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Survey of *Pasteuria*, the parasitic bacterial group to plant parasitic nematodes in Turkey

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Abstract

The present study was carried out in the agricultural areas of Northwestern Turkey with the purpose to assess the occurrence of *Pasteuria* spp. bacteria on plant-parasitic nematodes. The soil samples were collected from olive, cherry, peach, pear, almond, walnut, apple orchards, vineyards, vegetable, and sunflower fields, analyzed and the bacterium was detected on 30 nematode species belonging to 31 families. Nematode individuals from *Rotylenchus*, *Helicotylenchus*, *Pratylenchus*, *Tylenchorrhynchus*, and *Geocenamus* spp. were most frequently infected while the average height and width of the spore core and endospore ranged between 3.70–4.90 and 1.40–2.37 μm , respectively. The results indicated that *Helicotylenchus digonicus*, *Pratylenchus thornei*, *P. neglectus*, *Geocenamus brevidens*, *Tylenchorrhynchus cylindricus*, *Rotylenchus cypriensis*, *Meloidogyne javanica*, and *M. incognita* individuals were the most parasitized by the bacterium.

Keywords: Soil bacteria, Occurrence, *Pasteuria* spp., Plant parasitic nematodes, Turkey

Background

The soil ecosystem consists of various micro and macro-organisms. Nematodes from the phylum Nematoda, which includes more than 20,000 identified species, are the most abundant group with 4,100 plant-parasitic species feeding on plant roots and causing yield loss up to 77% (Abd-Elgawad and Askary 2015; Mitiku 2018). Yield reduction occurs as a result of root damage and secondary infections of disease agents that enter plant tissues from the nematode feeding sites (Brown 1997).

Nematodes living under the soil have mutualistic or parasitic interaction with many prokaryotic archaea and bacteria. The worldwide number of prokaryotic species was reported to be up to 10 million and only 4,000 were recorded in the soil. Among the bacterial prokaryotes, *Pasteuria* spp. from *Pasteuriaceae* are significant parasites of nematodes. *Pasteuria* spp. are gram (+)

endospore-forming obligate bacteria, which are considered as one of the most effective agents with higher tolerance to unfavorable temperature and soil conditions (Walker and Wachtel 1988). The parasites have several beneficial features such as the ability to survive for up to 70 years in the soil without losing pathogenicity, forming endospore rapidly, attaching to host within 24 h, being immune to different pesticides and being applicable with other natural enemies (Walker and Wachtel 1988; Bird et al. 2003; Chaudhary and Kaul 2011; and Stirling 2014). In addition, bacterium was able to suppress nematode populations under field conditions in a year of period (Tateishi and Sano 2001).

Pasteuria is the most specific obligate parasite that only adheres to the cuticle of nematodes without affecting other organisms. The bacterial endospores attached and sporulated inside the nematodes lead to loss of the reproduction ability by damaging the genital tract of females. The restrictive effect of bacterial infection on the movement of nematodes, results in a decrease of the

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number of specimens that reach the plant roots and cause damage (Kariuki and Dickson 2007).

Several species of bacteria are reported to infect all economically important nematode pests and multiply to a level that is adequate for population suppression. Throughout the world, 8 *Pasteuria* species including *P. thornei*, *P. penetrans*, *P. nishizawae*, *P. usgae*, *P. ramosa*, *P. hartismeri*, *P. goettingiana*, and *P. aldrichii* were identified on 323 nematode species from 116 genera in 80 countries (Giblin-Davis et al. 2003; Cho et al. 2005; Bishop et al. 2007; Giblin-Davis et al. 2011; and Stirling 2014). The only report on the presence of nematophagous bacteria in Turkey was in the East Mediterranean Region (Elekcioglu 1995).

The present study was carried out based on data on host association, distribution, and impact of climate and soil type in Northwestern Turkey. Also, the incidence map was created and host status by genus and species level were included.

Materials and methods

Survey and soil sample collection

An extensive nematode survey was carried out at Edirne (Keşan, Meriç, İpsala, Enez, Uzunköprü districts), Kırklareli (Merkez district), and Tekirdağ (Malkara, Süleymanpaşa, Şarköy, Hayrabolu districts) provinces of the Northwestern Marmara Region in Turkey, in order to collect soil samples and evaluate the presence of bacterial infected nematode species. Research was conducted between 2017-2019 years and each year field surveys started in April and lasted in late October. Sampled fields were randomly selected grapevine (*Vitis vinifera* L./143 vineyards), apple (*Malus domestica* Borkh/34 orchards), pear (*Pyrus communis* L./40 orchards), peach (*Prunus persica* (L.) Batsch/33 orchards), almond (*Prunus amygdalus* L./46 orchards), walnut (*Juglans regia* L./

51 orchards), cherry (*Prunus avium* L./66 orchards), fig (*Ficus carica* L./30 orchards), olive (*Olea europaea* L./26 orchards), sunflower (*Helianthus annuus* L./36 fields), wheat (*Triticum aestivum* L./11 fields), and vegetable fields (17 fields). A total of 5 subsamples with an amount of 1 kg were collected from each field at 10-40 cm soil depth.

Bacteria and nematode extraction, species identification

The nematodes were extracted from 200 g sub-soil by sieving with 60, 400 mesh sieves, followed by centrifugal flotation. The isolated nematodes were heat-killed at 55 °C for 1 min and fixed in a double strength formalin-triethanolamine solution (Seinhorst 1959; Jenkins 1964; Brown and Boag 1988). Prior to analysis, all samples were separated based on their vegetation type and district in order to determine the distribution and nematode associated to each host plant. Right after individuals attached with *Pasteuria* spp. were hand-picked from extracted nematode suspensions at × 100 magnification and mounted on slides by the wax-ring method described by (Hooper 1986). Prepared slides were examined with a Leica DM1000 microscope, the morphometric of nematodes and the endospore size of bacteria were measured, using the Leica Application Suite software. The images of infected individuals were taken by Leica ICC50 W camera. Colony shape and sporangia size of bacteria were used to confirm *Pasteuria*, while nematode identifications by genus and species level were performed with the published keys of Geraert and Raski (1987), Loof and Luc (1990), Brzeski (1991), Castillo and Vovlas (2005), and Handoo et al. (2007).

Results and discussions

Among 516 soil samples collected for the study, the occurrence rate of nematodes attached by *Pasteuria* was

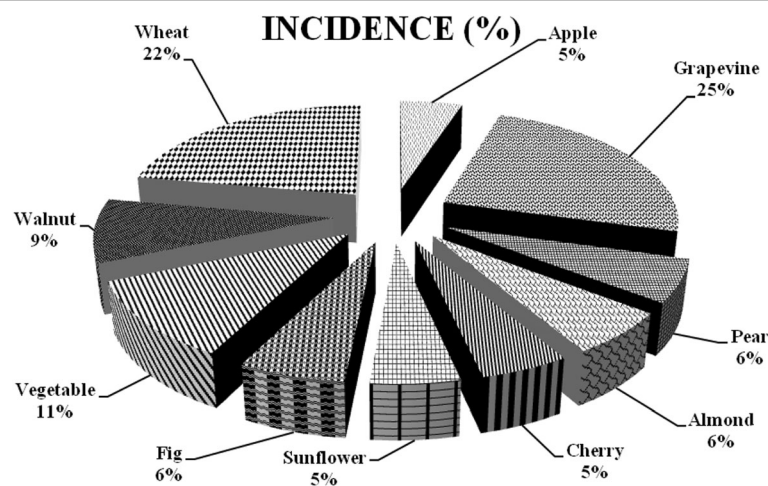


Fig. 1 Occurrence percentage of *Pasteuria* spp. in different vegetations

Table 1 Host nematode genera and occurrence of *Pasteuria* in Edirne, Kırklareli, Çanakkale and Tekirdağ provinces

GENERA		Edirne		Kırklareli		Tekirdağ	
		A	B	A	B	A	B
1	<i>Aphelenchus</i>	+	+	+	+	+	+
2	<i>Aphelenchoides</i>	+	-	+	+	+	-
3	<i>Basiria</i>	+	-	-	-	+	-
4	<i>Boleodorus</i>	+	-	+	-	+	+
5	<i>Coslenchus</i>	+	-	+	-	+	-
6	<i>Criconema</i>	+	-	+	-	+	+
7	<i>Ditylenchus</i>	+	-	+	-	+	+
8	<i>Dorylaimus</i>	+	+	+	-	+	-
9	<i>Filenchus</i>	+	-	+	-	+	+
10	<i>Geocenamus</i>	+	+	+	+	+	+
11	<i>Gracillacus</i>	-	-	-	-	+	-
12	<i>Helicotylenchus</i>	+	-	+	+	+	+
13	<i>Heterodera</i>	+	-	+	-	+	-
14	<i>Meloidogyne</i>	+	-	-	-	+	+
15	<i>Mesocriconema</i>	+	-	+	-	+	+
16	<i>Paratylenchus</i>	+	-	-	-	+	-
17	<i>Paratrophurus</i>	-	-	-	-	+	+
18	<i>Pratylenchus</i>	+	-	+	-	+	+
19	<i>Pratylenchoides</i>	+	-	-	-	+	-
20	<i>Psilenchus</i>	+	-	-	-	+	-
21	<i>Radophulus</i>	-	-	-	-	+	-
23	<i>Rotylenchus</i>	+	+	+	-	+	+
24	<i>Rotylenchulus</i>	-	-	-	-	+	+
25	<i>Scutylenechus</i>	-	-	+	-	-	-
26	<i>Trichodorus</i>	+	-	+	-	-	-
27	<i>Trophurus</i>	-	-	-	-	+	-
28	<i>Tylenchorrhynchus</i>	+	+	+	+	+	+
29	<i>Tylenchus</i>	+	-	-	-	+	-
30	<i>Xiphinema</i>	+	+	+	-	+	+
31	<i>Zygotylenchus</i>	-	-	-	-	+	-

A: Presence of nematode genera

B: Presence of *Pasteuria* spp

calculated as 19.3% (100 samples). Most of the *Pasteuria* positive samples were collected from the coastal Şarköy and Süleymanpaşa districts of Tekirdağ and Merkez district of Kırklareli. The more frequent incidence of bacteria is considered as the result of more moderate soil temperatures in Tekirdağ and sandiest soil conditions in

Kırklareli. In addition, a positive correlation between nematode population increase and bacterium infection was observed in all locations. Since there are many published research results related to the increase of nematode species and populations under temperate and sandy soil conditions, the highest parasitism is considered to

be the result of higher nematode abundance. The bacterium was found only in one or two samples in Uzunköprü, Meriç, İpsala, and Keşan districts.

There were significant variations of infection in the point of vegetation type. Most cases were observed in vineyards (57 vineyards) in both provinces. The other crop plants in infected areas were sunflower (7 fields), wheat (4 fields), vegetable fields (3 fields), walnut (8 orchards), fig (3 orchards), cherry (6 orchards), almond (5 orchards), apple (3 orchards), and pear (4 orchards) (Fig. 1). The main reason for this variation was the soil type. The vineyards in the region were mostly established in soils with lower clay and higher sand content, while in many studies *Pasteuria* was reported to transport more easily in these conditions (Dabiré and Mateille 2003). The other reason

for the infection was host plant susceptibility. The total number of healthy and infected nematodes varied between host crop plants, while the highest number of nematodes was observed in vineyard soils. The olive samples, collected only from Şarköy district and despite warmer soil temperatures and sandy soil type the bacterium, was not detected in any of the samples. Different sized cup-shaped bacterial endospores were observed adhering to the cuticle of nematode species from 32 families (Table 1).

The bacterial isolates were observed on 28 plant-parasitic nematode species listed in Table 2. In addition, the bacterium was found on *Criconema* and *Dorylaimus* juveniles. Based on feeding habitat, 4 species were classified as fungivorous nematode, 5 species as endoparasite, 1 specie as semi-endoparasite and the rest as ectoparasite. The

Table 2 The list of plant parasitic nematodes, the number of soil samples infected, attachment rate, nematode stage in Northwestern Marmara Region

Nematode species	Samples infected	Endospore numbers	Attachment	Nematode stage
<i>Aphelenchus avenae</i>	4,0	2,50	Cuticle	Female
<i>Aphelenchoides confusus</i>	1,0	1,0	Cuticle	Female
<i>Boleodorus thylactus</i>	1,0	2,0	Cuticle, within the body	Juvenile
<i>Criconema</i> sp.	1,0	5,0	Cuticle	Juvenile
<i>Dorylaimus</i> sp.	7,0	>50	Cuticle, within the body	Juvenile
<i>Ditylenchus dipsaci</i>	2,0	2->50	Cuticle, within the body	Female, juvenile
<i>Filenchus thornei</i>	5,0	2,0	Cuticle	Female
<i>Filenchus sheri</i>	1,0	1,0	Cuticle	Female
<i>Geocenamus brevidens</i>	6,0	3,0	Cuticle	Female, male
<i>Geocenamus microdorus</i>	5,0	3,0	Cuticle	Female
<i>Helicotylenchus canadensis</i>	4,0	2,50	Cuticle	Female
<i>Helicotylenchus varicaudatus</i>	2,0	3,50	Cuticle	Female
<i>Helicotylenchus digonicus</i>	4,0	9,0	Cuticle	Female
<i>Helicotylenchus multicinctus</i>	2,0	2,0	Cuticle	Female
<i>Helicotylenchus platyurus</i>	2,0	1,50	Cuticle	Female
<i>Helicotylenchus dihystrera</i>	3,0	3,0	Cuticle	Female
<i>Mesocriconema xenoplax</i>	2,0	3,0	Cuticle	Female
<i>Paratrophurus loofi</i>	1,0	3,0	Cuticle	Female
<i>Meloidogyne javanica</i>	1,0	10	Cuticle	Juvenile
<i>Meloidogyne incognita</i>	1,0	7,0	Cuticle	Juvenile
<i>Pratylenchus penetrans</i>	1,0	2,30	Cuticle	Female
<i>Pratylenchus neglectus</i>	3,0	3,0	Cuticle,	Female
<i>Pratylenchus thornei</i>	4,0	3,20 to>50	Cuticle, within the body	Female, juvenile
<i>Rotylenchulus anamictus</i>	2,0	>50	Cuticle, within the body	Female
<i>Rotylenchus cypriensis</i>	14,0	6,71	Cuticle	Female
<i>Rotylenchus glabratus</i>	1,0	1,0	Cuticle	Female
<i>Rotylenchus brevicaudatus</i>	1,0	1,0	Cuticle	Female
<i>Tylenchorrhynchus cylindricus</i>	7,0	10,70	Cuticle	Female, male
<i>Tylenchorrhynchus nudus</i>	1,0	2,0	Cuticle	Female
<i>Xiphinema pachticum</i>	2,0	1,50	Cuticle	Juvenile

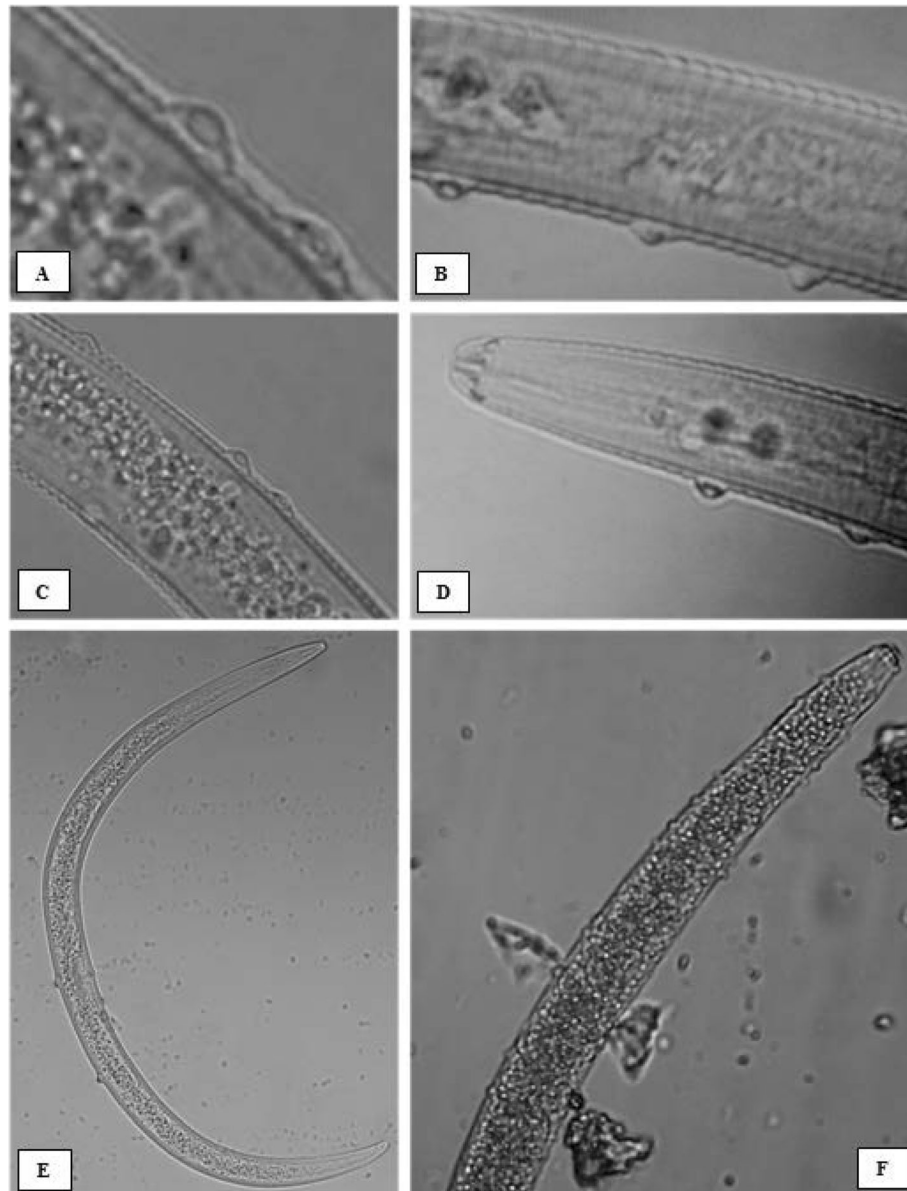


Fig. 2 a *Pasteuria* endospore attached to nematode cuticle. b, c, d Endospore on *Helicotylenchus digonicus*. e Endospore on *Geocenamus brevidens*. f Heavy infection on *Pratylenchus thornei*

individuals parasitized by bacteria and endospores were mostly detected in the outer cuticle of the head, middle body, and tail region of mature females (Fig. 2). Except for individuals from *Filenchus*, *Aphelenchus*, *Aphelencooides*, *Boleodorus*, and *Criconema* genera, which were mostly infected only from the head, most of the specimens from *Helicotylenchus*, *Tylenchorrhynchus*, *Meloidogyne*, *Pratylenchus*, and *Geocenamus* were found attached both from head, mid body, and tail region. Furthermore, the bacterium was observed in the head and tail region of two *Xiphinema pachtaicum* and *Mesocriconema xenoplax* samples (Table 3).

The impact of the degree of parasitism on nematode movement was observed only on *P. thornei*, *T. cylindricus*, *D. dipsaci*, *G. brevidens*, and *R. cyprinesis*. The number of endospores adhering to the cuticle of specimens ranged from 1 to more than 50. Lesion nematode *P. thornei*, onion bulb nematode *D. dipsaci*, omnivore *Dorylaimus* sp., and the reniform nematode *R. anamictus* had mature endospores filled inside nematode body and thus dead individuals were collected from 5 vineyards. The rate of totally filled specimens was 14.7%. In most locations, 17 species were found attached with at least 3 endospores per nematode, while *A. confusus*, *B. thylactus*, *F. thornei*, *F. sheri*, *H.*

Table 3 Endospore measurements of different *Pasteuria* isolates

Nematode species	Endospore diameter (µm)	Endospore height (µm)
<i>Aphelenchus avenae</i>	4,0±0,11	1,40±0,11
<i>Ditylenchus dipsaci</i>	4,0±0,08	1,73±0,12
<i>Filenchus thornei</i>	3,70±0,17	1,82±0,25
<i>Geocenamys brevidens</i>	4,90±0,25	1,96±0,04
<i>Helictylenchus varicaudatus</i>	4,17±0,17	2,0±0,15
<i>Mesocriconema xenoplax</i>	4,05±0,05	2,15±0,15
<i>Rotylenchus cypriensis</i>	4,22±0,17	2,37±0,22
<i>Pratylenchus thornei</i>	3,81±0,12	2,2±0,15
<i>Tylenchhorhynchus cylindricus</i>	7,0-8,40	2,10-2,50
<i>Xiphinema pachtaicum</i>	4,30±0,10	1,92±0,07

multicinctus, *H. platyurus*, *R. brevicaudatus*, *R. glabratus*, *T. nudus*, and *X. pachtaicum* had only one or two endospore. The endospore and central core diameter of specimens were quite similar and in most cases the mean average ranged between 3.70-4.90 and 1.40-2.37, respectively. Depending on higher morphometrics (7.0-8.40) of *T. cylindricus*, it was believed that it may belong to different *Pasteuria* species.

R. cypriensis (11.4%) was the leading nematode in the point of parasitism frequency, followed by *T. cylindricus* (7.1%). Isolated from 10 vineyards and 4 other vegetation, the nematode was attached by at least 6 bacterial spores that were only observed on the upper layer of cuticle.

According to the survey collected data, the bacterium gave promising results as potential biological control candidate of *D. dipsaci*, *P. thornei*, *T. cylindricus*, *R. cypriensis*, *R. anamictus*, and *Criconeema* sp. *D. dipsaci* is known to attack more than 450 different plant species and is reported to cause 50-60% yield loss in garlic and onion as *P. thornei* (Sturhan and Brzeski 1991). Meanwhile, *M. javanica* and *M. incognita* are two endoparasitic nematode that can cause 25-100% yield loss (Wesemael et al. 2011). The other species may accidentally be infected since the bacterium had a lower number of endospores on nematodes.

The previous study carried out to evaluate the *Pasteuria* species in the agricultural areas in Turkey was by Elekcioğlu (1995) in Southeastern Mediterranean region, and the bacteria were identified on nematodes from 10 genera. The identified species were *Aphelenchus avenae*, *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, *Geocenamys brevidens*, *Pratylenchus penetrans*, *P. thornei*, *Rotylenchulus macrosomus*, *R. parvus*, *Tylenchulus semipenetrans*. The results recorded in this study: *A.confusus*, *F. sheri*, *Dorylaimus* sp., *D. dipsaci*, *H. dihystra*, *H. digonicus*, *H. varicaudatus*, *H. platyurus*, *H. canadensis*, *M. microdorus*, *M. xenoplax*, *R. brevicaudatus*, *R. cypriensis*,

R. glabratus, *R. anamictus*, *P. loofi*, *T. cylindricus*, and *T. nudus* constitute new hosts for the country.

Conclusion

The *Pasteuria* spp. bacterial isolates were detected in 30 nematode species isolated from soil samples collected from the agricultural areas of Edirne, Kırklareli, and Tekirdağ provinces in Turkey. *Pasteuria* isolates have been recommended as the most beneficial bacteria with many advantageous features such as tolerance to higher temperatures.

Abbreviations

spp: several species

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Authors' contributions

All authors work in the study and prepare the manuscript together. All authors approved the final manuscript. The identification of bacterium was carried out by MM. The nematodes were identified by LÖ and IHE.

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Availability of data and materials

The available data is included in present manuscript. Results of this study can be used as a reference by other researchers.

Ethics approval and consent to participate

Not applicable. Ethical approval is not required for this study.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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