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Investigation note

Phytoparasitic nematodes in broad bean crops in eastern Puebla

Ángel Enrique Núñez-Sánchez[1]
María del Carmen Núñez-Camargo[1,§]
Carlos Yair Vázquez-González[1]
Alejandro Salinas-Castro[2]
Daniel López-Lima[1]

1 Facultad de Ciencias Agrícolas-Universidad Veracruzana. Circuito Gonzalo Aguirre Beltrán s/n, Zona Universitaria, Xalapa-Enríquez, Veracruz, México. CP. 91090. (anunez@uv.mx; chapa-12-12@hotmail.com; danielopez@uv.mx).

2 Centro de Investigación en Micología Aplicada-Universidad Veracruzana. Médicos núm. 5, Colonia Unidad del Bosque, Xalapa-Enríquez, Veracruz, México. CP. 91010. (asalinas@uv.mx).

Autora para correspondencia: marnunez@uv.mx

Abstract

The broad bean is one of the most important crops in the Central Mexican Plateau due to its adaptation to edaphoclimatic conditions and its high nutritional value in the diet of the inhabitants of this region. Nonetheless, the yields obtained are low compared to the world average. Phytoparasitic nematodes are one of the main constraints to broad bean production in other parts of the world; however, its presence in this crop is unknown in Mexico. In this work, phytoparasitic nematodes associated with broad bean crops were identified and quantified in 10 farms in the municipalities of Tepeyahualco and Cuyoaco, Puebla, and in plants germinated in sterile soil from seeds from different areas in 2022. The following six genera of phytoparasitic nematodes were found: *Criconemoides*, *Ditylenchus*, *Helicotylenchus*, *Hoplolaimus*, *Pratylenchus* and *Zygotylenchus*. The most abundant genera were *Ditylenchus* and *Pratylenchus*, with a population density of 11 and 7 specimens per 100 cm^3 of soil and 159 and 60 specimens per 100 g of plant tissue, respectively, and they were found in all sampling sites. The population density of *Ditylenchus* exceeds the economic threshold of the crop; in addition, specimens of this genus were found in plants germinated in sterile soil. The results show that *Ditylenchus* is distributed throughout the sampled area and can affect yield. Further studies are needed to determine the identity of nematode species and their distribution in other production areas.

Keywords:

Ditylenchus, Pratylenchus, Vicia faba, detection.

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The broad bean (*Vicia faba* L.) is a legume of the Fabaceae family, it is the seventh economically important legume in the world due to its nutritional qualities (Dhull *et al*., 2022). The leading broad bean-producing countries are China, Ethiopia, the United Kingdom, Algeria, and Egypt (FAO, 2022). Mexico is the seventh largest producer of green broad beans with more than 80 000 t per year, and the twenty-first producer of dried broad beans with more than 37 000 t per year. It has a sown area of 36 000 ha (SIAP, 2022).

The State of Mexico, Puebla and Michoacán are the leading green broad bean-producing states with 89% of the national production. Likewise, the states of Puebla, Veracruz and Tlaxcala are the largest producers of dried broad beans, with 94% of the national production. The average yield obtained in Mexico is 6.3 t ha^{-1} for green grain and 1.6 t ha^{-1} for dried grain (SIAP, 2022); nevertheless, this yield is low compared to other countries such as China, where 19 t ha^{-1} of green broad beans are obtained, or Argentina, where yields of 8.8 t ha^{-1} of dried broad beans are obtained.

Despite the low yields obtained in Mexico, the broad bean is a very important crop, mainly in the states of the Central Mexican Plateau because it is an alternative to corn and bean crops due to its tolerance to low temperatures and low humidity conditions (Rojas-Tiempo *et al*., 2012).

Worldwide, broad bean crops are affected by various pests, among which phytoparasitic nematodes stand out, which are considered one of the main constraints to the production of this plant in countries in Europe, Africa, and Asia (Sikora *et al*., 2018). Nematodes are difficult to detect as they cause symptoms that can be mistaken for nutrient deficiencies or for effects caused by other pathogens, such as stunted growth, reduction of the root system and deformation of stems, leaves, pods, and seeds (Vovlas *et al*., 2011).

In the eastern region of the state of Puebla, the broad bean is widely cultivated as it adapts to edaphoclimatic conditions and is a major component of the diet of the inhabitants; however, in recent years, the yield has been steadily decreasing, to the extent that it is currently below the national average (Rojas-Tiempo *et al*., 2012; SIAP, 2022). Symptoms corresponding to nematode infestation have been observed in field visits; nevertheless, no studies have been carried out to determine their presence and population levels, therefore, the objective of this work is to identify the genera of nematodes associated with broad beans crops in one of the main producing areas in the east of the state of Puebla.

Sampling was carried out on May 21, 2022, in 10 one-ha plots sown with *V. faba* var. major in the localities of Mancuernas and Xaltipanapan in the municipality of Tepeyahualco and the localities of Francisco I. Madero and Texcal in the municipality of Cuyoaco (Figure 1). All the study sites have a subhumid temperate climate with rainfall in summer, lower humidity, with an average temperature between 10 and 16 °C, regosol-type soil, 300-700 mm of precipitation, and between 2 500 and 2 600 masl (INEGI, 2010).

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Figure 1. Location of sampling sites in the state of Puebla.

At the time of sampling, the plants were two months old and between 10 and 15 cm tall. At each site, a sample composed of five complete plants with rhizospheric soil was taken in a zigzag pattern along the land.

The plants and soil were transferred to the Laboratory of Parasitology and Biological Control of the Faculty of Agricultural Sciences of the Veracruz University. To extract ectoparasitic nematodes, 100 cm^3 of rhizospheric soil was processed using the sieving-centrifuging technique (van Bezooijen, 2006). To extract the endoparasitic nematodes, 100 g of fresh plant tissue was processed, including the complete root system, part of the stem and foliage, by the macerating and sieving-centrifuging method.

After extraction, the nematodes were fixed with 4% formalin and cleared by the Sehinhorts method (van Bezooijen, 2006). Subsequently, the nematodes were quantified by separating the different morphotypes with the presence of stylets (phytoparasites) and free-living nematodes in a Sedwick-Rafter counting chamber at 100X in a Nikon Alphaphot YS2-T light microscope.

Once quantified, between 10 and 15 specimens of each morphotype were mounted on a paraffin ring to observe them under a microscope and identify the genus using specialized keys (Siddiqi, 2000). Additionally, we collected broad bean seeds from different places, used in the study area for sowing: Querétaro, Querétaro, Tenango del Valle, State of Mexico and San Salvador el Seco, Puebla. Twenty seeds from each site were placed in pots with sterile soil and left to develop for a period of 45 days.

Subsequently, the plants were removed from the pot and the total plant tissue was processed using the macerating, sieving-centrifuging technique. The samples were fixed in 4% formalin and then

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inspected under the 100X light microscope in a Sedwick-Rafter counting chamber. The nematodes found were processed by the Sehinhorts method and mounted on a paraffin ring for identification at the genus level (Siddiqi, 2000; van Bezooijen, 2006).

Six genera of phytoparasitic nematodes associated with soil and broad bean plants were found at the collection sites (Table 1). The characteristics of the observed specimens are described below: *Criconemoides* sp. Length 500 to 600 µm, cuticle strongly ringed. Rings with soft edges. Cephalic region and labial disc not protruding. Stylet robust, 80 µm, with anchor-shaped base (Figure 2a). Median bulb elongated and wide. The vulva is seen as a transverse groove, closed with the anterior lip not protruding.

Table 1. Genera of phytoparasitic nematodes associated with broad bean crops and their relative abundance in the east of the state of Puebla.

Family	Subfamily	Genus	Relative abundance
Family	Subfamily	Genus	Relative abundance
Criconematidae	Macroposthoniinae	*Criconemoides*	1.09
Anguinidae	Anguininae	*Ditylenchus*	68.83
Hoplolaimidae	Hoplolaiminae	*Helicotylenchus*	2.06
		Hoplolaimus	1.21
Pratylenchidae	Pratylenchinae	*Pratylenchus*	26.76
		Zygotylenchus	0.04

A single branch of the female's reproductive organs is functional. Tail conoid with truncated end. *Ditylenchus* sp. Length between 1 and 1.2 mm, cuticle with very fine transverse striation, lateral field with four incisures. Cephalic region low, without striae, slightly flattened and barely separated from the body. Stylet between 10 and 12 µm, with visible basal nodules (Figure 2b). Esophageal procorpus cylindrical, which narrows slightly when attached to the median bulb. Vagina distinctive at a right angle to the body, only the anterior branch of the female's reproductive organs developed and functional, with post-vulval uterine sac present and extended. Tail conoid, elongated, with a sharp, pointed end. *Helicotylenchus* sp. Length from 700 to 850 µm. Cuticle with well-defined striation and lateral field with four incisures.

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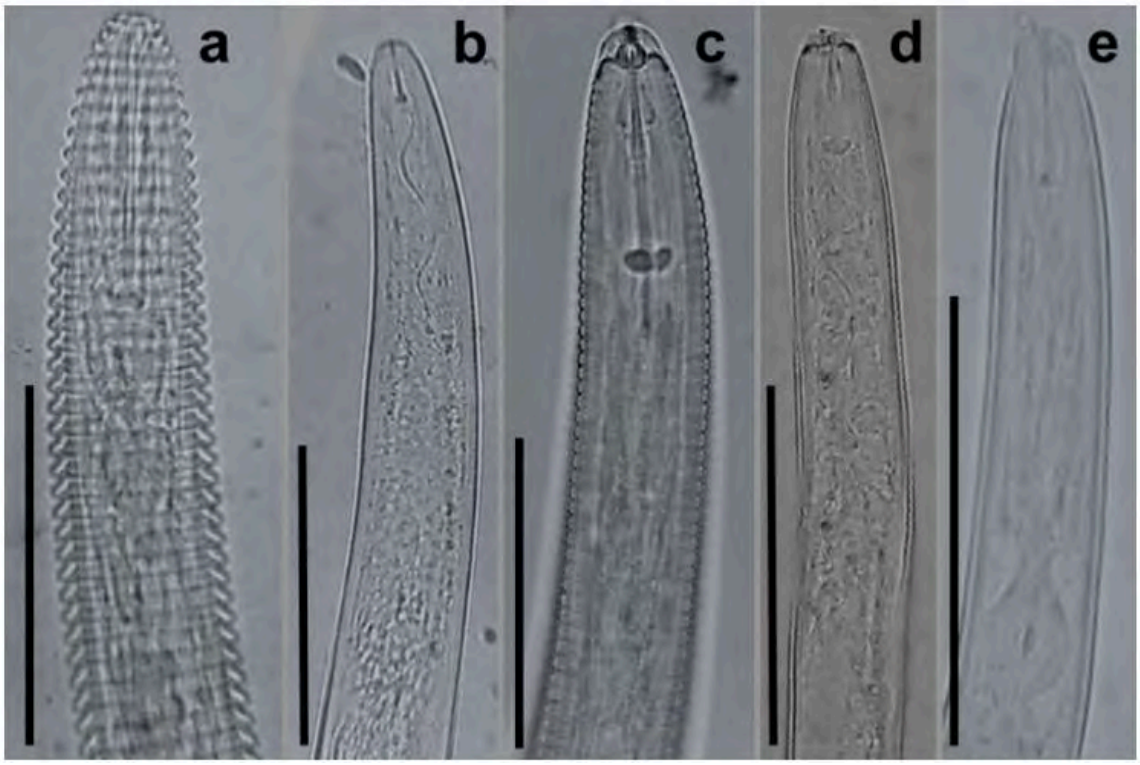
Figure 2. Anterior region of the body of a) *Criconemoides* sp.; b) *Ditylenchus* sp.; c) *Helicotylenchus* sp.; d) *Pratylenchus* sp.; and e) *Zygotylenchus* sp. (Scale bars= 50 µm).



Cephalic region elevated, hemispherical with four to five rings. Stylet well-developed, 25 to 27 µm, with prominent basal nodules (Figure 2c). Median bulb oval, the esophageal glands partially surround the anterior part of the intestine. Vagina in the last third of the body, both branches of the female reproductive organs well developed and functional. Tail dorsally convex, conoid with a mucro. *Hoplolaimus* sp. Length 1 to 1.1 mm. Cuticle with well-defined striation, lateral field with four incisures. Cephalic region hemispheric, four to six rings, well separated from the body by a constriction.

Labial frame well-developed and sclerotized. Stylet massive, well-developed, 40 to 45 µm, with compact basal nodules. Median bulb oval, occupying about half the width of the body. Esophageal glands with a nucleus, extending dorsally over the intestine. Vulva slightly posterior to the middle of the body. Both branches of the female's reproductive organs well developed and functional. Tail short and rounded. *Pratylenchus* sp. Length from 600 to 750 µm. Cuticle slightly striated, lateral field with four incisures. Cephalic region low, flattened, continuous with the contour of the body and strongly sclerotized.

Stylet robust, 15-17 µm, with round basal nodules (Figure 2d). Median bulb oval, esophageal glands extending ventrally over the intestine. Vulva in the posterior quarter of the body. Only the anterior branch of the female's reproductive organs is developed and functional, with the presence of a post-vulvar uterine sac. Tail rounded with no projections. *Zygotylenchus* sp. Length 500 µm. Cuticle slightly striated, lateral field with four incisures.

Cephalic region low, slightly narrow, and rounded, continuous with the body and strongly sclerotized. Stylet strong, 15 to 16 µm, with rounded basal nodules (Figure 2e). Esophageal glands extending over the intestine mainly ventrally. Vulva slightly posterior to the middle of the body. Both

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branches of the female's reproductive organs well developed and functional. Tail slightly narrow with rounded end.

The most abundant genus was *Ditylenchus*, which accounted for more than 68% of the specimens collected, followed by *Pratylenchus* with 26%. The remaining four genera had an abundance of less than 5% (Table 1). The genera found in this study coincide with those previously recorded in producing countries in Europe, Asia, and Africa (Vovlas *et al*., 2011; Feyisa, 2021). *Ditylenchus* and *Pratylenchus* are the most abundant phytoparasitic nematodes in broad bean crops (Sikora *et al*., 2018).

Likewise, *Helicotylenchus*, *Hoplolaimus* and *Zygotylenchus* have been recorded in some regions, although in low abundance (Azimi, 2017). On the other hand, the genus *Criconemoides* had not previously been recorded associated with broad bean crops, although it is common in the rhizosphere of various crops in Mexico (Velásquez-Valle *et al*., 2018). Globally, *Ditylenchus* is considered the most harmful pest nematode in broad bean crops, they are migratory endoparasites that feed on the stem, petiole, leaf tissue, pods, and seeds, but do not cause significant root damage (Sikora *et al*., 2018).

In countries in the Mediterranean region, the species *D. dipsaci* and *D. gigas* have been recorded causing severe damage to broad bean crops (Vovlas *et al*., 2011; Azimi, 2017). Likewise, nematodes of the genus *Pratylenchus* are migratory endoparasites that cause lesions and necrosis in the root tissue. Species such as *P. neglectus* and *P. thornei* are considered important pests for the crop in Italy, North Africa, and the Middle East (Di Vito *et al*., 2000).

The population density of phytoparasitic nematodes was 30.4 specimens in 100 cm^3 of soil and 221.2 in 100 g of fresh plant tissue (Table 2). The genera with the highest soil and root population density were *Ditylenchus* and *Pratylenchus* (Table 2). Nevertheless, it was lower than the population density in other production areas in Africa, where 150 individuals of *Ditylenchus* per 100 cm^3 of soil and 750 per 100 g of plant tissue and 150 *Pratylenchus* per 100 cm^3 of soil and 1000 per 100 g of root are recorded (Feyisa, 2021).

Table 2. Population density and frequency of appearance of nematodes associated with broad bean crops.

Genus	Soil (100 cm^3)		Fresh plant tissue (100 g)	
	Density of population	Frequency	Density of population	Frequency
Criconemoides	3 ±3	90	-	
Ditylenchus	11 ±14	100	159 ±82	100
Helicotylenchus	5 ±6	60	1 ±0.3	10
Hoplolaimus	1 ±0.8	50	2 ±2	60
Pratylenchus	7 ±5	100	60 ±23	100
Zygotylenchus	-	-	1 ±0.3	10
Total number of phytoparasitic nematodes	30.4 ±29.9		221.2 ±69	
Free-living nematodes	21 ±15	100	-	-

In this work, the population of *Pratylenchus* is below the economic threshold to cause damage to the broad bean crop, which is 200 juveniles and adults per 100 cm^3 of soil (Di Vito *et al*., 2000); however, for *Ditylenchus* it is above the economic threshold of 2 individuals per 100 g of soil (Subbotin and Ryley, 2012). The low population compared to other sites may be due to the drought conditions that prevailed at the time of sampling in the study area and the little development of the plants, it is necessary to carry out sampling in the different phenological stages of the crop.

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Specimens of *Ditylenchus* were found in plants germinated in sterile soil from all three sources. In seeds from Querétaro and the State of Mexico, an average of one specimen per germinated plant was found in 41% and 33% of the samples examined, respectively. In the seeds from Puebla, an average of 6 specimens per germinated plant were found in 91% of the samples.

It is likely that this nematode has a higher incidence in the state of Puebla, since the seeds harvested in the zone are more infested. Nonetheless, it is necessary to determine whether the variety of seeds used in different areas can influence the presence of the nematode. The seed infested with this nematode is the main means of dissemination in crops such as broad beans or garlic, since individuals of *Ditylenchus* can enter a state of anhydrobiosis and survive desiccation for long periods of time inside the seed, reactivating their life cycle once there are suitable conditions (Holajjer *et al*., 2020).

Conclusions

In the broad bean crops of the study area, there are populations of phytoparasitic nematodes that can affect the yield of the broad bean crop. In particular, nematodes of the genus *Ditylenchus* are found at all sampling sites at population densities above the economic threshold to affect yield. It is necessary to carry out broader samplings to determine the distribution and species of nematodes in the broad bean crop, mainly of the genera *Ditylenchus* and *Pratylenchus* in order to determine their impact on productivity and propose management strategies.

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