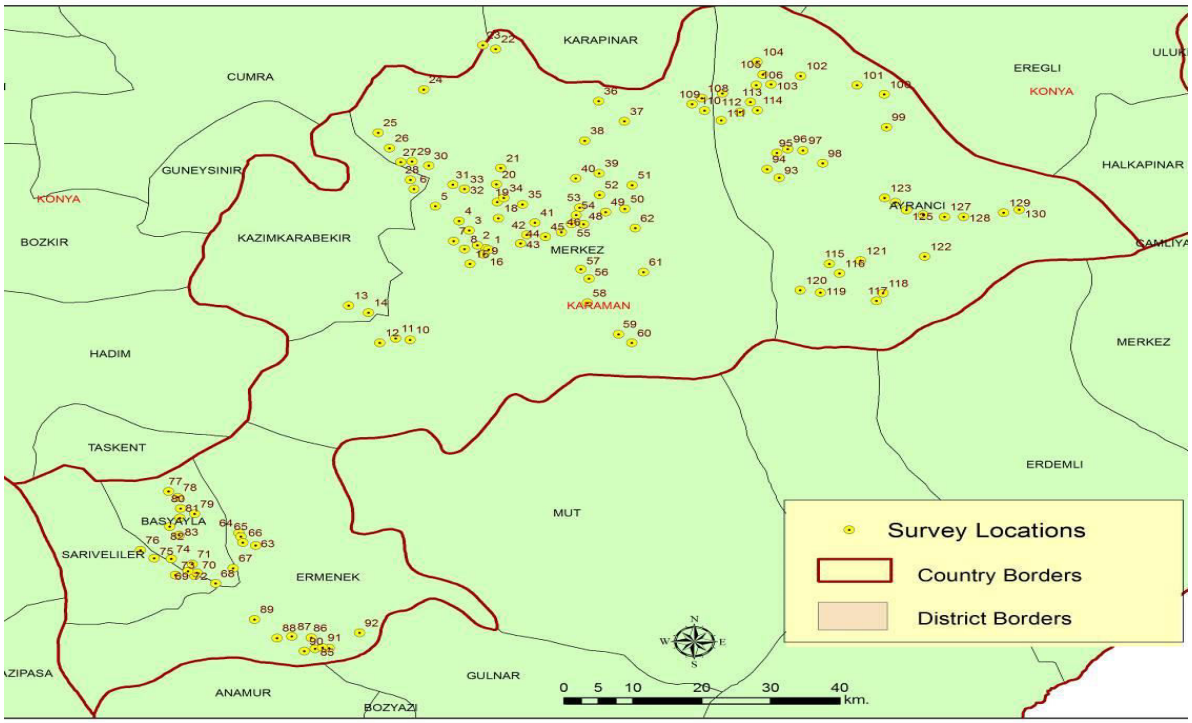


**Fig. 1.** Soil sampling locations for entomopathogenic nematode isolation in Karaman province during 2012-13.

Soil samples were collected systematically 2-3 km apart between apple orchards. Every apple orchard was sampled walking in a zigzag pattern. Every 10-15 steps, 4-5 cores of soil were taken around one apple tree horizontal projection area (Griffin *et al*., 2000). Soil samples were collected in a container and were composed of 2-3 kg of bulk sample for entomopathogenic nematode isolation and soil physicochemical analysis (Stock *et al*., 1999; Iraki *et al*., 2000). Soil samples were taken using a 2.5 cm diameter soil corer from a depth of 5-30 cm, after gently cleaning the upper layer of soil (Mracek *et al*., 1999; Sturhan & Liskova, 1999).

**Physicochemical analysis of soil samples:** Soil pH, electrical conductivity (EC), texture, and $CaCO_3$ and organic matter content were analyzed. pH and EC ($\mu$S/cm) analysis was carried out according to the 1:2.5 soil: water methodology described by Richards (1954). $CaCO_3$ content (%) was analyzed using Scheibler calcimeter methodology (Caglar, 1949). Organic matter (%) was determined using the Walkley-Black method (Walkley, 1946). Sand, silt and clay contents (%) were fractionated using Bouyoucos hydrometer methodology (Anonymous, 1990).

**Isolation of entomopathogenic nematodes from the soil:** Larvae of *G. mellonella* was grown on special growth medium (2000 ml bran, 200 ml honeycomb, 250 ml glycerine, 200 ml honey, 100 ml distilled water) in glass cups that were closed with a cloth to allow air entrance and to avoid escape of the larvae (Kaya & Stock, 1997).

On average, 500 g soil from each sample was mixed well and placed in plastic cups. Eight to ten last instar *G. mellonella* larvae were placed on the soil in the cups. Next, the plastic cups were reversed and the larvae left below the soil (Bedding & Akhurst, 1975; Griffin *et al*., 2000). Plastic cups including soil samples and the *G. mellonella* larvae were incubated at 22-25 $^{o}$C for 7 days (Stock *et al*., 1999).

Entomopathogenic nematodes were collected from dead *G. mellonella* larvae using White trap technique (White, 1927) at the end of the incubation period (Koppenhöfer, 2000). Collected nematodes were surface sterilized and stored in the flasks.

**Morphometric characterisation of isolates:** Morphological and morphometric characterization of the isolates was carried out using infective larvae and first-generation male nematodes according to Hominic *et al*., (1997). Each isolate was inoculated with *G. mellonella* larvae and first-generation infective juveniles and male individuals were collected. Twenty individual nematodes were used for morphometric measurements for each isolate identified. Measurements were made by using a compound Leica DM 1000 light microscope fitted with a drawing tube.

**Pathogenicity of the isolates against *G. mellonella*:** Experiments were conducted for the isolates of *H. bacteriophora* and *S. feltiae* against *G. mellonella* at 25 °C. Twelve healthy last instar larvae of *G. mellonella* were each inoculated with 50 infective juveniles of the isolates. The experiment was replicated five times and dead *G. mellonella* larvae were counted 24, 48 and 72 h after inoculation.

**Statistical analysis:** Statistical analysis was run on the combined data of two years of survey. Mean, maximum and minimum values of soil physicochemical properties, distribution of soil texture classes and isolation frequency of entomopathogenic nematodes were calculated using distribution analysis. Correlation between soil physicochemical properties and entomopathogenic nematode isolation frequency was investigated using multifactorial correlation analysis. The distribution of physicochemical properties of soil according to entomopathogenic nematodes was determined using multifactorial discriminant analysis. Pathogenicity of the isolates on *G. mellonella* was compared using analysis of variance depending on the time. The JUMP 5.0.1 statistical analysis program was used for all statistical analyses applied.

## Results

**Physicochemical properties of soil samples:** The pH values of soil samples varied between 7.05 and 8.38 (mean: 7.89). Electrical conductivity of soil samples was between 65.7 and 1445 $\mu$S/cm (mean: 195.81 $\mu$S/cm). The $CaCO_3$ content of the samples ranged between 1.06% and 74.7% (mean: 37.39%). Organic matter contents of soil samples were between 0.25% and 8.65% (mean: 2.92%). Mean sand, silt and clay contents of all soil samples were 35.57% (max: 65.34%; min: 16.45%), 28.72% (max: 52.4%; min: 2.13%) and 35.76% (max: 56.41%; min: 11.88%), respectively.

Four soil texture classes were identified according to sand, silt and clay content of soil samples. Sandy loam accounted for 1.53% of soil samples, 30% of soils were clay loam, 30.76% of soils were clay soil and 37.69% of soils were sandy clay loam soil.

**Frequency of EPN:** Entomopathogenic nematodes were obtained in 25 of 130 apple orchards (19.23%). Morphologic characteristics and morphometric measurements of the isolates confirmed that isolates from 22 apple orchards (16.92%) belonged to *Heterorhabditis bacteriophora* and three of the entomopathogenic nematode isolates belonged to *Steinernema feltiae* (2.3%) (Fig. 2).

**Relationship between soil physicochemical properties and EPN isolation frequency:** The isolation frequency of *H. bacteriophora* was dependent on physicochemical properties of soil at a rate of 79.29% according to multivariate discriminant analysis (Fig. 3). The isolation frequency of *S. feltiae* was not differentiated depending on the investigated soil physicochemical properties according to multivariate discriminant analysis.

Correlation analysis between soil physicochemical properties and *H. bacteriophora* isolation frequency values had a significant relationship. There was a negative relationship between soil sand and organic matter content, and a positive relationship between soil clay content and nematode isolation frequency ($P<0.05$; Fig. 4, 5 and 6). There was no significant relationship between the *S. feltiae* species and soil physicochemical properties.

**Pathogenicity of the isolates against *G. mellonella*:** Pathogenicity of both *H. bacteriophora* and *S. feltiae* against *G. mellonella* was 100% after 72 h. There was no significant difference between pathogenicity of the two species at either time; however, the mortality rate according to time was significantly different for both species ($P< 0.05$; Fig. 7).

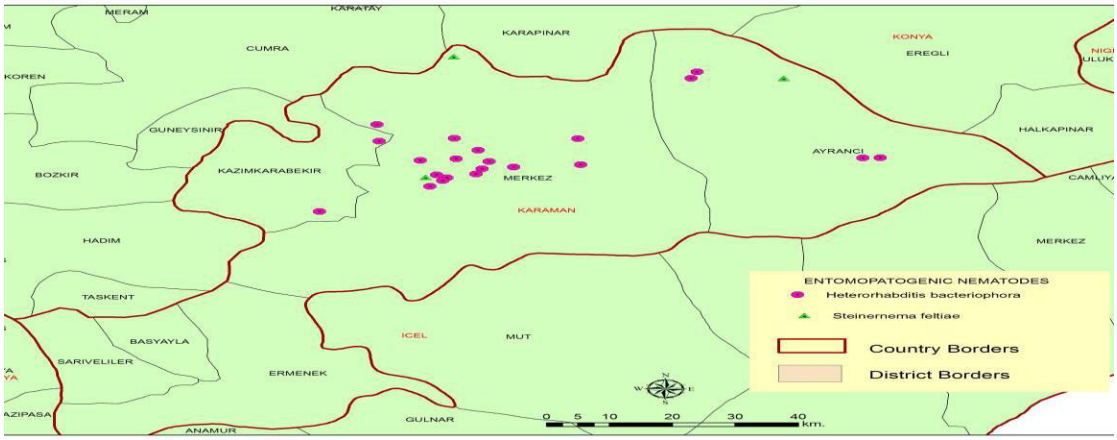


**Fig. 2.** Entomopathogenic nematode species in Karaman province.

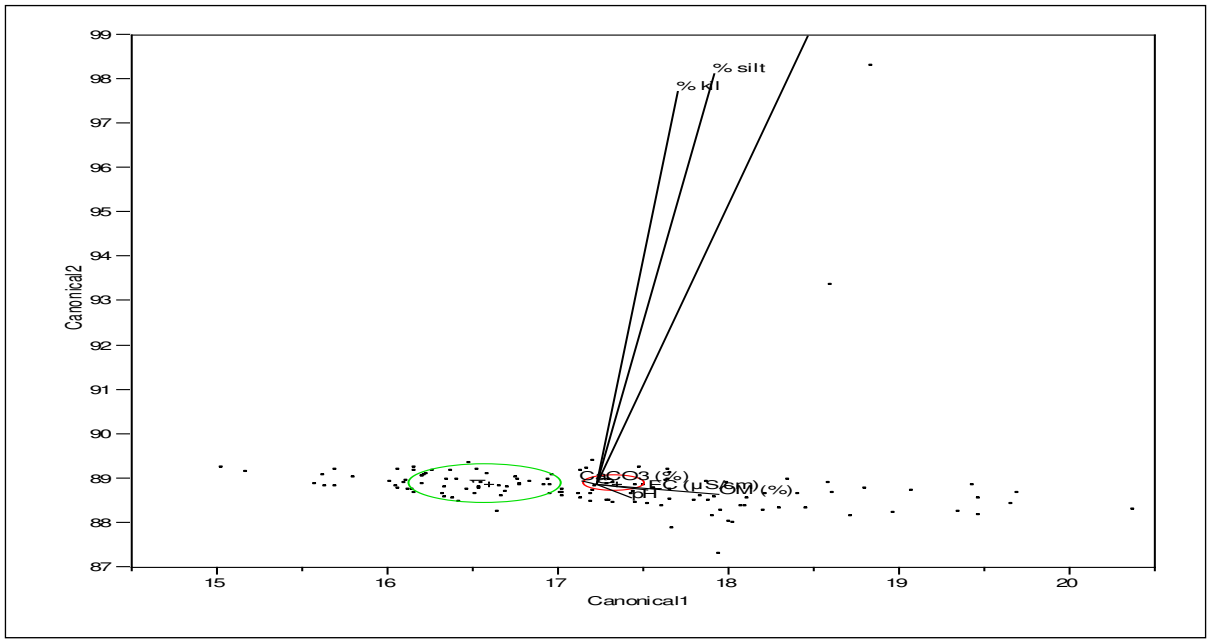


**Fig. 3.** Isolation frequency of *H. bacteriophora* according to soil physicochemical properties (+1 in circle: presence of *H. bacteriophora*, +0 in circle: absence of *H. bacteriophora*).

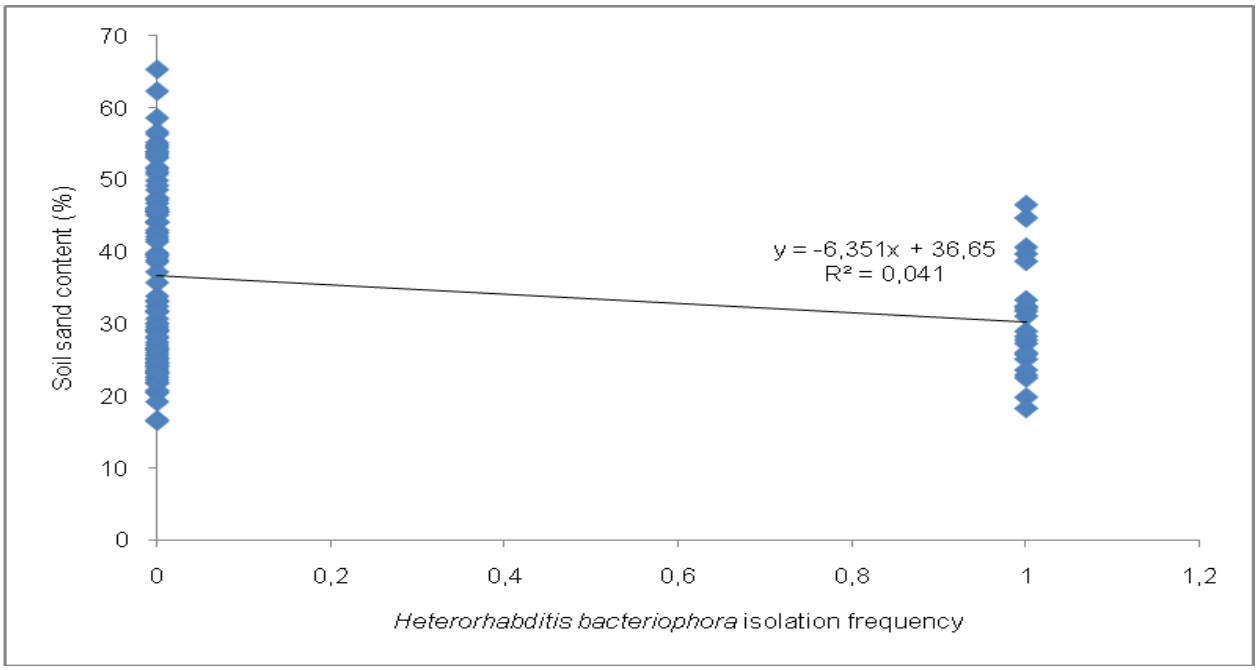


**Fig. 4**. Relationship between isolation frequency of *H. bacteriophora* and soil sand content (%).

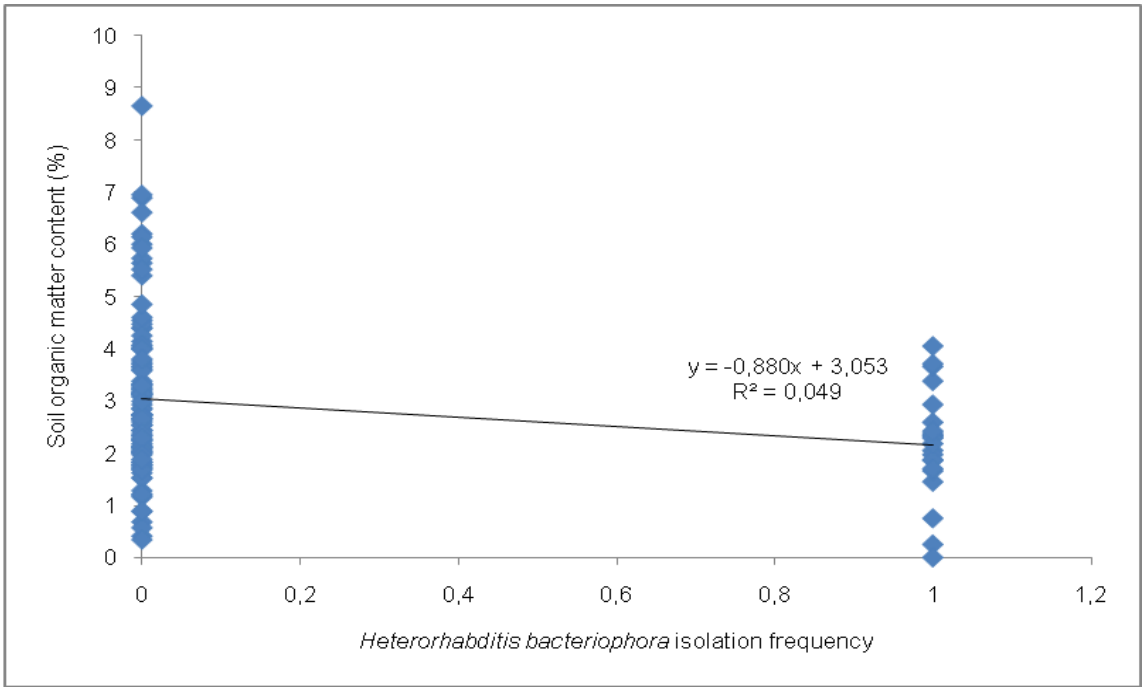


**Fig. 5.** Relationship between isolation frequency of *H. bacteriophora* and soil organic matter content (%).

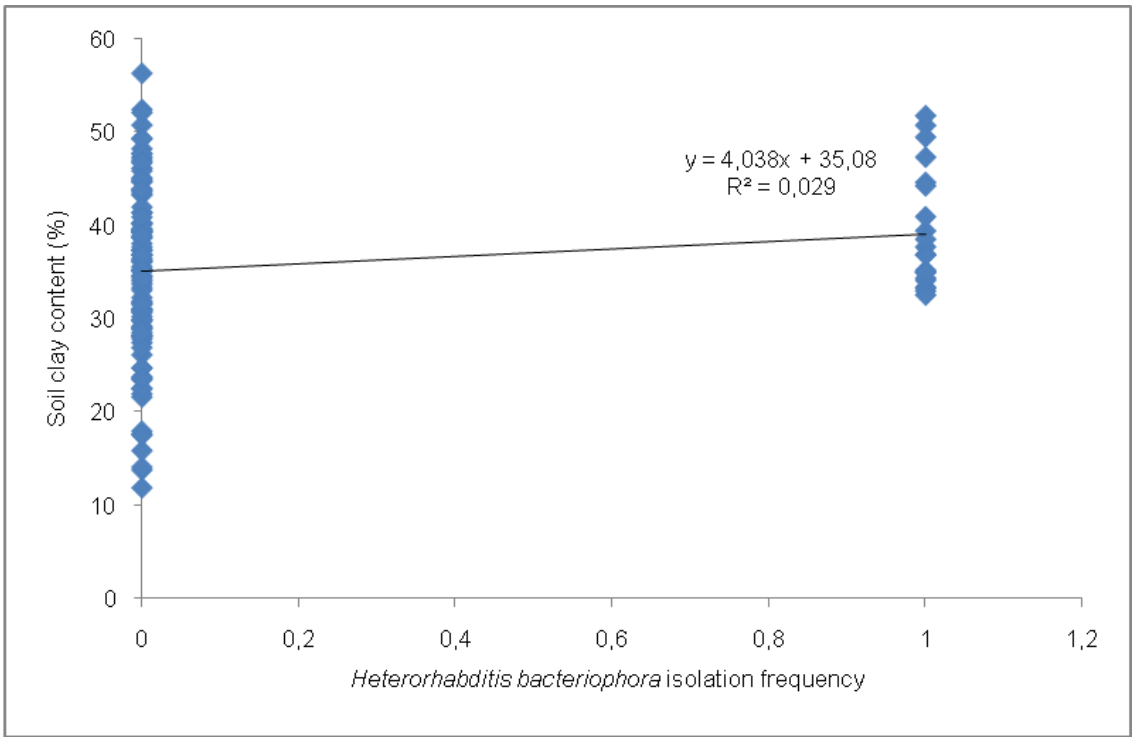


**Fig. 6.** Relationship between isolation frequency of *H. bacteriophora* and soil clay content (%).

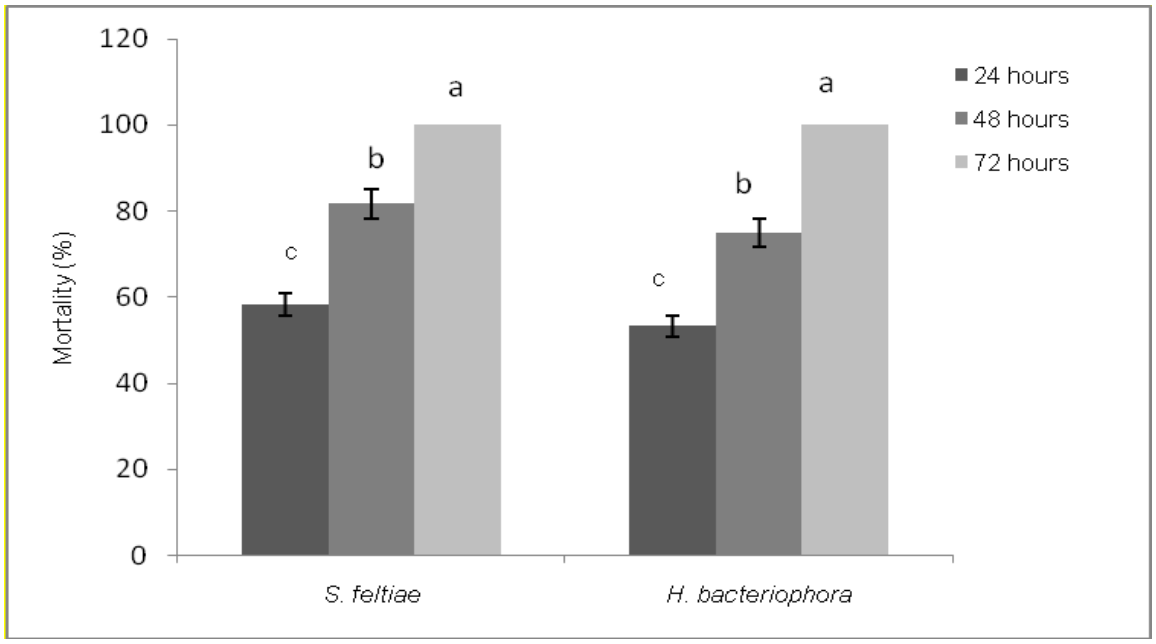


**Fig.7.** Mortality rate of *G. mellonella* after 24, 48 and 72 h exposure to *S. feltiae* and *H. bacterophora* (Different letters are significantly different).

## Discussion

Soil samples had the characteristic properties of Karaman province agricultural soils (Anonymous, 2007). Soil samples showed a weak alkali character and there was no salinity problem in the sampled area. Generally, the $CaCO_3$ content of soil samples was high and the soil was classified as calcareous soil. Generally, organic matter content of sampled orchards was low according to the reference values in Anonymous (2013a). Most of the soils had a sandy clay loam soil texture.

Isolated entomopathogenic nematode species *Heterorhabditis bacteriophora* and *Steinernema feltiae* in this study are the most widely distributed species in different habitats in Turkey (Ozer, 1995; Kepenekci, 1999; Kepenekci & Susurluk, 2000; Susurluk *et al*., 2001; Hazır *et al*., 2003a). Hazır *et al*., (2003a) identified *S. feltiae* as the most distributed species in Turkey, while *H. bacteriophora* was isolated at high frequency in Ankara and Aksaray by Kepenekci (1999), Kepenekci & Susurluk (2000) and Susurluk *et al*., (2001) and in Karaman apple-growing areas in this study. This shows that the *H. bacteriophora* species is more widely distributed in the Central Anatolian Plateau in Turkey. Isolation frequency of entomopathogenic nematodes in Karaman (19.23%) is higher than other regions of Turkey. For example, Hazır *et al*., (2003a) isolated entomopathogenic nematodes from 22 locations out of 1080 locations (2.03%). Aydın (2007) recorded 12% isolation frequency from different ecological conditions. Armagan *et al*., (2010) found entomopathogenic nematodes in 4.54% of soils. Therefore, Karaman province looks to have an important potential for entomopathogenic nematodes.

Organic matter content of soil could be the determinant for distribution of entomopathogenic nematodes according to the current results. However, there is no evidence regarding the amount of organic matter that affects the species of EPNs in the soil (Griffin *et*

*al*., 2000; Stock *et al*., 2008). Therefore, it is estimated that the pest species that can be used as food by nematodes in orchards is more effective on the rate of EPNs recovery in apple orchards in Karaman.

*Heterorhabditis* and *Steinernema* species were found in fruit orchards and pasture by Rosa *et al*., (2000). Soil is biologically balanced in the fruit orchards and pasture lands due to the low soil tillage and long-term cultivation of the same plant. This increases the isolation frequency of entomopathogenic nematodes. Likewise, isolation frequency of entomopathogenic nematodes was high in old and low-tillaged apple orchards in the current study.

*Heterorhabditis bacteriophora* was more effective than *S. feltiae* against *Melolontha melolontha* (Linnaeus, 1758) (Coleoptera: Scarabidae) (Erbas *et al*., 2014), opposite of our results being more mortality rate in 48 h with *S. feltiae*. However, pathogenicity of individual entomopathogenic nematode species could be different against different target pests. Therefore, future studies will include investigation of activity of the isolates against a range of pests affecting apple growing.

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