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The root-knot nematode, *Meloidogyne incognita*, profoundly affects the production of popular biofortified cassava cultivars

Aminat Korede AKINSANYA [1,2,*,**], Steve Olaoluwa AFOLAMI [1], Peter KULAKOW [2] and Danny COYNE [2]

[1] *Department of Crop Protection, Federal University of Agriculture, P.M.B. 2240, Abeokuta, Ogun State, Nigeria*
[2] *International Institute of Tropical Agriculture (IITA), Headquarters and West Africa Hub, P.M.B. 5320, Oyo Road, Ibadan 200001, Oyo State, Nigeria*

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Summary – Despite being the single largest cassava-producing country, yields in Nigeria remain consistently poor and among the lowest. Regionally, yields are also particularly low across Africa. Pests and pathogens, including plant-parasitic nematodes, play an important role in this current yield deficit. African countries are not only faced with the problem of food security but also that of nutritional deficiency, due to limited micronutrients in the diet. In this study, six biofortified cultivars were evaluated for their response to inoculation with approximately 30 000 root-knot nematode (*Meloidogyne incognita*) eggs in 30 l pots in Nigeria. All cassava cultivars proved highly susceptible to *M. incognita* infection after 6 months, with nematode reproduction factor ranging from 7.0 to 44.8. Galling was common on feeder roots and gall index scores were recorded between 4 to 5 (on a scale of 1-5 where 5 $\leqslant$ 100 galls). *Meloidogyne incognita* infection significantly reduced plant height, stem girth, fresh plant mass, fresh storage root number and storage root weight. Percentage yield loss of between 41.8-88.4% was recorded in *M. incognita*-infected plants compared with non-infected controls. Although *M. incognita* reduced storage root weight, it did not necessarily affect the nutritional quality (total carotenoid) or dry weight percentage of the biofortified cassava cultivars. Total carotenoid and dry weight contents of the control cultivar were similar to some of the biofortified cultivars. The high susceptibility of the biofortified cassava cultivars to *M. incognita* infection indicates that substantial yield losses are likely being experienced by farmers, as this nematode pest is prevalent across sub-Saharan Africa and the tropics.

Keywords – food security, *Manihot esculenta*, nematode susceptible, nutrition, plant-parasitic nematodes, sub-Saharan Africa, yield loss.

Cassava (*Manihot esculenta*), a crop particularly suited to conditions of low water and soil nutrient availability (Burelle, 2003), is among the most important staple foods across the humid tropics of Africa, Latin America and Asia. It plays a major role in efforts to alleviate the African food crisis because of its efficient production of food energy, year-round availability, tolerance to extreme stress conditions and suitability for small holder systems in Africa (Hahn, 1996). Among starchy staple crops, cassava provides a carbohydrate production approximately 40% higher than rice and 25% more than maize, with the result that cassava is the most cost-effective source of calories for both human nutrition and animal feed (Burelle, 2003). However, cassava is generally low in nutritional value, while over-reliance and consumption of cassava high in hydrogen cyanide content is associated with malnourishment and ill health conditions, such as nodding disease and bone disfigurement (Tanumihardjo *et al*., 2008). Given the importance of cassava as a cost-effective starch staple crop, its ability to thrive in marginal conditions and its geographical distribution, it has been receiving substantial attention towards improving the nutritional value and reducing the more harmful health effects from hydrogen cyanide (Montagnac *et al*., 2009).

The specific enhancement of nutritional elements through genetic improvement is referred to as biofortification (Tanumihardjo *et al*., 2008). Micronutrients that are especially targeted for bio-fortification in various crops

* Corresponding author, e-mail: korede.akinsanya@augustineuniversity.edu.ng
** Current address: *Department of Biological Sciences*, *Faculty of Science*, *Augustine University*, *P.M.B. 1010*, *Ilara-Epe 106101*, *Lagos State*, *Nigeria*

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include vitamin A, iron and zinc. Recently, biofortified cassava cultivars with significantly raised levels of provitamin A carotenoids (pVACs) and low hydrogen cyanide content have been developed by conventional plant breeding methods and released for use in Nigeria and the Democratic Republic of the Congo (HarvestPlus, 2011). These biofortified cultivars are aimed at addressing vitamin A deficiency (VAD) (Saltzman *et al*., 2013), an important public health problem in sub-Saharan Africa (SSA).

Despite being the single largest cassava-producing country, yields in Nigeria remain consistently poor and among the lowest. However, across SSA, yields are similarly low (approximately 10 t ha^{-1}) compared with India (approximately 33 t ha^{-1}) or Thailand (26 t ha^{-1}), for example (FAOSTAT, 2017). There are various reasons for this deficit, of which pests and pathogens play a key role, including losses due to plant-parasitic nematodes. Traditionally, nematodes have not been viewed as a constraint on cassava, partly due to their subterranean existence, an often poor correlation between nematode infection and aerial growth, and general folklorist consensus that cassava is immune to nematodes (Coyne & Affokpon, 2018). However, a number of studies have documented the considerable impact that nematodes can impose on cassava production, especially to newly introduced cultivars (Van den Oever, 1995; Coyne *et al*., 2004; Akinsanya & Afolami, 2018). Pot studies have shown that root-knot nematode (*Meloidogyne* spp.) infection affected above-ground fresh weight, plant height, tuber weight and tuber number in susceptible cultivars (Coyne & Talwana, 2000; Akinsanya & Afolami, 2019). Reduced sprouting and establishment of cuttings are also associated with high *Meloidogyne* spp. infection, which affects crop establishment and ultimate yields (Talwana *et al*., 1997; Makumbi-Kidza *et al*., 2000). Under field conditions, cassava yields were severely affected by naturally occurring *Meloidogyne* spp. densities in Nigeria (Abidemi, 2014), whilst almost complete losses have been recorded in experimental plots (Caveness, 1982; Theberge, 1985). Furthermore, as higher levels of storage root rots are associated with nematode damage (Bridge *et al*., 1991; Akinsanya & Afolami, 2018; Coyne & Affokpon 2018), this impacts on cassava in-ground storability, which currently has a strong influence on cassava breeding efforts (Maroya *et al*., 2012).

The current study was therefore undertaken to assess the susceptibility and impact of the root-knot nematode, *Meloidogyne incognita*, on the production and nutritional content of biofortified cassava.

Materials and methods

The experiment included two factors: nematodes (inoculated and non-inoculated) and cultivar (six biofortified cultivars and a susceptible standard) (Table 1). Cassava cultivars were selected from among the most popular biofortified cultivars cultivated in Nigeria with a popularly grown, root-knot nematode-susceptible, control (Akinsanya & Afolami, 2019). Treatments were arranged in a randomised complete block design with three replicates in the screenhouse at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (latitude: 7°22′39″N; longitude: 3°54′21″E; 181 m a.s.l.). Single 20 cm long stem cuttings were planted into 30 l pots filled with steam-sterilised sandy-loam topsoil, maintained for 3 weeks for roots to develop, before inoculating a suspension of approximately 30 000 *M. incognita* eggs pot^{-1}. Plants were irrigated daily and mean daily temperatures were 28-32°C. The experiment was conducted first in March 2017 and repeated in September 2017. The experiments were maintained for 6 months before harvesting in order to allow storage roots to develop for nutritional assessment, and, as cassava is a semi-perennial crop, this determines more effectively the impact of *M. incognita* on crop development over a period of time, which is more reflective of the field situation (Abidemi, 2014; Akinlesi, 2014; Akinsanya & Afolami, 2018).

A pure population of *M. incognita*, originally isolated from infected tomato plants, was maintained in the screenhouse at IITA on *Celosia argentea* (plumed cockscomb). Galled, infected roots of *C. argentea* were gently removed from pots and rinsed under running tap water to remove soil debris, chopped into 2-3 cm pieces and nematode eggs extracted using the Hussey & Barker (1973) sodium hypochlorite method. The eggs were collected on a 25 μm sieve and rinsed into a beaker, reduced to 30 ml and the egg suspension density quantified using 5 × 1 ml aliquots in a Doncaster (1962) ringed counting dish under a stereomicroscope (×40). The volume was then adjusted to enable delivery of similar volumes of inoculum across the experiments. The nematode suspension was agitated and 25.4 ml and 23.7 ml used to inoculate plants in Experiments 1 and 2, respectively. Inoculum was delivered into a furrow made around each stem using a trowel and then the soil replaced after inoculation.

Data were collected 3 months after planting (MAP) for plant height and stem girth and at harvest (6 MAP) for plant height, stem girth, storage root fresh weight, feeder root fresh weight and aerial fresh weight for each plant. The number of galls on 5 cm feeder roots per

Table 1. Resistance evaluation of biofortified cassava cultivars following inoculation with 30 000 *Meloidogyne incognita* in 30 l pots[1].

Cultivar	*M. incognita* juveniles and eggs (10 g root)$^{-1}$	Galling index[2]	RF[3]	Inoculated plants yield (a) (g plant^{-1})	Control plants yield (b) (g plant^{-1})	Yield difference (a-b) (g plant^{-1})	Degree of resistance[4]
'IITA-TMS- IBA011368'	43 040	4.67	20.3	13.26	29.01	−15.75	Susceptible
'IITA-TMS-IBA011412'	45 970	5.00	33.2	37.00	68.19	−31.19	Susceptible
'IITA-TMS-IBA011371'	34 742	5.00	31.9	11.74	20.17	−8.43	Susceptible
'IITA-TMS-IBA070593'	46 082	5.00	44.8	22.21	92.22	−70.01	Susceptible
'IITA-TMS-IBA070539'	19 038	4.67	13.9	5.00	43.08	−38.08	Susceptible
'NR 07/0220'	11 062	4.00	7.0	11.43	57.92	−46.49	Susceptible
'IITA-TMS-IBA30572' (control)	41 760	5.00	18.8	22.76	59.14	−36.38	Susceptible

[1]n = 6: means of three replications × two experiments. Non-inoculated control pots recorded no galling.
[2]Gall Index = (1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 ⩾ 100 galls) (after Taylor & Sasser, 1978).
[3]RF = nematode Reproduction Factor (Oostenbrink, 1966).
[4]Resistance rating based on modified scheme of Sasser *et al.* (1984) and Afolami (2000); Susceptible: RF > 1, GI > 2, significant yield loss; Tolerant: RF > 1, GI ⩽ 2, no significant yield loss; Resistant: RF ⩽ 1, GI ⩽ 2, no significant yield loss; Hypersusceptible: RF ⩽ 1, GI > 2, significant yield loss.

plant, removed randomly at harvest, was counted and galling index (GI) per plant root assessed using the 1-5 gall index (Taylor & Sasser, 1978) (1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 $\geqslant$ 100 galls).

Nematode eggs and second-stage juveniles (J2) were extracted and their density calculated from roots as above, using a 10 g sub-sample after chopping and thoroughly mixing all roots for each plant. The total number of nematodes per root system was then calculated. Nematodes were extracted from 250 g soil sub-samples using the Whitehead & Hemming (1965) tray method, thoroughly mixing the soil from each pot and using a double-ply extractor sandwiched between two plastic sieves with 250 ml water, after spreading out the soil in the sieve. Nematode extracts were removed after 24 h, allowed to settle for 5 h and the volume adjusted to 30 ml by siphoning off the excess (Caveness, 1975). The nematode density was assessed under the microscope (3 $\times$ 1 ml aliquots) and the total number of soil nematodes per pot calculated. Total number of nematodes per pot from soil and root data was used to calculate the nematode reproduction factor (RF) (Oostenbrink, 1966):

$$\text{RF} = \frac{\text{Final population } (P_f) \text{ per pot}}{\text{Initial population } (P_i) \text{ per pot}}$$

Storage roots harvested from each pot were rinsed under running tap water immediately after harvest, peeled and further rinsed in sterile distilled water. Storage roots from each pot were randomly divided, one for fresh and one for dried analysis for total carotenoid and dry matter content, respectively. Roots were chopped into *ca* 0.5 cm^3 cubes and 100 g samples of each cultivar were randomly removed to determine the total carotenoid content using the iCheck™ method (BioAnalyt). Total carotenoid content and dry matter were conducted only for Experiment 1 due to the high cost of this procedure.

For dry matter, the 100 g fresh root cubes were oven-dried at 70°C for 72 h, then milled to obtain a homogeneous powder, stored in moisture-free plastic containers and dry matter calculated for each cultivar (Rodriguez-Amaya & Kimura, 2004):

$$\text{Dry matter (\%)} = \frac{\text{Final weight}}{\text{Fresh weight}} \times 100$$

Statistical analyses of data

Data was subjected to a factorial Analysis of Variance (ANOVA) using SAS 9.4 (2012) statistical package and means were separated using Least Significant Difference (LSD) at 5% level of probability. A resistance rating (Susceptible: RF > 1, GI > 2, significant yield loss; Tolerant: RF > 1, GI $\leqslant$ 2, no significant yield loss; Resistant: RF $\leqslant$ 1, GI $\leqslant$ 2, no significant yield loss; Hypersusceptible: RF $\leqslant$ 1, GI > 2, significant yield loss), based on a modified scheme of Sasser *et al.* (1984) and Afolami (2000), was used for assigning crop cultivar reaction into resistance categories based on crop yield, RF and GI. As ANOVA showed no significant ($P \leqslant 0.05$) differences (interaction) between the two experiments, the data for the two experiments were pooled for analysis.

Results

All biofortified cassava cultivars were susceptible to *M. incognita* infection, although the level of susceptibility differed among the cultivars. All cultivars were heavily galled, with GI ranging from 4 to 5 in both experiments (Table 1; Fig. 1B). All cultivars were rated susceptible to *M. incognita* infection, based upon the GI, RF and crop yield with significant ($P \leqslant 0.05$) yield losses and levels of nematode damage (Table 1).

Generally, *M. incognita* infection caused stunting of the aerial growth of the biofortified cassava cultivars at 3 MAP, which became more pronounced at 6 MAP when compared with their respective controls (Fig. 1A). The effect of *M. incognita* infection on the growth of most cultivars was significant ($P \leqslant 0.05$) (Table 2). At 3 MAP, plant height of all cultivars except 'IITA-TMS-IBA011412' and 'NR 07/0220' were significantly ($P \leqslant 0.05$) suppressed, while at 6 MAP all inoculated cultivars were significantly ($P \leqslant 0.05$) shorter. Significant ($P \leqslant 0.05$) differences were observed in stem girth of 'IITA-TMS-IBA070593', 'IITA-TMS-IBA30572' and 'NR 07/0220' at both 3 MAP and 6 MAP.

Fresh shoot weight was significantly ($P \leqslant 0.05$) lower for *M. incognita*-infected plants of all cultivars, except 'IITA-TMS-IBA070539' (Table 3). Fresh storage root number was significantly ($P \leqslant 0.05$) lower for *M. incognita* infected plants of 'IITA-TMS-IBA011368', 'IITA-TMS-IBA070539' and 'NR 07/0220'. Fresh storage root weight was also significantly ($P \leqslant 0.05$) lower for plants infected with *M. incognita* for all cultivars except 'IITA-TMS-IBA011371' (Table 3; Fig. 1C, D). The total

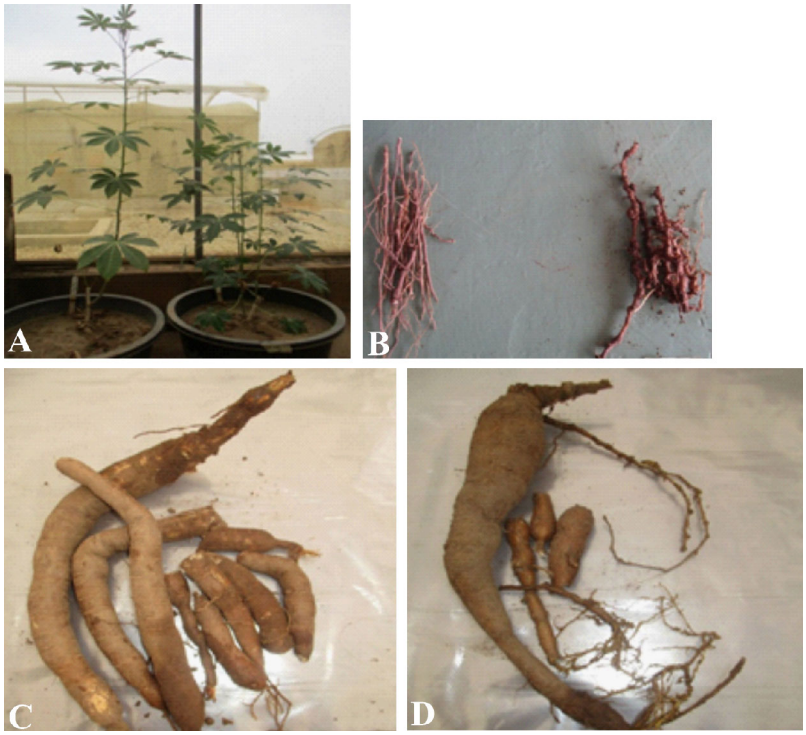

Fig. 1. Reaction of biofortified cassava cultivars to *Meloidogyne incognita* infection in pots after 6 months growth; A: Non-inoculated (left) and inoculated 'IITA-TMS-IBA01138' plants; B: Healthy (left) and galled feeder roots of 'IITA-TMSIBA011371'; C: Non-inoculated 'IITA-TMS-IBA011412' storage roots; D: Inoculated IITA-TMS-IBA011412 storage roots.

carotenoid and dry matter contents of biofortified cultivars, although relatively lower in inoculated pots, were not significantly ($P \leqslant 0.05$) affected by nematode infection (Table 4).

Discussion

The current study demonstrates the magnitude of the potential damage that root-knot nematodes can inflict on biofortified cassava cultivars, in particular, but also on cassava production in general. Although this study was conducted under controlled conditions in pots, after just 6 months of growth, feeder root galling damage was severe and storage root production was significantly affected. Although high, the *M. incognita* inoculum level is, however, not uncommon, and readily reflects prevailing conditions that are experienced in tropical and sub-tropical regions (Sikora *et al*., 2018).

Cassava is also traditionally viewed as not being afflicted by nematodes, yet, when specifically studied, impact of plant-parasitic nematodes on production has been demonstrated repeatedly (*e.g.*, Coyne & Affokpon. 2018). The biofortified cultivars evaluated within the current study were shown to be susceptible to *M. incognita*. The cultivars were selected from those proving popular, further to their being officially released in Nigeria. As this nematode, and tropical root-knot nematode species in general, are pervasive pests, infection by these nematodes is expected to result in high levels of damage and low production. In Nigeria, *M. incognita* infection caused significant suppression in the growth and yield of elite cassava cultivars after 12 months, despite the low level of nematode field infestation (Akinsanya & Afolami, 2018). Using data from farmers' fields in Uganda, Coyne & Talwana (2000) and Coyne *et al*. (2004) directly correlated root galling on cassava roots with yield, showing that substantial yield losses due to root-knot nematodes were being experienced by farmers. Indeed, root-knot nematodes have been indicated as the greatest biotic threat to crop productivity in tropical and sub-tropical regions (Trudgill & Blok, 2001; Coyne *et al*., 2018).

Other *Meloidogyne* species have been recorded infecting cassava, including *M. javanica* and *M. arenaria*

Table 2. Growth evaluation of biofortified cassava cultivars following inoculation with 30 000 *Meloidogyne incognita* in 30 l pots[1].

Cultivar	Treatment	Plant height (cm)		Stem girth (cm)	
		3 MAP	6 MAP	3 MAP	6 MAP
'IITA-TMS- IBA011368'	Control	275.70[a]	468.83[a]	1.90[a]	1.97[a]
	Infected	204.77[b]	271.37[b]	1.60[a]	1.93[a]
	LSD ($P \leqslant 0.05$)	68.63	183.31	0.35	0.50
'IITA-TMS-IBA011412'	Control	251.90[a]	525.83[a]	1.80[a]	2.37[a]
	Infected	251.53[a]	314.47[b]	1.80[a]	2.20[a]
	LSD ($P \leqslant 0.05$)	200.92	132.16	0.32	0.74
'IITA-TMS-IBA011371'	Control	347.63[a]	506.73[a]	2.28[a]	2.48[a]
	Infected	296.80[b]	411.57[b]	2.13[a]	2.40[a]
	LSD ($P \leqslant 0.05$)	47.22	92.03	0.60	1.38
'IITA-TMS-IBA070593'	Control	299.13[a]	445.03[a]	2.10[a]	2.73[a]
	Infected	235.00[b]	275.77[b]	1.78[b]	2.37[b]
	LSD ($P \leqslant 0.05$)	60.27	99.24	0.31	0.26
'IITA-TMS-IBA070539'	Control	311.07[a]	495.77[a]	2.02[a]	2.47[a]
	Infected	284.93[b]	331.13[b]	1.83[a]	2.30[a]
	LSD ($P \leqslant 0.05$)	20.71	40.09	0.55	1.22
'NR 07/0220'	Control	167.33[a]	357.90[a]	1.67[a]	1.93[a]
	Infected	138.67[a]	189.37[b]	1.25[b]	1.60[b]
	LSD ($P \leqslant 0.05$)	100.49	135.90	0.40	0.30
'IITA-TMS-IBA30572' (control)	Control	287.37[a]	439.37[a]	2.08[a]	2.43[a]
	Infected	226.40[a]	333.80[b]	1.82[b]	2.08[b]
	LSD ($P \leqslant 0.05$)	145.15	96.92	0.20	0.31

[1]n = 6: means of three replications × two experiments.
Values between the two inoculation treatments followed by the same letter are not significantly different ($P \leqslant 0.05$).
MAP = months after planting.

(Coyne & Affokpon. 2018) and recently *M. enterolobii* (Akinsanya, unpubl. data), which can occur as multiple infections, in combination with *M. incognita*, or individually. These nematodes are prevalent in cropping systems across SSA and consequently pose a substantial threat to cassava production. However, it is not reduced productivity alone that is important. Improved in-ground storability is a key trait that cassava breeders seek to breed for, as cassava is prone to deteriorate, especially following harvest (Teeken *et al*., 2018). Nematode-infected roots are known to be more susceptible to rot organisms (Theberge, 1985). The deformations and the physical damage caused by the nematodes facilitates and predisposes the host to secondary pathogens, such as fungal and bacterial root and tuber rot pathogens increasing the extent of disease complexes and root and tuber rots. In the presence of root-knot nematodes, it was demonstrated how storage root rot was significantly increased by over 48%, resulting in substantial losses due to rots (Akinlesi, 2014). Bridge *et al*. (1991) also associated a possible secondary fungal root rot with severe nematode infestation in farmers' cassava fields in Uganda, impacting heavily on production. On yam tubers, the resulting complex of nematodes and rot pathogens could destroy the entire tuber in the field and during storage (Bridge, 1973).

In the field, potato plants infected with *Pratylenchus* spp. were stunted, along with greater levels of rot and deteriorated roots than non-infected plants, leading to yield losses up to 50% (Ferraz, 1999; Castillo & Vovlas, 2007). Breeding for nematode resistance may, therefore, indirectly improve cassava in-ground storability.

From our current study, it is evident that *M. incognita* will infect popularly used biofortified cassava cultivars and result in yield loss. This needs to be confirmed for other *Meloidogyne* species and from field studies, but the high RF values recorded for most cultivars clearly demonstrate the susceptibility of these cultivars to *M. incognita*. Studies elsewhere have shown how nematode infection impacts the uptake of certain nutrients or reduction of their concentrations in different parts of plants

Table 3. Yield evaluation of biofortified cassava cultivars following inoculation with 30 000 *Meloidogyne incognita* in 30 l pots[1].

Cultivar	Treatment	Fresh shoot weight (g plant^{-1})	Fresh storage root number	Fresh storage root weight (g plant^{-1})
'IITA-TMS- IBA011368'	Control	2803.3[a]	2.67[a]	29.01[a]
	Infected	1166.7[b]	1.33[b]	13.26[b]
	LSD ($P \leqslant 0.05$)	539.01	1.31	15.61
'IITA-TMS-IBA011412'	Control	2526.7[a]	2.33[a]	68.19[a]
	Infected	1720.0[b]	1.00[a]	37.00[b]
	LSD ($P \leqslant 0.05$)	329.03	2.93	124.81
'IITA-TMS-IBA011371'	Control	2366.7[a]	1.67[a]	20.17[a]
	Infected	1650.0[b]	1.67[a]	11.74[a]
	LSD ($P \leqslant 0.05$)	1008.5	2.62	33.85
'IITA-TMS-IBA070593'	Control	2340.0[a]	2.67[a]	92.22[a]
	Infected	1133.3[b]	2.00[b]	22.21[b]
	LSD ($P \leqslant 0.05$)	422.69	2.93	22.58
'IITA-TMS-IBA070539'	Control	1816.7[a]	2.00[a]	43.0[a]
	Infected	1133.3[a]	0.33[b]	5.00[b]
	LSD ($P \leqslant 0.05$)	812.11	0.93	32.67
'NR 07/0220'	Control	2166.7[a]	1.67[a]	57.92[a]
	Infected	690.0[b]	0.33[b]	11.43[b]
	LSD ($P \leqslant 0.05$)	109.11	1.27	44.43
'IITA-TMS-IBA30572' (control)	Control	2746.7[a]	2.00[a]	59.14[a]
	Infected	1490.0[b]	1.33[a]	22.76[b]
	LSD ($P \leqslant 0.05$)	644.06	1.85	34.76

[1]n = 6: means of three replications × two experiments.
Values between the two inoculation treatments followed by the same letter are not significantly different ($P \leqslant 0.05$).

(Sijmons *et al*., 1991). Following infection cell disruption, root damage and disintegration occur, interfering with water and nutrient supply to the crop. Talwana *et al*. (2002) reported a decrease in nutrient concentrations in the leaves of banana as a result of nematode inoculation, whilst Rao *et al*. (1984, 1988) reported nutritional deficiencies in total sugar, protein, cytokinin, thiamine and phenol in rice plants due to infection by *Heterodera oryzicola* and *M. graminicola*. The cyanogenic potential of cassava storage roots in some cultivars following *M. incognita* inoculation has been shown to be higher compared with the same cultivars in non-inoculated soil (Makumbi-Kidza, 2001). Results in the current study indicated that although storage root weights were affected by nematode infection, the nutritional quality (total carotenoid) or dry matter percentage were not necessarily affected. However, these results should be treated as tentative and would be worth assessing using a larger sample size and repeated, as this assessment was limited to one experiment in the current study due to the high costs involved.

An effective nematode control strategy for cassava and for the improvement of biofortified cassava could be through breeding for resistance to root-knot nematodes. Cassava cultivars with resistance against root-knot nematodes have been identified (Coyne *et al*., 2004; Udo *et al*., 2008; Abidemi, 2014) and so it should be possible to introgress this resistance into new improved, biofortified cultivars. In the current study *M. incognita* was used as a candidate species to assess its potential damage on biofortified cassava. The crop is affected by other species of *Meloidogyne*, however, which can impose similar lev-

Table 4. Nutritional quality of biofortified cassava cultivars following inoculation with 30 000 *Meloidogyne incognita* in 30 l pots[1].

Cultivar	Treatment	Total carotenoid (μg (g fresh weight)$^{-1}$)	Dry matter (%)
'IITA-TMS- IBA011368'	Control	3.60[a]	9.09[a]
	Infected	2.00[a]	9.82[a]
	LSD ($P \leqslant 0.05$)	6.33	37.15
'IITA-TMS-IBA011412'	Control	5.55[a]	16.71[a]
	Infected	3.62[a]	10.00[a]
	LSD ($P \leqslant 0.05$)	18.40	54.07
'IITA-TMS-IBA011371'	Control	1.81[a]	6.18[a]
	Infected	0.73[a]	4.74[a]
	LSD ($P \leqslant 0.05$)	5.42	21.64
'IITA-TMS-IBA070593'	Control	4.07[a]	15.36[a]
	Infected	1.07[a]	5.34[a]
	LSD ($P \leqslant 0.05$)	3.04	26.43
'IITA-TMS-IBA070539'	Control	1.87[a]	4.07[a]
	Infected	0.00[a]	0.00[a]
	LSD ($P \leqslant 0.05$)	5.20	11.31
'NR 07/0220'	Control	1.92[a]	5.88[a]
	Infected	1.59[a]	3.54[a]
	LSD ($P \leqslant 0.05$)	6.93	19.06
'IITA-TMS-IBA30572' (control)	Control	0.68[a]	5.56[a]
	Infected	0.17[a]	3.92[a]
	LSD ($P \leqslant 0.05$)	1.07	18.88

[1]n = 3: means of three replications × one experiment.
Values between the two inoculation treatments followed by the same letter are not significantly different ($P \leqslant 0.05$).

els of damage (Coyne & Affokpon, 2018), whilst resistance against one species may not necessarily be effective against other species. When breeding for resistance against root-knot nematodes, therefore, it will be necessary to build in the assessment of multiple species.

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