RESEARCH ARTICLE

Soil chemical properties influence abundance of nematode trophic groups and *Ralstonia solanacearum* in high tunnel tomato production [version 1; peer review: 2 approved with reservations]

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Abstract

Background: Plant parasitic nematodes (PPNs) and bacterial wilt (*Ralstonia solanacearum*) are serious soil-borne pests in tomato (*Solanum lycopersicon* L) production in high tunnels. This study was undertaken to determine effects of soil chemical properties on their abundance.
Method: Soil samples were collected from 32 high tunnels in the sub-counties: Gatundu North, Gatundu South, Juja, Thika, Ruiru and Kiambu, Kenya, from January to November 2016. Nematodes genera, *R. solanacearum* and soil chemical properties were evaluated from composite soil samples collected from the high tunnels.
Results: The soil pH and N, P, K, Ca, Mg, Na and Cu varied across sub-counties. Twenty-four nematode genera including 14 PPNs, 5 bacterivores, 3 fungivores and 2 predators were recovered from soil samples. The genera *Meloidogyne*, *Alaimus*, *Aporcelaimus* and *Mononchus* were the most abundant PPNs, bacterivores, fungivores and predators, respectively, and differed across sub-counties. The abundance of *Meloidogyne* spp. and *R. solanacearum* was higher in Gatundu North than in the other sub-counties. There was a strong, positive correlation between *Meloidogyne* spp. (second stage juveniles counts) population and *R. solanacearum* (cfu · mL^{-1}) with soil N and P, and a weak negative correlation with soil pH, EC, Zn and Cu. Fungal feeders exhibited a strong negative correlation with soil pH and Ca; predators, bacterial feeders, and PPNs had similar correlations with N, P and Ca, respectively.
Conclusion: Soil chemical properties affect abundance of beneficial and phytoparasitic nematodes and *R. solanacearum*, which varies with location.

Keywords

Solanum lycopersicum, bacterial wilt, plant parasitic nematodes, small holder farmers

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Introduction

Tomato (*Solanum lycopersicon* L) is an important vegetable due to its mineral, vitamin, amino acid, fiber, lycopene, choline, folic acid, α-lipoic acid, lutein and β-carotene contents (Naika *et al.*, 2005; Seifried *et al.*, 2004), some of which could prevent chronic diseases and have other additional health benefits (Sharoni *et al.*, 2012). Livelihoods of 40–60% of small- and medium-scale farmers in Kenya is dependent on open field tomato production, and recently under high tunnels (Wachira *et al.*, 2014). High tunnel farming faces challenges from pests and pathogens that thrive due to ideal conditions of high temperature and relative humidity (Gullino *et al.*, 2000; Ireri *et al.*, 2018).

Plant parasitic nematodes (PPNs) are of economic importance in agriculture worldwide (Bird *et al.*, 2009) and estimated to exceed US$100 billion/year in crop loss (Nicol *et al.*, 2011). Many species of PPNs have been identified (Decraemer & Hunt, 2006), while others are continually being described as cropping patterns change (Nicol, 2002). Economically important PPNs are grouped into restricted specialized groups that affect crops through feeding on plant parts either above- or below-ground (Nicol *et al.*, 2011). In addition to direct feeding and host wounding, nematode feeding facilitates subsequent infection by secondary pathogens, such as fungi and bacteria (Powell, 1971).

In high tunnel tomato production, root knot nematode (RKNs) are the most prevalent PPNs (Ireri *et al.*, 2018). Root knot nematodes engage in host seeking behavior during the J2 vermiform life stage. The infective J2 uses a hollow, protrusible syringe-like stylet to penetrate plant cell walls, to secrete proteins from their esophageal glands into the cell and withdraw nutrients from the cell cytoplasm (Berg *et al.*, 2009). In response to effectors secreted by the nematode, infected cells differentiate into hypertrophied, multinucleated giant cells that sustain the nematode feeding, further development and reproduction (Bartlem *et al.*, 2014; Truong *et al.*, 2015). The giant cell in the vascular system hinders uptake and translocation of water and nutrients. Infected plant parts also show galling and crop loss can be 100% (Ireri *et al.*, 2018; Onkendi *et al.*, 2014; Perry *et al.*, 2009).

The bacterium, *Ralstonia solanacearum*, which is often endemic in soil, penetrates plants through the root system and eventually causes irreversible wilting and death (Agrios, 2005; Hayward, 1991; Kelman, 1965). It primarily enters plants through natural openings as a result of either lateral root emergence, from wounds caused by soilborne organisms (e.g., the root-knot nematode), transplanting, cultivation or insects (Agrios, 2005).

Free-living nematodes are the most abundant metazoans in soil, constituting an important component of the soil fauna which impacts nutrient cycling and primary productivity in diverse ecosystems (Liu *et al.*, 2006). Increased microbial activity in soil leads to an increased proportion of fungal and bacterial feeders (Bongers & Ferris, 1999). This is important for decomposition of soil organic matter and mineralization of plant nutrients (Hunt *et al.*, 1987; Ingham *et al.*, 1985). Nematode diversity in high tunnel production systems offers possibilities for use as biological indicators of agricultural practices, soil characteristics, and degree of conservation of soils, especially in continuous cropping of the same soil (Liu *et al.*, 2011). Previous reports indicated an adverse trend between free-living nematode populations and second stage juveniles (J2s) of *Meloidogyne* spp (RKNs) in different continuously cropped soils (Wu & Shi, 2011).

Soil chemical characteristics influence abundance and diversity of soil pathogens (Spann & Schumann, 2010). When calcium ammonium nitrate fertilizer is applied, the positive charge on the ammonium ion (NH_4+) allows it to be adsorbed by plant roots, resulting in release of positively charged hydrogen ions into the surrounding area lowering the soil pH (Barak *et al.*, 1997). Diseases more common in acidic soils increase in severity. Despite the importance of bacterial wilt and RKNs in tomato farming in Kenya, little is known about the impact of soil chemical characteristics in high tunnel tomato production. This study was undertaken to determine effects of soil chemical properties on abundance of nematodes and *Ralstonia solanacearum* in high tunnel tomato production.

Methods

Sample collection

The study was carried out from January to November 2016 in the sub-counties Thika, Juja, Ruiru, Kiambu, Gatundu South and Gatundu North of Kiambu County, Kenya, a major high tunnel tomato growing area in Kenya (Anonymous, 2014). Mean temperature in the region is 26°C and relative humidity ranges from 54–100% (Anonymous, 2017a).

Soil samples were collected from 32 high tunnels (measuring: 18–25 m long × 8 m wide × 2 m high) covered with translucent UV-treated plastic sheeting. Seven tunnels were sampled in Thika, 5 in Juja, 2 in Ruiru, 10 in Kiambu, 5 in Gatundu South and 3 in Gatundu North. The sub-counties were identified for the study during focus group discussions. The location of each high tunnel was identified using a GPS device (Magellan, triton windows CE core 5.0 00039_272_446_822 X11_15302, Integritytech, Chicago, IL) (Table 1). Each high tunnel was divided into 4 quadrats to collect soil samples. Five sub-samples per quadrat were collected with a soil auger to a depth of 15 cm in a cross-diagonal pattern (Coyne *et al.*, 2014). The 5 sub-samples were mixed in a plastic basin to make a composite sample of ~1 kg and placed in a labelled plastic bag. A similar procedure was repeated across quadrats with 4 samples (comprised 20 sub-samples) collected from each high tunnel and constituting a total of 128 samples for the 32 high tunnels. Soil samples were placed in cooler boxes and transported to the laboratory at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Juja, Kenya (latitude 0°10′48″ S, longitude 37°7′12″ E, altitude 1525 m a.s.l.) and stored at 10°C for 1–2 weeks for *R. solanacearum,* nematode and soil chemical analyses. In the laboratory, each soil sample was divided into two 500 g portions, 1 for nematode and the other for *R. solanacearum* analyses. For nematode extraction and quantification, samples were transported in insulated boxes to a laboratory at the International Center of Insect Physiology and Ecology, Duduville Campus, Kasarani, Nairobi, Kenya. Abundance of *R. solanacearum* and soil chemical properties were analyzed at JKUAT.

Table 1. Self-help farmer groups operating high tunnels in sub-counties of Kiambu County and their location coordinates.

		Location	
Sub-county	**Organization operating high tunnel**	**Latitude**	**Longitude**
Gatundu North	Gitwe United	0° 57' 03.1'' S	36° 53' 29.2'' E
	Kihururu	0° 57' 29.1'' S	36° 54' 04.2'' E
	Ngaraka	0° 53' 25.2'' S	36° 52' 39.6'' E
Gatundu South	Kahuguini Dairy	1° 02' 42.2" S	36° 55' 12.2" E
	Kianyoni Dairy	0° 57' 20.1" S	36° 46' 04.0" E
	Kimunyu	1° 02' 56.6'' S	37° 56' 42.7'' E
	Mwirutiri	1° 04' 06.9'' S	36° 53' 23.4'' E
	New Gitwe	0° 56' 48.1" S	36° 48' 47.9" E
Juja	Focal Area	1° 09' 54.3'' S	37° 05' 54.8'' E
	Jokumo Kihuria	1° 03' 27.0" S	37° 00' 29.1'' E
	Juja Botanical	1° 06' 43.5" S	37° 00' 46.5'' E
	Mirimaini Primary	1° 04' 24.8'' S	36° 59' 30.3'' E
	Mwinjoyo	1° 09' 40.7'' S	37° 07' 38.7'' E
Kiambu	By Grace	1° 17' 03,4" S	36° 81' 71,2" E
	Agricultural booster	1° 17' 29.1'' S	36° 80' 12.8'' E
	By Faith	1° 09' 28.2'' S	36° 50' 27.7' E
	Gikirithia	1° 17' 18.7'' S	36° 84' 59.1'' E
	Horticulture Investors	1° 07' 13.1'' S	36° 49' 42.8'' E
	Kahuguini	1° 02' 42.5"S	36° 55' 12.6" E
	Kanene GH	0° 57' 23.9" S	36° 46' 03.5" E
	Kilimo Bishara	1° 09' 22.3'' S	36° 48' 55.6'' E
	Urban Farming	1° 79' 03.2'' S	36° 83' 78.1' 'E
	Wendi Mwega	1° 88' 79.7'' S	36° 33' 45.3'' E
Ruiru	Membeley Park	1° 09' 32.8'' S	36° 55' 22.2'' E
	Ruiru Baptist	1° 08' 52.0'' S	36° 57' 48.0'' E
Thika	Digital Women	1° 07' 49.3" S	37° 08' 39.9" E
	Gatuanyaga Youth Focus	1° 02' 52.9"S	37° 10' 29.4'' E
	Kirigu	1° 06' 21.7'' S	37° 20' 26.1'' E
	Thogoto	1° 06' 06.2'' S	37° 19' 45.4'' E
	Upendo	1° 03' 29.0'' S	37° 15' 17.3'' E
	Ushirikiano Booster	1° 06' 07.5'' S	37° 09' 13.6'' E
	Vision Farmer	1° 04' 29.1'' S	37° 05' 38.7'' E

Soil sample analysis

Soil samples from high tunnels for each sub-county were air dried on the bench in the laboratory (25±2°C) for 3 days and their chemical properties determined using the following protocols: total nitrogen (N) by Kjedahl's method (Kjeldahl, 1883; McGeehan & Naylor, 1988); available phosphorus (P) using the double acid extractable P (HCl-H_2SO_4) method (Mehlich, 1953; Olsen, 1954); potassium (K), calcium (Ca) and magnesium (Mg) using the ammonium acetate extraction method (Jones Jr., 1999; Normandin *et al.*, 1998); iron (Fe), copper (Cu) and zinc (Zn) using the EDTA extraction method (Lindsay & Norvell, 1978); electrical conductivity (EC) by the 4 electrode method (Nadler & Frenkel,1980) and soil pH using an electric pH meter (Conkling & Blanchar, 1988).

Nematode analysis

Nematodes were extracted from 100 mL of soil from each composite sample using a modified Baermann's technique (Coyne *et al.*, 2014) and fixed for identitifcation (Seinhorst, 1962). Briefly, 50 mL Falcon centrifuge tubes containing nematodes were immersed in heated water (55°C) for 2 min to kill them. Two drops of formalin glycerol (obtained by mixing 10 mL of 40% formaldehyde, 1 mL of glycerol and 89 mL of distilled water) were added and tubes were stored at 20°C to allow fixed nematodes to adequately settle to the bottom of the vial. Nematodes were identified to genus level based on morphological features using at least 100 nematodes per sample with a compound microscope (Carl Zeiss Primo Star iLED, Carl Zeiss Promenade 10, Jena, Germany) (Hunt *et al.*, 2005). Identified nematodes were assigned to trophic groups as PPNs (Mai & Lyon, 1975), bacterial feeders (Overgaard Nielsen, 1949), fungal feeders (Thorne & Swanger, 1936) and predators (Small, 1987) following methods described by Yeates *et al*. (1993).

R. solanacearum analysis

To isolate *R. solanacearum*, 1 g of soil from each composite sample was placed in a 28 mL universal glass bottle filled with 10 mL of double distilled water and mixed thoroughly using a laboratory shaker (Stuart Lab-Scale Linear Reciprocating Shaker, Model SSL2, Cole Parmer Ltd, Staffordshire, UK) to yield a stock solution concentration which was serially diluted to concentrations of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8},10^{-9} and 10^{-10} mg·mL^{-1}. Of these, 50 µL were obtained from the 10^{-5} (1:100,000), 10^{-6} (1:1000,000) and 10^{-7} (1:10,000,000) mg·mL^{-1} dilutions and subsequently streaked using a wire loop on Kelman's Tetrazolium Chloride (TZC) medium (Kelman, 1954). For each concentration, 2 Petri dishes were used to grow *R. solanacearum* from soil obtained from respective high tunnels. Inoculated Petri dishes were incubated (Laboratory incubator PC-IB-150, Xi'an HEB Biotechnology Co., Ltd, Shaanxi, China) at 32°C for 30–48 h in an upside-down position using existing protocols (Hayward, 1991; Schaad *et al.*, 2001). Virulent colonies of *R. solanacearum* were identified by their large and elevated size, fluidal nature, and if they were either entirely white, or with a pale red center (Buddenhagen & Kelman, 1964). Mutant, or non-virulent, type colonies of *R. solanacearum* are uniformly round and dark red, smaller in size, and butyrous or dry on TZC. Bacteria colonies (virulent and non-virulent) were counted on a daily basis for 3 consecutive days to produce a cumulative amount. Average colony count was used to determine colony forming units per mL (cfu·mL^{-1}) which was calculated according to Sieuwerts *et al.* (2008). Gram staining, potassium hydroxide solubility, Kovac's and Levan tests (Rahman *et al.*, 2013) were used to detect presence of *R. solanacearum* in soil.

Data analysis

To determine if high tunnel location influenced soil chemical characteristics, parameters in each sub-county were subjected to analysis of variance (ANOVA) and means separated using Tukey's HSD test. The abundance of respective nematode trophic groups, *Meloidogyne* spp. and *R. solanacearum* were correlated with respective soil chemical properties. All data analyses were with the package "vegan" in R statistical software, ver. 3.2.3 (Anonymous, 2017b).

Results

The soil pH was slightly acidic to neutral and differed between the sub-counties; EC, which was within the normal range, was not different across sub-counties (Table 2). Although high levels of N were recorded in soils from Gatundu North, significantly lower levels were found in Juja, Ruiru and Thika sub-counties. The P level was lower in all sub-counties except Gatundu North, which was within normal range (Table 2). The Mg level was 3 times higher in Gatundu North relative to the other sub-counties, and above the normal range across all sub-counties (Table 2).

There were 4 trophic groups, from 24 genera, of nematodes identified from soils collected in the high tunnels in the sub-counties, they were plant parasitic nematodes (PPN), bacterial feeders, fungal feeders and predators (Table 3, Table 4). Of the PPNs, the genus *Meloidogyne* was the most abundant across the sub-counties with higher populations in Gatundu North than in the other sub-counties (Table 4). Among bacterial feeders, the genus *Alaimus* was the most abundant in >50% of the sub-counties with higher populations in Juja and Ruiru relative to the other sub-counties (Table 4). Of fungal feeders and predators, the genera *Aporcelaimus* and *Mononchus* were higher in Gatundu North and Ruiru sub-counties, respectively. The abundance of *R. solanacearum* in Gatundu North was 2- and 4-fold higher than in Gatundu South and Juja sub-counties respectively, and ~1.7 times higher than in Kiambu, Ruiru and Thika sub-counties (Figure 1).

There were mainly weak correlations between nematode trophic groups and soil chemical characteristics (Table 5). However, a few strong correlations were found: fungal feeders had a strong negative correlation with soil pH and Ca, while predators, bacterial feeders and PPNs had a similar correlation with N, P and Ca, respectively (Table 5). The PPNs and fungal feeders had a strong positive correlation with N, P and Mg (Table 5). There were weak positive, negative, correlations between *Meloidogyne* spp. and *R. solanacearum* populations with soil pH, EC, K, Na, Zn, Fe, Cu and Mn; there was a strong positive correlation with soil N and P (Table 5). Calcium had strong and weak negative correlations with *Meloidogyne* spp. and *R. solanacearum*, respectively (Table 5).

Discussion

The mineral composition of nutrient fertilizers may alter reactions of tomato plants to pathogenic agents. The soil chemical properties, which differed across sub-counties, influenced nematodes and *R. solanacearum* populations. Gatundu North sub-county supported high populations of *Meloidogyne* spp and *R. solanacearum*, but these soil pathogens were low in Gatundu South, Thika, Kiambu, Ruiru and Juja sub-counties, which could be attributed to location differences (Jaetzold *et al.*, 2007). For instance, the soil pH in Gatundu North was slightly acidic relative to the other sub-counties, which consequently led to high *Meloidogyne* spp and *R. solanacearum* populations. These results agree with those of Li *et al*. (2017) who reported that acidified soils (pH <5.5) increased multiplication and infestation of *R. solanacearum* in solanaceous crops. Increased soil acidity (pH 4.5–5.4) led to faster reproduction of *Meloidogyne* spp leading to high crop loss (Wang *et al.*, 2009). Acidic soils increase populations of soil microbial communities particularly

Table 2. Chemical[a] characteristics of soil collected in high tunnels in Gatundu North, Gatundu South, Juja, Kiambu, Ruiru and Thika sub-counties.

		Sub-county						
Characteristic	**Normal**	**Gatundu**	**Gatundu**	**Juja**	**Kiambu**	**Ruiru**	**Thika**	**P value**
Soil pH	5.5-6.8	5.3b[b]	5.6b	7.0a	6.6b	6.6b	6.7b	<0.001
Soil EC[c] (μS·cm^{-1})		0.4a	0.7a	0.5a	0.6a	0.4a	0.6a	0.144
N (%)		0.6a	0.3c	0.1c	0.4b	0.2c	0.2c	<0.001
P (mg·kg^{-1})		0.01b	0.004a	0.002b	0.005a	0.002b	0.003b	<0.001
K [cmol(+)·kg^{-1}]		0.6a	0.4c	0.3c	0.6a	0.5b	0.5b	<0.001
Ca (mg·kg^{-1})		254c	120d	338a	204c	293b	186d	<0.001
Mg (mg·kg^{-1})		884a	192c	168c	315b	169c	187c	<0.001
Na (mg·kg^{-1})		11.6a	10.1ab	8.3c	11.2a	10.9b	8.3c	<0.001
Zn (mg·kg^{-1})		303.1a	444.6a	415.8a	383.0a	486.6a	320.3a	0.561
Fe (mg·kg^{-1})		4291.0a	2828.0a	2540.0a	3874.00a	555.00a	4832.00a	0.336
Cu (mg·kg^{-1})		19.2b	15.8b	21.6a	20.5a	25.1a	19.2b	<0.001
Mn (mg·kg^{-1})		3122.0a	3039.0a	3029.0a	3038.0a	547.0a	2807.0a	0.723

[a]Data on soil chemical characteristic in each sub-county was run with ANOVA and means separated with Tukey's HSD test, P≤ 0.05.

[b]Means in rows followed by the same letter are not significantly different.

[c]EC = Electrical conductivity; N = Nitrogen; P = Phosphorus; K= Potassium; Ca = Calcium; Mg = Magnesium; Na = Sodium; Zn =Zinc; Fe = Iron;Cu = Copper; Mn = Manganese;μS = micro-Siemens; cmol(+)·kg^{-1} = centimoles of positive charge·kg^{-1} soil

Table 3. Trophic group of nematodes found in sub-counties of Kiambu County.

Trophic group	**Sub-county**						
	Gatundu North	**Gatundu South**	**Juja**	**Kiambu**	**Ruiru**	**Thika**	**P-value**
Plant parasitic nematodes	2465a[a]	1105b	997b	1493b	740b	1181b	0.001
Bacterial feeders	56.7a	101a	159.7a	81.9a	129.1a	223.5a	0.34
Fungal feeders	45.74a	16.21b	2.27c	18.07b	3.45c	3.09c	<0.001
Predators	9.32c	6.78c	25.21b	9.46c	43.11a	19.55b	0.001

[a]Means in rows followed by the same letter are not significantly different, Tukey's HSD test, P≤ 0.05.

Table 4. Population densities of nematode genera, assigned to different trophic groups, in rhizosphere soil from tomato grown in high tunnels.

	Sub-county						
Trophic group and genus	**Gatundu North**	**Gatundu South**	**Juja**	**Kiambu**	**Ruiru**	**Thika**	**p-value**
Plant parasitic							
Meloidogyne	2225.0a[a]	905.0ab	796.5a	1267.5b	575.0a	1007.1ab	<l0.001
Pratylenchus	42.5a	37.0a	23.3 a	37.8a	17.5a	26.1a	0.431
Tylenchus	13.3a	16.0a	13.5 a	22.3a	0.0 a	10.0a	0.211
Filenchus	8.3c	40.0a	18.5bc	8.8c	12.5bc	31.4b	<0.001

	Sub-county						
Trophic group and genus	**Gatundu North**	**Gatundu South**	**Juja**	**Kiambu**	**Ruiru**	**Thika**	**p-value**
Radopholus	12.5a	10.0a	1.5a	5.0a	0.0a	8.6a	0.06
Hoplolaimus	2.5a	2.5a	6.0a	8.3a	20.0a	8.6a	0.332
Rotylenchus	8.3a	8.0a	0. 0c	7.3b	0.0c	0.0c	0.067
Helicotylenchus	25.0a	24.0a	33.0a	43.0a	18.8a	13.9a	0.137
Tylenchorynchus	14.2bc	8.0c	27.5b	39.5a	18.8bc	12.9bc	0.028
Trichodorus	12.5b	0.0 c	3.5c	18.0a	1.3c	2.9c	0.008
Xiphinema	10.8bc	19.0ab	17.0ab	7.3c	33.8a	20.4b	0.038
Longidorus	44.2a	20.5b	18.0bc	12.8c	11.3c	14.6bc	0.005
Ditylenchus	3.0c	2.0c	21.9a	9.4b	9.7b	11.3b	<0.001
Aphelenchoides	12.0b	10.8b	3.8c	18.3a	5.5c	1.1c	<0.001
Bacterial feeders							
Alaimus	11.7d	43.6c	121.0a	36.3c	90.6b	19.3d	<0.001
Acrobeles	5.0c	11.4b	7.1bc	6.0bc	14.4ab	15.5a	0.018
Leptolaimus	5.0c	5.6c	33.6a	13.3ab	21.5b	1.7c	<0.001
Plectus	12.1c	35.1b	4.3d	22.5ab	15.0c	41.9d	0.041
Desmodora	27.0a	2.5c	2.6c	11.8ab	3.0c	16.9b	0.002
Fungal feeders							
Dorylaimus	19.6b	0.0c	17.8ab	13.5ab	26.0a	7.0c	<0.001
Aphelenchus	23.4a	16.1ab	2.6c	18.9b	0.0c	3.4c	<0.001
Aporcelaimus	29.8a	9.5b	7.0b	5.1b	4.4b	5.0b	0.001
Predators							
Mononchus	2.6d	1.7d	18.3b	8.4c	35.2a	5.7c	0.001
Discolaimus	7.5b	5.5c	8.9b	3.8c	8.8b	14.8a	0.014

[a]Means in rows followed by the same letter are not significantly different; Tukey's HSD test, P≤ 0.05

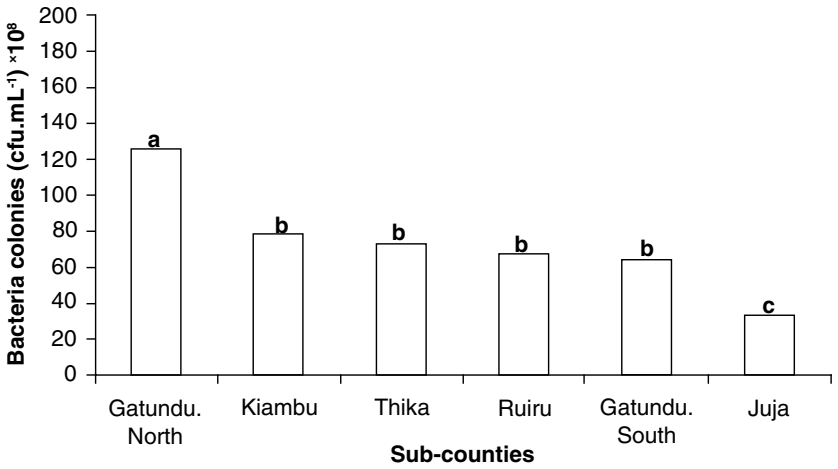

Figure 1. Abundance of *Ralstonia solanacearum* populations in soil samples collected in high tunnels in which tomato was grown in Gatundu North, Gatundu South, Juja, Kiambu, Ruiru and Thika sub-counties of Kiambu County.

Table 5. Correlation co-efficient (r) of abundance of nematode trophic groups, *Meloidogyne* spp. and *Ralstonia solanacearum* relative to soil chemical property.

Soil chemical property	Plant parasitic nematodes	Bacterial feeders	Fungal feeders	Predators	*Meloidogyne* spp.	*R. solanacearum*
Soil pH	-0.3765	0.2469	-0.5882**	0.2837	-0.3681	-0.4203
Soil Ec	-0.1269	-0.2	0.1259	-0.3247	-0.1573	-0.0811
Nitrogen	0.6291**	-0.4306	0.7793**	-0.5052	0.6256**	0.7793**
Phosphorus	0.5857**	-0.5262	0.6986**	-0.6334**	0.5963**	0.8717**
Potassium	0.3497	-0.4032	0.3848	-0.332	0.3918	0.3197
Calcium	-0.573**	0.2724	-0.5289	0.2493	-0.6043**	-0.4752
Magnesium	0.5756**	-0.3458	0.7096**	-0.2092	0.5804**	0.3583
Sodium	0.2173	-0.3748	0.4105	-0.2721	0.1899	0.111
Zinc	-0.1042	-0.1484	-0.036	0.0023	-0.1158	-0.2318
Iron	0.2035	-0.1387	0.3149	-0.3037	0.1728	0.155
Copper	-0.1716	0.1316	-0.12	0.1471	-0.152	-0.0391
Manganese	0.2583	0.0754	0.2921	-0.2677	0.3238	0.3859

** = significant correlation at 0.01 probability level

R. solanacearum and survival and reproduction of root knot nematodes (Kesba & Al-Shalaby, 2008).

Meloidogyne spp. and *R. solanacearum* had positive and negative relationships with soil characteristics indicating their role in pathogen-host interactions (Desaeger & Rao, 2000; Wang *et al.*, 2004). Soil chemical characteristics are known to influence the abundance and diversity of soil pathogens i.e., plant parasitic nematodes and *R. Solanacearum* (Spann & Schumann, 2010). For example, when ammonium nitrogen fertilizer such as calcium ammonium nitrate, is applied, the positive charge on the ammonium ion (NH_4^+) allows it to be adsorbed by plant roots, resulting in the release of positively charged hydrogen ions into the surrounding that lowers the soil pH (Barak *et al.*, 1997). Despite the fact that the soil pH had weak positive, and negative, correlations with abundance of PPNs, bacterial feeders, predators and *R. solanacearum*, the results indicate their role in influencing nematode diversity (Ingham *et al.*, 1985; Zhong & Cai 2007). Soil pH had a strong negative correlation with the fungal feeders indicating that a decrease in the former may lead to an increase in the latter. The soil pH may alter soil microbiota by affecting soil microbial activities as reported by Rocha *et al*. (2006). Previous studies reported that continuous use of mineral fertilizers decreased soil pH (Adamtey *et al.*, 2016), affecting soil microbes and nematodes population and diversity (Zhong *et al.*, 2010). Differences in soil chemical properties and their subsequent effect on nematode and *R. solanacearum* populations could be attributed to changes in farmer practices and varying environmental conditions in high tunnels which were not measured in this study and deserve further examination.

The strong positive correlation between PPNs, fungal feeders and *Meloidogyne* spp. with N, P and Mg indicate that any increase in these mineral elements may lead to similar effects in the nematode trophic groups. Our results concur with previous studies that reported that increased N levels led to high populations of PPNs (de Melo Santana-Gomes *et al.*, 2013). In addition, P as potassium phosphate, increases hatchability of *Meloidogyne exigua* (Salgado *et al.*, 2007) leading to high populations of *M. exigua* in soils with high levels of potassium or whose major component in rock phosphate. Micronutrients, i.e., Mg that exhibited a positive correlation with PPNs in our study, has been reported to reduce production of some plant metabolites that protect plants from nematode attack, thus increasing their prevalence (Fancelli, 2008). Previous reports indicated that fungal feeders are associated with mycorrhiza that facilitate recycling of nutrients in soil by forming a carbon sink (Teotia *et al.*, 2017). An increase in N and P fertilizers may not impact negatively on abundance of fungal feeders that facilitate availability of these nutrients to plants. However, further research is required concerning this.

The negative correlation between PPNs, fungal feeders and *Meloidgyne* spp. with Ca indicated that increases in this element in the soil lowers populations of these nematode trophic groups. This agrees with studies that indicated application of calcium based components in soil reduced root galling, egg masses and growth of juveniles and susceptibility of plants to nematode attack (Hurchanik *et al.*, 2003; Mohamed & Youssef, 2009; Rocha *et al*. 2006). The fact that *R. solanacearum* and *Meloidogyne* spp. populations were high in Gatundu North, relative to other sub-counties, suggests that disease complexes exist in high tunnel tomato farming (Agrios, 2005; Begum *et al.*, 2012). There was a strong positive correlation between *R. solanacearum* and *Meloidogyne* spp. abundance indicating that a high abundance of the former increases the severity of the latter pathogen. This agrees with previous studies (Agrios, 2005). Whether the

Meloidogyne species and *R. solanacearum* strain in the open fields are similar to those in high tunnel tomato production in Kiambu County need to be investigated further.

Conclusion

The findings indicate that soil chemical characteristics influence soil pathogens in high tunnels and specific knowledge of the interaction could be used to design effective nematode and bacterial wilt prevention strategies for small holder tomato high tunnel farmers.

Data availability

Underlying data

Open Science Framework: Manipulating the soil ecosystem using fertilizer for improved management of root-knot nematodes and bacterial wilt in smallholder 'greenhouse' tomato production in Kenya, https://doi.org/10.17605/OSF.IO/68W4P (Murungi *et al*, 2019).

Grant information

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We thank the staff and tomato farmers of Kiambu County who provided vital information that guided the development of this work.

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Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 20 February 2019

https://doi.org/10.21956/aasopenres.14008.r26725

?

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The positive point about the manuscript is the idea, which is very relevant. But authors analysis regarding the abundance value of Ralstonia solanacearum is not full proof.

Identifying Ralstonia solanacearum from soil by observing the colony phenotype on TZC plate is not enough. Additional experiments such as multiplex PCR, virulence test will be important to confirm these are indeed R. solanacearum, which is the main point of the paper.

In addition it will be interesting to do a comparative study regarding the abundance of the pathogen and occurrence of the disease caused by the pathogen

I am not able to comment on Nematode part as I do not have expertise.

I am attaching some of our references which may be useful to the authors.

1. Singh N, Kumar R, Ray SK. (2018) An innovative approach to study *Ralstonia solanacearum* pathogenicity in 6 to 7 days old tomato seedlings by root dip inoculation[1].
2. Singh N, Phukan T, Sharma P L, Kabyashree K, Barman A, Kumar R, Sonti RV, Genin S, Ray SK. (2018). An innovative root inoculation method to study *Ralstonia solanacearum* pathogenicity in tomato seedlings[2].
3. Kumar R, Barman A, Phukan T, Kabyashree K, Singh N, Jha G, Sonti RV, Genin S, Ray SK. (2017) *Ralstonia solanacearum* virulence in tomato seedlings inoculated by leaf clipping. Plant Pathol[3].
4. Kumar R, Barman A, Jha G, Ray SK (2013) Identification and establishment of genomic identity of *Ralstonia solanacearum* isolated from a wilted chilli plant at Tezpur, North East India[4].

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2. Singh N, Phukan T, Sharma PL, Kabyashree K, Barman A, Kumar R, Sonti RV, Genin S, Ray SK: An Innovative Root Inoculation Method to Study Ralstonia solanacearum Pathogenicity in Tomato Seedlings. *Phytopathology*. 2018; **108** (4): 436-442 PubMed Abstract | Publisher Full Text
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Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 04 February 2019

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?

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This work presents valuable real-world survey data uniting different subdisciplines (nematology, soil chemistry, plant bacteriology). The study addresses a set of serious problems confronting high tunnel tomato producers in Kenya. Yields of this high-value cash crop are reduced by poor soil fertility, by root-knot and other pathogenic nematodes, and by bacterial wilt disease caused by *Ralstonia solanacearum*. Although the data presented here are descriptive rather than experimental, they are likely to be useful. Very little information about this region or cropping system is available in the scientific literature. Another valuable aspect of this work is the number of different grower production sites sampled across the Kenyan highlands.

The writing in this manuscript is generally clear and the content is well organized. One phrase does need clarifying however: it is not clear what the authors mean when they write: “There were weak positive, negative, correlations between Meloidogyne spp. and ….” What are weak positive negative correlations? (Results, paragraph 3)

Major comments
1. We have substantial concerns about the quantification of *R. solanacearum* in soil samples. The *R. solanacearum* population size data presented in Figure 1almost certainly over-represent the actual populations by several orders of magnitude. These data were obtained by streaking serially-diluted soil samples onto TZC medium and counting mucoid colonies with white borders and red centers and small round dark red colonies. Unfortunately, TZC medium is not at all selective for *R. solanacearum*. In our considerable experience, soil contains many bacteria that have these colony morphologies. Most of them are not *R. solanacearum*. Moreover, the dilution samples were streaked onto the plates. This is a non-quantitative method; dilution samples must be spread onto plates to quantify bacterial populations. Further, the population sizes described are inconsistent with many other studies which find *R. solanacearum* populations do not exceed 10e6 CFU/gm soil, even in fields undergoing active bacterial wilt outbreaks. It is not plausible that the soils sampled here contained 10e9 CFU *R. solanacearum*/gm.

This serious methodological problem means that the authors must remove the data in Figure 1 and all references to them in the text and title.To obtain some information about this pathogen, we suggest:

- If the samples are still available, they could be tested qualitatively for the presence of *R. solanacearum* using a diagnostic immunostrip (detection limit ~10e5 CFU), or semi-quantitatively with a PCR-based *R. solanacearum* detection assay.
- Alternatively, growers could be asked to estimate bacterial wilt disease incidence on each sampled site. A simple scale like: 0=never saw tomato wilt symptoms in this plot, 1= fewer than 1 in ten plants had wilt symptoms; 2=more than a tenth but fewer than half the plants had wilt symptoms; 3=more than half the plants had wilt symptoms.

2. Please re-examine the statistical analyses presented in Table 4. It doesn’t seem possible that *Meloidogyne* populations of 2225 and 575 could be in statistical cluster (a) while 1267.5 is in statistical group (b) and 905 is in group (ab).

Please note that we do not have the technical expertise to evaluate the soil chemistry or nematology aspects of this manuscript. Other reviewers should be asked to address these findings.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
No source data required

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I'm a plant pathologist with expertise in the biology of the bacterial wilt pathogen, Ralstonia solanacearum. I've worked with outreach specialists and growers trying to manage bacterial wilt of tomato in the developing tropics. Importantly, I'm not a nematologist or a soil scientist.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.