Applying Aqueous Crude Leaf Extracts and Organic Soil Amendments Manages *Meloidogyne incognita* on Hot Pepper

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Abstract

The root-knot nematode, *Meloidogyne incognita*, can severely damage hot pepper plants (*Capsicum annum* and *C. frutescens*). Therefore, it is crucial to develop effective management strategies. The impact of aqueous crude leaf extracts from *Vernonia amygdalina* and *Lantana camara* and organic soil amendments with vermicompost and poultry manure was investigated in an open-field pot experiment on three pepper genotypes (Oda Haro, Acc.03, and Melka Awaze). The growth performance, yield, and nematode population were assessed. Twenty-seven treatments, including the untreated checks, were arranged in a factorial RCBD in three replications. Plots treated with leaf extracts and/or organic soil amendments resulted in significantly lower number of root galls, egg masses, and nematode populations than the untreated checks in all the pepper genotypes. The combined treatments were more effective than individual treatments in all the genotypes. The lowest nematode population was on the genotype Melka Awaze *Lantana camara* with vermicompost on genotype Melka Awaze, *L. camara* with poultry manure on genotype Oda Haro, and *V. amygdalina* with vermicompost on genotype Acc.03 sustained fewer root galls, egg masses and nematode populations. On the genotype 'Oda Haro', the untreated control gave significantly lower values for the number of pods, marketable pods, number of branches and plant height than the remaining treatments except *V. amygdalina* applied without soil amendments for number of branches. Similarly, on the genotype'Acc.03', except *L. camara* without soil amendments for number of branches and *V. amygdalina* and poultry manure for plant height, values were significantly lower in the untreated control than the other treatments. Vermicompost enhanced the growth and yield of pepper and reduced the number of root galls, egg masses and nematode populations. Therefore, applying leaf extracts and organic soil amendments appeared promising to control *M. incognita*.

Keywords*:* Botanicals, cultural management, root gall, root-knot nematodes, egg mass

Introduction

The worldwide presence of the root-knot nematodes (RKN) (*Meloidogyne* spp.) poses a significant threat to vegetable production, causing substantial yield loss (Moosavi, 2015). This damage is more severe in tropical countries due to favorable environmental conditions (De Waele and Elsen, 2007). Identifying the damage caused by RKN is challenging because the typical signs remain underground, and above-ground symptoms, such as stunted growth, chlorosis, and yield deterioration, resemble water and nutrient deficiencies (De Waele and Elsen, 2007). Hot pepper, a high-value crop vital to the Ethiopian diet, frequently falls victim to RKN. The matured red pods are processed into a dry powder and used as a colorant and flavoring spice, while the fresh green pods are consumed as vegetables (Shumeta, 2012).

A sustainable integrated nematode management strategy is needed to avoid the negative impact on the environment, public health, and economy caused by the excessive use of chemical nematicides. A cost-effective and ecologically safe option is to use botanicals and organic soil amendments alone or in combination (Khalil and Darwesh, 2018). Previous studies have shown that

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aqueous leaf extracts of plants such as *Lantana camara* and *Vernonia amygdalina* can be toxic to nematodes, primarily by preventing egg hatching or killing infective juveniles (Taye et al., 2013; Karim et al., 2019). Adding organic matter to soil can protect plants from severe damage caused by plant-parasitic nematodes (PPN). This is achieved by releasing neurotoxic substances like hydrogen sulphide, ammonia, nitrates, and inorganic acids or changing the soil's physical structure, making it less conducive for nematode reproduction, hatching, and movement (Oka, 2010). Furthermore, organic soil amendment stimulates microbial activity and proliferation, creating antagonism with the nematodes (Renco, 2013). Animal manures, sawdust, and various composted crop residues are commonly used organic materials for soil amendment to suppress PPN (Akhtar and Malik, 2000).

Vermicompost is the excreta of earthworms, which is rich in humus, macronutrients, and micronutrients and largely influences the root penetrability, potential rooting volume, nutrient uptake and mobility, soil aeration, and water availability, thereby improving the nutrient content of different plant components such as the roots, shoots, and pods (Rekha et al., 2018). It also contains plant growth regulators such as auxins, gibberellins and cytokinins that result from the interactions between the microflora and the earthworm's gut (Adhikary, 2012). It increases water-holding capacity compared to conventional compost due to its humus content (Hussain and Abbasi, 2018). Even though there is variability among different findings, the suppressive effect of vermicompost on PPN has been reported (Kumar et al., 2011; Mishra et al., 2017). Several studies reported on the effect of poultry manure treatment or in combination with other nematode management methods (Abdeldaym et al., 2014; Shiferaw et al., 2014; Osman et al., 2018).

Finding safe and affordable alternatives for managing RKN has become a significant concern, especially in countries like Ethiopia, where agriculture is the primary source of the economy. Studies have been conducted in Ethiopia to manage RKN on tomatoes using botanicals and poultry manure (Taye et al., 2013; Shiferaw et al., 2014). However, these studies did not consider the integrated application of leaf extracts and organic soil amendments. Moreover, the efficacy of leaf extracts and organic soil amendments for managing RKN on hot peppers has not been studied so far. This study evaluated the effectiveness of integrating *Lantana camara* and *Vernonia amygdalina* crude leaf extracts and organic soil amendments (vermicompost and poultry manure) for managing *M. incognita* on three hot pepper genotypes.

Materials and Methods

Experimental site

The experiment was conducted at Tony Farm of Haramaya University, located in Dire Dawa, Ethiopia. Dire Dawa is situated at 9.26°N and 41.8°E, with an altitude of 1210 m.a.s.l. On average, the area receives 650 mm of rainfall each year. The highest temperature usually reaches 34°C, and the lowest is around 18°C. The soil in this area is sandy loam and has a pH range of 5.8 to 6.8 (Berhe et al. 2012).

Experimental materials

The experiment used three different hot pepper genotypes namely: Melka Awaze (*C. frutescens*), Acc. 03 (*C. annum*), and Oda Haro (*C. frutescens*). These genotypes had varying degrees of susceptibility to *M. incognita* (Abegaz et al., 2019). Two types of aqueous leaf extracts, *V. amygdalina* and *L. camara,* and two types of soil amendments, poultry manure and vermicompost were used. The vermicompost used as organic soil amendment was prepared from wheat straw, cow dung, and *L. camara* using the earthworm *Eisenia fetida.*

Pots with 10 L capacity that were filled with 6 L of solarized soil were used for the experiment.

Preparation of aqueous leaf extracts

Fresh mature leaves of *V. amygdalina* and *L. camara* plants were collected at the Haramaya University experimental field station (Raree), dried under shade and powdered separately using an electric grinder. Afterwards, 20 g of each powder was soaked in 100 ml of distilled water for 24 hours in 500 ml Erlenmeyer flasks. After 24 hours of soaking, the solution was filtered through cheesecloth, and the resulting filtrate was considered a stock solution "SS" (100% concentration) and stored in the refrigerator at 4^{o}C for two days (Ahmad et al. 2010; Taye et al. 2013).

Preparation of nematode inoculum

A population of *M. incognita* originating from tomatoes in Ethiopia (Seid et al., 2019) was multiplied on a susceptible tomato cultivar "Moneymaker". After eight weeks, the tomato plants were uprooted, and their roots were rinsed gently. The roots were then cut into 1 cm pieces and placed on a modified Baermann tray for 48 hours (Hooper et al., 2005). The infective second-stage juveniles (J2) were collected, suspended in a glass beaker containing 100 ml tap water and stored in a refrigerator at 4 ^{o}C for a week to be used for inoculation. The nematode population density was calculated and adjusted to 8 J2 per g of soil (Abegaz et al., 2019).

Preparation of soil and amendments

A sandy loam field soil mixed with sand at a 3:1 (v/v) ratio was covered with a polythene plastic sheet and stirred weekly for eight weeks at Haramaya University experimental station located in Dire Dawa (Tony Farm) to free it from pathogens that might interfere with the experiment. Before planting, a soil sample was taken from the solarized soil and examined for the presence of PPN using the Modified Baermann technique (Hooper et al., 2005), and it was confirmed that the soil was free of PPN. The poultry manure was obtained from the Haramaya University poultry farm, and the vermicompost was prepared from a combination of wheat straw, cow dung, and *L. camara* at Haramaya University experimental field station. Both were sterilized separately. Depending on the treatment combination, the solarized soil was mixed with vermicompost or poultry manure and filled into 10 L capacity pots to make a total of 6 L soil per pot. The rate of 20 t ha^{-1} was used for both vermicompost (Arancon et al., 2002) and poultry manure (Shiferaw et al., 2014). The control and leaf extract treatment pots were filled only with solarized soil.

Application of crude leaf extracts

The stock solutions of the botanicals were diluted to 5% by mixing 5 ml of the concentrated extracts with 95 ml of distilled water (Taye et al., 2013). The soil in the pot was drenched with the diluted extracts one day before the *M. incognita* was introduced. The control plants received an equal volume of water.

Inoculation

Four-leaf stage pepper seedlings of three different genotypes were transplanted into separate pots. One week later, the seedlings were inoculated with the infective J2 of *M. incognita*. Prior to the inoculation, the soil around the roots was carefully removed, and the prepared suspension of *M. incognita* J2 was applied near the roots and then covered with the removed soil.

Experimental design

The experiment consisted of 27 treatments arranged in a factorial randomized complete block design (RCBD) with three replications. Factors were three hot pepper genotypes, three levels of leaf extracts, and three levels of soil amendments (Table 1).

Table 1. Treatment combinations of the crude leaf extracts and soil amendments on three hot pepper genotypes

T1=OH NENA (C)	T10=A03 NENA(C)	T19=MA NENA(C)
T2= OH NEVC	T11= A03 NEVC	T20= MA NEVC
T3= OH NEPM	T12= A03 NEPM	T21= MA NEPM
T4= OH LCNA	T13= A03 LCNA	T22= MA LCNA
T5= OH LCVC	T14= A03 LCVC	T23= MA LCVC
T6= OH LCPM	T15= A03 LCPM	T24= MA LCPM
T7= OH VANA	T16= A03 VANA	T25= MA VANA
T8= OH VAVC	T17= A03 VAVC	T26= MA VAVC
T9= OH VAPM	T18= A03 VAPM	T27= MA VAPM

OH= Oda Haro; A03= Accession 03; MA= Melka Awaze; NE=No Extract; NA=Not Amended LC=*Lantana camara*; VA=*Vernonia amygdalina*; VC=Vermicompost; PM=poultry manure; C= Control

Data collection

Data were collected weekly on the total number of pods and marketable pods per plant. After 135 days of transplanting, plant growth data such as height, number of branches, and fresh root weight were recorded. The roots from the pot were carefully removed and rinsed with tap water to remove the soil, and the number of galls and egg masses per gram of roots was counted after staining with Phloxine B solution (Daykin and Hussey, 1985). The J2 and eggs of *M. incognita* were dislodged from the root using 0.5% NaOCl solution (Hussey and Barker, 1973). *Meloidogyne incognita* J2s were also extracted from a subsample of 100 g soil per pot using the modified Baermann method (Hooper et al., 2005). The root and soil nematode populations were estimated by counting the *M. incognita* eggs and J2s in the suspension extracted from the root and soil. To determine the final nematode population (*Pf*), the nematode data from both the root and soil were combined. The reproduction factor (RF) was then calculated by dividing the final population (*Pf*) by the initial population (*Pi*).

Data analysis

The data collected were subjected to ANOVA using GenStat 64-bit Release 17.1 (GenStat, 2015). Significant means were separated using Duncan's Multiple Range Test (DMRT) at p=0.05. Nematode parameter data were $Log_{10}(x+1)$ transformed before analysis. Growth and yield parameters were compared with two-way ANOVA, while nematode parameters used three-factor factorial ANOVA in the general model.

Results

The analysis of variance indicated a significant ($p \leq 0.05$) interaction between the genotypes, leaf extracts and soil amendments in decreasing the gall, egg mass formation and nematode reproduction (Table 2).

A significant interaction was also observed between leaf extracts and soil amendment for growth and yield components except for marketable pods per plant for Melka Awaze (Table 3). The main and interaction effects were significant (at $p \leq 0.05$) for the total number of pods per plant, number of marketable pods per plant, plant height and number of branches per plant of the genotype Oda Haro. Similarly, for genotype Acc.03, both main and interaction effects were significant for all plant parameters except the main effect of the soil amendments on the number of branches (Table 3).

Table2. Mean squares of ANOVA for the nematode parameters on Oda Haro, Acc. 03 and Melka Awaze pepper genotypes according to DMRT

Source of variation	DF	GA/g	EM/g	*Pf*	RF
Genotype	2	1251.58***	1272.58***	901500000000***	391.28***
leaf extract	2	321.71***	314.57***	323600000000***	140.46***
Soil amendment	2	144.98***	98.37***	42330000000***	18.37***
Genotype x leaf extract	4	104.71**	103.06***	45260000000^{NS}	19.64***
Genotype x Soil amendment	4	46.53^{NS}	38.95**	9550000000***	4.15**
Leaf extract x Soil amendment	4	42.65**	46.48***	52200000000***	22.65***
Genotype x Leaf extract x Soil amendment	8	22.65**	13.39**	8515000000**	3.69***
Error	54	16.60	13.27	2269000000	0.98
CV (%)		4	5.9	1.3	16.7

, *- significant at 0.01, and 0.001 probability levels, respectively. NS= Not significant, CV= Coefficient of variation, D.F. =Degree of freedom, LE= leaf extracts, SA= Soil amendment, GA/g=Number of galls per 1g root, EM/g= Number of egg masses per 1g root, Pf=final nematode population, RF=Reproduction factor

Effect on galls, egg masses and nematode multiplication

Both the sole and combined treatments resulted in fewer root galls than the corresponding control on Melka Awaze (Table 4). The lowest number of root galls was on the genotype Melka Awaze (1.52), which was recorded from the combined treatment of *L. camara* with vermicompost. Similarly, genotype Acc.03 exhibited the smallest number of galls from a treatment combination of *L. camara* with vermicompost (8.62). On Oda Haro, the lowest number of galls (7.33) was recorded from the treatment combination of *L. camara* with poultry manure.

The decrease in egg mass was consistent across all treatments in Melka Awaze except with the vermicompost treatment (Table 4). However, on genotype Acc. 03, the application *V. amygdalina* with vermicompost and *L. camara* with vermicompost significantly decreased the egg masses. On the contrary, all treatments except vermicompost reduced egg masses on Oda Haro compared to the control. Application of vermicompost on genotypes Acc. 03 and Oda Haro did not affect the number of egg masses.

The application of leaf extracts and/or soil amendments significantly decreased the final nematode population (Table 3). On Melka Awaze, either sole or combined treatments markedly lowered the final nematode populations. A similar trend was observed on Acc. 03. On Oda Haro, the highest number of egg masses and the least reduction was from the integration of *V. amygdalina* with vermicompost (176713) and vermicompost treatment (563402), respectively (Table 4).

The leaf extracts and/or soil amendments, except vermicompost, significantly lowered the reproduction factor (RF) for all three genotypes compared to the control. The maximum decline in RF was from Melka Awaze for all treatments except vermicompost. On the other hand, Acc. 03 treated with vermicompost exhibited the highest RF (12.1), which is not significantly different from the control (Table 4).

Effect on growth and yield components of hot pepper genotypes

a. Genotype Oda Haro and genotype Acc.03

Sole applications of leaf extracts and soil amendments or in combinations significantly improved the number of pods, number of marketable pods, number of branches, and plant height on Oda Haro and Acc.03 compared to their respective control plants. The integrated treatment of *V. amygdalina* with vermicompost had the highest total and marketable pods per plant (19.33). The highest plant height was recorded from *V. amygdalina* with vermicompost (69.33). Oda Haro also exhibited a significant increase in the number of branches with the treatment of *V. amygdalina* with poultry manure, which had the highest number of branches (5.67) (Table 5).

Sole or combined application of leaf extracts and soil amendments resulted in higher yield and yield parameters. *Lantana camara* with vermicompost resulted in the highest number of pods per plant (Table 5). Similarly, higher marketable yield was recorded from the integrated application of *L. camara* with vermicompost on both Oda Haro and acc. 03 genotypes (Table 5) Plant height was higher in pepper treated with vermicompost (Table 5). The application of leaf extracts and soil amendments notably increased the number of branches per plant (Table 5).

b. Genotype Melka Awaze

Except for the number of marketable pods per plant, the leaf extracts combined with the soil amendments significantly affected all other growth and yield parameters on Melka Awaze. However, only the main effects were significant for the number of marketable pods per plant (Table 4). The combined application of leaf extracts and soil amendments gave higher numbers of pods, branches, and plant height than the control (Table 6). Leaf extracts with vermicompost gave more pods than the rest of the treatments. Plant height was lower in the control than in the rest of the treatments (Table 6). The number of branches tended to be lower in plants treated with leaf extracts without soil amendments (Table 6).

Both the leaf extract and soil amendment treatments gave significantly higher marketable pods than their respective untreated control (Fig 1. and 2).

Table 3. Mean squares of ANOVA for growth and yield parameters Oda Haro, Acc. 03 and Melka Awaze pepper genotypes according to DMRT

Genotype	Source of variation	DF	№ pods/plant*	№ of marketable pods/plant	Plant height	BR/Pl
Oda Haro	Leaf extracts	3	160.52***	252.5***	131.07**	14.81***
	soil amendments	3	22.84***	17.88***	109.43**	2.89***
	Leaf extracts x soil amendments	3	11.09**	5.46**	104.79**	1.42**
	Error	20	3.37	1.53	25.1	0.34
	CV (%)		11	10.2	7.8	11.9
Acc.03	Leaf extracts	3	23.49***	10.55NS	69.43***	0.67*
	soil amendments	3	11.06**	10.54NS	72.41***	0.45**
	Leaf extracts x soil amendments	3	12.79**	16.42*	38.27**	0.72**
	Error	20	3.37	3.47	11.38	0.16
	CV (%)		6	6.4	5.6	8
Melka Awaze	Leaf extracts	3	150.9***	173.36***	17.89**	1.06***
	soil amendments	3	12.62NS	34.39***	24.07***	2.67***
	Leaf extracts x soil amendments	3	13.16*	4.42NS	16.07**	1.26***
	Error	20	4.17	4.97	4.292	0.2
	CV (%)		7.8	9.2	3.8	9

*,**,***=significant at 0.05, 0.01, and 0.001 probability levels, respectively. NS= Not Significant, DF= Degree of freedom, BR/pl= number of branches per plant

Table 4. The impact of leaf extracts and organic soil amendments on the number of galls and egg masses per gram of root, the final nematode population (*Pf*) and reproduction factor (RF) of *M. incognita* on different hot pepper genotypes.

Genotype	Leaf extract	Soil amendment	Root gall	Egg mass	*Pf*	*RF*
		Vermicompost	1.52^{a}	1.14^{a}	60265^{a}	1.3^{a}
		Poultry manure	3.18^{cd}	1.75^{ab}	61827^{a}	1.3^{a}
	Lantana camara	Not amended	3.17^{bcd}	1.66^{ab}	53519^{a}	1.1^{a}
Melka Awaze		Vermicompost	2.8^{abcd}	1.72^{ab}	59346^{a}	1.2^{a}
		Poultry manure	2.29^{abc}	1.54^{a}	54157^{a}	1.1^{a}
	Vernonia amygdalina	Not amended	1.75^{ab}	1.19^{a}	52314^{a}	1.1^{a}
		Vermicompost	4.25^{de}	2.64^{b}	107393^{b}	2.2^{ab}
	No extract	Poultry manure	2.14^{abc}	1.81^{ab}	59749^{a}	1.2^{a}
	Control		6.58^{ef}	6.13^{c}	153892^{c}	3.2^{b}
		Vermicompost	8.62^{fgh}	8.31^{c-f}	266211^{d}	5.5^{c}
		Poultry manure	13.80^{g-j}	14.67^{g-j}	346087^{d-g}	7.2^{cde}
	Lantana camara	Not amended	11.94^{f-i}	12.72^{f-i}	343541^{d-g}	7.2^{cde}
		Vermicompost	8.74^{fgh}	7.40^{cde}	283088de	5.9^{cd}
Acc.03		Poultry manure	10.11^{fgh}	11.14^{e-h}	290290de	6.0^{cd}
	Vernonia amygdalina	Not amended	11.96^{f-i}	9.08^{c-g}	288508de	6.0^{cd}
		Vermicompost	12.91^{g-j}	13.12^{f-j}	582316^{hi}	12.1^{fg}
	No extract	Poultry manure	14.15^{hij}	13.06^{f-j}	424715^{g}	8.8^{e}
	Control		19.72^{ijk}	19.67^{ijk}	660161^{hi}	13.8^{g}
		Vermicompost	10.36^{fgh}	10.84^{d-g}	274670de	5.7^{c}
		Poultry manure	7.33^{fg}	6.18^{cd}	408323^{fg}	8.5^{e}
	Lantana camara	Not amended	10.34^{fgh}	9.47^{c-g}	354473^{d-g}	7.4^{cde}
		Vermicompost	9.43^{fgh}	10.41^{efg}	176713^{c}	3.7^{b}
Oda Haro		Poultry manure	15.2^{hij}	14.28^{g-j}	320094^{d-g}	6.7^{cd}
	Vernonia amygdalina	Not amended	23.74^{ijk}	19.34^{hij}	307904^{def}	6.4^{cd}
		Vermicompost	24.37^{jk}	22.42^{jk}	563402^{h}	11.7^{f}
	No extract	Poultry manure	15.71^{g-j}	15.66^{g-j}	372770^{efg}	7.8^{de}
	Control		30.38^{k}	29.48^{k}	757861^{i}	15.8^{h}

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

Table 5. The effect of leaf extracts and organic soil amendments (SA) on the number of pods per plant, marketable pod per plant, plant height and number of branches per plant of Oda Haro and Acc.03 hot pepper genotypes after infestation by *M. incognita*

Genotype	Leaf extract	Soil amendment	№ pods/plant*	№ of marketable pods/plant	Plant height(cm)	No of branches/plant
		Vermicompost	18.00^{b}	14.33^{b}	65.00^{ab}	4.83^{bcd}
		Poultry Manure	17.00^{b}	14.67^{b}	63.67^{ab}	4.50^{cde}
	L. *camara*	Not amended	17.33^{b}	14.00^{b}	60.00^{b}	4.50^{cde}
		Vermicompost	19.33^{b}	16.00^{b}	69.33^{ab}	5.33^{bc}
Oda Haro		Poultry Manure	16.67^{b}	14.33^{b}	64.00^{ab}	5.67^{b}
	V. *amygdalina*	Not amended	17.67^{b}	14.00^{b}	68.67^{ab}	3.67^{ef}
		Vermicompost	17.00^{b}	14.33^{b}	69.00^{ab}	4.33^{de}
	No extract	Poultry Manure	16.33^{b}	14.00^{b}	60.33^{b}	4.50^{cde}
	Control		12.00^{c}	10.00^{c}	50.00^{c}	3.00^{f}
	L. *camara*	Vermicompost	33.00^{ab}	30.00^{a}	64.50^{a}	5.33^{ab}
		Poultry Manure	30.67^{b}	29.33^{a}	60.00^{abc}	4.83^{b}
		Not amended	30.67^{b}	28.67^{a}	62.00^{ab}	4.67^{bc}
		Vermicompost	30.67^{b}	29.67^{a}	64.33^{a}	4.83^{b}
Acc. 03		Poultry Manure	30.67^{b}	28.67^{a}	64.33^{a}	5.00^{ab}
	V. *amygdalina*	Not amended	30.67^{b}	29.67^{a}	54.67^{cd}	5.00^{ab}
		Vermicompost	31.00^{b}	30.00^{a}	61.67^{ab}	4.83^{b}
	No extract	Poultry Manure	30.67^{b}	29.67^{a}	56.33^{bcd}	5.33^{ab}
	Control		25.33^{c}	23.67^{b}	53.00^{d}	4.00^{c}

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

Table 6. The effect of leaf extracts and soil amendments on the number of pods per plant, plant height and number of branches per plant of Melka Awaze genotype

Leaf extract	Soil Amendment	Number of pods/plants*	Plant height (cm)	Number of branches/plants
Lantana camara	Vermicompost	26.67^{b}	56.33^{a}	5.67^{a}
	Poultry manure	25.67^{b}	56.33^{a}	5.00^{ab}
	Not amended	25.00^{b}	56.33^{a}	4.67^{bc}
Vernonia mygdalina	Vermicompost	25.00^{b}	56.33^{a}	5.33^{ab}
	Poultry manure	25.67^{b}	56.67^{a}	5.67^{a}
	Not amended	25.00^{b}	51.67^{b}	4.00^{c}
No extract	Vermicompost	26.67^{b}	57.00^{a}	5.67^{a}
	Poultry manure	25.00^{b}	53.33^{ab}	4.00^{c}
Control		20.00^{c}	50.00^{b}	4.00^{c}

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

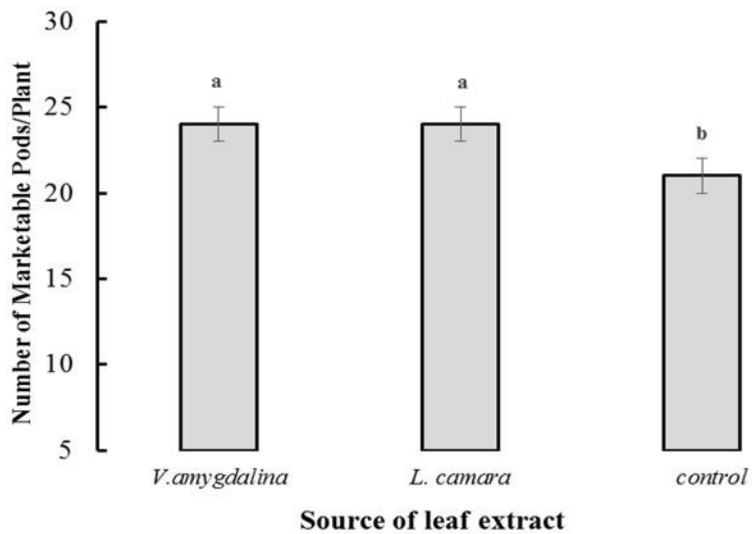

Figure 1. Effect of leaf extracts on the number of marketable pods per plant on genotype Melka Awaze

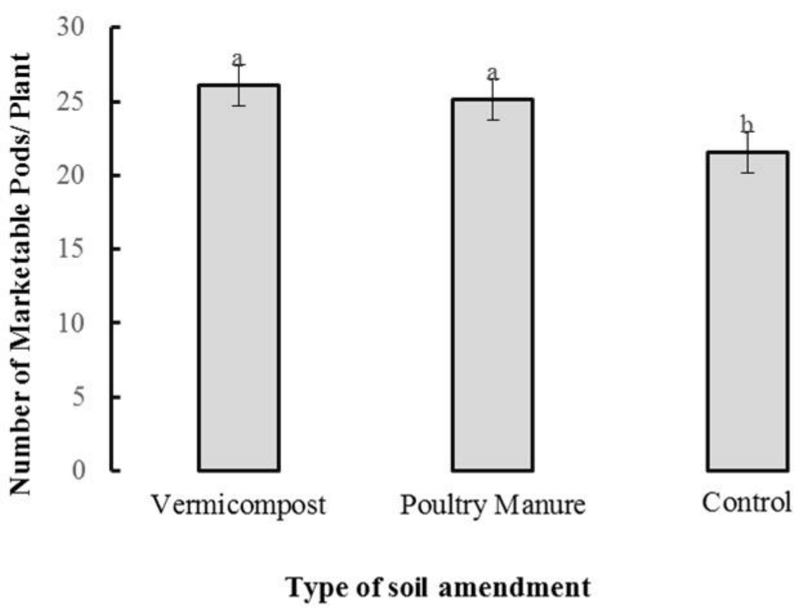

Figure 2. Effect of organic soil amendments on the number of marketable pods per plant on genotype Melka Awaze

Discussion

This study revealed that different genotypes have different abilities to suppress nematode reproduction. Specifically, the genotype Melka Awaze showed a higher resistance to nematode reproduction than Acc. 03 and Oda Haro. The effectiveness of the leaf extracts and soil amendments varied depending on the specific hot pepper genotype. Nevertheless, these treatments significantly reduced the number of galls and egg masses in the three genotypes over the control. The application of vermicompost alone did not result in a substantial decline in the nematode population or reproductive potential.

Boran et al. (2017) reported that exposing vermicompost to a one-hour thermal treatment at 70°C significantly decreases total bacteria, fungi, and enzyme activities. This could explain why treating vermicompost alone was not as effective in decreasing the nematode population, as sterilization may have depleted the beneficial microorganisms in the vermicompost that could have combated the RKN and reduced their population. The findings of an in vitro experiment to examine the suppressive effect of vermicompost on *Fusarium oxysporum* f. sp. lycopersici (Szczech, 1999) showed that the vermicompost, which was autoclaved for 30 minutes, lost its ability to suppress the fungus's mycelial growth. Similarly, Simsek-Ersahin et al. (2009) reported that vermicompost's ability to suppress *Rhizoctonia solani* was more microbiological, and the effect was lost upon heat sterilization in both Petri dish and pot experiments using cucumber.

Combined application of vermicompost and *V. amygdalina* proved to be the most effective treatment for reducing nematode population and root damage in the genotypes Melka Awaze and Oda Haro. On the other hand, *L. camara* with vermicompost was the most effective treatment on the genotype Acc.03. In another experiment, using vermicompost with garlic extract and neem oil resulted in the best control of *M. incognita* on cauliflower (Nath and Singh, 2011). Udo et al. (2014) found that a combination of *L. camara* with *Paecilomyces lilacinus* was the best treatment for inhibiting root galling and increasing yield in tomato plants, while Shiferaw et al. (2014) reported combining poultry litter with rapeseed cake significantly reduced root galling and nematode populations in tomatoes under field conditions.

A study by Taye et al. (2013) showed that using extracts from *L. camara* and *V. amygdalina* can reduce RKN due to their nematicidal potential. The same authors reported that applying these leaf extracts to tomato plants significantly reduced root galling and was more effective than soil amendments. Treating soil with powdered leaves of *V. amygdalina* also improved plant growth and significantly reduced the nematode population (Abulosoro et al., 2018). In vitro studies by Ghimire et al. (2015) showed that *L. camara* at varying concentrations had a hemostatic and nematotoxic effect, immobilizing and killing *Meloidogyne* infective juveniles. Similarly, integrating *L. camara* leaf extracts with *T. harzianum* demonstrated a considerable reduction in nematode reproduction in a greenhouse experiment on tomatoes (Feyisa et al. 2019). Our study revealed that poultry manure treatment significantly limited nematode reproduction in all genotypes. In line with this, Lopez-Perez et al. (2005) found that biofumigation by poultry manure minimized nematode infestation on tomatoes under greenhouse conditions. Shiferaw et al. (2014) also reported that poultry litter at the rate of 10 tons ha^{-1} and above significantly suppressed nematode multiplication.

Xiao et al. (2016) found that applying vermicompost to susceptible and resistant tomato cultivars can suppress root gall formation caused by *M. incognita*. While vermicompost reduced gall and egg mass formation in all genotypes except Acc.03, it was not as effective in suppressing nematode reproduction. Mishra et al. (2017) also experimented on the effect of vermicompost tea and found irregularities in the reduction of nematode population- some treatments reduced root penetration and hatching in laboratory experiments. However, not all forms of vermicompost tea suppressed nematodes in the root and soil under greenhouse conditions. In another study, Xiao et al. (2016) reported that vermicompost treatment had differential efficacy on cultivars in reducing the number of galls formed by *M. incognita.*

The current study investigated the effects of leaf extracts and soil amendments on plant growth. The outcomes differed depending on the genotype, with the combination of vermicompost and either *V. amygdalina* or *L. camara* producing better results. The integration of poultry manure with *V. amygdalina* resulted in more branches. Although vermicompost alone did not reduce the nematode population, it had a similar impact on the growth and yield of pepper. Previous studies have shown that vermicompost can enhance the growth and yield of different plants when integrated with other management options. For instance, Karagöz et al. (2019) found that autoclaved vermicompost applied alone or with plant growth-promoting bacteria at a rate of 60 kg ha^{-1} improved the growth of *Gladiolus grandiflorus* in terms of leaf number, stem diameter, and shoot height. Ansari and Sukhraj (2010) also observed that a combination of vermiwash and vermicompost significantly increased the plant height, stem circumference, number of leaves per plant, and marketable pod yield of Okra plants. Even after thermal treatment at 121^{o}C, Boran et al. (2017) reported that vermicompost's elemental and chemical composition remained unchanged, except for the humic-fulvic acid and total organic carbon. The use of vermicompost can supplement plants with extra nutrients and modify the soil's physico-chemical properties, such as water-holding capacity, which helps the plants overcome disease pressure and stress caused by a higher population of *M. incognita* in the soil. According to Osman et al. (2018), integrating poultry/chicken manure with inorganic NPK fertilizer, *Trichoderma harzianum*, or *Bacillus thuringiensis* can decrease the nematode population and improve eggplant yield. Similarly, Abdeldaym et al. (2014) found that poultry manure alone and in combination with the nematophagous fungus *Paecilomyces lilacinus* maximized Melon production.

In addition to integration, applying vermicompost, *V. amygdalina*, *L. camara*, and poultry manure can also enhance the growth and yield of pepper genotypes. According to Arancon et al. (2005), commercial vermicompost from cattle manure, food, and paper waste significantly improved the growth and pod yield of pepper. This is likely attributed to the vermicompost's ability to increase soil fertility with macro and micronutrients and stimulate plant growth-promoting hormones (Hussain and Abbasi, 2018). Taye et al. (2013) and Feyisa et al. (2019) reported an increase in plant biomass due to *L. camara* application, while Shiferaw et al. (2014) and Abdeldaym et al. (2014) observed significant improvements in plant growth and yield using poultry manure.

Conclusion and Recommendations

Combining aqueous crude leaf extracts from *L. camara* and *V. amygdalina* with vermicompost and poultry manure effectively reduces the population of *M. incognita* in pepper. While vermicompost promotes growth, its use alone does not limit the nematode build-up. Integrating vermicompost with crude leaf extracts has a significant role in nematode suppression and enhanced pepper yield. However, further research is needed to evaluate the efficacy of different concentrations of the crude leaf extracts and types of composted material against nematodes.

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