Evaluation of hot pepper (*Capsicum annum* and *C. frutescens*) genotypes for resistance and hatch stimulation to *Meloidogyne incognita* and *M. javanica* populations from Ethiopia

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Summary. The reaction of eighteen hot pepper genotypes to *Meloidogyne incognita* and *M. javanica* populations from Ethiopia was evaluated under glasshouse condition. In addition, a laboratory experiment was conducted to examine the effect of root exudates on hatching of the nematode populations. None of the pepper genotypes were found immune. However, 'Acc.003' and 'Melka Awaze' were found resistant, while 'Oda Haro' was susceptible to all populations tested. The hatching assay indicated that the root exudates had no significant effect on cumulative hatching. However, the time in which half of the population hatched varied significantly among the genotypes. After validation under field condition, the genotypes that were found resistant to both *M. incognita* and *M. javanica* could be used in areas infested with species complex in the country to enhance hot pepper productivity.

Key words: host resistance, reproduction factor, root exudate, root-knot nematode.

Hot pepper (*Capsicum* spp.) is one of the most important vegetable crops in the Solanaceae family. It is widely cultivated worldwide (Bosland & Votava, 2000). Hot pepper is an excellent source of compounds, such as capsasinoids, carotenoids, flavonoids and vitamins A, C and E that stimulate the immune system, and prevent cardiovascular and cancer diseases (Materska & Perucka, 2005; Chuah *et al.*, 2008; Shotorbani *et al.*, 2013). The introduction of hot pepper to Ethiopia is believed to date back to the early 17th century (Huffnagel, 1961). Since then, it is extensively grown for home consumption as a fresh vegetable and a dried powder spice for seasoning different dishes. Moreover, it is also a source of income for small scale farmers on domestic and export markets (Marame *et al.*, 2009). According to CSA (2017), 75.41% of the area under vegetable production in the country is covered by hot pepper. The total coverage of green and red pepper was 9,832.28 and 180,701.46 ha, while the annual production is about 61,794.33 and 329,804.29 tons of green and red pepper respectively (CSA, 2017). Despite its high nutritional and economic significance in the country, the productivity is low due to various reasons including diseases, scarcity of improved varieties and lack of proper crop management practices (Fekadu & Dandena, 2006; Abrham *et al.*, 2017). Root-knot nematodes (RKN) were reported among the important pests of hot pepper in the country (Tefera & Hulluka, 2000; Mandefro & Mekete, 2002).

Root-knot nematodes (*Meloidogyne* spp.) are one of the most economically important plant-parasitic nematodes (PPN) with a wide host range. They cause up to 20-40% yield losses on vegetable crops (Sasser, 1979; Sikora & Fernández, 2005). Both *Meloidogyne incognita* and *M. javanica* are known to cause a significant yield loss in pepper worldwide (Thies & Ferry, 2000). In Ethiopia, damage potential up to 100% was recorded from pot experiment on hot pepper and tomato due to *M. javanica* (Mekete *et al.*, 2003; Seid, 2016). Since they are endoparasitic in nature, they cause

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qualitative and quantitative yield losses by disturbing the normal physiology of the plants (Ralmi *et al.*, 2016). About 101 species of *Meloidogyne* are described to date, of which 22 species are known to occur in Africa (Onkendi *et al.*, 2014; Seid *et al.*, 2015). In Ethiopia, root-knot nematodes are the most frequently reported PPN. Earlier findings described *M. incognita*, *M. javanica* and *M. ethiopica* among the species known to occur in the country and infect vegetables, including hot pepper (Stewart & Yirgou, 1967; O'Bannon, 1975; Tefera & Hulluka, 2000; Mandefro & Mekete, 2002; Seid *et al.*, 2017). However, recently *M. hapla* has been also detected from cut flowers and tomato production areas of the country (Meressa *et al.*, 2014; Seid, 2016).

Although management of PPN through synthetic nematicides was among the effective methods employed worldwide, the health hazard to human beings and other non-target organisms, cost feasibility, and phytotoxicity have limited their application and many have been withdrawn from the market (Cook & Starr, 2006; Ornat & Sorribas, 2008; Katooli *et al.*, 2011; Abd-Elgawad & Askary, 2018). The sustainable management of PPN and reduction of damage to cultivated crops involves the integrated approach that encompasses different methods including crop rotations, soil amendments, use of resistant cultivars, biological controls and reduced use of nematicides (Roberts, 1993; Ntalli & Caboni, 2012; Mashela *et al.*, 2017).

The use of host plant resistance (HPR) in crops is one of the most effective and eco-friendly components of integrated disease management (Djian-Caporalino *et al.*, 2007; Abebe *et al.*, 2015). It ensures augmented crop productivity in the presence of PPN. The expression of HPR against PPN includes a reduced rate of nematode reproduction, resulting in lower nematode population densities (Roberts, 1995). Although Thies & Ferry (2002) described resistance in bell-pepper to *Meloidogyne* species as owing to a single dominant gene *N*, other authors have reported the presence of several dominant genes, called *Me* genes, that control resistance in pepper (Castagnone-Sereno *et al.*, 2001; Djian-Caporalino *et al.*, 2007). HPR remains a very important potential integral solution to many nematode problems of tropical agriculture, especially for the resource poor and low input small-scale farmers when used in combination with cultural practices (Luc *et al.*, 2005).

To exploit the genetic potential of crops as part of the nematode management strategy either as a rotation or cover crop in areas where the nematode infestation is prevalent, it is useful to know the reaction of each genotypes towards PPN species of interest (Robertson *et al.*, 2006; Brito *et al.*, 2007). In Ethiopia, research on PPN is very scarce. Furthermore, information on the reaction of hot pepper genotypes to RKN is lacking. Therefore, the objectives of this research were to evaluate the resistance status of hot pepper genotypes to root-knot nematodes (*M. incognita* and *M. javanica*) populations from Ethiopia and to examine the effect of root exudates of hot pepper genotypes on hatching of *M. incognita* and *M. javanica* populations.

MATERIAL AND METHODS

The screening experiment was conducted in 2016/2017 under glasshouse conditions at Haramaya University experimental field station (Raree) and the hatching assay was performed at Haramaya University Plant Protection Laboratory in 2018. The temperature of the glasshouse was between 25°C and 38°C with a mean of 28°C during the experimental period.

Resistance screening experiment. Eighteen hot pepper genotypes belonging to the species *C. annum* and *C. frutescens* were used for the current study (Table 1). Among these, eight of them were registered Ethiopian varieties that are currently under production; nine genotypes were on variety trial level and one is a registered commercial hybrid pepper variety (Table 1).

Seedlings were raised on seedling trays filled with sterilised field soil and sand (3:1 v/v) in the glasshouse. The texture of the field soil was sandy loam. When seedlings reached to four true leaf stage, they were transplanted to 3 l-sized pots filled with steam sterilised field soil and sand (3:1 v/v).

Nematode inoculum preparation. Six *Meloidogyne* populations (Table 2) collected and identified from three tomato producing locations (Babile, Koka and Jittu) in Ethiopia using molecular (DNA and isozyme based) methods (Seid, 2016; Seid *et al.*, 2019) were tested for their pathogenicity and aggressiveness on hot pepper. Then two most aggressive populations with the highest number of gall and egg mass from each species were selected. Each population was maintained on tomato cultivar 'Moneymaker'. All the four populations were used for the hatching experiment. However, only three populations (two *M. incognita* and one *M. javanica*) were tested for the glasshouse screening. The two *M. incognita* populations (JIT5 and JIT10), were originally collected from Jittu farm found at Tikur wuha, whereas the *M. javanica* populations (BGS4 and KOK2) were collected from Babile and Koka areas, respectively.

Table 1. Status, registration year, disease reaction and source of the pepper genotypes used in the experiment against root-knot nematodes at Haramaya, Ethiopia during 2016-2018.

Genotypes	Species	Status	Registration year	Adaptation altitude (m a.s.l.)	Days to maturity	Yield on research field (ton ha^{-1})	Disease reaction*	Breeder/ Maintaining institution
'Melka Shote' (PBC 223)	*Capsicum frutescens*	Registered	2006	1000-2200	110-120	2.00-3.00	Tolerant to foliar diseases	MARC
'Melka Awaze' (PBC 600)	*C. frutescens*	Registered	2006	1000-2200	100-110	2.50-2.80	Tolerant to soil borne and foliar diseases	MARC
'Oda Haro'	*C. frutescens*	Registered	2005	1400-2200	139	1.10-1.25	Resistant to bacterial leaf spot, fungal leaf diseases, *Phytophtora* root disease and virus diseases	BARC
'Melka Zala' (PBC 972)	*C. frutescens*	Registered	2004	1200-2200	130-150	1.50-2.50	Resistant to bacterial leaf spot, fungal leaf diseases *Phytophtora* root disease and virus diseases	MARC
'Melka Dima' (Papri King)	*C. annum*	Registered	2004	less than 1900	120-140	1.30-2.00	Resistant to bacterial leaf spot, fungal leaf diseases and *Phytophtora*	MARC
'Melka Eshet' (Papri Queen)	*C. annum*	Registered	2004	less than 1900	100-120	1.50-2.00	Resistant to bacterial leaf spot, fungal leaf diseases and *Phytophtora*	MARC
'Mareko Fana'	*C. annum*	Registered	1976	1400-2200	110-130	1.50-2.00	–	MARC
'Bako Local'	*C. annum*	Registered	1976	1200-1900	130-145	2.00-2.50	–	BARC
'Acc.001'	*C. frutescens*	Under trial	–	–	–	–	–	MARC
'Acc.002'	*C. frutescens*	Under trial	–	–	–	–	–	MARC
'Acc.003'	*C. frutescens*	Under trial	–	–	–	–	–	MARC
'Acc.004'	*C. frutescens*	Under trial	–	–	–	–	–	MARC
'Acc.01'	*C. annum*	Under trial	–	–	–	–	–	MARC
'Acc.02'	*C. annum*	Under trial	–	–	–	–	–	MARC
'Acc.03'	*C. annum*	Under trial	–	–	–	–	–	MARC
'Acc.04'	*C. annum*	Under trial	–	–	–	–	–	MARC
'Acc.05'	*C. annum*	Under trial	–	–	–	–	–	MARC
'Saidah'	*C. annum*	Registered hybrid variety	2013	500-2500	–	52.90	Resistant to powdery mildew, Tobamovirus: pathotype P0, and Potato Virus Y (PVY): pathotype 0	Syngenta

BARC = Bako Agricultural Research Centre, MARC = Melkassa Agricultural Research Centre, m a.s.l. = meters above sea level.

*Disease reaction was indicated for genotypes whose disease status was described on the crop variety register of the corresponding year of release.

Table 2. Root-knot nematode (*Meloidogyne*) species tested for their pathogenicity towards pepper genotypes from Ethiopia (Seid *et al.*, 2019).

No.	Species	Population code	Collection area
1	*M. incognita*	JIT5	Jittu
2	*M. incognita*	JIT10	Jittu
3	*M. incognita*	JIT8	Jittu
4	*M. javanica*	BGS1	Babile
5	*M. javanica*	BGS4	Babile
6	*M. javanica*	KOK2	Koka

Table 3. Rating scale of genotypes for resistance based on the egg mass index and reproduction factor (Sasser *et al.*, 1984).

Plant damage (Gall or Egg mass index)	Host efficiency (R factor)	Degree of resistance (DR) designation
≤ 2	≤ 1	Resistant
≤ 2	> 1	Tolerant
> 2	≤ 1	Hypersusceptible
> 2	> 1	Susceptible

Nematode inoculum was prepared from heavily galled tomato roots. The roots were chopped into 2 cm pieces and placed on a modified Baermann tray and incubated for 48 h (Coyne *et al.*, 2007). Hatched second-stage juveniles (J2) were collected into glass bottles and stored in a refrigerator at 4°C until used for inoculation. A day before inoculation, the stock nematode suspensions of the three populations were brought to room temperature. The population density was then determined and the volume of the stock solutions was adjusted to 200 J2 ml^{-1}. Then, inoculation of the pepper plants was done by taking 10 ml suspension containing 2000 J2 from the stock suspensions of the three nematode populations and pipetting into the soil around the roots of the plants in each pot separately. Pots without *M. incognita* and *M. javanica* juveniles received 10 ml water and served as controls.

Fifty-four treatments (18 genotypes × three nematode populations) were arranged on a raised glasshouse bench in a factorial randomised complete block design (RCBD) with four replications. Watering was done regularly as required. No fertiliser was applied to the treatments during the experimental time.

The experiment was terminated ten weeks after inoculation to favour the optimum egg mass formation for these populations (Seid *et al.*, 2017). The shoots were cut off at soil level and fresh weight was recorded. Roots were also washed free of adhering soil, carefully blotted on tissue paper and weighed. The galls on the plant roots were counted. Data on the number of egg masses per root system were also taken after staining by dipping the roots in Phloxine B solution (0.15 g l^{-1} of water) for 20 min and rinsing with tap water as described by Daykin & Hussey (1985).

Measurements. Nematode density from the root was determined by dislodging eggs using 0.52% sodium hypochlorite (NaOCl) solution as described by Hussey & Barker (1973). The total nematodes from the soil were estimated from aliquots of 100 ml soil using a modified Baermann tray (Hooper *et al.*, 2005). The nematode suspension was collected after 72 h into glass and stored in a refrigerator at 4°C until counting. The final nematode population density (Pf) was estimated from nematode populations in the root and soil. Number of eggs per egg mass was determined as an average count of the eggs from five randomly taken egg masses per plant in a pot.

The gall index was computed by converting the number of galls from plant roots in to 0 to 5 scales as follows: 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 = more than 100 galls (Taylor & Sasser, 1978). Resistant, tolerant, susceptible and hypersusceptible classification of pepper genotypes were rated based on gall index (GI) as a measure of plant damage and reproduction factor (RF) as an indicator of host efficiency (Sasser *et al.*, 1984) (Table 3). The galls that are developed on the host roots were taken as indicator of plant damage since the change on the structure of the roots results in physiological disturbance of the plant, thereby affecting the productivity. Sasser *et al.* (1984) also recommended the use of gall index as a measure of damage caused to the host in rating plants for resistance/ susceptibility when a known susceptible cultivar is not available for comparison. The host efficiency, which is the potential of the nematode populations to multiply within the host tissue, on the other hand, was measured through RF. The genotypes were rated as resistant when the nematodes' reproduction rate as well as the damage were minimal; tolerant where there were less damage to the plant irrespective of high nematode reproduction rate; susceptible when both reproduction of the nematodes and the damage were high; and hypersusceptible when substantial damage on the host was observed at the slightest nematode reproduction.

Hatching assay. The effect of root exudates was tested in order to see whether there are differences among the tested genotypes in hatching stimulation. Seeds of eighteen pepper genotypes were sown on pots of 5 l filled with sterilised soil and allowed to grow for 8 weeks. Root exudates were collected following the method described by Yang *et al.* (2016) with modification. Ten plants from each of the genotypes were uprooted, the roots were washed in running tap water to remove adhering soil and rinsed with sterile water. Then, the roots were suspended in

500 ml sterile water for 48 h on a laboratory bench to allow the root exudates to leach into the water. The suspension then was collected and filtered through a folded filter paper to remove dirt and pieces of plant parts. The exudate was stored at 4°C in a refrigerator and used for hatching assays. The egg masses of *M. incognita* and *M. javanica* populations were collected from 10 week-old previously infested tomato roots. Then single egg masses were randomly handpicked and individually placed in 2 ml root exudates into Petri dishes of 1.2 cm × 5.1 cm and kept for 2 weeks on a laboratory bench at room temperature and 12 h light/dark condition to stimulate hatch of J2. Egg masses in distilled water served as control. The Petri dishes containing the treatments were replicated six times and arranged in a completely randomised design (CRD).

The suspension containing J2 were collected from each Petri dish in intervals of 2 days beginning from 48 h and pipetted into a 2 ml Eppendorf tube, while retaining the egg masses in the Petri dish, followed by addition of the same volume (2 ml) of root exudates/distilled water to the Petri dishes after the suspension was taken every time. The suspension was stored in a refrigerator at 4°C until counting. The experiment continued for two consecutive weeks. After collecting the suspension on the last day (14^{th} day), the remaining contents in the Petri dishes were covered with 2 ml 0.52% NaOCl solution and carefully shaken to dislodge the remaining eggs (Hussey & Barker, 1973). Then all the collected suspensions were transferred separately into a nematode counting dish and the number of J2 in 2 ml suspension and the number of eggs in the final solution were counted under a stereomicroscope. The maximum and minimum temperature in the laboratory was recorded daily throughout the experimental period.

Measurements. The number of J2 hatched from the egg mass at each time of data collection and the number of remaining unhatched J2 were the data taken from the experiment. The total hatching percentage was calculated by dividing the total hatched J2 to the sum of hatched and unhatched J2. The sum of hatched and unhatched J2 at the end of the experiment was taken as the total nematode population and the half nematode population was calculated by dividing the total population by two. Then the data collection day at which the hatched population reached 50% was taken as T_{50}.

Data analysis. One-way analysis of variance (ANOVA) was computed for the plant biomass data (root and shoot fresh weight). Nevertheless, the nematode data from both the screening and hatching experiments was subjected to two-way ANOVA using GenStat 64-bit Release 17.1 (PC/Windows 8), VSN International Ltd., 2014. Treatment means were separated using Duncan's Multiple Range Test (DMRT) at $P = 0.05$ whenever the ANOVA was significant. The final population (Pf), egg mass per root system (EMRS) and egg per egg mass (E/EM) data were Log_{10} (x + 1) transformed before analysis.

RESULTS

Resistance screening. Among the eighteen genotypes screened for resistance to *M. incognita* and *M. javanica*, based on the gall index (GI) and reproduction factor (RF), three genotypes (*viz.* 'Melka Awaze', 'Acc.002' and 'Acc.003') and four genotypes ('Melka Awaze', 'Acc.003', 'Acc.03' and 'Acc.04') could be considered resistant to *M. incognita* populations JIT5 and JIT10, respectively. However, for *M. javanica* (BGS4), with the exception of 'Mareko Fana', 'Melka Shote' and 'Oda Haro', all genotypes were found resistant (Table 4). These genotypes did not support nematode reproduction; hence, they had RF less than or equal to one and least gall formation (GI ≤ 2). 'Mareko Fana' and 'Oda Haro' were susceptible for *M. javanica* population (BGS4). 'Melka Shote' was hypersusceptible for *M. javanica* population (BGS4) exhibiting considerable damage (GI = 2) with the minimal nematode reproduction (RF < 1). On the other hand, two genotypes, 'Acc.004' and 'Acc.03', were tolerant to *M. incognita* population JIT5. These genotypes supported considerable nematode reproduction (RF > 1) but with minimal damage to the root (GI < 2). However, none of the genotypes was found tolerant for JIT10 and BGS4 population.

Both nematode populations and pepper genotypes revealed a highly significant effect on the final nematode population density ($P < 0.001$). The interaction between the populations and genotypes was also highly significant at $P < 0.001$. All the three nematode populations reproduced efficiently on the 'Oda Haro', while their multiplication was highly reduced on 'Melka Awaze' (Table 5). The peak final population was for JIT5 on 'Oda Haro' (55,248), while the least was for BGS4 on 'Melka Awaze' (30). Among the two *Meloidogyne* species, *M. incognita* (JIT5 and JIT10) maintained the greatest nematode population build-up as compared to *M. javanica* (BGS4) (Table 5).

The main effects (pepper genotypes and nematode populations) as well as the interaction effect of genotypes × populations on the egg mass formation were highly significant at $P < 0.001$. Other genotypes, except 'Oda Haro' and 'Mareko Fana', suppressed the egg mass formation significantly

Table 4. Egg mass index (EMI), reproduction factor (RF) and degree of resistance (DR) of the eighteen pepper genotypes against two *Meloidogyne incognita* and one *M. javanica* populations under glasshouse conditions in 2016/2017.

Genotypes	Nematode populations								
	M. incognita						*M. javanica*		
	JIT5			JIT10			BGS4		
	GI	RF	DR	GI	RF	DR	GI	RF	DR
'Acc.001'	4.25±0.25	7.94±0.59	S	2.75±0.25	2.16±0.30	S	0.00±0.00	0.39±0.03	R
'Acc.002'	0.50±0.29	1.27±0.25	R	3.00±0.00	2.37±0.18	S	0.50±0.29	0.25±0.03	R
'Acc.003'	1.50±0.29	0.93±0.07	R	1.25±0.25	0.82±0.09	R	1.25±0.25	0.36±0.04	R
'Acc.004'	1.00±0.00	2.11±0.06	T	3.00±0.00	4.60±0.36	S	1.00±0.00	0.28±0.03	R
'Acc.01'	4.00±0.00	6.52±0.44	S	2.00±0.00	0.67±0.08	S	1.00±0.00	0.15±0.05	R
'Acc.02'	4.00±0.00	5.27±0.20	S	3.75±0.25	3.18±0.60	S	0.00±0.00	0.27±0.03	R
'Acc.03'	1.75±0.25	1.22±0.20	T	1.50±0.29	0.81±0.07	R	0.00±0.00	0.14±0.07	R
'Acc.04'	4.00±0.00	4.63±0.47	S	2.00±0.00	0.55±0.05	R	0.00±0.00	0.35±0.05	R
'Acc.05'	3.75±0.25	2.72±0.35	S	3.00±0.00	1.94±0.11	S	0.50±0.29	0.29±0.06	R
'Bako Local'	5.00±0.00	16.03±1.76	S	4.00±0.00	5.32±0.31	S	1.00±0.00	0.39±0.03	R
'Melka Awaze'	1.25±0.25	0.68±0.02	R	0.50±0.29	0.03±0.00	R	0.00±0.00	0.02±0.01	R
'Melka Dima'	4.25±0.25	7.04±0.31	S	5.00±0.00	11.82±0.82	S	0.00±0.00	0.34±0.03	R
'Melka Eshet'	3.75±0.25	4.69±0.30	S	5.00±0.00	8.90±0.46	S	1.25±0.25	0.44±0.03	R
'Mareko Fana'	3.75±0.25	4.83±0.43	S	4.00±0.00	9.61±0.46	S	2.75±0.25	1.80±0.12	S
'Melka Shote'	3.50±0.29	4.57±0.33	S	3.75±0.25	4.23±0.32	S	2.00±0.00	0.57±0.09	HS
'Melka Zala'	3.25±0.25	2.73±0.26	S	4.00±0.00	5.05±0.70	S	2.00±0.00	0.61±0.04	R
'Oda Haro'	5.00±0.00	27.62±0.96	S	4.00±0.00	11.28±1.69	S	3.25±0.25	2.92±0.23	S
'Saidah'	4.00±0.00	5.14±0.41	S	4.00±0.00	4.82±0.33	S	0.75±0.25	0.25±0.04	R

R = resistant, S = susceptible, HS = hypersusceptible. GI and RF values are mean of four replicates ± standard error.

for BGS4 (Table 5). 'Acc.002', 'Acc.004', 'Melka Awaze', 'Acc.003' and 'Acc.03' displayed fewer egg mass numbers for the JIT5 population while 'Bako Local' and 'Oda Haro' were the genotypes with highest number of egg masses. For JIT10, 'Melka Awaze' inhibited development of egg masses. By contrast, 'Melka Dima' and 'Melka Eshet' supported highest egg mass formation (Table 5).

The effect of population, genotype and population × genotype on number of eggs contained per egg mass was highly significant ($P < 0.001$). The smallest number of eggs per egg mass was obtained from 'Melka Awaze', 'Acc.003' and 'Acc.01' for JIT5, JIT10 and BGS4 nematode populations, respectively. On the other hand, JIT5 and BGS4 populations produced the highest number of eggs per egg mass on 'Oda Haro', while the maximum egg formation per egg mass for JIT10 was recorded from 'Acc.004' (Table 5).

In some of the genotypes, greater number of eggs per egg mass were recorded regardless of the few egg masses observed on their roots and *vice versa*. For example, 'Acc.004' with the smallest egg mass number had the highest number of eggs per egg mass, which was nearly equal to 'Oda Haro' that had the highest egg mass per root system for JIT10 population. Similarly, 'Acc.001' had higher egg number per egg mass for both JIT5 and JIT10 populations. By contrast, 'Melka Eshet', with a significantly higher number of egg masses per root system, had relatively smaller egg number for JIT10 (Table 5).

Pepper genotypes exhibited disparity in root fresh weight among nematode infected plants over controls. Most of the plants with nematode infection showed a generally decreasing trend for root fresh weight over their control except for *M. javanica* population (Table 5). However, this variation was not significant except in genotypes 'Bako Local', 'Melka Dima', 'Melka Eshet', 'Melka Shote', 'Mareko Fana', 'Melka Zala', 'Oda Haro' and 'Saidah'. Among these genotypes, only 'Melka Shote' displayed a reduction in root fresh weight for all the three populations. Conversely, the nematode populations JIT5 and JIT10 significantly reduced root fresh weight on genotypes 'Melka Dima', 'Melka Zala', 'Oda Haro' and 'Saidah'. Furthermore, 'Bako Local' and 'Melka Eshet' had a

substantial drop in fresh root weight for JIT5 and JIT10, respectively.

There was also a variation in shoot fresh weight between the nematode infected plants and their respective controls (Table 5), but only four genotypes revealed a significant reduction over the control. The nematode population JIT5 caused a pronounced decline of shoot fresh weight on 'Oda Haro' and 'Saidah'. JIT10 populations decreased the shoot fresh weights on 'Mareko Fana' and 'Saidah'. BGS4 nematode population resulted in reduced shoot fresh weight on 'Melka Shote' only. In comparison, the two *M. incognita* (JIT5 and JIT10) populations were significantly different from the control in their effect on root and shoot fresh weights than *M. javanica* (BGS4) populations.

Evaluation of root exudates for hatching of *M. incognita* and *M. javanica* populations. The temperature in the laboratory (25.5°C) was nearly constant during the experimental period. In the *in vitro* experiment the root exudates did not have an effect on the total hatch percentage of the nematode for all population. The total percentage of hatching on root exudates ranged from 63.8% (on control for BGS4) to 83.7% (on 'Melka Shote' for JIT10) (Table 6). However, there was a significant interaction between the pepper genotypes and the nematode populations in the number of days at which 50% of the J2 hatched (Table 6). The root exudates of pepper genotypes showed variation in their effects on hatching. Genotypes 'Acc.001', 'Acc.003', 'Acc.01' and 'Acc.03' slowed down the time required for hatching to reach 50% as compared to the control (water) for JIT10 population. On the other hand, all the other genotypes caused hatching to reach 50% earlier than the control. The exudate from the roots of 'Saidah' significantly reduced the hatching time of half of the nematode population by 59.09% for JIT10.

DISCUSSION

It is necessary to generate information on the degree of the inherent potential of the hot pepper genotypes in inhibiting nematode infection and reproduction in order to consider plant genetic resistance as a nematode management option. In the present study, the eighteen evaluated hot pepper genotypes varied in their resistance to *M. incognita* and *M. javanica* populations. None of the tested genotypes were found to be immune. Two genotypes, 'Melka Awaze' and 'Acc.003', were found resistant to all the three nematode populations restricting the nematode reproduction, thereby resulting in the least egg mass formation and low RF value. By contrast, 'Oda Haro' was the only variety that was equally susceptible to all the nematode populations with highest egg mass proliferation and maximum RF. The remaining genotypes showed variable reactions to the three nematode populations.

The reason for the RF not to be totally zero on the resistant cultivars regardless of their ability to suppress the nematode reproduction is that the final nematode population was computed from both organic (root) and soil fractions. Consequently, there were J2 recovered from the soil that might have been from either a few egg masses that accidentally detached from the root or from inoculums survivors that hatched during extraction.

There was variation in resistance among the hot pepper genotypes to nematode species and populations. The *M. incognita* populations from Jittu were found to be more aggressive on the tested genotypes than the *M. javanica* population from Babile. Most of the hot pepper genotypes assessed were resistant to *M. javanica*. Pepper genotypes more susceptible to *M. incognita* than *M. javanica* were also reported by many other authors (Khan & Khan, 1991; Thies & Ferry, 2000; Oka *et al.*, 2004; Soares *et al.*, 2018). Robertson *et al.* (2006) found that pepper genotypes they evaluated were resistant to *M. javanica*. Rosa *et al.* (2013) also indicated that *Capsicum* species were found immune to *M. javanica*. Even though most species of RKN have wide host ranges and it is difficult to find crop species that are completely immune or resistant, this species-specific resistance to *M. javanica* in hot pepper, if confirmed under field conditions, makes it a better alternative crop to be grown under heavy *M. javanica* infestation with minimum yield loss. Moreover, it can also be used to reduce the population build-up of this species in the soil to protect the succeeding crops from damage.

The intraspecific variation was observed in four pepper genotypes under the variety trial for the two populations of the same *Meloidogyne* species, *M. incognita* (JIT5 and JIT10). Seid *et al.* (2015) reported the occurrence of differential pathogenicity of nematode populations of the same species on a specific tomato cultivar. The same authors also confirmed this incidence through the screening experiment they conducted on breeding lines and commercial tomato cultivars to Ethiopian *M. incognita* and *M. javanica* populations (Seid *et al.*, 2017). Since these two populations of *M. incognita* are from the same location exhibiting different pathogenicity on the aforementioned genotypes, the possible reason might be either the previously non-pathogenic individuals of a population overcame the

Table 5. Final nematode population (Pf), number of egg mass per root system (EM/RS), number of eggs per egg mass (E/EM), root fresh weight, and shoot fresh weight of the pepper genotypes under greenhouse conditions in 2016/2017 (n = 4).

Genotypes	Nematode population	Pf (Root and soil)	EMRS	E/EM	Root fresh weight	Shoot fresh weight
'Acc.001'	Control	–	–	–	6.95^{a}	11.99^{a}
	JIT5	15885^{c-f}	41.00^{d-g}	362^{ab}	5.99^{a}	11.42^{a}
	JIT10	4311^{kl}	11.00^{j}	312^{bc}	6.30^{a}	11.60^{a}
	BGS4	780^{p-s}	0.00^{r}	–	6.48^{a}	11.25^{a}
'Acc.002'	Control	–	–	–	4.20^{a}	7.99^{a}
	JIT5	2535^{lm}	0.00^{r}	–	4.27^{a}	6.32^{a}
	JIT10	4748^{jk}	17.50^{i}	146^{k-n}	3.56^{a}	6.31^{a}
	BGS4	502^{s}	0.00^{r}	–	4.24^{a}	6.67^{a}
'Acc.003'	Control	–	–	–	6.45^{a}	10.09^{a}
	JIT5	1868^{mn}	2.50^{mn}	123^{nop}	6.17^{a}	10.08^{a}
	JIT10	1632^{mno}	2.50^{mn}	112^{op}	6.25^{a}	10.21^{a}
	BGS4	724^{qrs}	1.00^{pq}	117^{op}	6.08^{a}	9.98^{a}
'Acc.004'	Control	–	–	–	5.40^{a}	8.26^{ab}
	JIT5	4215^{kl}	1.00^{pq}	127^{mno}	5.91^{a}	8.59^{ab}
	JIT10	9197^{f-i}	14.25^{ij}	403^{a}	5.65^{a}	6.83^{b}
	BGS4	557^{s}	0.80^{q}	112^{op}	6.19^{a}	8.91^{a}
'Acc.01'	Control	–	–	–	5.44^{a}	7.71^{a}
	JIT5	13039^{c-f}	47.50^{cde}	269^{cd}	5.02^{a}	6.17^{a}
	JIT10	1349^{m-q}	6.25^{kl}	205^{fgh}	5.31^{a}	7.52^{a}
	BGS4	306^{t}	1.30^{opq}	111^{op}	5.51^{a}	7.69^{a}
'Acc.02'	Control	–	–	–	6.43^{a}	10.15^{a}
	JIT5	10545^{d-g}	52.30b^{-e}	182^{g-j}	6.16^{a}	9.29^{a}
	JIT10	6357^{g-k}	32.00^{gh}	168^{h-k}	5.75^{a}	9.69^{a}
	BGS4	548^{s}	0.00^{r}	–	6.33^{a}	10.50^{a}
'Acc.03'	Control	–	–	–	5.77^{a}	6.53^{a}
	JIT5	2437^{lm}	5.00^{l}	184^{g-j}	5.50^{a}	7.43^{a}
	JIT10	1622^{mno}	1.75^{nop}	123^{nop}	5.88^{a}	6.60^{a}
	BGS4	270^{t}	0.00^{r}	–	5.38^{a}	6.47^{a}
'Acc.04'	Control	–	–	–	4.27^{a}	7.54^{a}
	JIT5	9256^{f-i}	45.80c^{-f}	189^{g-j}	4.26^{a}	6.49^{a}
	JIT10	1089^{n-r}	5.00^{l}	130^{mno}	4.10^{a}	7.40^{a}
	BGS4	705^{rs}	0.00^{r}	–	4.85^{a}	7.57^{a}
'Acc.05'	Control	–	–	–	6.19^{a}	12.33^{a}
	JIT5	5443^{ijk}	33.00^{fgh}	146^{k-n}	5.17^{a}	11.47^{a}
	JIT10	3876^{kl}	18.00^{j}	165^{i-l}	5.95^{a}	11.55^{a}
	BGS4	585^{s}	0.00^{r}	–	6.05^{a}	11.42^{a}

Table 5. (continued) Final nematode population (Pf), number of egg mass per root system (EM/RS), number of eggs per egg mass (E/EM), root fresh weight, and shoot fresh weight of the pepper genotypes under greenhouse conditions in 2016/2017 (n = 4).

Genotypes	Nematode population	Pf (Root and soil)	EMRS	E/EM	Root fresh weight	Shoot fresh weight
'Bako Local'	Control	–	–	–	6.36^{ab}	10.85^{a}
	JIT5	32054^{b}	125.00^{a}	240^{def}	5.08^{c}	9.56^{a}
	JIT10	10646^{d-g}	37.50^{e-h}	253de	5.46^{bc}	9.36^{a}
	BGS4	787^{p-s}	0.00^{r}	–	6.77^{a}	10.28^{a}
'Melka Awaze'	Control	–	–	–	6.96^{a}	12.45^{a}
	JIT5	1367^{m-p}	1.80^{nop}	103^{p}	6.95^{a}	12.27^{a}
	JIT10	52^{u}	0.00^{r}	–	6.71^{a}	12.58^{a}
	BGS4	30^{v}	0.00^{r}	–	6.92^{a}	12.61^{a}
'Melka Dima'	Control	–	–	–	10.57^{ab}	21.07^{a}
	JIT5	14069^{c-f}	72.0^{b}	183^{g-j}	9.45^{b}	21.40^{a}
	JIT10	23634^{bc}	118.50^{a}	192^{ghi}	7.43^{c}	18.96^{a}
	BGS4	675^{rs}	0.00^{r}	–	10.74^{a}	20.21^{a}
'Melka Eshet'	Control	–	–	–	12.72^{a}	20.99^{a}
	JIT5	9387^{e-i}	29.3g^{h}	181^{g-j}	12.52^{a}	19.28^{a}
	JIT10	17797^{b-e}	112.00^{a}	154^{j-m}	11.32^{b}	19.39^{a}
	BGS4	870^{o-s}	0.00^{r}	–	12.65^{a}	20.09^{a}
'Mareko Fana'	Control	–	–	–	12.81^{a}	25.38^{a}
	JIT5	9664^{e-i}	28.30^{h}	190^{g-j}	12.37^{ab}	23.13^{ab}
	JIT10	19227^{bcd}	71.30^{b}	213^{efg}	11.49^{b}	20.52^{b}
	BGS4	3598^{kl}	10.30^{j}	123^{nop}	12.71^{ab}	23.00^{ab}
'Melka Shote'	Control	–	–	–	6.84^{a}	13.29^{a}
	JIT5	9146^{f-i}	29.30^{gh}	166^{ijk}	5.60^{b}	10.99^{ab}
	JIT10	8454^{f-j}	27.30^{h}	272^{cd}	5.02^{b}	11.14^{ab}
	BGS4	1129^{n-r}	3.50^{lm}	115^{op}	4.96^{b}	10.76^{b}
'Melka Zala'	Control	–	–	–	12.71^{a}	25.53^{a}
	JIT5	5456^{h-k}	26.50^{h}	137^{l-o}	11.17^{b}	23.93^{a}
	JIT10	10098^{e-i}	50.50^{b-e}	174^{h-k}	11.19^{b}	23.30^{a}
	BGS4	1213^{n-r}	3.50^{lm}	121^{nop}	12.33^{a}	26.43^{a}
'Oda Haro'	Control	–	–	–	6.50^{a}	15.46^{a}
	JIT5	55248^{a}	125.00^{a}	408^{a}	4.24^{b}	12.17^{b}
	JIT10	22563^{bc}	67.30^{bc}	272.00^{cd}	5.07^{b}	14.03^{ab}
	BGS4	5839^{g-k}	27.00^{h}	131^{mno}	6.07^{a}	14.14^{ab}
'Saidah'	Control	–	–	–	12.72^{a}	26.62^{a}
	JIT5	10275^{e-h}	49.00^{cde}	178^{g-j}	11.06^{b}	22.76^{b}
	JIT10	9639^{e-i}	55.50^{bcd}	155.00^{f-l}	11.00^{b}	20.50^{b}
	BGS4	495^{k-o}	0.00^{r}	–	12.67^{a}	26.41^{a}

Means in a column sharing the same letter are not significantly different from each other at $P = 0.05$ according to DMRT.

Table 6. Final hatching percentage, days to 50% hatch (T_{50}) and percent T_{50} reduction (Red., %) of two species of *Meloidogyne* exposed to root exudates of pepper genotypes under laboratory conditions.

Genotypes	*M. incognita*						*M. javanica*					
	JIT5			JIT10			BGS4			KOK2		
	Final hatch (%)	T_{50}	Red. (%)	Final hatch (%)	T_{50}	Red. (%)	Final hatch (%)	T_{50}	Red. (%)	Final hatch (%)	T_{50}	Red. (%)
'Acc.001'	77.38	6.33^{b-g}	13.64	69.20	8.67^{a}	-18.19	79.72	6.33^{b-g}	13.64	78.55	6.33^{b-g}	20.84
'Acc.002'	80.17	7.33^{a-d}	0.00	80.49	5.33^{d-h}	27.27	80.40	5.33^{d-h}	27.27	82.08	4.67^{f-i}	41.66
'Acc.003'	81.75	7.33^{a-d}	0.00	77.39	7.67^{abc}	-4.55	66.55	7.33^{a-d}	0.00	79.72	5.67^{c-h}	29.16
'Acc.004'	79.9	5.67^{c-h}	22.72	79.86	6.33^{b-g}	13.64	81.26	5.33^{d-h}	27.27	79.77	7.00^{a-e}	12.50
'Acc.01'	78.72	7.00^{a-e}	4.54	80.77	8.00^{ab}	-9.10	80.92	6.00^{b-h}	18.18	80.61	6.00^{b-h}	25.00
'Acc.02'	81.78	5.33^{d-h}	0.27	81.22	6.00^{b-h}	18.18	80.2	6.33^{b-g}	13.64	79.97	6.33^{b-g}	20.84
'Acc.03'	78.44	7.33^{a-d}	0.00	81.56	7.00^{a-e}	-9.10	79.53	6.00^{b-h}	18.18	78.87	6.00^{b-h}	25.00
'Acc.04'	79.85	6.67^{b-f}	9.08	80.94	5.00^{e-h}	31.82	78.16	7.00^{a-e}	4.54	80.03	4.67^{f-i}	41.66
'Acc.05'	77.45	6.33^{b-g}	13.64	81.71	5.33^{d-h}	27.27	79.55	6.33^{b-g}	13.64	80.64	6.67^{b-f}	16.66
'Bako Local'	65.19	6.67^{b-f}	9.08	81.09	5.00^{e-h}	31.82	80.41	7.00^{a-e}	4.54	80.63	6.00^{b-h}	25.00
'Melka Awaze'	80.35	6.67^{b-f}	9.08	80.42	5.33^{d-h}	27.27	81.54	6.33^{b-g}	13.64	79.51	5.67^{c-h}	29.16
'Melka Dima'	80.52	6.00^{b-h}	18.18	79.35	4.33^{ghi}	40.91	79.68	5.67^{c-h}	22.72	68.72	5.67^{c-h}	29.16
'Melka Eshet'	81.78	5.67^{c-h}	22.72	78.76	4.00^{hi}	45.45	77.04	6.33^{b-g}	13.64	68.52	6.00^{b-h}	25.00
'Mareko Fana'	82.54	4.67^{f-i}	36.36	77.07	4.33^{ghi}	40.91	79.33	5.00^{e-h}	31.82	77.01	5.33^{d-h}	33.34
'Melka Shote'	77.85	6.00^{b-h}	18.18	83.72	4.33^{ghi}	40.91	80.43	6.00^{b-h}	18.18	81.07	5.00^{e-h}	37.50
'Melka Zala'	78.71	5.67^{c-h}	22.72	78.48	6.00^{b-h}	18.18	77.56	5.33^{d-h}	27.27	77.5	5.67^{c-h}	29.16
'Oda Haro'	82.65	4.67^{f-i}	36.36	80.87	5.33^{d-h}	27.27	80.26	5.67^{c-h}	22.72	81.83	5.00^{e-h}	37.50
'Saidah'	82.38	5.33^{d-h}	27.27	77.93	3.00^{i}	59.09	81.15	6.33^{b-g}	13.64	81.78	5.33^{d-h}	33.34
Water (Control)	74.95	7.33^{a-d}	0.00	78.13	7.33^{a-d}	0.00	78.57	7.33^{a-d}	0.00	63.83	8.00^{ab}	0.00

Means sharing the same letter are not significantly different from each other at $P = 0.05$ according to DMRT. Values are means of six replicates.

resistance genes in the pepper genotypes and segregated themselves into a distinct population or race differences might exist that were not previously investigated among the populations at that specific location for which the genotypes showed differential reaction. In this regard, Khan & Khan (1991) also observed similar difference on reaction of a pepper genotype 'Pusa Jwala' for four *M. incognita* races. Therefore, the need for examination of species and population composition is emphasised before using host resistance for root-knot nematode management (Khan & Khan, 1991; Seid *et al.*, 2017; Coyne *et al.*, 2018). Due attention must be given to these discrepancies of genotypes in further planning to use genetic potential of these hot pepper genotypes against RKN. Mandefro & Mekete (2002) reported that *Meloidogyne* spp. exist in the country as species mixture of *M. incognita* with either *M. ethiopica* or *M. javanica* on pepper. Hence, this co-existence of nematode species should be taken into consideration during selection of the hot pepper genotypes for nematode management as the degree of resistance of the hot pepper genotypes depends on nematode species. The hot pepper genotypes with variation in reaction to the two *M. incognita* populations are not yet released for production, so it is worth checking their susceptibility against different *M. incognita* populations in the country with other selection parameters during the varietal trial.

Interestingly, the resistant and the tolerant genotypes to the two populations of *M. incognita* were found from genotypes that are on variety trial, except for 'Melka Awaze' that is currently under production. Therefore, these genotypes would be potential candidates for production of hot pepper in highly nematode-infested areas of the country to overcome the damage and yield loss.

'Melka Awaze', which is registered as a tolerant variety to other foliar and soil borne diseases in the country, as well as 'Acc.003' that is under variety trial could be potential sources of resistant genes for Ethiopian *M. incognita* and *M. javanica* populations as part of resistance management strategy for RKN. The imported commercial hybrid variety 'Saidah' was found susceptible to both populations of *M. incognita*. Thus, in areas of high nematode pressure,

its cultivation needs to be coupled with alternative nematode management strategies.

The root and shoot fresh weight in most of the hot pepper genotypes did not exhibit significant reduction over the controls for all nematode populations. A significant decline was only recorded from eight and three genotypes for root and shoot fresh weights, respectively. Four genotypes ('Bako Local', 'Melka Dima', 'Melka Eshet' and 'Melka Shote') did not demonstrate difference in shoot fresh weight from the control regardless of the decline in their root fresh weight. Moreover, some of the susceptible genotypes did not show a significant difference in fresh root and shoot weights from the controls. Mekete *et al.* (2003) showed that there was no reduction in fresh weight of 'Mareko Fana' below 0.36 J2 cm^{-3} of soil for *M. javanica*. Ahmed *et al.* (2013) also indicated that no significant reduction was observed on plant growth parameters of chilli pepper at the inoculum density less than 1000 J2 per plant.

The cumulative hatching percentage ranged from 63.8 to 83.7%. The presence of more than 10% unhatched J2 indicates that the eggs might need more time to develop than the duration of the experiment, or diapause existed in the population. In line with this finding, De Guiran (1979) pointed out that hatching of *M. incognita* ended 20 days after incubation under favourable condition leaving some unhatched J2 that could not even be stimulated by host root exudates. He also associated this condition with diapause, which is the survival mechanism of PPN. The nematode populations did not show a significant difference in their cumulative hatch in root exudates and the control (water). Even though the cumulative hatching percentage is generally higher in root exudate than in water, the difference was not statistically significant. The host root exudate is not a prerequisite for root-knot nematode hatching as long as the temperature and moisture are favourable, although hatching might be stimulated by the presence of root diffusates (Wesemael *et al.*, 2006). Wesemael *et al.* (2006) also reported that tomato root exudates did not have influence on the cumulative final hatch of *M. fallax* and *M. chitwoodi* from eggs from young tomato plants but increased hatching of *M. chitwoodi* from eggs from senescing plants. Inserra *et al.* (1983), in their experiment on effect of host plant and temperature on hatching, also found that the cumulative hatching of *M. chitwoodi* and *M. hapla* to be greater than in distilled water at 15 and 25°C even though the effect was inconsistent in other temperatures tested. By contrast, Gaur *et al.* (2000) detected enhancement of total percentage hatch by root exudate of rice on *M. triticoryzae*.

The time in which the hatched J2 reached 50% also differed among the treatments. Fourteen of the root exudates increased the rate of hatching by reducing the required number of days for the J2 to reach half of the final population, while only four delayed the hatching time compared to the control. Variation in T_{50} within species was also observed in root exudates of 'Acc.001' for *M. incognita* and 'Acc.04' for *M. javanica*. This result differs from the finding of Wesemael *et al.* (2006) who reported that there was no difference in time for 50% hatching of *M. fallax* and *M. chitwoodi* for eggs from young tomato plants (13 weeks). Huang & Pereira (1994) associated the delay in hatching with the type and age of the host plant from which the egg masses were collected and incubation temperature after observation of difference in hatching of *M. javanica* egg masses. Khokon (2009) examined the effect of the source of root exudates (host) and host plant age on *M. chitwoodi* hatch and found that among six tested host plant root diffusates only those from tomato and carrot delayed the hatching time to 50%; they confirmed that the variability in the time taken for hatching of nematode population depends on type and age of the host plant. Nevertheless, as most of recent studies emphasise the role of temperature and host plant age in hatching (Curtis *et al.*, 2009), the hatch stimulation of the eighteen hot pepper genotypes at different ages of the host plant and ranges of temperature needs further investigation.

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Beimnet Abegaz, Awol Seid, Beira H. Meressa, Chemeda Fininsa, Mashilla Dejene and Kebede Woldetsadik. Оценка генотипов острого перца (*Capsicum annum* и *C. frutescens*) на устойчивость и стимуляцию вылуплений в популяциях *Meloidogyne incognita* и *M. javanica* из Эфиопии.

Резюме. Реакцию 18 генотипов стручкового перца к поражению эфиопскими популяциями *Meloidogyne incognita* и *M. javanica* оценивали в условиях защищенного грунта. Также проводили лабораторные эксперименты по оценке действия экссудатов корней перца на вылупление личинок в этих популяциях нематод. Все изученные генотипы перца оказались чувствительными к галловой нематоде. Однако генотипы ‘Acc.003’ и ‘Melka Awaze’ оказались сравнительно устойчивыми, тогда как генотип ‘Oda Haro’ был восприимчив к заражению всеми популяциями мелойдогин. Эксперименты по вылуплению показали, что экссудаты корней не оказывают существенного воздействия на общие показатели вылупления. Тем не менее, время, за которое вылуплялась половина личинок, существенно варьировало при использовании экссудатов от разных генотипов. После проверки в полевых условиях устойчивые генотипы могут применяться для повышения продуктивности плантаций перца.