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Prevalence of plant parasitic nematodes in tomatoes (*Lycopersicon esculentum*) grown in different soil pH and texture conditions

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Abstract

The present study was carried out from March to May 2017 at Dema Growth Point in Seke District of Mashonaland East Province, Zimbabwe to investigate the prevalence of plant parasitic nematodes in tomatoes grown in different soil pH and texture conditions. The field soil was classified as sandy loam, whilst the garden soil was classified as clay loamy. Out of a total of 235 nematodes identified, 70.6% were from the fields, while 29.4% were from the gardens. The plant parasitic nematodes, *Meloidogyne* spp, *Helicotylenchus* spp and *Pratylenchus* spp were identified. The *Meloidogyne* spp accounted for 50.6% of the total population, while 26.4% were *Helicotylenchus* spp and 23.0% were *Pratylenchus* spp. A strong negative correlation ($r = -0.935$) between nematode population and soil pH was observed. High nematode infestation was found in root samples of tomatoes grown in acidic soil (pH 5.8) and low infestation was in samples grown in alkaline soil (pH7.7).

Keywords: nematode, tomato, *Meloidogyne*, *Helicotylenchus* and *Pratylenchus*

1. Introduction

Plant parasitic nematodes (Phylum: Nematoda) are small microscopic roundworms living in the soil attacking the roots of plants, thereby presenting a major threat to agriculture throughout the world [1]. They are considered as the invisible enemies of crops because of the nonspecific visible symptoms that they cause on crops such as chlorosis and early senescence and the difficulties with their diagnosis [2]. The nematode infection acts as an energy sink, absorbing photosynthates needed by the plant for growth and fruit production, hence crop yields are reduced and produce is of poor quality and reduced storage life [3-5].

According to Noling [3], the most widespread and economically important nematode species can be classified into three broad categories. These are the root-knot nematodes, root lesion nematodes and sting (spiral) nematodes. Root-knot nematodes have been reported as the major pests of tomatoes which interact with soil borne pathogens thereby increasing their infectivity [6]. *Meloidogyne* spp, the most economically important nematode in tropical and subtropical agriculture has been reported to reduce yield by 30 – 50% [7, 8].

Root lesion nematodes are nematodes that use a syringe-like stylet to extract nutrients from the roots of plants [9]. According to Smiley [10], damage from root lesion nematodes can easily be mistaken for nutrient deficiencies whereby the affected plants display stunting and yellowing of older leaves. *Pratylenchus neglectus* and *P. thornei* have been reported to be the most common species that have been shown to cause significant yield losses [11].

Sting nematodes are among the most destructive plant parasitic nematodes on a wide range of plants. Adults can reach lengths greater than 3 mm, making them one of the largest plant-parasitic nematodes. Yeates and Wouts [11] report that in most plants with which sting nematodes are associated, the damage is usually insidious because these types of nematodes usually permit secondary infection by fungi and bacteria. Some spiral nematodes such as *Helicotylenchus paxillini* have been shown to reduce root length by causing necrotic lesions. [12].

Nematodes respond to extremes in soil and plant environments [13]. Most plant parasitic nematodes have particular soil and climatic requirements. Changing soil conditions causes direct and indirect effects on nematode populations [4]. Factors such as soil moisture, texture temperature and pH influence nematode population and distribution. Popovici and Ciobanu [14] observed a difference in the composition of nematode communities which could have been caused by environmental variables such as soil pH, humus content and soil type.

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Although much is known about the life cycle of most plant parasitic nematodes, less is known about the influence of soil conditions on nematodes' life cycles. With different agricultural practices being carried out in Zimbabwe, an in-depth knowledge on the effects of soil characteristics on nematode survival is important. Therefore, the objective of the present study was to investigate the influence of soil pH and texture on the prevalence of plant parasitic nematodes.

2. Materials and Methods

2.1 Study Area

The present study was conducted at Dema Growth Point from March to May 2017. Dema Growth Point (Altitude 1448m above sea level; 18° 5' S latitude and 31° 13' E longitude) lies about 45 km south of the capital city Harare. It is the administrative centre of Seke district (Fig 1), one of the nine districts of Mashonaland East Province of Zimbabwe. The climate in Dema is warm to temperate and the average annual temperature is 18.3°C. Rainfall averages 774 mm per year [15].

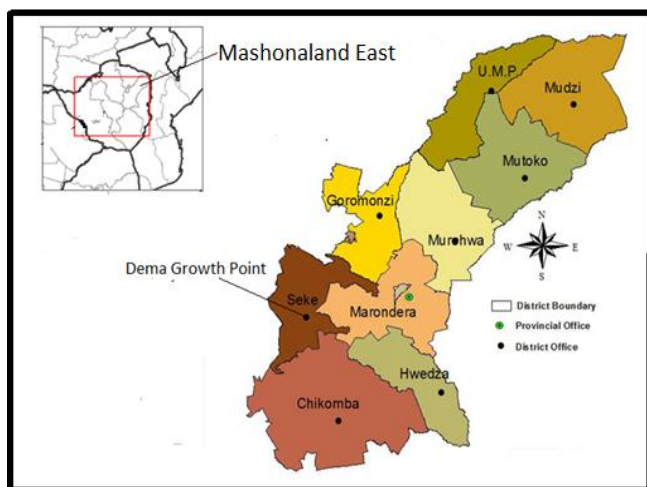


Fig 1: Map showing the study area Dema Growth Point in Seke District.

2.2 Planting of tomatoes

Soil preparation was carried out at depths of 200 to 400mm. The tomato seedlings of the Rodade cultivar were planted in five beds in the garden and five beds in the field. The inter-row spacing of 75cm and intra-row row spacing of 35cm was used.

All the tomato plants were well watered to field capacity for a period of three months. No pesticides were applied for disease control in order to avoid interference with the nematode populations in the soil. After three months, the tomato plants from the field and those from the garden were uprooted and taken for nematode extraction and identification.

2.3 Measurement of soil pH

Five soil samples, each weighing 20g were randomly taken from the field and the garden. The soil samples from the field were thoroughly mixed, removing any rocks and pieces of debris. Four replicates of the sample soil were made. The procedure was repeated for soil samples from the garden.

The four replicates of soil from the field and four from the garden were taken for pH tests. Distilled water was added in each soil sample. The soil-water mixture was shaken vigorously and then left to settle for five minutes. The pH of both field and garden soil was measured using an Orin 720 digital pH meter.

2.4 Determination of soil texture

The United States Department of Agriculture textural triangle [16] was used to classify the textural category by quantities of sand, silt and clay. The percentage composition of soil components was calculated using the formula:

$$\text{Percentage volume} = \frac{\text{Volume of soil component}}{\text{Total soil volume}} \times 100$$

2.5 Nematode extraction from roots

Root systems were carefully uprooted 9 to 10 weeks after planting, washed free of soil and placed in plastic bags. Five root samples from garden soil and five from field soil were taken. Roots were taken for galling assessment, extraction, identification and counting of nematodes.

2.5.1 Gall index

Gall assessment, using Taylor and Sasser [17], gall index scale of 0-5 (Table 1) was performed. A hand lens was used in the counting of galls. The roots were then kept in a refrigerator at 8°C awaiting nematode extraction.

Table 1: Taylor and Sasser (1978) Gall Index

| Number of galls | Root knot index | Description |
|-----------------|-----------------|-----------------------------|
| 0 | 0 | Immune |
| 1-2 | 1 | Resistant |
| 3-10 | 2 | Low susceptibility |
| 11-30 | 3 | Intermediate susceptibility |
| 31-100 | 4 | Susceptible |
| >100 | 5 | High susceptibility |

2.5.2 Nematode extraction

The blender centrifugal flotation technique by van Bezooijen [18] was used in extracting nematodes. The method was used to extract active nematodes from tomato roots. Tomato roots were sliced into small portions of about 0.5-1cm. Then 5g gram root samples were randomly selected and macerated in 100 ml water and centrifuged at about 1200 revolutions per minute for 30 seconds using a domestic blender. Then, the nematode water suspension was collected in a 500 ml beaker. The suspension obtained was passed through a set of sieves with the 250 nested on top of the 150 and 45µm aperture sieves. The debris which was collected from 250 and 150µm sieve was discarded. The nematodes on the 45µm sieve were transferred into a glass beaker. The nematodes were then counted within a counting slide at X40 magnification. Nematodes were identified using morphology. The number of nematodes in each root sample was recorded.

2.5.3 Nematode Identification

Plant-parasitic nematodes were identified to genus level using the identification key by Mekete *et al.*, [19]. The identification involved the use of the following standard keys:

1. Oesophagus 1 or 2 parts/Oesophagus 3 or 4 parts;
2. Stoma with stylet/Stoma without stylet;
3. Lip region without setae/Lip region with setae;
4. Oesophagus 4-part, median bulb present/ Oesophagus 3-part, median bulb absent;
5. Oesophagus not overlapping intestine/Oesophagus overlapping intestine
6. Female nematode body cylindrical, mobile/Female nematode body swollen, globose or saccate;
7. Vulva located near middle of the body/Vulva located in posterior third of the body;
8. Basal bulb not overlapping intestine/Basal bulb overlapping intestine;

9. Stylet long about 3X body width at stylet base/Stylet short about 2X or less body width at stylet base;
10. Tail terminus pointed /Tail terminus not pointed;
11. Tail filiform, terminus may be clavate/Tail not filiform, terminus not clavate;
12. Lip region offset by constriction from body, more than 1/2 higher than wide/Lip region not offset by constriction from body, or slightly offset; less than 1/2 as high as wide;
13. Stylet massive and short, large stylet knobs/Stylet thin, very long (3 or more times the body width at stylet base), small stylet knobs;
14. Body C-shaped/Body spiral shaped;
15. Lip region without striation, epiptygma present/Lip region with striation, epiptygma absent;
16. Both scutella (phasmids) located from tail terminus to anal region/Scutella not so located
17. Body short, 0.5 to 0.8 mm/Body long, 0.9 to 4.2 mm
18. Cuticle prominently annulated, base of stylet in or almost in median bulb/Cuticle not prominently annulated, base of stylet is not in median bulb
19. Cuticular sheath present/Cuticular sheath absent
20. Stylet knobs anchor shaped, forward directed
21. Body elongate, cylindrical, tail elongate/Body stout, usually fusiform
22. Annules with spines or scale like extension/Annules plain without spines or scale like extensions
23. Body after death spiral /Body death position straight or slightly curved
24. Median bulb, its valve and stylet well developed, lip region flattened short ventraloverlap, monovarial; low flat lip/Median bulb and its valve small, stylet usually small, its length almost equal to body width at stylet base
25. Mature female mostly obese/Mature female slender
26. Swollen female with pointed tail /Swollen female without pointed tail
27. Mature female kidney shaped, with short pointed tail/Mature female not kidney shaped, with long pointed tail
28. Mature female white, without eggs inside body/ Mature female creamy or brown with eggs inside body
29. Lip region smooth and offset/Lip region annulated and not offset

2.6 Data analysis

Statistical Package for Social Sciences (SPSSv16) was used to establish the relationship between pH and nematode population. Descriptive statistics were used to show the distribution of nematode population in different soil texture.

3. Results

3.1 Soil pH

Results on pH test (Table 2) showed that the field soil samples had an average pH of 5.8. The pH of the garden soil had an average pH of 7.7.

Table 2: pH of the field and garden soils.

| Sample | pH reading | |
|---------|------------|-------------|
| | field soil | garden soil |
| 1 | 5.5 | 7.5 |
| 2 | 5.7 | 7.6 |
| 3 | 5.8 | 7.7 |
| 4 | 5.9 | 7.8 |
| 5 | 6.1 | 7.9 |
| average | 5.8 | 7.7 |

3.2 Soil texture

Field soil had a sand percentage volume of 76%, 16% silt and 8% clay. In contrast, the garden soil had silt percentage volume of 40%, 38% clay and 22% sand. The soil texture triangle (Fig 2), showed that the texture of field soil was sandy loam while the garden soil was clay loam.

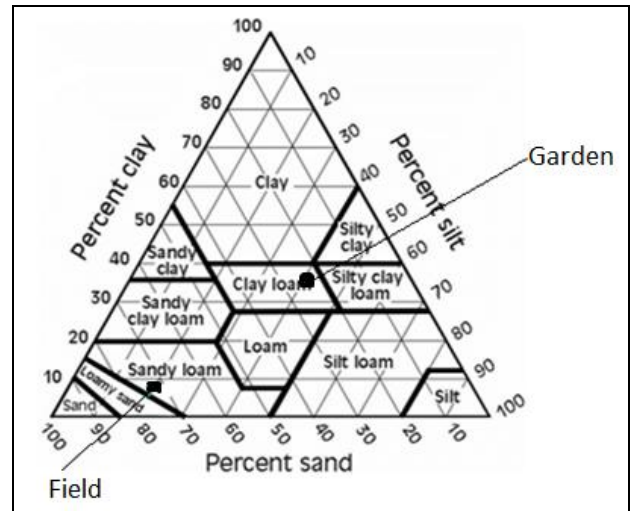


Fig 2: Soil Triangle showing classification of garden and field soils.

3.3 Gall index

The gall index for the garden gall index ranged from 2 to 3 (Table 3), while the gall index for field soil ranged from 3 to 4.

Table 3: Number of galls and gall index for tomatoes from the garden and field

| Sample | Site | Number of galls | Gall index |
|--------|--------|-----------------|------------|
| 1 | Garden | 6 | 2 |
| | Field | 84 | 4 |
| 2 | Garden | 9 | 2 |
| | Field | 69 | 4 |
| 3 | Garden | 5 | 2 |
| | Field | 35 | 4 |
| 4 | Garden | 11 | 3 |
| | Field | 31 | 4 |
| 5 | Garden | 15 | 3 |
| | Field | 26 | 3 |

3.4 Nematode identification and count

The total number of nematodes identified from the soil samples was 235 (Table 4). The percentage of nematodes contributed by the field soil was 70.6% while that contributed by the garden soil was 29.4%. Three genera identified were *Meloidogyne* spp, *Helicotylenchus* spp and *Pratylenchus* spp. Of the total number of nematodes, 50.6% were *Meloidogyne* spp, while 26.4% were *Helicotylenchus* spp and 23.0% were *Pratylenchus* spp.

Table 4: Populations of identified nematode genera identified from the field and garden.

| Sample | <i>Meloidogyne</i> spp | | <i>Helicotylenchus</i> spp | | <i>Pratylenchus</i> spp | |
|--------|------------------------|--------|----------------------------|--------|-------------------------|--------|
| | Field | Garden | Field | Garden | Field | Garden |
| 1 | 18 | 7 | 5 | 5 | 11 | 9 |
| 2 | 23 | 9 | 9 | 8 | 6 | 5 |
| 3 | 21 | 4 | 5 | 3 | 9 | 0 |
| 4 | 15 | 5 | 11 | 0 | 4 | 3 |
| 5 | 9 | 8 | 13 | 3 | 7 | 0 |
| Total | 86 | 33 | 43 | 19 | 37 | 17 |

3.5 Relationship between soil pH and nematode population.

The Pearson's correlation coefficient of -0.935 showed a strong negative relationship between pH and nematode population. As the pH increased, the nematode population decreased (Fig 4).

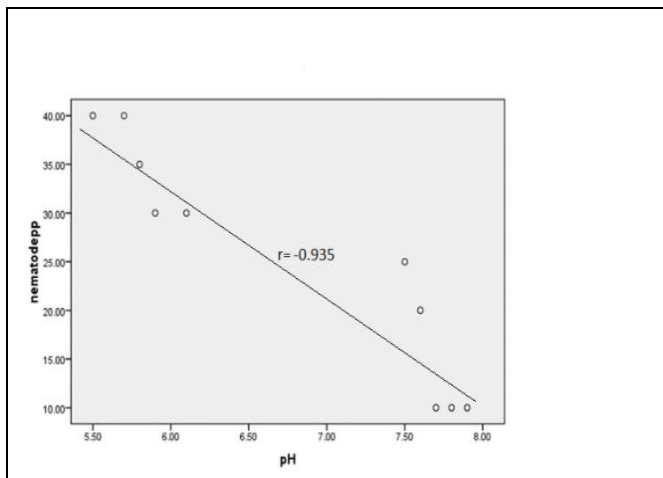


Fig 4: Scatter plot showing the relationship between pH and nematode population

4. Discussion

In this study, three genera of plant parasitic nematodes, *Meloidogyne* spp, *Helicotylenchus* spp, and *Pratylenchus* spp, identified. These same species were also reported by Chitamba *et al.*,^[20] in banana plants in Rusitu Valley of the Eastern Highlands of Zimbabwe. These nematode genera have been reported by Suyadi and Rosfiansyah^[21] as some of the most frequent plant parasitic nematodes in agricultural ecosystems, affecting most fruit and vegetable crops. Similar results were also reported by Bafokuzara^[22] who also found out that same three genera were the most abundant in the soils of Tororo district in Uganda.

In terms of abundance, *Meloidogyne* spp had the highest population density followed by *Pratylenchus* spp and lastly *Helicotylenchus* spp. However, in this study, *Helicotylenchus* spp was ranked second while *Pratylenchus* spp was in third position. Suyadi and Rosfiansyah^[21], in their ranking of three plant parasitic nematode genera affecting tomatoes and bananas in East Kalimantan Province of Indonesia also placed *Meloidogyne* spp in first position and *Pratylenchus* spp in third position. The most prevalent genus was the *Meloidogyne* spp which was reported by Seid *et al.*,^[23] as the universal pest of tomatoes.

Soil type and texture have been demonstrated to have a very strong bearing on plant parasitic nematodes. Several previous studies suggest that sandy soils provide favourable conditions for the nematode to survive, move, find and infect the host (Barvercheck^[24], Gardner *et al.*,^[24] have indicated that sandy soils, because of their higher thermal conductivity than soils with higher clay content, tend to show significant relationships with plant parasitic nematodes. These results are in agreement with those of Taylor and Sasser^[17] who report that root knot nematodes are more prevalent in sandy soils than clay soils. However, in the case of root-lesion nematodes which are migratory plant parasitic nematodes, Smiley^[10] reports that they are not strongly restricted by soil as they have been detected in silt loams, clay loams, and irrigated sandy loams. Because of their migratory behaviour, this could explain why they were most abundant in field than garden soil

where they could easily move between the soil particles.

The study indicates that plant parasitic nematode populations are negatively correlated with soil pH. A decrease in soil pH resulted in an increase in nematode population. Similar findings have also been reported by Ortiz *et al.*,^[26] and Neher^[27] who observed that areas with high nematode population had low pH. However, the results for *Pratylenchus* spp which was abundant in the field soil are contrary to those of Thompson *et al.*,^[28] who reported maximal incidence of *Pratylenchus thornei* and *P. neglectus* in soils of pH of 8.4 and 8.2 respectively. Soil pH could play an important role in nematode reproduction presumably through providing an optimum pH for the organism's enzyme machinery.

The high gall index in field indicates that the tomato plants grown in low pH and coarse textured soil were more susceptible to plant parasitic nematodes. Because sandy soils tend to harbour larger populations of plant parasitic nematodes they move with ease through the root zone and easily prey on the tomato plants. Garden samples, with a gall index of 2 indicated that the nematode populations were below the damage threshold and so the plants were less susceptible to infestation.

4. Conclusion

The findings of this research conclude that soil physicochemical factors such as texture and pH are some of the factors driving plant parasitic nematode population dynamics. Soils with an acidic pH tend to favour high nematode infestation. *Meloidogyne* spp, *Helicotylenchus* spp and *Pratylenchus* spp are the major plant-parasitic nematodes associated with tomatoes at Dema Growth Point. Considering the destructive nature of these nematode species in various tomato producing communities, sound research and management practices have to be in place to minimize crop damage to improve income and well-being of the tomato dependent community of Dema.

Given the ubiquitous nature of nematodes that are capable of causing serious yield reduction in tomato production, there is need for research on methods that can cheaply and effectively control the pests. Farmers should be advised to take their soils for pH test before they engage in crop production and choose appropriate cultivars. Organic matter could be applied in soils especially sandy loam to increase organic content of the soil thereby increasing pH which may consequently reduce root knot nematode populations.

5. Acknowledgements

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