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*Research article*

# Determination of Plant Parasitic Nematodes in Potato Growing Areas in Bolu Province

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**Abstract.** Potato (*Solanum tuberosum* L.) is one of the most important crops grown for food in the world, but many potato-yield limiting biotic factors are present such as plant parasitic nematodes (PPN). This study was conducted to determine the nematodes associated with potato growing areas during 2014-2016 growing seasons in Bolu province. Overall, Root lesion nematode, *Pratylenchus* spp. were the most common nematode genus occurring in potato production fields with an incidence of over 52.5%. The Cyst nematode, *Globodera* spp. was found in 35% and Root knot nematode, *Meloidogyne* spp. was in 20% of the samples whereas potato cropping very frequently with low rotation, incidences of plant-parasitic nematodes were 62.5% of *Ditylenchus* spp., 50% *Geocenamus* spp., 40% for *Helicotylenchus* spp. or *Rotylenchus* spp., 30% for *Paratylenchus* and *Amplimerlinus* spp., 9% for *Pratylenchoides* spp. and *Tylenchorhynchus* spp. The survey results suggested that the presence and the abundance of plant-parasitic nematodes were found relatively high in potato growing areas in Bolu and may have a potential to cause dramatic yield losses. The comprehensive surveys are indispensable to define the distribution, frequency as well as more accurate identification of plant parasitic nematodes species, particularly *Globodera* spp., *Meloidogyne* spp. and *Pratylenchus* spp. species in potato growing areas of Bolu province.

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# Bolu İli Patates Üretim Alanlarında Bitki Paraziti Nematodların Belirlenmesi

**Anahtar kelimeler:**
Yaygınlık, yoğunluk, bitki paraziti nematodlar, patates, survey

**Özet.** Patates (*Solanum tuberosum* L.) dünyada insan beslenmesinde kullanılmak amacıyla yetiştiriciliği yapılan önemli tarımsal ürünlerden biri olup, başta bitki paraziti nematodlar (BPN) olmak üzere, birçok biyotik faktör patates yetiştiriciliğini önemli ölçüde sınırlandırmaktadır. Bu çalışma, Bolu ili patates üretim alanlarında patateste zararlı nematodların belirlenmesi amacıyla 2014-2016 yılları arasında yürütülmüştür. Çalışma sonucunda, Kök yara nematodları *Pratylenchus* spp. %52.5 oranla patates üretim alanlarında en fazla bulunan nematod olarak tespit edilmiştir. Kist nematodu, *Globodera* spp. %35, Kök-ur nematodu *Meloidogyne* spp. ise %20 oranında bulunmuş olup, münavebenin yapılmadığı lokasyonlarda bitki paraziti nematodların bulunma oranları: *Ditylenchus* %62.5 *Geocenamus* spp. %50, *Helicotylenchus* spp. or *Rotylenchus* spp. %40, for *Paratylenchus* ve *Amplimerlinus* spp. %30, *Pratylenchoides* spp. ve *Tylenchorhynchus* spp. %9 olarak tespit edilmiştir. Survey sonuçları, Bolu ili patates yetiştiriciliği yapılan alanlarda bitki paraziti nematodların yüksek yoğunlukta bulunduğu ve ekonomik ürün kayıplarına neden olabilecek potansiyele sahip olduğunu göstermektedir. Bu nedenle, Bolu ili patates üretim alanlarında bitki paraziti nematodların yaygınlık ve yoğunluğunu belirlemek amacıyla daha kapsamlı surveylerin yapılması, patateste zararlı nematodların, özellikle *Globodera* spp., *Meloidogyne* spp. ve *Pratylenchus* spp. türlerinin teşhislerinin yapılması gerekmektedir.

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## INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important commercial crop and produced worldwide in 19 million ha, at the global production capacity of 368 million ton/year. Turkey is among the leading potato-producer countries in the worldwide and production mostly supplied from Nigde, Nevsehir, Izmir, Bolu and Afyon provinces. Only the Bolu province supplies 160 000 Mg year$^{-1}$ (Turkstat 2015) but it has been reduced due to the severe infections of pests and disease such as plant parasitic nematodes (Kepenekci 2012).

Plant parasitic nematodes (PPNs) cause significant yield reduction of agricultural products with a cost of >$120 billion p.a. in the world (Chitwood 2011). PPNs are ubiquitous in many cropping systems including potato agro-ecosystem in Turkey (Kepenekci *et al.,* 2012). It has been reported that yield and quality traits of the potato are reduced by PPN infestations (Coyne *et al.,* 2003, Olabiyi 2007). The occurrence of PPN in potato cultivated areas in Turkey becoming an important yield limiting factors and the situation may be worsen if proper management programs will not be put forward (Kepenekci *et al.,* 2012). The most frequently encountered plant-parasitic nematodes in potato, such as potato rot nematode (*Ditylenchus destructor*), potato cyst nematodes (*Globodera rostochiensis* and *G. pallia*), root-knot nematodes (*Meloidogyne chitwoodi*), the stem and bulb nematode (*Ditylenchus dipsaci*), and root lesion nematodes (*Pratylenchus* spp.) have also been reported by Jones *et al.* (2013).

The most important potato cyst nematode, *Globodera rostochiensi* has been reported for the first time by Enneli (1996) in Bolu, Turkey. Since the detection of this potato cyst nematode species in Bolu potato growing areas, the strict quarantine measures have been implemented to prevent nematode spread and contamination. However, the potato cyst nematode species was later detected in main potato growing province such as Niğde and İzmir (Evlice 2012; Ulutas 2012). Because of its capacity of quickly spreading to and colonizing new areas (Bebber *et al.,* 2014) and wide host range, root-knot nematode (RKN, *Meloidogyne* spp.), is positioned as the most financially harming (Jones *et al.,* 2013). Nine *Meloidogyne* species occur in Turkey with *M. incognita*, *M. javanica* and *M. chitwoodi* being the most prevalent (Kepenekci *et al.,* 2012). Moreover, Root lesion nematode, (RLN; *Pratylenchus* spp.) are the most encountered plant parasitic nematodes in Turkey, infest potato (*Solanum tuberosum* L.), other agronomic crops, perennials, ornamentals and hardwoods (Kepenekci *et al.,* 2012).

There is a very limited number of literature present about plant parasitic nematodes especially root knot nematode, cyst nematode and root lesion nematode affecting potatoes in Bolu. The objective of this work is to investigate the occurrence and distribution of plant parasitic nematodes associated with potato in Bolu.

## MATERIAL AND METHOD

### *Nematode survey*

The study was conducted to investigate occurrence of frequently encountered plant parasitic nematode genera in potato growing areas in Bolu. Soil samples and potato tubers were randomly sampled from selected locations in main potato producing areas of Dortdivan and Central District in Bolu province (Figure 1). Several potato fields in which symptoms such as wilting, slow growth, stunting and yellowing of leaves were observed during the surveys. Soil samples from the rhizospheric regions of the plants were collected at a depth of 10-15 cm (Barker 1985). A minimum of 10 roots and soil sub-samples were collected from each field and were stored in polythene bags in the laboratory for processing. Altogether, 40 soil samples and tubers were collected from 40 different localities belonged to the 3 districts in Bolu province (Table 1).

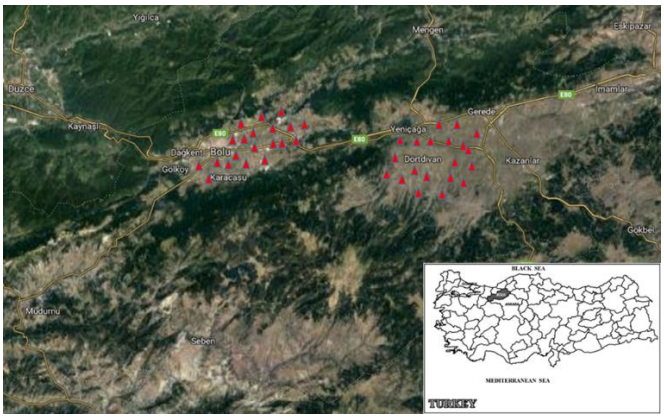


**Figure 1.** A map of Bolu province showing surveyed regions.
*Şekil 1. Bolu ilinde survey yapılan bölgelerin haritası.*

### *Nematode Extraction (Soil)*

Soil samples were processed for the extraction of nematodes by Baermann's funnel and sieving-decanting method as described by (Hooper *et al.,* 2005).

***Baermann's funnel method*:** This extracting method was used to isolate motile nematodes from the soil. A sub-sample of 200 ml soil was taken from the samples to extract nematodes by using the modified Baermann funnel technique (Hooper *et al.,*

**Table 1.** The location of soil samples collected from the Bolu province of Turkey.
*Çizelge 1. Türkiye'de Bolu ilinde toplanan örneklerin lokasyonları.*

| No | County | Location | Coordinates | |
|---|---|---|---|---|
| | | | Latitude | Longitude |
| 1 | Dortdivan | County Center | 40° 44' 08'' N | 32° 05' 56'' E |
| 2 | Dortdivan | County Center | 40° 44' 03'' N | 32° 05' 40'' E |
| 3 | Dortdivan | County Center | 40° 43' 55'' N | 32° 05' 30'' E |
| 4 | Dortdivan | County Center | 40° 43' 54'' N | 32° 05' 39'' E |
| 5 | Dortdivan | County Center | 40° 43' 54'' N | 32° 05' 39'' E |
| 6 | Dortdivan | Dogancılar Village | 40° 43' 54'' N | 32° 05' 54'' E |
| 7 | Dortdivan | Dogancılar Village | 40° 43' 54'' N | 32° 05' 54'' E |
| 8 | Dortdivan | Cardak Village | 40° 43' 30'' N | 32° 06' 18'' E |
| 9 | Dortdivan | Yavalar Village | 40° 43' 13'' N | 32° 05' 50'' E |
| 10 | Dortdivan | Yavalar Village | 40° 43' 26'' N | 32° 05' 42'' E |
| 11 | Dortdivan | Yavalar Village | 40° 43' 39'' N | 32° 05' 22'' E |
| 12 | Dortdivan | Yavalar Village | 40° 43' 50'' N | 32° 05' 15'' E |
| 13 | Dortdivan | Deveciler Village | 40° 43' 43'' N | 32° 05' 01'' E |
| 14 | Dortdivan | Deveciler Village | 40° 43' 43'' N | 32° 05' 01'' E |
| 15 | Dortdivan | Adakınık Village | 40° 42' 33'' N | 32° 04' 20'' E |
| 16 | Dortdivan | Adakınık Village | 40° 42' 33'' N | 32° 04' 20'' E |
| 17 | Dortdivan | Adakınık Village | 40° 42' 28'' N | 32° 04' 03'' E |
| 18 | Dortdivan | Adakınık Village | 40° 42' 24'' N | 32° 03' 56'' E |
| 19 | Dortdivan | Kadılar Village | 40° 42' 45'' N | 32° 03' 20'' E |
| 20 | Dortdivan | Kadılar Village | 40° 42' 41'' N | 32° 03' 08'' E |
| 21 | Provincial center | Ogulduruk Village | 40° 45' 15'' N | 31° 43' 15'' E |
| 22 | Provincial center | Kasaplar Village | 40° 42' 09'' N | 31° 33' 22'' E |
| 23 | Provincial center | Dogancı Village | 40° 41' 16'' N | 31° 34' 15'' E |
| 24 | Provincial center | Dogancı Village | 40° 41' 16'' N | 31° 34' 30'' E |
| 25 | Provincial center | Karaagaç Village | 40° 44' 46'' N | 31° 39' 39'' E |
| 26 | Provincial center | Tatlar Village | 40° 45' 47'' N | 31° 42' 26'' E |
| 27 | Provincial center | Vakıfgecitveren Village | 40° 43' 57'' N | 31° 40' 09'' E |
| 28 | Provincial center | Demirciler Village | 40° 43' 10'' N | 31° 39' 33'' E |
| 29 | Provincial center | Hacıbey Village | 40° 42' 17'' N | 31° 39' 56'' E |
| 30 | Provincial center | Okcular Village | 40° 43' 09'' N | 31° 38' 13'' E |
| 31 | Provincial center | Örencik Village | 40° 42' 16'' N | 31° 38' 37'' E |
| 32 | Provincial center | Ilıcakınık Village | 40° 41' 51'' N | 31° 37' 59'' E |
| 33 | Provincial center | Karacasu Village | 40° 41' 53'' N | 31° 37' 47'' E |
| 34 | Provincial center | Sultanbey Village | 40° 41' 47" N | 31° 34' 15" E |
| 35 | Provincial center | Cakır Village | 40° 41' 48" N | 31° 34' 15" E |
| 36 | Provincial center | Kopruculer Village | 40° 41' 39" N | 31° 35' 1" E |
| 37 | Provincial center | Campınar Village | 40° 41' 23" N | 31° 35' 35" E |
| 38 | Provincial center | Campınar Village | 40° 41' 12" N | 31° 35' 28" E |
| 39 | Provincial center | Campınar Village | 40° 41' 10" N | 31° 35' 21" E |
| 40 | Provincial center | Campınar Village | 40° 41' 10" N | 31° 35' 22" E |

2005). Nematodes were collected after 24 hours in a 100 ml measure and transferred into a 2 ml Eppendorf tube. Extracted nematodes were counted using a stereomicroscope.

***Sieving-decanting method*:** This extracting method was used for the isolation of sedentary nematodes in soil. By mixing the soil, a sub-sample of 250 $cm^3$ was taken to extract nematodes by using a modified sieving-decanting technique (Fenwick 1940). Cysts were collected on 250 µm pore-sized sieves, then, counted and collected by hand using a dissecting needle under a stereo-binocular microscope (Zeiss, V20) at 12 × magnification.

***Nematode Extraction from Tubers***

Nematodes were extracted within 48 hours after tubers were collected from the field. Modified Baermann technique was used for extraction. Potato tubers were rinsed and peeled thinly with knife, then; peelings were chopped to 3-5 cm sizes and mixed thoroughly. The sample was gently poured onto a tissue paper on a sieve, 24 hours later nematode containing solutions were collected in 100 ml measures, allowed to settle in the measures, removed the excess water from the measures, then, they were transferred into 2 ml Eppendorf tubes. Extracted nematodes were counted using a stereomicroscope.

Root and tubers were also examined visually under a stereo-binocular microscope. The females of the root knot nematodes were removed from the galled roots for species identification. The assessment of nematode abundance in 10 g root samples and 100 g soil sample was performed in a chambered counting dish under a stereoscopic binocular microscope.

***Plant Parasitic Nematode Community Analyses***

The community structure of plant parasitic nematodes was analysed in relation to the site differences especially the distance between the sampling sites and locations in potato fields. The population densities of nematode species in the samples were calculated using the formulae (Norton 1978).

$$\text{Absolute frequency} = \frac{\text{Number of samples containing a genus}}{\text{Number of samples collected}} \times 100$$

$$\text{Relative frequency} = \frac{\text{Frequency of a genus}}{\text{Sum of frequency of all genus}} \times 100$$

$$\text{Absolute density} = \frac{\text{Number of individuals of a genus in a sample}}{\text{Volume or mass or units of the samples}} \times 100$$

## RESULTS

The abundance of plant parasitic nematode population 1000 $cm^{-3}$ associated with potato plant in 40 different localities belonging to 3 district in Bolu (Table 2). The total survey area represented 12% of the potato hectare in Bolu province. Plant parasitic nematodes were present in 90% of soil samples. The plant parasitic nematode abundances in the investigated areas had an average value of 820 individuals 100 $g^{-1}$ soil in sample. Ten genera of plant-parasitic nematodes were identified. Most abundant and common genera were: *Geocenamus*, *Ditylenchus*, *Pratylenchus*, *Helicotylenchus* or *Rotylenchus*, *Amplimerlinus*, *Globodera, Paratylenchus, Pratylenchoides*, *Tylenchorhynchus* and *Meloidogyne* (Table 2).

**Table 2.** Community analysis of plant parasitic nematodes on potato plant in 40 different localities of Bolu.
*Çizelge 2. Bolu ilinde 40 farklı lokasyonda patates bitkisinde bitki paraziti nematodlarının topluluk analizi.*

| No | Nematode | Absolute Frequency | Relative Frequency | Absolute Density |
|---|---|---|---|---|
| 1 | *Globodera* spp. | 27.5 | 3.8 | 7.7 |
| 2 | *Meloidogyne* spp. | 10.0 | 10.0 | 2.8 |
| 3 | *Pratylenchus* spp | 52.5 | 89.0 | 14.8 |
| 4 | *Paratylenchus* spp. | 27.5 | 25.5 | 7.7 |
| 5 | *Pratylenchoides* spp. | 20.0 | 22.5 | 5.6 |
| 6 | *Tylenchorhynchus* spp. | 20.0 | 28.8 | 5.6 |
| 7 | *Geocenamus* spp. | 65.0 | 40.0 | 18.3 |
| 8 | *Amplimerlinus* spp. | 30.0 | 25.8 | 8.5 |
| 9 | *Helicotylenchus* spp. or *Rotylenchus* spp. | 40.0 | 47.5 | 11.3 |
| 10 | *Ditylenchus* spp. | 62.5 | 40.0 | 17.6 |

Economically important plant parasitic nematodes found within the potato rhizosphere were root knot nematode, *Meloidogyne* spp., root-lesion nematode, *Pratylenchus* species and cyst nematode, *Globodera* species. The nematodes were unevenly distributed across the sampled area. The population of lesion nematode, *Pratylenchus* species tends to be generally higher than the other plant parasitic nematodes after *Geocenamus* species within the potato rhizosphere. The population of root knot nematode, *Meloidogyne incognita* was the lowest than the other nematodes in the potato growing regions. Moreover, potato cyst nematode, *Globodera* spp. were detected in many samples, however there was no full cyst. Additionally, free-living nematodes from bacterivorous group were detected in all soil and root samples (Table 2).

An analysis of nematode communities is also shown in Table 2 and revealed the presence of 10 genera of plant parasitic nematodes. The most frequently occurring nematode was *Geocenamus* spp. having an absolute frequency of 65%, followed by *Ditylenchus* spp. with 62.5% and *Pratylenchus* spp. with 52.5%. The lowest absolute frequency was recorded in *Meloidogyne* spp. with 10%. Likewise, the highest relative frequency was recorded in *Pratylenchus* spp. with 89% followed by *Helicotylenchus* or *Rotylenchus* spp. with 24.39% and *Geocenamus* spp. with *Ditylenchus* spp. with 40%, the lowest being recorded in *Meloidogyne* spp. with 3.8% (Table 2).

## DISCUSSION

The main goal of the present study was to investigate the distribution of plant-parasitic nematodes in potato farming in Bolu, the key potato-producing province, north-western Turkey. Survey results revealed that more than 90% of the potato-sampled stocks were infected with by *Geocenamus*, *Ditylenchus*, *Pratylenchus*, *Helicotylenchus* or *Rotylenchus*, *Amplimerlinus*, *Globodera, Paratylenchus, Pratylenchoides*, *Tylenchorhynchus* and *Meloidogyne*, and approximately 10% of the stocks were co-infected by more than one of these nematodes. Knowledge is limited about the extent and damage potential by many of these species to potato. However, *Globodera* spp., *Meloidogyne* spp., and *Pratylenchus* spp. can be damaging the potato (Olabiyi 2004; Kepenekci *et al.,* 2014).

On a worldwide basis, the ten most important nematode genera include *Meloidogyne, Pratylenchus, Heterodera, Ditylenchus, Globodera, Tylenchulus, Xiphinema, Radopholus, Rotylenchulus* and *Helicotylenchus* (Sasser and Freckman 1987; Whitehead 1998) and some of which are economic nematode pests of potato (Ames *et al.,* 1997; Coyne *et al.,* 2003). Sasser (1989) in a worldwide research survey, ranked root knot nematode *Meloidogyne* species, root lesion nematodes *Pratylenchus* species and cyst nematodes *Globodera* species as the world's top three plant parasitic nematodes. Moreover, *Meloidogyne chitwoodi*, *Globodera rostochiensis*, *Ditylenchus destructor*, *D. dipsaci*, and *Pratylenchus* spp. have been confirmed as the nematode pests of potato in Turkey (Ulutas *et al.,* 2011; Evlice *et al.,* 2012). Our result is significant to note that the majority of pathogenic nematodes are soil inhabiting and endo-parasitic in nature in potato farming in Bolu, Turkey.

Root knot nematodes are ubiquitous and over 126 species have been described (Moens *et al.*, 2009) but only a very few are economically important in agriculture (Sasser 1980). The most economically important ones are *M. incognita*, *M. indica*, *M. javanica*, *M. hapla*, *M. graminicola*, *M. arenaria*, and *M. triticoryzae* (Mangala and Mauria 2006; Kepenekci *et al.,* 2014). However, root-knot nematodes were not identified species level in sampled areas in present study. The symptoms caused by root lesion nematodes, *Pratylenchus* spp. are non-specific and often confused with nutrient deficiency, water deficits, salinity or other soil disorders. There are variations in the symptoms expressed based on species of plant parasitic nematodes involved, initial nematode population density, age of the host, plants and various ecological factors (Mangala and Mauria 2006). A more comprehensive study is needed to identify *Pratylenchus* species in surveyed areas in this study. *Globodera* spp. were detected in studied areas, but the nematode cysts were found empty. It should be re-evaluated in a detailed survey to determine species level of frequency and density of *Globodera* spp. In addition, it is specifically needed to determine the reason behind the empty cysts of *Globodera* spp.

Management strategies of plant parasitic nematodes in potato growing areas in the Bolu will require an integrated approach. Although nematicides will still be used, other control strategies, which include crop rotations, cultural practices, sanitation and quarantine, are expected to integrate into management practices.

## CONCLUSION

By this study, a survey conducted in three main sweet potato growing areas in Bolu, Turkey, the plant-parasitic nematodes associated with this crop have a low diversity since only ten genera of parasitic nematodes were found in the rhizosphere of the plants. They are widespread in the main potato growing areas with a relatively high abundance. However, their effects on the growth and yield of potato has not been determined, yet. Therefore, it is necessary to study its bio-ecology and pathogenicity to potatoes, particularly, detected by this study such as *Globodera* spp., *Meloidogyne* spp., and *Pratylenchus* spp. Moreover, it needs a comprehensive study in sampled areas to determine these nematode species and their significances.

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