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Diversity of plant parasitic nematodes associated with common beans (*Phaseolus vulgaris*) in the Central Highlands of Kenya

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Abstract

Common beans (*Phaseolus vulgaris* L.) are the most important legume staple food in Kenya coming second to maize. In Central Highlands of Kenya, the 0.4-0.5ton ha^{-1} output is below the genetic yield potential of 1.5-2ton ha^{-1} partly due pests and diseases. Plant parasitic nematodes (PPN) have been reported to cause yield losses of up to 60% on beans. Though bean production is important in the Central highlands of Kenya, information on PPN associated with the beans in the region is lacking. This study was therefore undertaken to establish the diversity of PPN associated with common beans and to assess the root knot nematode damage on beans in the region. The study covered 50 farms (32 in Kirinyaga and 18 in Embu Counties) distributed in eight localities namely Kibirigwi (L1), Makutano (L2), Kagio (L3), Mwea (L4) and Kutus (L5) in Kirinyaga County and Nembure (L6), Manyatta (L7) and Runyenjes (L8) in Embu County and covering three Agro Ecological Zones (AEZs); UM2 (L1, L2, L3 & L4), UM3 (L5, L7 & L8) and UM4 (L6) AEZs. Manyatta (L7) and Nembure (L6), had the highest and second highest gall indices, respectively, while Kibirigwi (L1), Makutano (L2) and Mwea (L4) had some of the lowest gall indices. The most common PPN in bean roots were *Meloidogyne* spp. *Pratylenchus* spp. and *Scutellonema* spp. with a frequency of 94.38%, 78.25% and 59.13%, respectively. This further confirm the importance of these nematodes in bean production systems. Upper Midland 3 (UM3) AEZs and UM4 had higher nematode population densities and diversity than UM2. Disease severity and nematode composition and distribution were notably low in the irrigated areas Kibirigwi, Kagio and Mwea compared to rain-fed areas such as Makutano, Nembure and Manyatta.

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Introduction

Common beans (*Phaseolus vulgaris* L.) are the most important legume staple food in Kenya providing up to 65 percent of the country's national dietary protein intake and 32 percent of caloric intake (Wortmann & Allen 1994). Due to their capacity to fix nitrogen they are frequently used in maize cropping systems as an intercrop and reduces dependence on inorganic N fertilizers (Rheenen *et al.*, 1981). In the central highlands of Kenya, though bean production is an importance economic activity where it covers over 40000 hectares and, the yield of 0.5ton ha^{-1} is far below the genetic yield potential of 2ton ha^{-1} (Katungi *et al.*, 2010). These low yields are attributed to low soil fertility, diseases and insect pests as revealed by a study conducted in 2015 in parts of the region (Mwaniki 2015). The bean diseases were ranked as the most important (87.5%), followed by low soil fertility (52.5%), insect pests (50%), lack of quality seed (17.5%), low market prices (12.5%) and erratic weather patterns (10%) (Mwaniki, 2015). Although a study on the distribution and population densities of PPN associated with beans in Western Kenya revealed that PPN are a big threat to bean production (Karanja *et al.*, 2003), studies conducted in the central highlands of Kenya often overlook the impact on PPN on beans production. In other regions, plant parasitic nematodes have been reported to cause yield losses of up to 60% with root knot nematodes being rated as the most important (Talwana *et al.,* 2016; Kavuluko *et al.*, 2010; Kimenju *et al.*, 1999). In addition to direct pathogenic effects on plants, the nematodes act synergistically with other plant pathogens and pests to form disease complexes that further impact negatively on the crops. The nematodes also suppress nodulation and therefore affect nitrogen fixation (Kimenju *et al.*, 1999). Information on PPN associated with common beans in Central Highland of Kenya is lacking. Further, due to the growing importance of bean production in the region, it is imperative that a study be undertaken assess the diversity of PPN associated with beans in an effort to applying sound management and control strategies.

This study was therefore undertaken to establish the occurrence and diversity of plant parasitic nematodes associated with common beans and assess the root knot nematode damage on beans in the Central highlands of Kenya.

Materials and methods

Study Area

The study was conducted in Kirinyaga and Embu Counties in the Central Highlands of Kenya. These counties lie on the foot of Mt. Kenya at 1280 - 2500m above sea level. The climate is cool and humid\sub-humid with an annual mean temperature of 20-21°C. This region has a bimodal rainfall pattern with peaks in May and November and a hot dry spell between January and April. Average annual rainfall for this area is 1250mm in the higher altitudes. In lower altitudes the rainfall decreases and the rainy seasons become shorter. Soils are deeply weathered volcanic types mainly andosols in Upper Midland 2 (UM2) and nitosols in Upper Midland 3 (UM3) and Upper midland 4 (UM4) agro-ecological zones (AEZ) (Jaetzold and Schmidt, 1983).

The study covered 50 farms (32 in Kirinyaga and 18 in Embu Counties) distributed in eight localities namely Kibirigwi (L1), Makutano (L2), Kagio (L3), Mwea (L4) and Kutus (L5) in Kirinyaga County and Nembure (L6), Manyatta (L7) and Runyenjes (L8) in Embu County. The farms were concentrated in the UM2 (L1, L2, L3 & L4), UM3 (L5, L7 & L8) and UM4 (L6) AEZs where cultivation of bean is most common. Though bean production is mainly rain fed, farms in three localities; L1, L3 and L4 irrigate their crops since most of them are under Kibirigwi and Mwea Irrigation Schemes. In most farms, beans were inter-cropped with maize.

Sampling

Samples were collected in the middle of the short rain season October - November 2015. Ten bean and soil samples per farm were taken at mid-season when the bean plants were in the early poding stage. The farms were selected with the assistance of the County Executive Committee (CEC) offices in charge of agriculture in the two counties. The plants were gently dug out with a hand hoe taking care not to damage the roots. Soil adhering on the roots was gently shaken off into a plastic bag, then the root system was separated from the shoot system and cleaned before assessing the gall index and extracting the nematodes. The soil samples for each farm were bulked, mixed thoroughly before taking a 1000cm^3 for nematode assays

Assessment of disease severity

Nematode damage on the bean plant was assessed by visually rating the roots for galls using the Bridge and Page (1980) gall rating of 0-10 where 0= No knots on roots; 1= Few small knots that are difficult to find; 2=Small knots only but are clearly visible and main roots clean; 3= Some larger knots are visible and main roots clean; 4=Larger knots pre-dominate but main roots clean; 5=50% of roots infested and knotting on parts of main root and reduced root; 6=Knotting on main root; 7=Majority of main roots knotted; 8=All main roots knotted but few clean roots visible; 9=All roots severely knotted and plants usually dying and 10=All roots severely knotted and no root system and plant usually dead.

Extraction of Nematode from bean roots

The cleaned root systems from each farm were cut into 1cm long pieces, were well mixed before taking two 5g sub samples. One sub -sample was oven dried for 72 hours at 60°C while the second one was used for nematodes extraction using Maceration-Filtration method (Hooper *et al*., 2005). The nematodes were harvested after 48 hours and concentrated into 20ml in vial bottles. Nematode population from each sample was determined using a 1ml aliquot of a well-mixed suspension pipetted into a Hawksley's nematode counter and enumerated under a Leica MS 15 dissecting microscope. Counting was repeated in three aliquots and the mean recorded. Nematodes extracted from the roots were expressed per gram dry root weight. Nematodes were identified to genus level using the morphological features. A photographic record of the nematode genera was obtained using a digital camera (Leica DFC 280) mounted on a compound microscope.

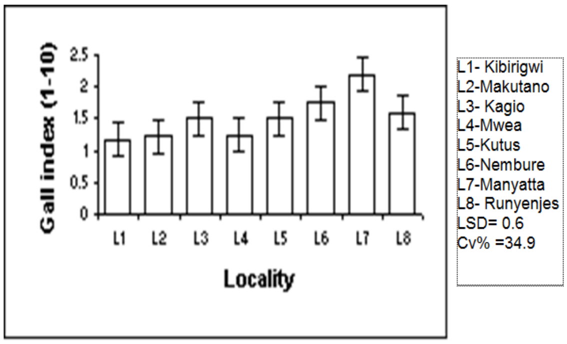
Nematode extraction from the soil

Nematodes were extracted from a 200cm^3-soil sub-samples using the Modified Extraction Tray method (Hooper *et al.,* 2005) Nematode suspension from the tray was sieved through a 38μm sieve every day for 3 days. The nematodes were backwashed into a beaker and concentrated into a 20ml suspension and placed in vials. Nematodes were enumerated under a dissecting microscope and density and frequency of occurrence determined.

Results and discussion

Nematode galling index

The most distinct symptom of nematode damage on the bean plants was root galling by *Meloidogyne* spp. Root Knot nematode damage levels varied in the 8 localities with Manyatta (L7) and Nembure (L6), having the highest and second highest gall indices, respectively. Kibirigwi (L1), Makutano (L2) and Mwea (L4) had some of the lowest gall indices of 1.17, 1.25 and 1.3, respectively, that differed significantly (p<0.05) from those in locality 7. Farms in localities 3, 5 and 8 had gall indices of 1.5 (Fig. 1).



‡ Gall index according to Bridge and Page (1980) galling scale of 0-10 where 1= No galls; 2=1-5%; 3=6-10%; 4=11-20%; 5=21-30%; 6=31-40%; 7=41-50%; 8=51-75% and 9=76% of root system and 10=All roots severely knotted and no root system and plant usually dead.

Fig. 1. Mean Gall indices‡ on bean roots from farms in eight localities of Kirinyaga and Embu Counties.

The significantly higher RKN gall indices Manyatta could be due to the mixed crop farming (beans, tomatoes, squash, egg plants) that is a common practice in the area with most of the crops grown being good hosts to root knot nematodes (Silva dos Santos *et al*., 2012; Waceke & Chege In Press; Weselmael & Maurice 2012). Further, the high RKN disease severity in Manyatta and Nembure could be attributed to the susceptibility of the bean varieties grown in the areas. GLP 2 (Rose coco), GLP24 and GLP1004 bean varieties that are commonly grown in the area are more susceptible to RKN than GLP 585 (Wairimu) and GLP 92 (Mwitemania) (Omwega *et al.*, 1989; Silva dos Santos *et al.*, 2012; Weselmael & Maurice 2012) grown in other localities. The relatively low galling indices some localities could be due to the relatively poor bean host of RKN namely GLP 585 (Wairimu) and GLP 92 (Mwitemania) that are grown in the area besides the high use of pesticides and organic and inorganic fertilizers. Farming in these localities is semi – commercial unlike in other areas where farming is purely subsistence.

The high nematode damage and population densities could be due to poor agricultural practices (low or no fertilizer or agricultural inputs and continuous bean cropping (twice a year)) in the areas. This contributes to the build -up of nematode levels over the seasons beyond damage thresholds (Omwega *et al.*, 1989). In farms where fertilizers (organic or inorganic) were used, lower gall indices were recorded. It has been reported that application of organic amendments and fertilizers increases growth and vigor of nematode infected plant thus allowing the host to tolerate nematode attack (Rheenen *et al.*, 1981) while organic matter in the soil allows nematode antagonists to compete successfully and attack nematodes (Waceke and Waudo 2002).

Diversity and Density of PPN associated with beans roots

The most common plant parasitic nematodes encountered in bean roots were *Meloidogyne* spp. *Pratylenchus* spp. and *Scutellonema* spp. with a frequency of 94.38%, 78.25% and 59.13%, respectively (Table 1).

Table 1. Percent Frequency of occurrence of plant parasitic nematodes in bean roots obtained from 8 localities in Kirinyaga and Embu Counties.

Nematode genus	Kibirigwi	Makutano	Kagio	Mwea	Kutus	Nembure	Manyatta	Runyenjes
Meloidogyne	100	100	100	78	98	85	100	94
Pratylenchus	80	86	88	37	93	84	80	78
Scutellonema	-	63	24	62	90	74	80	80
Hemicycliophora	26	-	21	-	-	-	-	-

Hemicycliophora spp was present in two localities; Kibirigwi and Kagio with a frequency of occurrence of up to 26%. *Meloidogyne, Pratylenchus* and *Scutellonema* spp. have been reported to be the major nematode genera associated with beans in different parts of the country and so the findings of this study corroborates with the findings by Kimenju *et al.*, 1999.

Roots of bean supported higher densities of *Meloidogyne* spp than *Pratylenchus* spp.in all the localities except Nembure where the density of lesion nematodes was more than double that of the root knot nematodes (Table 2). The differences in the densities of the two nematodes in bean roots could be attributed to the difference in their mode of feeding (lesion nematodes are migratory endoparasites while root knot nematodes are sedentary endoparasites and to the susceptible bean genotype. The bean genotypes grown in the area are more susceptible to root knot nematodes than lesion nematodes (Waceke *et al.,* 2007; Waceke and Chege 2017 In Press).The relatively high densities of *Pratylenchus* in Nembure could be attributed to the presence of coffee and maize in most of the farms where beans were intercropped with them (Waceke and Arim 2017; Arim *et al.*, 2006; Katungi *et al.*, 2010). Coffee and maize are good hosts of *Pratylenchus* spp and coffee being a perennial crop, it support the continuous growth and reproduction of the lesion nematodes. Further, lesion nematodes are polyphagus except *P. goodeyi* which infects only bananas. The impact of the two ectoparasites; *Scutellonema* and *Hemicycliophora* on bean production needs to be further investigated.

Overall farms in Kutus, Runyenjes and Manyatta had some of the highest nematode densities in bean roots while Mwea, Kibirigwi and Kagio had some of the lowest densities (Table 2).

Most of the farmers in the irrigation schemes concentrated in Mwea and Kibirigwi localities are semi commercial farmers and use pesticides, organic and inorganic fertilizers and practice good crop husbandry in their production systems that could have reduced the nematode population (Wortmann & Allen 1994).

Disease severity and nematode composition and distribution were notably low in the irrigated areas (L1, L3 and L4) compared to rain-fed areas (L2, L5, L6, L7 and L8). This supports the observation that disease severity is more pronounced under conditions of water stress (Rheenen *et al*, 1981; Wortmann & Allen 1994).

Table 2. Mean Nematodes densities per g dry root weight of bean root in 8 localities in Kirinyaga and Embu Counties.

Nematode genus	Kibirigwi	Makutano	Kagio	Mwea	Kutus	Nembure	Manyatta	Runyenjes
Meloidogyne	33.6	76	47	18.4	113.4	36.2	97.4	104.8
Pratylenchus	8.4	16.6	16.2	12.6	29.8	84	17.4	34.8
Scutellonema	-	13.8	2.4	6.8	20.6	7.4	20.2	12.8
Hemicycliophora	5.2	-	4.2	-	-	-	-	-
Total	47.2	106.4	69.8	37.8	163.8	127.6	135	152.4

Diversity and density of PPN in soil

Eleven PPN genera namely *Meloidogyne* spp. *Pratylenchus* spp. and *Scutellonema* spp., *Helicotylenchus* spp., *Paratylenchus* spp., *Tylenchorynchus* spp., *Tylenchus* spp., *Hemicycliophora* spp., *Hoplolaimus* spp., *Trichodorus* spp. and *Xiphinema* were encountered in bean rhizosphere soils (Table 3) underscoring the importance of PPN in crop production systems. *Pratylenchus* spp, *Meloidogyne* spp, *Scutellonema* spp and *Paratylenchus* spp, some of the most frequently encountered had an overall percent occurrence of 100%, 97%, 73% and 63%, respectively (Table 3). All the 11 genera were present in three of the localities; Kibirigwi, Kutus and Nembure.

This could be attributed to the diversity of the crops grown in these localities (Katungi *et al.,* 2010). Kagio had the lowest diversity of nematodes with seven out of the 11 genera being isolated from the farms in this locality (Table 3).

Table 3. Percent frequency of occurrence of PPN in soil obtained from 8 localities in Kirinyaga and Embu Counties.

Nematode genus	Kibirigwi	Makutano	Kagio	Mwea	Kutus	Nembure	Manyatta	Runyenjes
Meloidogyne	100	100	100	78	100	100	100	100
Pratylenchus	100	100	100	100	100	100	100	100
Scutellonema	70	63	51	78	81	78	80	60
Helicotylenchus	80	38	23	22	82	62	40	21
Paratylenchus	50	78	50	57	46	60	80	81
Tylenchohynchus	70	25	-	38	82	40	62	42
Tylenchus	81	18	-	12	48	79	60	-
Hemicycliophora	31	16	22	-	63	15	-	-
Hoplolaimus	33	-	-	36	46	23	60	96
Trichodorus	72	24	-	-	100	62	63	40
Xiphinema	30	17	50	20	65	38	19	43

Six of the 11 genera; *Meloidogyne, Pratylenchus, Scutellonema, Helicotylenchus, Paratylenchus* and *Xiphinema* were encountered in all the farms underscoring the importance of PPN in agriculture. The rich biodiversity could be due to mixed cropping systems in most of the localities (Kavuluko *et al.*, 2010).

The three localities in Embu (Nembure, Manyatta & Runyenjes) had some of the highest nematode densities in the soils and nematode diversity (Table 3) while Kagio and Mwea had some of the lowest densities (Table 4).

Table 4. Mean population densities of dominant plant parasitic nematodes recovered from 200cm^3 of soil samples.

Nematode genus	Kibirigwi	Makutano	Kagio	Mwea	Kutus	Nembure	Manyatta	Runyenjes
Meloidogyne	266	227	184	158	264	838	602	487
Pratylenchus	272	224	201	175	222	491	359	285
Scutellonema	38	75	28	60	77	155	174	69
Paratylenchus	34	74	29	37	27	101	97	92
Helicotylenchus	80	43	11	8	72	72	18	14
Total	690	643	453	438	662	1657	1250	947

The high nematode densities in these areas could be attributed to the mixed cropping practiced in the area while the low nematode densities in Kagio and Mwea could be attributed to the application of pesticides and other inputs. Crop production in this area is done on a semi- commercial basis and so application of pesticides and other inputs is relatively a more common practice than in other study areas. Some of the farms in these areas were formerly rice paddies and therefore the flooding previously done could have contributed to the low nematode densities. It is notable that these areas had also the lowest nematode diversity (Table 3).

High counts of lesion nematodes (*Pratylenchus* spp.) recovered from both the soil and root samples further confirm their association with bean plants. (Wortmann & Allen 1994)). *Scutellonema* spp., found in most of the localities is a polyphagous genus associated with a wide range of plant species including maize and beans (Rheenen *et al.,* 1981). Dominance of *Meloidogyne* species in all the localities may be attributed to the presence of beans, a suitable host and other alternative hosts (including weeds) and continuous cropping. The wide diversity of nematodes identified from the sampled farms could be due to mixed cropping systems (Rheenen *et al.,* 1981).

Considering the AEZs in relation to the species composition and structure of the nematode communities, UM3 and UM4 had higher nematode population densities. This could be due to differences in soil properties and mixed cropping systems common in these AEZs as previously reported by (Wortmann & Allen 1994). Disease severity and nematode composition and distribution were notably low in the irrigated areas Kibirigwi, Kagio and Mwea compared to rain-fed areas such as Makutano, Nembure and Manyatta. This supports the observation that disease severity is more pronounced under conditions of water stress (Rheenen *et al.,* 1981).

Conclusion

Manyatta (L7) and Nembure (L6), had the highest and second highest gall indices, respectively while Kibirigwi (L1), Makutano (L2) and Mwea (L4).

The study revealed high densities of lesion nematodes (*Pratylenchus* spp.) and root knot nematodes (*Meloidogyne* spp) in bean roots and soil. Upper Midland 3 (UM3) AEZs and UM4 had higher nematode population densities and diversity than UM2. Disease severity and nematode composition and distribution were notably lower in the irrigated areas compared to areas where bean production relies on rain

Recommendation

Continuous production of susceptible bean varieties should be minimized to reduce PPN population build up and minimize the detrimental effects on the beans. Further selection of the varieties to be grown in the area should be done carefully. Appropriate measures should be incorporated in the bean production systems to reduce the high densities of lesion nematodes (*Pratylenchus* spp.) and root knot nematodes (*Meloidogyne* spp).

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