International Journal of Agriculture Innovations and Research
Volume 6, Issue 4, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy):
Received: 06/01/2018 | Accepted on: 13/01/2018 | Published: 03/02/2018

Response of Common Beans Varieties to a Mixed Population of Root Knot Nematodes (*Meloidogyne spp*) in Central Highlands of Kenya

Waceke J.W.
Department of Agricultural Science and Technology, Kenyatta University, P.O. Box 43844, Nairobi - 00100 Kenya
Corresponding author email id: wanjohi.waceke@ku.ac.ke

Abstract **– Response of eight bean varieties to *Meloidogyne javanica* and *M. arenaria* was evaluated under greenhouse and field conditions. The bean varieties were GLP2 (Rosecoco), GLP24 (Canadian wonder/Gituru), GLPX92 (Mwitemania), GLP585 (Wairimu), GLP1004 (Mwezi moja), GLP1127 (New mwezi moja), and two climbing beans (Vuninkingi and Gisenyi). .In the greenhouse test, two weeks old seedlings established on sterilized sandy loam soil were inoculated with 4000 nematodes comprising of second stage juveniles (J2) and eggs obtained from beans in the Central highlands of Kenya and maintained on tomato cv money maker in the greenhouse. Each treatment was replicated six times in a Randomized Complete Block Design (RCBD). Five bean varieties namely GLP2 (Rose coco), GLPX92 (Mwitemania), GLP585 (Wairimu), GLP1127 (New mwezi moja) and Gisenyi that were moderately resistance or resistant as revealed in the greenhouse experiments were evaluated under field conditions. Treatments were replicated four times and arranged in a RCBD. Greenhouse test was terminated 60 days after inoculation while the field test was terminated 90 days after planting. Plant growth, nematode damage as measured by gall indices and egg mass indices and reproductive factor were assessed. Based on these parameters, the host responses to the nematodes were categorized as resistance, moderately resistant, susceptible and moderately susceptible. GLP 585 was categorized as resistance or a poor host while Vuninkingi was rated the most susceptible among the eight varieties. The moderately resistant varieties were (GLPX92, GLP2, GLP1127 and Gisenyi, while GLP24 and GLP1004 were moderately susceptible**.

Keywords **– Resistant, Susceptible, Root Knot Nematodes, Reproductive Factor, Gall Index, Egg Mass Index, Host Suitability.**

I. INTRODUCTION

Common beans (*Phaseolus vulgaris* L.) are the most important legume staple food in Kenya coming second to maize. Beans are rich in protein (lysine tryptophane, methiomine), vitamins (vitamin B, nicotine acid) and minerals (Ca and iron) making it a cheap source of protein especially for the rural poor who cannot in most cases afford the relatively expensive animal protein [6]. They provide up to 65 percent of the country's national dietary protein intake and 32 percent of caloric intake [6]. Half a cup, for example, provides the recommended daily allowance of folic acid and B vitamins; supplies 25-30% of the recommended levels of iron, meets 25% of magnesium and copper needs and 15% of the potassium and zinc [6]. In addition, N-fixation through root nodules of beans improves soil fertility status and reduces amount of N used in intercropping systems [6], [8]. For this reason, its common practice to intercrop beans with maize in small holder farming systems in Kenya.

Though bean yields are on the decline, their demand is on the rise, being at 5-10% per annum [8]. The production constraints include diseases, insect and nematode pests, low soil fertility and poor agricultural practices [11], [18], [19]. Among nematode pests, root knot (*Meloidogyne* spp) are the most important [9], [19]. Root knot nematodes which are polyphagus nature, are reported to cause yield losses of up to 60% [4], [18]. In addition to direct pathogenic effects on plants, the nematodes act synergistically with other plant pathogens to form disease complexes that further impact negatively on the crops [18]. The nematodes also suppress nodulation and therefore affect nitrogen fixation [15], [23].

Poor agronomic practices including continuous cultivation of beans, a common practice among resource poor farmers further aggravate the nematode menace as the nematode population builds up beyond the economic threshold levels [19]. Though effective, use of nematicides in small holder farming systems is confined to high value crop and thus they are not a first choice option for nematode management on beans [18]. Though use of resistant varieties is an effective way of controlling nematodes [13], [14], the long periods required to develop and release resistant varieties and the existence of pathotypes that break the resistance remain a great challenge to the use of resistant varieties in the management of nematodes. In spite of these shortcomings, use of resistant varieties has great potential in nematode management in low – input agriculture according to [10], [15], [17], [20], [21]. Information on response of bean varieties to *Meloidogyne javanica* and *M. arenaria* in the Central highlands of Kenya is lacking. There is therefore need to screen the bean varieties grown in the area for resistance against the mixed population of the two RKN species *(Meloidogyne javanica* and *M. arenaria*) commonly found in the area of study.

This study was therefore carried out to evaluate the response of bean varieties to *M. javanica* and *M. arenaria* in the Central highlands of Kenya with the overall aim of identifying a variety for inclusion in integrated management strategies.

II. MATERIALS AND METHODS

Greenhouse and field experiments were conducted to evaluate the response of bean varieties to root knot nematodes in Kirinyaga and Embu Counties of the Central highlands of Kenya.

Greenhouse Experiment

Test Plants

Reaction of eight bean varieties to a mixed population of *M. javanica* and *M. arenaria* was evaluated under greenhouse conditions. The varieties were GLP2 (Rosecoco), GLP24 (Canadian wonder/Gituru), GLPX92 (Mwitemania), GLP585 (Wairimu), GLP1004 (Mwezi moja), GLP1127 (New mwezi moja), and two climbing beans (Vuninkingi and Gisenyi). The varieties selected were commonly grown as intercrops with maize in the study area. The seeds were surface sterilized before sowing them into a 15cm- diameter pots filled with steam sterilized soil made up of Sandy-loam in the ratio of 1:3. Thinning was done one week after sowing so as to have 1 seedling per pot.

Inoculum Preparation and Inoculation Procedure

Two - weeks old seedlings were inoculated with 4,000 nematodes. The inoculum consisted of a 20ml suspension of second stage juvenile (J2) and eggs. The nematodes were obtained from infected bean root system collected from infested farms in the study area and maintained on tomato *Solanum esculentum* cv. moneymaker in the greenhouse. In preparing the inoculum, the nematodes were extracted from the tomato roots using the method according to [5]. The inoculum was pipetted into a depression made around the bean roots and covered with soil. Treatments in which no nematodes were added served as controls. The treatments were arranged in a Randomized Complete Block Design (RCBD) and replicated 6 times. The experiment was terminated 60 days after inoculation and plant growth and disease assessment data collected.

Data Collection

Plant Growth Assessment

Plants were gently uprooted and the root system separated from the shoot system at the first basal node. The root systems were carefully and thoroughly washed before taking their fresh weights. Fresh and dry shoot weights and dry pod weights were obtained. The dry shoot and pod weights were obtained after drying them at 70C for 72 hours in an oven.

Disease Assessment

Nematode disease severity and damage were assessed using gall indices, egg mass indices and number of nematodes in root and in the soil and the reproductive indices [14], [16], [17], [22]. To determine the gall index, the root system was visually rated using a gall-rating index of 0-10 by [1] 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.

In determining the egg mass indices, the root system was washed free of soil and blotted dry before immersing 5g fresh roots sub samples in Phloxine B (Fluka, Germany) (0.15 g/liter tap water) for 15–20 min to stain the egg masses as described by [2]. The stained roots were rinsed with running tap water and blotted dry. Egg-masses were enumerated under a stereo microscope using a manual Counter and scored using a 0-5 egg-mass rating index according to [12] where; 0 = no egg-masses; 1 = 1-2 egg-masses; 2 = 3-10 egg-masses; 3 = 11-30 egg-masses; 4 = 31-100 egg-masses and 5 ≥ 100 egg-masses per root system.

To obtain the nematode final population in roots, a 10g root sub - sample was obtained from the remaining unstained roots that had been cut into 1 cm long pieces. The root sub sample was blended for 30 seconds in 200 ml of 0.5% sodium hypochlorite and passed through a 400 mesh sieve (0.038 mm) and washed in tap water, according to the methods by [3], [5]. The nematode suspension was adjusted to 20ml, and a 1ml aliquot placed in a Hawksley's Counter and nematodes counted under a Leica MS 5 stereo microscope at 40X. The final nematode population per root system was the average number of nematodes counted from three-1ml aliquots taken from the 20ml nematode suspension.

The nematode Reproduction Factor (RF) was calculated as RF = Pf/Pi, where PF = final nematode population in the roots and Pi = initial nematode population. Host Suitability was categorized based on RF according to [14], [22], [24] as good (susceptible) when RF > 5.0, fair (Moderately susceptible) if 5.0 >RF >1, poor (Resistant) if 1 > RF > 0, and non - host (immune) when RF = 0.

In determining the final nematode population in the soil, the soil was thoroughly but gently mixed and a 200 cm^3 sub sample was used to extract the nematode using the method according to [7] and countered under a Stereo microscope using Hawksley's nematode counter. The data were transformed by log l0 (X + 1) to standardize variances before analyses.

Field Experiments

The field test was conducted at Kenyatta University located in Nairobi County. The university lies between latitudes 1° 10' South of the Equator and Longitude 36° 55'East and at an altitude of 1790m above the sea level. The climate is fairly cool with temperature ranges of 10°C to 29ºC. The rainfall pattern is bi-modal experiencing long rains between March and May with a mean rainfall of 899 mm while the short rains are experienced between October and December with a mean rainfall of 638 mm. The mean annual rainfall is 786.5 mm. The soils are deep friable loamy-clay type. The field, naturally infested with RKN was demarcated into forty 3x4m micro plots with a 1m alley between them. The initial RKN second stage juveniles' population was determined before planting. To obtain the initial nematode population in the soil, ten soil cores were taken at random from each plot and thoroughly mixed before extracting nematodes from a 200cm^3 sub-sample using the method according to [3].

Five bean varieties namely GLP2 (Rose coco), GLPX92 (Mwitemania), GLP585 (Wairimu), GLP1127 (New mwezi moja), and Gisenyi that were resistant or moderately resistant as revealed in the greenhouse test were used in this test. Seeds were sown at the recommended spacing of

15x50cm within and between rows. The experiment was arranged in a Randomized Complete Blocks Design (RCBD) with four replications (2 outer rows served as the guard rows). Plots treated with a Tellon II (pre-plant soil fumigant) at recommended rate and planted with the respective bean variety served as the controls. The plots were manually kept weed free throughout the experimental period and watered using overhead irrigation as was needed.

Data Collection

Plant Growth Assessment

Plant and soil samples for nematode assays were collected at the end of the experiment, 90 days after planting. Ten plants were randomly selected from each micro plot. All mature pods were harvested from the ten selected plants before gently uprooting them to minimize damage of the root system. The soil adhering to the roots systems was gently shaken off into a plastic bag before separating the root from the shoot system at the first basal node. The soil from each of the ten plants was composited and a 200cm^3 composite sub sample taken for nematode extraction. Both the soil and the plant materials were transported to the laboratory in cool boxes for further analyses. The roots were carefully but thoroughly washed and fresh root weights obtained. Dry pod weight were obtained to determine the yield.

Disease Assessment

The roots were washed in running tap water and nematode damage assessed by visually rating the galling using the gall rating index of 1-10 [1] and an egg mass index of 0-5 [2] as described in details for the greenhouse test.

Nematodes were extracted from a 200cm^3 soil sub sample. The J2 were then concentrated in water in 20ml vials. One ml aliquot of a well-mixed nematode suspension was pipetted into a Hawksley nematode counting slide and nematodes counted under a dissecting microscope.

Data Analysis

Data obtained were subjected to Analysis of Variance (ANOVA). Means were separated using Fisher's Least Significance Difference (LSD) ($p<0.05$). In addition, Student T-test was carried out to compare means of crop performance in the greenhouse test.

III. Results and Discussion

Green House Test

Plant Growth

There were significant differences ($p< 0.05$) in the plant growth parameters for five bean varieties, namely GLP 2, GLP 24, GLPX92, GLP 585 and GLP1127 (Table 1). The fresh and dry shoot weights and the fresh root weight of the uninoculated GLP 24 were significantly ($P<0.05$) lower than those of the controls. The dry shoot weights, fresh root weights and dry pod weights of inoculated GLP585 were significantly ($P<0.05$) lower than the uninoculated. The fresh shoot weights of inoculated GLP1127 and GLPX92 were significantly ($P<0.05$) lower than the uninoculated varieties. There were no significant differences between inoculated and uninoculated GLP 1004, Vuninkingi and Gisenyi bean varieties as depicted in Table 1.

Table 1. Mean† Fresh Shoot Weight (FSW), Dry Shoot Weight (DSW), Fresh Root Weight (FRW) and Dry Pod Weight (DPW) of beans, Greenhouse Test

Bean varieties									
Parameter		**GLP2**	**GLP24**	**GLPX92**	**GLP585**	**GLP1004**	**GLP1127**	**Vuninkingi**	**Gisenyi**
FSW	T	30.7	45.35*	35.65*	29.4	28.51	27.58*	23.65	47.54
	C	32.97	63.36	25.36	35.25	35.74	35.6	27.3	48.35
DSW	T	5.3	6.12*	3.95	4.22*	4.68	4.34	4.58	8.36
	C	6.17	9.06	2.91	5.39	5.67	3.92	4.22	8.61
FRW	T	6.9*	8.4*	7.13	4.1*	7.13	4.8	5.09	11.8
	C	10.3	11.46	7.66	5.75	7.49	6.12	6.13	14.6
DPW	T	4.8	5.5	6.37	5.76*	5.89	6.17	5.82	8.03
	C	7.54	5.96	6.76	9.07	5.75	5.61	5.29	9.39

†Data are means of six replications

* Significant differences ($p< 0.05$) between the inoculated and control plants in the respective growth parameter by Student's T test.

The reduced growth of the various bean varieties could be attributed to the effects of nematodes on growth. The relatively superior performance of inoculated plants in some cases could be attributed to the ability of root knot nematodes to stimulate nitrogen fixation and thereby improve growth [15] or to the bean genotype [6].

Nematode Disease Severity and Damage

There were significant differences ($P<0.05$) in gall indices and number of J2 in the soil among the treatments as depicted in Table 2. Vuninkingi revealed the highest nematode damage as had the highest gall indices and number of nematodes in the soil followed by GLP1004 and

GLP24. GLP 585 revealed the lowest gall indices and number of nematodes in the soil followed by Gisenyi, GLPX92, GLP1127 and GLP2 (Table 2). Though there were no significant differences in egg mass and reproductive indices, Vuninkingi had the highest egg mass and reproductive indices while GLP 585 had the lowest (Table 2).

Based on the reproductive indices GLP585 was the most resistant while Vuninkingi was the most susceptible bean variety. The other varieties were moderately resistant (GLPX92, GLP2, GLP1127 and Gisenyi) and moderately susceptible (GLP24 and GLP1004).

Table 2. Mean† gall rating indices, egg mass indices and numbers J2 in the soil, RF and Host reaction of different bean varieties to RKN nematodes, Greenhouse test

Treatment	GRI[a]	EMI[b]	J2/200cm^3 [c]	RF	Host Reaction
GLP2	4.66b	2.34	73c	0.38	MR
GLP24	6.18c	2.18	179d	1.43	MS
GLPX92	4.01b	1.17	53b	0.27	MR
GLP585	2.52a	0.68	34a	0.17	R
GLP1004	6.75cd	2.51	181d	1.61	MS
GLP1127	4.02b	1.83	77c	0.39	MR
Vuninkigi	7.67d	3.19	212e	5.56	S
Gisenyi	3.82ab	2.65	46ab	0.21	MR
$LSD_{0.05}$	1.34	NS	18.99	NS	
Se	11.1	1.3	16.2	1.52	
Cv%	22.3	51.4	11.1	3.07	

† Data are means of six replications. [a] Gall indices according to [1]
[b] Egg mass indices according to [12]

Field experiment

Plant growth

Bean varieties in the plots that were treated with the nematicide had higher dry pod weights than those grown in micro plots that were not treated with the nematicide (Fig. 1). There were no significant differences however in dry pod weights between the treatments and the control. (Fig. 1). The difference in the performance of the various bean varieties could be attributed to their genotypic differences.

Nematode Disease Damage

There were significant differences ($P<0.05$) in Gall indices (GI) and Egg mass indices (EMI) among the bean varieties (Table 3). GLP585 and GLP1127 had some of the lowest GI (1.15 and 1.87, respectively).

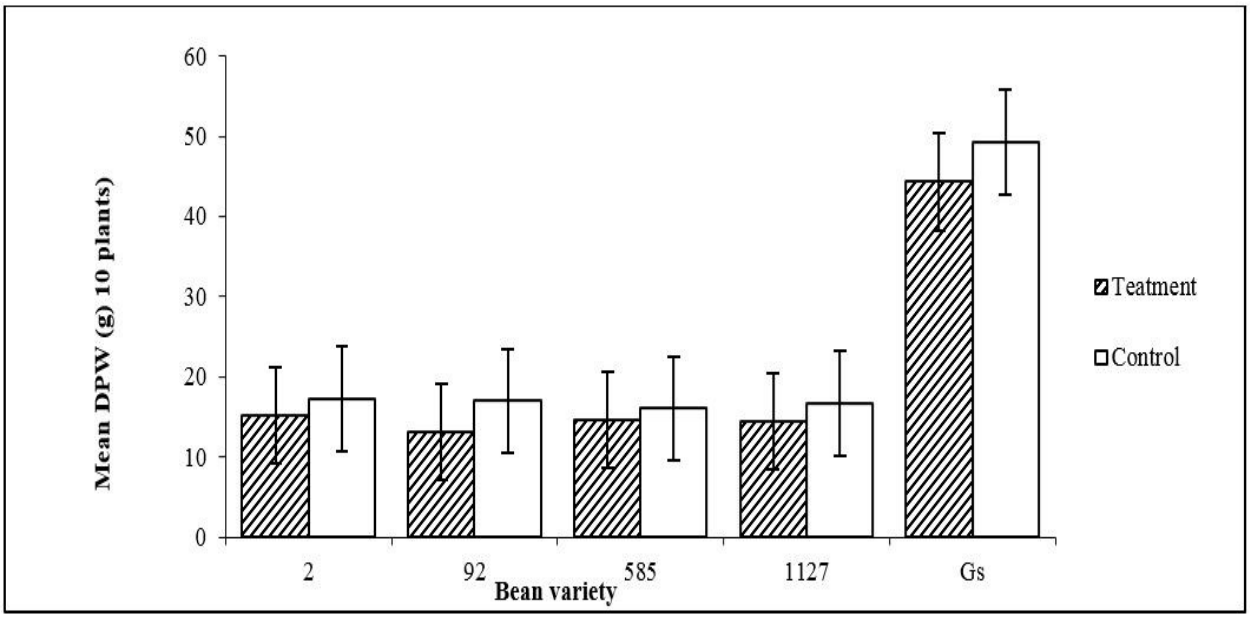

Fig. 1. Mean dry pod weight (DPW) of different bean varieties grown in root knot nematode- infested and nematicide treated plots, Field Test

while Gisenyi had the highest (Table 3). While most of the varieties revealed low gall mass indices, GLP 585 revealed no gall masses in their roots. Gisenyi had the highest egg masses which were significantly higher ($P<0.05$) than those of the other varieties. These results are consistent with the finding in the greenhouse results which revealed that GLP585 was the most resistant cultivar.

Table 3: Mean† gall index, Egg mass index and number of J2 in the soil different bean varieties grown in root knot nematode- infested field; Field test

Treatment	GRI[a]	EMI[b]	J2/ 200cm^3
GLP2	2.15	0.1	270
GLPX92	2.2	0.2	422
GLP585	1.15	0.0	335
GLP1127	1.87	0.15	210
GISENYI	3.2	0.8	575
$LSD_{0.05}$	1.23	0.39	NS
SE	0.19	0.11	21.3
CV%	21.5	36.5	8.9

† Data are means of four replications.
[a] Gall rating indices according to [1]
[b] Egg mass indices according to [12]

The wide range of responses of the bean varieties to the mixed population of root knot nematodes indicate the potential presence of resistance genes within the germplasm, with GLP585 having the highest potential. The type of resistance, whether polygenic or monogenic needs to be elucidated by further studies. Root exudates from plant with resistance genes are known to suppressive to root knot nematodes [10], [13], [15]. The stability and mechanism of resistance needs to be determined in future studies as nematode pathotypes and environmental factors especially temperature affects the stability [10], [13], [15].

IV. Conclusion

The study revealed that GLP 585 was a poor host to a mixed population of root-knot nematodes while Vuninkingi was the best host to the nematodes. GLPX92, GLP2, GLP1127 and Gisenyi were moderately resistance while GLP24 and GLP1004 were moderately susceptible. A study on the mode or mechanism of resistance needs to be elucidated and whether its controlled by single or multiple genes.

Acknowledgements

We acknowledge the Rockefeller Foundation for financially supporting this project, the farmers for allowing us to collect galled bean roots from their farms and Kenyatta University for providing office and laboratory spaces for the researchers.

References

[1] Bridge J. and Page R. (1980): Estimation of Root-Knot Nematode Infestation Levels on Roots Using a Rating Chart. Tropical Pest Management, 26(3): 296-298.

[2] Holbrook CC, Knauft DA, Dickson DW. (1983): A technique for screening peanut for resistance to *Meloidogyne arenaria.* Plant Disease, 67: 957-958.

[3] Hooper DJ, Hallmann J, Subbotin SA. (2005): Methods for extraction, processing and detection of plant and soil nematodes. In Plant Parasitic Nematodes in Subtropical and Tropical Agriculture 2nd edition. Edited by Luc M, Sikora RA, Bridge J. CAB International Oxfordshire: UK, 53-80.

[4] Hussey RS, Janssen GJW. (2002): Root-knot nematodes: *Meloidogyne* species. In Plant resistance to plant parasitic nematodes edited by Starr JL, Cook R, Bridge J. Wallingford: CABI Publishing, 43–70.

[5] Hussey RS, Barker KR. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Report, 12: 1025-1028.

[6] International Center for Tropical Agriculture (CIAT) (2004). *Atlas of common Beans (Phaseolus vulgaris L.) production in Africa*, C.S. Wortmann, R.A. Kirkby, C.A. Eledu & D.J. Allen (Eds) Cali, Colombia 133p

[7] Jenkins WR. (1964): A rapid centrifugal flotation technique for separating nematodes from the soil. Plant Disease Report, 48: 692.

[8] Katungi E, Farrow A, Mutuoki T, Gebeyehu S, Karanja D, Alemayehu F, Sperling L, Beebe S, Rubyogo JC, Buruchara R. (2010): Improving common bean productivity: an analysis of socio-economic factors in Ethiopia and Eastern Kenya. Baseline Report Tropical legumes II. Centro Internacional de Agricultura (Agriculture International) Tropical - CIAT. Cali, Colombia.

[9] Kavuluko JM, Gichuki C, Waceke JW, Runo SM (2010): Characterization of root-knot nematodes (*Meloidogyne* spp.) from selected legumes in Mbeere District Kenya using isoenzyme phenotypes. In Proceedings of the 12th KARI Biennial Scientific Conference on Transforming Agriculture for improved livelihoods through Agricultural Product Value Chains: 8- 12 November 2010; Nairobi. Edited by Wasilwa L. 92- 97.

[10] Omwega CO, Thomason IJ, Roberts P, Waines CG. (1989): Identification of new sources of resistance to the root knot nematodes in *Phaseolus*. Crop science, 29: 1463-1468.

[11] Otsyula R.M., Ajanga S. I., Buruchara R.A. Wortmann C. S. (1998): Development of an integrated bean root rot control strategy for western Kenya. African Crops Science Journal 6: 61-67.

[12] Quesenberry KH, Baltensperger DD, Dunn RA, Wilcox CJ, Hardy SR (1989): Selection for tolerance to root-knot nematodes in red clover. Crop Science, 29:1362-1365.

[13] Roberts PA. (1992): Current status of the availability, development and use of host plant resistance to nematodes. Journal of Nematology, 24: 213-227.

[14] Sasser JN, Carter CC, Hartman KM. (1984): Standardization of host suitability studies and reporting of resistance to root-knot nematodes. Raleigh: North Carolina State University Press.

[15] Shree PS, Schwartz HF. (2011): Breeding common bean for resistance to insect pests and nematodes: A review. Canadian Journal of Plant Sciences, 91: 1-12.

[16] Silva dos Santos LN, Alves FR, Belan LL, Cabral PDS, Matta FP, Junior WCJ, Moraes WB. (2012): Damage quantification and reaction of bean genotypes (*Phaseolus vulgaris* L.) to *Meloidogyne incognita* race 3 and *M. javanica*. Summa Phytopathology, Botucatu, 38: 24-29.

[17] Sydenham GM, McSorley R, Dunn RA. (1996): Effects of resistance in *Phaseolus vulgaris* on development of *Meloidogyne* species. Journal of Nematology, 28**:** 485-491.

[18] Talwana H, Davis KG, Waceke JW, Sibanda Z, Manzanilla-Lopez R, Kimenju JW, Nessie DL, Masawe C. 2016. Agricultural nematology in East and Southern Africa: Problems, Management Strategies and Stakeholder linkages. Pest Management Science 72:226-245 DOI: 10.1002/ps.4104

[19] Waceke JW. Diversity of plant parasitic nematodes associated with common beans (*Phaseolus vulgaris*) in the Central Highlands of Kenya. Accepted for publication, International Journal of Agronomy and Agricultural Research.

[20] Walters SA, Wehner TC, Barker KR. (1990): Resistance of the cucumber to the root-knot nematode, *Meloidogyne hapla*. Cucurbit Genetics Cooperation. Report, 13: 10-11.

[21] Wehner TC Walters SA, Barker KR. (1991). Resistance to root-knot nematode in cucumber and horned cucumber. Journal of Nematology 23, (Suppl 4S): 611-614.

[22] Wehner TC, Walters SA, Barker KR. (1992): Use of Reproduction Factor and Gall Index in Determining Resistance in *Cucumis* spp. Cucurbit Genetics Cooperative Report, 15:28-30.

[23] Wesemael WM, Maurice M. (2012): Screening of common bean (*Phaseoulus vulgaris* L.) for resistance against temperate root-knot nematodes (*Meloidogyne* spp). Pest Management Science, 68: 702-708.

[24] Zhang F, Schmitt DP. (1994): Host Status of 32 Plant Species to *Meloidogyne konaensis.* Journal of Nematology 23, (Suppl 4S): 744 -748.