

Growth Performance Evaluation of Nine Accessions and a Local Variety of Pepper Inoculated with Single and Mixed Populations of *Helicotylenchus multicinctus* and *Meloidogyne incognita*

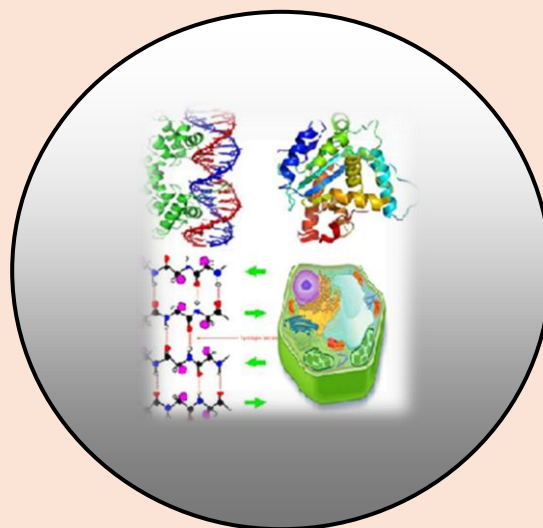
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RESEARCH PAPER

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Growth Performance Evaluation of Nine Accessions and a Local Variety of Pepper Inoculated with Single and Mixed Populations of *Helicotylenchus multicinctus* and *Meloidogyne incognita*

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ABSTRACT

Plant parasitic nematodes are serious pathogens of pepper throughout production areas in Nigeria, impacting negatively on both the quantity and quality of marketable yields. In addition, nematodes interaction with each other or with other plant pathogens, result in increased damage caused by these diseases. The objective of this study was to evaluate nine accessions and a local variety of pepper, for resistance and or susceptibility to pathological effect of *Helicotylenchus multicinctus* and *Meloidogyne incognita* singly or in combination. The pepper accession and the local variety namely; NGB00574, NGB00581, NGB00586, NGB00587, NGB00624, NGB00629, NGB00631, NGB00684, NGB00702 and LV were evaluated in Screen-House trial, laid out in 4x10 factorial arrangement fitted into completely randomized design with four replications. Each of the accessions and the variety were inoculated with approximately 1000 juveniles each of *H. multicinctum* and *M. incognita*; and 2000 mixed population, 1000 each of the nematodes on the root and adhering soil. Data on plant height, leaf area, root length and weight of the test plant were recorded and analysed. The results showed that nematodes infection had significant effect on pepper growth; however, the decrease in the various growth indexes was not consistent, which indicated that nematodes infection on different organs of pepper accessions and a variety was inhibited to varying degrees. The accessions NGB00587, NGB00702 NGB00624, NGB00631 NGB00629 and variety LV, recorded minimum decrease in growth with inoculation with the nematodes compared to their uninoculated control plant.

Keywords: *H. multicinctus*, *M. incognita*, Accessions, Variety and Nematode.

INTRODUCTION

Pepper (*Capsicum spp*) is an essential agricultural plant as it provides income to the farmer and the fruits are utilized for nutritional and medicinal purposes. The production of pepper in Nigeria is on the decline such that Nigeria imports pepper to make up for the low production (Abubakar, 2015). Yields obtained by farmers are often very low (Adigun, 2001). The decline according to Abdulmalik, *et al.*, (2012), has been attributed to biotic and abiotic factors like weeds, insects' pests, diseases and environmental stress. *Capsicum* suffers from numerous diseases and insects' pests. Diseases of *Capsicum* plants include virus, fungal and bacterial and Nematodes diseases. Plant-parasitic nematodes (PPNs) can also cause substantial damage to pepper plants. Yellowing of the leaves of pepper plants caused by *Rddphulos similis* and *Meloidogyne* spp. has been reported (Koshy *et al.* 2005). *R. similis* can cause the so-called "yellow(s) disease" and "slow wilt disease" in black pepper plants (Koshy *et al.* 2005).

Plants with damaged root systems result in poor foliage growth and consequently yield is reduced. The extent of damage ranges from no visible damage to significant damage depending on the species and density of nematodes present, the susceptibility of the crop being grown, and environmental conditions. Many plants can tolerate moderate densities PPNs without sustaining significant losses in production. When nematodes are a problem, patches of poor plant growth often become evident within a field that otherwise may appear normal. The quality of the marketable product is reduced by nematode infestations that cause tissue breakdown, deformation, or discoloration. Root systems may be deformed, and underground organs such as potato tubers and carrot taproots may be damaged and become unmarketable (Kimaru *et al.*, 2014). The estimated yield of 9t/ha obtained on the farmers' field is low compared with the estimated yields of 15t/ha obtained in developed countries or those obtained from research field in Nigeria (Anonymous, 2012). Up to date, nematode resistant genes in the cases of several wild and cultivated species of the family *Solanaceae* have been identified. Therefore, developing resistant cultivars might be effective and environmentally safe alternative ways of nematode management in pepper (Thies et Fery, 2003; Oka *et al.*, 2004). The objective of this study was to evaluate nine accessions and a variety of pepper, for resistance to pathological effect of *H. multicinctus* and *M. incognita* singly and combination.

MATERIALS AND METHODS

Description of the Study Location

The experiment was conducted at the screen house of the Federal University of Technology, Minna. According to (Federal Airport Authority of Nigeria [FAAN], 2012), Niger State is located in the Southern Guinea Savanna agro-ecological zone of Nigeria and lies between latitude 6° 8'E and longitude 8° 44'N of the equator. The State experiences distinct dry and wet seasons with an annual rainfall ranging from 1100 mm in the northern part to 1600 mm in the southern part with a mean of 1350 mm. The rainfall which peaks in September normally begins in April and ends in October. The temperature ranges between 35 and 37.5°C with relative humidity of between 60 and 80 % in January. The vegetation of the area is mainly short grasses and shrubs with scattered trees due to deforestation. Soils in Minna were found to have originated from basement complex rocks and generally are classified as Alfisols (Adeboye, *et al.*, 2011).

Sources of Planting Materials

Nine accessions and a variety of the red pepper seeds (*Capsicum* species) which belong to the *Capsicum frutescens*: sweet pepper (Atarodo) bird pepper (Ata wewe) and *Capsicum frutescens* (cayenne pepper) (Ata sombo) and *Capsicum annum* were used in the study. Nine of the accessions: NGB00574, NGB00581, NGB00586, NGB00587, NGB00624, NGB00629, NGB00631, NGB00684 and NGB00702 were obtained from the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan and the local variety was purchased from Adalinci Agrochemical store, Mokwa, Niger State, Nigeria.

Raising of Pepper Seedlings in the Nursery and Transplanting

Nurseries for each of the accession/variety were raised separately in plastic pots of 14 cm × 15 cm filled with 3 kg heat-sterilized soil as described by (Paiko *et al.*, 2019). Two seedlings each of the pepper accession/variety were transplanted at four weeks old into 3 kg plastic pots containing heat sterilized soil, shaded and watered as required.

Preparation of Nematode Inocula

Helicotylenchus multicinctum was isolated from pepper plants during a survey of PPNs in Niger state, Nigeria and cultured on plantain plants in IITA, Ibadan as described by Speijer and De Waele (1997), and was used for the nematode treatment. Nematodes were collected from the plantain infected roots in distilled water using White head tray method and applied on the roots and adhering soils. For the *M. incognita*, infected galled roots were also collected from pepper during a survey of PPNs in Niger state, Nigeria and cultured on tomato. The galled tomato roots were uprooted, washed, chopped and the egg mass extracted. Second-stage juveniles (J2) were recovered from hatched eggs by incubation in sterile distilled water at $28 \pm 1^\circ\text{C}$. The suspensions were made up with distilled water until 1000 juveniles/adults nematodes were counted for each.

Inoculation

A week after the establishment of the seedlings, each pot was inoculated with approximately the following nematodes population: 1000 *H. multicinctum*, 1000 juveniles *Meloidogyne* spp juveniles and a 2000 mixed population, 1000 each of *H. multicinctum* and *M. incognita* nematode. The 20 mL aqueous nematode suspension of the treatment was poured into three holes, 2-4 cm deep around the base of the plants. The plant in the control pots with no nematodes received 20 ml distilled water.

The trial was laid in Completely Randomized Design (CRD) and replicated 4 times in a screen house with a photoperiod of 12 h and an ambient temperature of $24 \pm 4^\circ\text{C}$, and irrigated as required.

Parameters Measured

The data on plant height and leaf area on all accessions and the variety in the pots was taken at every three weeks' interval. The fresh weights on all the treatment in pots were also recorded 120th days after planting. The galling index rating was assessed using a chart illustrated by Bello *et al.*, (2015) with a scale ranging between 1 -10 where 1 indicated no galling and 10 indicated severe galling.

Table 1. Plant heights of pepper accessions and a variety as affected by single and mixed population of *H. Multicinctum* and *Meloidogyne spp* in the Screen House Screen house.

Treatments	3WPI	6WPI	9WPI	12WPI
Nematodes				
<i>H. multicinctum</i>	52.09 ^c	73.22 ^c	83.16 ^c	89.47 ^c
<i>Meloidogyne spp</i>	55.57 ^{bc}	73.95 ^c	83.79 ^c	91.35 ^c
<i>H. multicinctum</i> and <i>Meloidogyne spp</i>	58.95 ^b	77.05 ^b	89.97 ^b	102.69 ^b
Control	69.42 ^a	89.69 ^a	97.58 ^a	109.95 ^a
SE _±	1.31	1.02	0.84	1.05
Accession/Varieties				
NGB00574	54.99	77.23 ^{ab}	89.13 ^{abc}	96.51 ^{bcd}
NGB00581	57.72	80.06 ^a	87.41 ^{bcd}	96.55 ^{bcd}
NGB00586	56.94	75.84 ^{ab}	83.06 ^d	93.98 ^{cd}
NGB00587	61.41	82.71 ^a	93.53 ^a	107.05 ^a
NGB00624	62.23	79.57 ^a	91.24 ^{ab}	100.24 ^{bc}
NGB00629	61.03	77.03 ^{ab}	84.30 ^{dc}	91.31 ^d
NGB00631	59.67	79.28 ^a	89.01 ^{abc}	98.45 ^{bc}
NGB00684	55.01	72.49 ^b	87.04 ^{bcd}	99.71 ^{bc}
NGB00702	57.85	78.37 ^{ab}	89.64 ^{abc}	98.51 ^{bc}
LV	63.23	82.20 ^a	91.91 ^{ab}	101.81 ^b
SE _±	2.07	1.61	1.32	1.66
Interaction				
Nematodes * Variety	NS	***	***	***

After assessing the galling index, the nematodes were extracted by use of the modified White head tray methods Whitehead and Hemmen, (1965) to identify and count the nematodes. The egg mass index using a scale of 1-5 was also assessed. Galling index, egg mass index and the juveniles in the soil samples from each plant were used to rate nematode infection and levels of infection for the nine accessions and a variety to single and combined nematodes and determine those which were susceptible or resistant to the nematodes.

Data Analysis

Data on nematode counts was subjected to analysis of variance (ANOVA) using SAS statistical software package. Significance of the differences between treatments was measured, while the treatment means were compared using least significant difference (Lsd) at p=0.05.

RESULTS

Infected plants with both single and mixed populations of *H. multicinctum* and *M. incognita* nematodes recorded reduced plant heights compared to the untreated controls. Mixed population of *H. multicinctum* and *Meloidogyne spp* supported lowest plants heights

reduction at 3WPI, with mean value of 58.95 and the highest reduction was recorded in *H. multincinctum* with 52.09. When compared to the uninoculated control plants, the trend was the same all through. The lowest plant reduction among the treatments was recorded in NGB00587 with 82.71, LV with 82.20, NGB00624 with 79.57, NGB00631 with 79.728 and which were significantly ($P \leq 0.05$) different from untreated controls (Table 1).

Table 2. Leaf area per plant of pepper accessions and a variety infected with *H. multincinctus* and *Meloidogyne spp.*

Treatments	3WPI	6WPI	9WPI	12WPI
Nematodes				
<i>H. multincinctum</i>	16.71 ^b	20.28 ^c	24.42 ^c	35.49 ^b
<i>Meloidogyne spp</i>	18.34 ^b	22.06 ^c	26.71 ^{bc}	40.58 ^b
<i>H. multincinctum</i> and <i>Meloidogyne spp</i>	18.83 ^b	23.79 ^b	29.14 ^b	37.67 ^b
Control	25.09 ^a	34.36 ^a	44.22 ^a	67.30 ^a
SE ₊	0.75	0.79	1.29	2.72
Accession/Variety				
NGB00574	18.34 ^{ab}	23.25 ^{dc}	26.65 ^d	36.35 ^b
NGB00581	18.47 ^{ab}	21.78 ^{dc}	26.51 ^d	40.65 ^{ab}
NGB00586	16.56 ^b	20.63 ^d	24.84 ^d	43.06 ^{ab}
NGB00587	18.84 ^{ab}	22.76 ^{dc}	30.03 ^{bcd}	36.12 ^b
NGB00624	18.03 ^{ab}	21.91 ^{dc}	26.65 ^d	40.19 ^{ab}
NGB00629	20.42 ^{ab}	23.54 ^{dc}	28.88 ^{cd}	50.24 ^{ab}
NGB00631	19.81 ^{ab}	26.36 ^{bc}	35.18 ^{abc}	52.62 ^{ab}
NGB00684	21.06 ^{ab}	28.89 ^{ab}	36.41 ^{abc}	46.56 ^{ab}
NGB00702	22.56 ^a	31.41 ^a	38.66 ^a	50.23 ^{ab}
LV	23.34 ^a	30.69 ^a	37.39 ^{ab}	56.59 ^a
SE ₊	1.19	1.25	2.04	4.30
Interaction				
Nematodes * Variety	**	***	***	***

The highest plant height reduction was recorded in NGB00684 with 72.49 and were significantly ($P \leq 0.05$) different from the untreated controls (Table 1). Similar trend was observed throughout the experimental period. Plants infected with these nematodes recorded reduced leaf area compared to the untreated controls. Two accessions recorded lowest leaf area reduction; LV with 23.34 and NGB00702 with 22.56 hence had significant difference compared with the untreated controls while NGB00586 with 16.56 had the highest leaf area reduction showing significant ($P \leq 0.05$) differences (Table 2). A similar trend was observed throughout the experimental period (Table 2).

Table 3. Root length (RL), Root weight (RW), Reproductive Factor (RF) and Gallings index (GI) of pepper accessions and a variety as affected by single and mixed populations of *H. multincinctum* and *Meloidogyne* spp in the Screen house, Minna, Niger State.

Treatments	RL	RW	GI	RF	
Nematodes					Reaction
<i>H. multincinctum</i>	17.38	14.43 ^{ab}	0.00	1.03	
<i>Meloidogyne</i> spp	16.49	15.10 ^{ab}	6.07	1.01	
<i>H. multincinctum</i> and <i>Meloidogyne</i> spp	15.14	16.75 ^a	5.80	1.09	
Control	15.68	12.53 ^b	6.13	1.08	
SE±	0.66	0.83	0.9	0.03	
Accession/Varieties					
NGB00574	17.04	14.25 ^{ab}	6.94 ^{abc}	1.15 ^a	susceptible
NGB00581	17.79	16.37 ^{ab}	8.82 ^{ab}	1.15 ^a	susceptible
NGB00586	15.76	13.87 ^b	9.50 ^a	1.20 ^a	susceptible
NGB00587	14.69	12.69 ^b	9.87 ^a	1.16 ^a	susceptible
NGB00624	16.63	13.37 ^b	6.31 ^{bcd}	1.17 ^a	susceptible
NGB00629	16.04	19.31 ^a	2.31 ^e	0.99 ^b	resistant
NGB00631	14.79	13.00 ^b	3.63 ^d	0.99 ^b	resistant
NGB00684	17.73	13.25 ^b	4.00 ^{cde}	0.96 ^b	resistant
NGB00702	15.41	15.81 ^{ab}	4.68 ^{cde}	0.88 ^b	resistant
LV	15.84	15.06 ^{ab}	4.63 ^{cde}	0.91 ^b	resistant
SE±	1.03	1.32	1.46	0.03	
Interaction					
Nematodes * Variety	NS	**	NS	NS	

Means in a column of any set of treatment(s) Followed by different letter(s) are significantly different, WPI = Weeks after Planting, LV=Local variety, SE = Standard Error, LS = Level of Significance, NS = Not Significant, *** = Significant at P≤0.001, ** = Significant at P≤0.01, level of probability.

Means in a column of any set of treatment(s) followed by different letter(s) are significantly different, WPI = Weeks after Planting, LV= SE = Standard Error, LS = Level of Significance, NS

Means in a column of any set of treatment(s) Followed by different letter(s) are significantly different, WPI = Weeks after Planting, LV= SE = Standard Error, LS = Level of Significance, NS = Not Significant, *** = Significant at P≤0.001, ** = Significant at P≤0.01, level of probability.

Root length (RL), Root weight (RW), Reproductive Factor (RF) and Gallings index (GI) of pepper accessions and a variety as affected by single and mixed populations of *H. multincinctum* and *Meloidogyne* spp

The effect of single and mixed populations of *H. multincinctum* and *Meloidogyne* spp on Root length (RL), Root weight (RW), Reproductive factor (RF) and Gallings index (GI) of pepper accessions and the variety is presented in Table 3.

The result shows that there was no significant ($P \leq 0.05$) difference in root length of both the single and mixed populations of the nematodes as well on the pepper accessions and the variety studied. For the root weight, mixed populations of the nematodes supported lower weights reduction in comparison to the single nematodes populations that were statistical similar but was significantly ($P \leq 0.05$) different from the untreated controls. Table 3 also shows the accessions and variety responses to single and mixed populations of *H. multicinctum* and *Meloidogyne* spp on root weights of pepper plants. The result showed that NGB00629 had recorded higher root weight of 19.31 g, after the control plant, while others were statistically similar.

The parasitic effect of single and mixed populations of *H. multicinctum* and *Meloidogyne* spp on the reproductive factor (RF) of pepper accessions and the variety showed that there was no significant ($P \leq 0.05$) difference in reproductive factor of both the single and mixed populations. The accessions and variety responses to single and mixed populations of *H. multicinctum* and *Meloidogyne* spp on reproduction of the nematodes, however, showed that, NGB00574, NGB00581, NGB00586, NGB00587 and NGB00624 were statistical similar and supported higher multiplication of nematodes, which show their level of susceptibility and the rest showed lower reproduction, which showed the ability of the latter to exhibit resistance.

The effect of single and mixed populations of *Meloidogyne* spp on the galling index (GI) on pepper accessions and the variety roots showed that there was no significant ($P \leq 0.05$) difference in galling index of both the single and mixed populations of the nematodes. The accessions and local variety responses to single and mixed populations of *Meloidogyne* spp with *H. multicinctum* on gall production on the roots of pepper showed non-significance. The result however showed high accessions and varietal ($P \leq 0.001$) differences on galling index (GI) on the roots of pepper plants, with NGB00586 and NGB00587 recording high galling index. The lowest GI was however found on NGB00629. There was no significant ($P \leq 0.01$) difference in interaction between nematodes and accessions (N * A) all through the parameters, during the period of the study.

DISCUSSION

The results from the present investigation showed that there were significant ($P \leq 0.05$) differences in both the single and mixed nematodes population on plant height, leaf area, root length and weight, reproductive factor and galling index throughout the period of the study in the screen house trial. These results agree partly with the findings of Karssen and Moens, (2006), who found the length of plants to decrease in the nematode infected plants, which was likely due to damage caused by the increasing numbers of nematodes that invaded the plant roots and possibly ceased uptake of nutrient and water. Sikora and Fernandez (2005), are of the view that initial density of the nematodes in the soil directly influence increase in the nematode populations and the subsequent reduction in the growth and yield of crops. The results corroborate the works of Maleita *et al.* (2012); who observed that plants heavily infected with nematodes exhibited stunted growth and poor yield, in some cases plants die even before attaining maturity (Singh and Khurma, 2007).

However, there was high accessions and variety ($P \leq 0.001$) difference on plant height, leaf area, fresh weight, fresh root weight throughout the period of the study. According to the results of the experiments, the accessions and the local variety vary in the degree to which they are susceptible or resistant to the single or mixed nematodes population of *H. multicinctum* and *Meloidogyne* spp. The growth indexes of plant height, leaf area, fresh root length, and root weight of the 9 pepper accessions and the variety inoculated with *H. multicinctum* and *Meloidogyne* spp singly and combined differed in the present study. The differences in the magnitude of height decrease among the accessions and the local variety evaluated may be linked to the differences in their genetic architecture, hypoplasia or hyperplasia.

The growth in all the plants decreased following inoculation with single and mixed populations of the nematodes. The results showed that nematodes infection had significant effect on pepper growth; however, the decrease in the various growth indexes was not consistent, which indicated that nematodes infection on different organs of the pepper accessions and a variety was inhibited to varying degrees. The accessions NGB00587, NGB00702 NGB00624, NGB00631 NGB00629 and variety LV, recorded minimum decrease in growth with inoculation with the nematodes compared to their uninoculated control plants. These results agree with the findings of Akpan *et al.* (2016), who reported genetic variation in gene pool as being vital for successful selection and yield improvement in all crop species. The workers observed that diverse genetic population provides huge desirable traits from which selection can be made for crop improvement. The significant differences observed among the parents at the vegetative growth stage are indication of their genetic diversity. An earlier study on these parents showed similar diversity. The results obtained by evaluating different resistance indexes differ, making them difficult to compare. Because nematode infestation had different effects on different evaluation indexes, (Wang *et al.*, 2013) it was difficult to accurately assess plant resistance.

The varying plant height recorded from the inoculated pepper plants in this study might be as a result of the activities of the two nematodes. Stunted growth and reduced height of the plants in the inoculated pepper were associated with the nematode infestation. These results are in tandem to those of Kimaru *et al.* (2014), who reported suppressed plant growth on crops that host nematode.

The root length and root weights of pepper accessions NGB00629, NGB00631, NGB00684, NGB00702 and variety LV were high despite the single and mixed nematodes infection implying resistance to these nematodes. These accessions and the local variety are able to withstand the damage caused by nematodes probably due to their large root weights. NGB00574, NGB00581, NGB00586, NGB00587 and NGB00624 on the other hand had relatively low length and weights of roots indicating susceptibility to the nematodes infection. The reduced fresh and dry shoot weight on the susceptible accessions and the local variety may be due to the fact that nematode infection interferes with water, minerals and nutrients absorption and translocation thus interfering with photosynthesis (Wesemael and Moens, 2008). The infected plants become stunted and leaves turn yellow, wilt and eventually die.

The present results agree with the findings of Kayani *et al.* (2016), who reported that the stunted and reduced growth of foliar parts subsequently results in reduced plant biomass and productivity. Due to disruption of the xylem vessels, the upward uptake of water and nutrients is greatly reduced. Nematode infection also greatly affects permeability of roots to water, owing to the induction of nurse cell systems by females of root-knot nematodes for incessant feeding in infected roots, there is therefore, greater translocation of photosynthesis towards these infection sites, while the aboveground parts experience acute deficiency of nutrients (Wyss 2002, Di Vito *et al.* 2004). As the infected plants face insufficient supply of nutrients, photosynthesis, energy, water among others, therefore, development and growth of leaf tissues and their essential constituents particularly chlorophyll pigments, are greatly hampered (Khan and Khan 1997). Thus, reduction in root and shoot lengths was probable a result of nematodes feeding on giant cells, halting root growth and tips to swell as reported by Kayani *et al.* (2016).

The high galling indices observed in the accession NGB00574, NGB00581, NGB00586, NGB00587 and NGB00624 implied that these crops were more susceptible to the root knot nematodes compared to NGB00629, NGB00631, NGB00684, NGB00702 and variety LV that recorded lower indices, showing their level of resistance. There were significant different ($P \leq 0.05$) in the final nematode population across the nine accessions and the local variety as there were significant differences in the root and soil populations across the test pepper crops, significant difference ($P \leq 0.05$) was also observed in the multiplication rate of both *H. multicinctum* and *Meloidogyne* spp. on the nine pepper accessions and the local variety, with a high Reproductive factor (RF) recorded in the susceptible ones compared to the resistant ones.

Similarly, a significant galling index caused by *Meloidogyne* spp. was observed across the test plants. Root damage was higher in susceptible accessions and the local variety, than in resistant ones. The differential response of different peppers to nematodes infection had been reported in other crops like tomato (Almeida and Santos, 2002), cassava (Coyne and Talwana, 2000), sweet potato (Cervantes-Flores *et al.*, 2002) and cowpea (Olowe, 2007). Castillo *et al.* (2001) identified susceptibility in pepper with root damage shown by damaged root systems, and galls (DiVito *et al.*, 2004). However, few reports on evaluating of pepper had shown the availability of resistant sources against *Meloidogyne incognita* (Bello *et al.*, 2015), which is believed to act as a metabolic sink, causing the redistribution of nutrients from plant leaves to the nematodes developing inside the roots (Kayani *et al.*, 2016).

CONCLUSION

Screening of the nine accessions and local variety has demonstrated that they are infected by and react differently to single and combined effect of *H. multicinctum* and *Meloidogyne* inoculation. Accessions NGB00629, NGB00631, NGB00684, NGB00702 and local variety LV were the most resistant, whereas NGB00574, NGB00581, NGB00586, NGB00587 and NGB00624 were susceptible to infection by the nematodes. The infection reduced crop growth through shortening and deforming the roots and lowers the biomass yield required for consumption. This information will enable the development of effective strategies for *H. multicinctum* and *Meloidogyne* management through selection of appropriate crop cultivars/species for nematode infected soils.

Author contributions

This work was conducted in collaboration with all the authors:

Author ASP, designed the study, performed the statistical analysis and wrote the first draft, LYB, MTS and ACW managed the analyses of the study. ACW edited and managed the literature searches, while ASP collected data and implemented the study. All the authors read and approved the final manuscript for submission by author ACW.

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