Phytochemical Screening and Nematicidal Activity of Cinnamon and Ginger Extracts Against Root-knot Nematode *(Meloidogyne incognita)* Infecting Tomato

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Abstract

The purpose of this work was to assess the nematicidal and antioxidant activities of different extracting solvents from cinnamon (*Cinnamomum zeylanicum*) and ginger (*Zingiber officinale*) against *Meloidogyne incognita* infecting tomato. Three solvents namely n-hexane, ethyl acetate and methanol were used. Phytochemical analysis of such extracts revealed the presence of more components in ethyl acetate and methanolic extracts than *n*-hexane extract of either cinnamon or ginger. The highest amount of total phenolics and flavonoids were detected in ethyl acetate extract of both ginger and cinnamon reaching 105.6; 93.6 mg gallic acid /g and 54.2; 45.1 mg quercetin/ g dry weight, respectively. Additionally, ethyl acetate and methanolic extracts of ginger exhibited the highest antioxidant activity (DPPH free radicals) reaching 90.0 and 89.1%, respectively. The efficacy of cinnamon and ginger using three solvents at two concentrations (50 and 10ul) on juveniles mortality of *Meloidogyne incognita* was studied *in vitro*. Ethyl acetate extracts gave better results than did methanol or hexane extracts. Ginger extracts gave promising results after 72hr of exposure compared to cinnamon ones. Hence, among ginger extracts the maximum mortality in nematode juveniles was achieved with ethyl acetate (68.0 %) followed by methanol (42.0%) and hexane extracts (30%) @ the concentration of 50µl after 72 hours of exposure. Ginger ethyl acetate (300 ppm) proved to be the best for enhancing total plant fresh weight with percentage of increase over control amounted to 196.0 %. Population densities, root galling and number of egg masses were significantly suppressed with such tested extracts. Leaves of tomato were assayed for NPK, total cholorophyll, proteins, and phenols. Activities of polyphenol oxidase (PPO), peroxide oxidase (PO) were also evaluated in roots of tomato infected with *M. incognita*. PO activity was much greater in ginger ethyl acetate at lower concentration (100 ppm). Conversely, PPO activity was increased in cinnamon ethyl acetate (100 ppm).The present study revealed high potential antioxidants and nematicidal properties in both cinnamon and ginger. The biological activities of these plants might be attributed to the various kind of secondary metabolites.

Key words: *Cinnamomum zeylanicum*, *Zingiber officina*le, nematicidal activity, antioxidant, total phenolics, flavonoids, polyphenol oxidase, peroxide oxidase, *Meloidogyne incognita*.

Introduction

Now a day's most of the researches focus on medicinal plants because of high pharmacological activity and low toxicity. It is mostly used in folklore medicine. Rhizome or root part of ginger is extensively employed in medicine for the management of different diseased conditions like nausea, vomiting, diabetes, fever, gastrointestional ulcers, arterial tension **(Ghasemezadeh et al., 2011)**. Ginger (*Zingiber officinale*) include extractable oleoresins, many fats, carbohydrates, vitamins, minerals and flavonoids **(Shukla and Singh, 2007)**. Flavonoids are large family of polyphenolic components synthesized by plants. They are functioned to reduce blood lipid, glucose and enhance human immunity Flavonoids were also a kind of natural antioxidant capable of scavenging free superoxide radical, anti aging and reducing the risk of cancer **(Atoui et al., 2005)**.

Bark cinnamon (*Cinnamomum zeylanicum*) is a favorite spice around the world because of its health benefits, flavors and preserves food. The most favorite chemical constituents of cinnamon are volatile oils (cinnamaldehyde, eugenol, cinnamic acid and weitherhin), mucilage, diterpens and proanthocyanidius.

In developing countries 65-80% of population depends upon herbal medicines for primary health care (**Oladele and Ayoola, 2015)**. Different categories of bioactive compounds are being isolated and characterized since the middle of 19^{th} century. Herbal medicines provide rich amount of tannins, alkaloids, flavonoids, phenolic compounds and so forth, so these can be used in the treatment of several degenerative disorders **Ali et al. (2015)**.

Phytochemicals i.e. various secondary metabolites of plants are an essential component of our diet. Phytochemicals are responsible for the beneficial properties which plants possess like antimicrobial, anti-inflammatory, antioxidant, insecticidal and fungicidal properties (**Shreya et al., 2015)**. Phenolic compounds are known to behave as reducing agents, hydrogen donators and singlet oxygen quenchers because of redox properties responsible for their antioxidant activity (**Pinho et al., 2014)**. Antioxidants are of interest to biologists and clinicians because they protect human body against damages induced by reactive free radicals generated in atherosclerosis, ischemic heart diseases, cancer and even in aging process (**Aruoma, 2003)**. DPPH is a free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (**Soares et al., 1997)**.

Tomato *(Solanum lycopersicum* Mill.) is one of the most important vegetables crops in the Egyptian Agriculture, cultivated in over 739038 feddans with a production of about 1287957 tones in 2016/2017 and it is a major component of daily meals in many countries. It constitutes an excellent source of health-promoting compounds due to the balanced mixture of minerals and antioxidants including ascorbic acid, vitamin E, lycopene, beta-carotene, xanthopyll and flavonoides. The root–knot nematode *Meloidogyne incognita* (**Kofoid & White**) **Chitwood** is among

the most important nematode infecting tomato. Efforts to protect such crop from root-knot nematodes infection are crucial. Because of the lack of plant resistance to most species of root-knot nematode as well as the environmental restrictions on nematicidal use for controlling plant parasitic nematodes, biological control and other eco-friendly disease control measures have received greater interest. The potential of plant extracts for the control of *Meloidogyne* spp. was undertaken by several researchers (**Zawam et al., 2003; Abbas et al., 2009; Khairy, 2016 and El Deriny, 2016).** In recent years, the activation of defense related enzymes through plant extracts has been considered as a focus of research only for the control of plant pathogens. Therefore the aim of the present study was to investigate the effect of ginger and cinnamon extracts using different polarity solvents on plant growth of tomato infected with *Meloidodyne incognita*. Nematicidal and antioxidant activities as well as chemical composition of studied plants were also undertaken.

Materials and Methods

1. Plant materials

Plant parts i.e bark of cinnamon (*C. zeylanicum*) and rhizome of ginger (*Z. officinale*) were obtained from the Egyptian local market.

2. Preparation of n. hexane extract

One hundred grams of each plant part understudy were macerated three times for a 3-day-period in n-hexane and filtered. The combined filtrate was evaporated under reduce pressure to dryness (crude oil matter). The crude oil was put in a dark bottle and kept at 4°C until use **(Peskin and Toroglu, 2011**). However, the percentage yield of n-hexane was calculated as follows:

%Yield = (Weight of extract recovered / Weight of dry powder) × 100

3. Preparation of ethyl acetate and methanolic extracts

One hundred grams of defatted plant were extracted using soaking method with ethyl acetate followed by 80% methanol separately for 6 hours. The extracts were filtered through filter paper (Whatman No.1). The solvents were evaporated to dryness using water bath set at 60°C. The residues were weighed and stored at 4°C until use (**Ahmad et al., 2013**). However, the percentage yield (ethyl acetate or methanolic extract) was calculated as follows:

%Yield = (Weight of extract recovered / Weight of dry powder) × 100

4. Phytochemical analysis

Phytochemical tests were carried out on *n*.hexane, ethyl acetate and 80% methanol extracts of tested plants to detect the major constituents. The extracts were dissolved in dimethyl sulfoxide (DMSO). The filtrates were used for the phytochemical examinations. The tests were performed for alkaloids (Mayers/

Wagners test), flavonoids (lead acetate test), tannins (ferric chloride test), saponins (foam test), steroids and terpenoids (Salkowski test) and glycosides (keller-killiani test), according to the protocol described by **Jonathan et al. (2012)**.

5. Determination of total phenolic content

The amount of total phenolic in *n*-hexane, ethyl acetate and methanolic extracts of cinnamon and ginger was determined by the Folin-Ciocalteu reagent, according to **Maurya and Singh (2010)**. One mlliliter of the dissolved extract was mixed with 0.5ml of Folin-Ciocalteu reagent and 7.5ml of distilled water. The mixture was kept at room temperature for 5min. Ten milliliters of sodium carbonate 7% were added to the mixture and incubated at room temperature for 90 min. All samples were done in triplicate. After incubation, the absorbance against the reagent blank was determined at 760nm. Total phenolic content of the extracts was expressed as gallic acid equivalent (g/100g dry weight)

6. Determination of total flavonoids

Aluminum chloride method was used to determine total flavonoid content (TFC) in ethyl acetate and methanolic extracts of cinnamon and ginger according to **Olajire and Azeez (2011)**, as follows: One milliliter containing 100µg/ml extract was diluted with 4ml of distilled water in a 10ml volumetric flask. 0.3ml of 5% of NaN_{O3} solution was added to each the volumetric flask. After 5 minutes 0.3ml of 10%$AlCl_3$ was added to mixture. Then after 6 minutes, 2ml of 1M NaOH was added and the volume made up to 10ml with distilled water. The absorbance was noted at 510nm using UV-visible spectrophotometer. TFC was determined as quercetin equivalent (g/100g of dry weight).

7.Calculated of non-flavonoids substances.

Non-flavonoids substances was calculated according to **Kyselvora (2011)**, as follows:

Non-flavonoids = Total phenolic – Total flavonoid

8. Antioxidant activity:

The 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

The antioxidant activity of the hexane, ethyl acetate and methanolic extracts of cinnamon and ginger was assessed by their ability to scavenge DPPH stable radicals as reported earlier (**Mimica-Dukic et al., 2003**). Each extract (250µg/ml) was mixed with DPPH solution (1ml; 90µM) and then with methanol 95% to a final volume of 4ml. Synthetic antioxidant butylated hydroxyl toluene (BHT) was used as control. After 1h incubation period at room temperature, the absorbance was recorded at 515nm. Percent radical scavenging concentration was calculated using the following formula:

%radical scavenging= (A (blank) - A(sample) / A (blank)) × 100

Where: A (blank) = Absorbance of the control; A (sample) = Absorbance of the test sample.

9. Preparation of *Meloidogyne incognita* inocula:

***M. incognita* juveniles inoculum:**

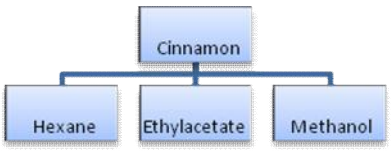
Fresh second stage juveniles of the root-knot nematode, *M. incognita* were obtained from pure culture maintained on coleus (*Coleus blumei*) roots. Roots were incubated in a modified Baermann technique for hatching at room temperature for 5-7 days.

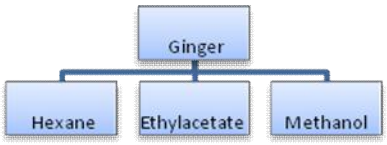
10. Laboratory Experiments:

10.1. Impact of cinnamon and ginger extracts on juveniles mortality of root-knot nematode *Meloidogyne incognita*

Three different solvents i.e. Hexane, Ethyl acetate and Methanol were used for cinnamon and ginger extracts. Such extracts were tested against second stage juveniles (J_2) of the root-knot nematode, *M. incognita*, comparing by DMSO solvent and nematodes alone in Petri dishes (5 cm-d) where there are three replicates for each extract. Five ml of DMSO solvent were added to 0.025 g of each plant species (i.e. cinnamon and ginger). Two concentrations (10 & 50 µl) were taken from each solvent. Four ml of each plant extract were added to one ml of *M. incognita* (100 J2) inocula per Petri dish. The dishes were examined under a microscope after 24, 48 and 72-hours, and then left for 7 days to study the effect of such extracts on the activity of second stage juveniles of root-knot nematode under laboratory conditions.

Tested Bio-agents concentrations





@ 50 & 10 µl for each treatment.

11. Greenhouse Experiments:

11.1. Evaluating of cinnamon and ginger extracts against *Meloidogyne incognita* infecting tomato plants under greenhouse conditions (27±5ºC):

Extraction of cinnamon and ginger using three solvents i.e. Hexane, Ethyl acetate and Methanol) was singly tested at the concentration of 100, 200 and 300

ppm in comparison with oxamyl for controlling *M. incognita* infecting tomato seedlings under greenhouse conditions (27±5ºC). Forty tomato seedlings cv. Castle Rock (21 days- old) were each inoculated with 2000 eggs of *M. incognita*. In addition to another five tomato seedlings, one seedling/ pot were included as plant free of nematode and without any of the treated compounds to serve as check. Therefore, treatments were as follows:

1. Cinnamon ethyl acetate (100 ppm) @ 10ml/pot
2. Cinnamon ethyl acetate (200 ppm) @ 10ml/pot
3. Cinnamon ethyl acetate (300 ppm) @ 10ml/pot
4. Ginger ethyl acetate (100 ppm) @ 10ml/pot
5. Ginger ethyl acetate (200 ppm) @ 10ml/pot
6. Ginger ethyl acetate (300 ppm) @ 10ml/pot
7. Oxamyl (O) @ 0.3ml/pot,
8. Untreated Uninoculated plants and
9. Nematode alone (N).

Plastic pots were arranged in a randomized complete block design on a greenhouse bench maintained at 27±5ºC. Plants received water as needed. Plants were harvested after 55 days from nematode inoculation. Data dealing with plant length and fresh weights of shoot and root and shoot dry weight were determined and recorded. Infected tomato roots were separately washed by tap water, fixed in 4% formalin for 48 hrs and stained in acid fuchsin lactic acid (**Byrd, 1983**) and examined with stereoscopic microscope for counting the numbers of galls, egg masses, developmental stages and females for *M. incognita* and recorded. *M. incognita* (j_2) was extracted from soil through sieving and modified Baermann technique (**Goodey, 1957**), counted and recorded.

N, P, K and chlorophyll content in tomato leaves treated with the tested extracts and the untreated plants as well were determined and recorded. Data were subjected to analysis of variance (ANOVA) **Gomez and Gomez, 1984**, followed by Duncan's multiple range tests to compare means (**Duncan, 1955**).

Determination of total phenols:

Total phenols were determined after harvesting in fresh whole plant using Folin-Ciocalteau reagent (**Kaur and Kapoor 2002**). Total content of phenolic compounds in plant ethanolic extracts was calculated as catechol equivalents by the following equation:

$$T = \frac{C \times V}{m} \times 100$$ where:

T: Total content of phenolic compounds (mg of catechol/100 g of fresh weight material).

C: The concentration of catechol established from the calibration curve (mg/ml).

V: The volume of extract (ml).

m: The weight of pure plant ethanolic extract (g).

***Preparation of enzyme extract*:**

Enzyme extracts were prepared following the method described by **Maxwell and Bateman (1967)**. Dry root tissues (0.5 g) of each treatment were ground in 3 ml Na-phosphate buffer at pH 6.8 in a mortar and then centrifuged at 1.500 g / 20 min at 6 °C. The resultant supernatant fluids were processed for enzyme assays.

Peroxidase activity (PO)

Peroxidase was assayed using photochemical method as described by **Amako et al. (1994).** The reaction mixture was added as the following sequences, 1500 ml phosphate buffer.,480 ml hydrogen peroxidase., 1000 ml pyrogallol, 20 ml sample extract. The increasing in the absorbance at 430 nm was recorded against blank with phosphate buffer instead of enzyme extract. One unit of enzyme activity was defined as the amount of the enzyme, which changing the optical density at 430 nm per min. at 25°C under standard assay conditions. Specific activity was expressed in units by dividing it to mg protein.

Polyphenol oxidase (PPO)

Polyphenol oxidase was assayed using photochemical method as described by **Coseteng and Lee (1987).** The reaction mixture was added as the following sequences: 2.7 ml potassium phosphate buffer 90.05M, pH 6.2, 0.25 ml of 0.25 M catechol, 0.05 ml of enzyme extract. The increasing in absorbance at 420 nm was measured. One unit of enzyme activity is defined as the amount of the enzyme that causes an increase of 0.001 absorbance unit per minute at 25°C.

Results and Discussion

1. Yield percentage of plant extracts:

Cinnamon bark extracts yielded the highest percentage reaching 14.3, 13.2and 8.0 in case of ethyl acetate, methanolic and n-hexane, respectively (Table 1). While, the highest yield in ginger was recorded in ethyl acetate extract (7.3%). This finding is logical since n-hexane extract (non polar) represents lower percentage compared to ethyl acetate and methanol 80% (polar part) which represent high percentage of the tested plant extracts.

Table (1): Yield percentage of ginger and cinnamon extracts.

Extracts	(%) yield
Ginger	
n.Hexane	6.5
Ethyl acetate	7.3
Methanolic	5.4
Cinnamon	
n.Hexane	8.0
Ethyl acetate	14.3
Methanolic	13.2

The differences in composition of certain plants might be attributed to the environmental variations, age of the plant, extraction process, nature of the extraction solvent and part of the plant used (**Womeni et al., 2013).** The amount of extractable components from *C. zeylanicum* using methanol was revealed in the literature as 8.4% **(Voukeng, 2011)** and *Z. officinale* as 9.2% **(Khalaf et al., 2008)**. Moreover, **Womeni et al. (2013)** measured the yield of *C. zeylanicum* and *Z. officinale* methanolic extract and found it to be 25.0 and 8.25%, respectively. Variations in yield extracts from different plant materials might be attributed to the availability of different extractable components defined by the chemical composition of the plant, nature of the soil and agro-climatic conditions **(Hsu et al., 2006).**

2. Phytochemical screening:

Extracts were subjected to phytochemical analysis to ascertain the various secondary metabolites present in plants under study (Table 2). Alkaloids, tannins, steroids, terpenoids were found to be present in ethyl acetate and methanolic extracts of *C. cassia* bark. However, saponins were absent in all extracts. These results are in agreement with those reported by **Ahmad et al., (2013).**

Table (2): Chemical constituents of *n*-hexane, ethyl acetate and methanolic extracts of cinnamon (*Cinnamomum zeylanicum*) bark.

Extracts / Components	*n*-Hexane	Ethyl acetate	Methanolic
Saponins	-	-	-
Flavonoids	-	+	+
Alkaloids	-	+	+
Steroids	+	+	+
Phenols	+	+	+
Terpenoids	+	+	+
Glycosides	-	+	+
Tannins	+	+	+

Where: +: detected -: not-detected

Cinnamon and ginger have a wide range of biological activities that are attributed to its active constituents that increase the quality of the medicinal plants **(Shukla and Singh, 2007)**. The rhizomes had the maximum amount of active components. The product of active component is also varied in the different method of solvent extraction **(Rubila and Ranganathan, 2014)**. Herein**,** phytochemical analysis of *Z. officinale* rhizome extracts showed the presence of alkaloids, saponins, flavonoids, glycosides, tannins, terpenoids and phenols in ethyl acetate and methanolic extracts (Table 3). Results recorded the absence of steroids in all extracts. While, *n*-hexane extract of ginger contains tannins, phenols and terpenoids only. These results support the findings of **Riaz et al. (2015)** and **Bhargava et al. (2012).**

Table (3): Chemical constituents of *n*-hexane, ethyl acetate and methanolic extracts of ginger (*Zingiber officinale*) rhizome.

Extracts / Components	*n*-Hexane	Ethyl acetate	Methanolic
Saponins	-	+	+
Flavonoids	-	+	+
Alkaloids	-	+	+
Steroids	-	-	-
Phenols	+	+	+
Terpenoids	+	+	+
Glycosides	-	+	+
Tannins	+	+	+

Where: +: detected -: not-detected

3. Total phenols and flavonoids contents

Total phenols, flavonoids and non-flavonoids content found in ginger and cinnamon extracts as mg/g dry weight are shown in table (4). Results demonstrated that ethyl acetate extract both of ginger and cinnamon contains the highest amount of phenolic and flavonoids, reaching 105.6; 93.6mg gallic acid/g and 54.2; 45.1mg quercetin/g dry weight, respectively. While, the lowest amount of phenolic and not flavonoids contents were detected in *n*-hexane extract both of ginger and cinnamon. In methanolic extracts, total phenolic and flavonoids contents were higher in ginger than cinnamon, reaching 85.4mg gallic acid/ g extract and 32.4mg quercetin /g extract, respectively.

Table (4): Quantitative of total phenolic, flavonoids and non-flavonoids in ginger and cinnamon extracts.

Components / Extracts	Total phenolic (gallic acid mg/g)	Total flavonoids (Quercetin mg /g)	Non-flavonoids
Ginger			
n.Hexane	73.4	n.d.	73.4
Ethyl acetate	105.6	54.2	51.4
Methanolic	85.4	32.4	53.0
Cinnamon			
n.Hexane	61.3	n.d.	61.3
Ethyl acetate	93.6	45.1	48.5
Methanolic	65.1	24.8	40.3

n.d.: not detected

The highest amount of non-flavonoids substances was detected in *n*-hexane extract of ginger which recorded 73.4mg/g dry weight. On the other hand, the lowest amount was detected in case of methanolic extract of cinnamon reaching 40.3mg/g dry weight, as shown in table (4). These results are in agreement with **Womeni et al. (2013)** who found that total phenols in methanolic extract of *Z. officinale* are higher than those of *C. zeylanicum* reaching 17.72 and 4.31 g GAE/100g extract, respectively. Differences between the results were likely due to genotopic and environmental differences namely climate, location, temperature, fertitility diseases and pest exposure within species choice of parts tested, time of taking samples and determination methods (**Shan et al., 2005**).

4. Antioxidant activity.

The reduction capability of DPPH radical was determined by the decrease in absorbance induced by plant extract antioxidants (Table 5).

Table (5): Antioxidant activity of ginger and cinnamon extracts.

Tested sample	DPPH radical scavenging (%)
Ginger	
n-Hexane	63.3
Ethyl acetate	88.0
Methanolic	85.2
Cinnamon	
n-Hexane	45.5
Ethyl acetate	84.0
Methanolic	73.6
BHT	
	88.5

BHT=Butyl Hydroxy Toluene

Ethyl acetate and methanolic extracts of ginger exhibited the highest antioxidant activity on DPPH free radicals, similar to that of BHT (88.5%), reaching 88.0 and 85.2%, respectively. While, the lowest antioxidant activity was observed in *n*.hexane extract both of ginger (63.3%) and cinnamon (45.5%). It was observed that the extracts showing greater amounts of total phenolic also exhibited higher antioxidant activity. These results are in line with the findings of **Womeni et al. (2013).**

Results obtained in this study are not far to those found in the literature: Antioxidant activity reached 90.1% for methanolic extract for *Z. officinale* **(Stoilova et al., 2007)** and 94.4% for essential oil of *C. zeylanicum* **(Schmidt et al., 2006)**. The difference observed might be attributed to be nature of the extraction solvent, to the part used and also to the method used. Antioxidant activity was determined by DPPH assay for different solvents extracts of ginger according to **Qadir et al. (2017)**. They found the maximum activity in aqueous extract (32.5µ g/ml) followed by acetone (28.3µg/ml) and methanol (24.6µg/ml). The lowest activity was detected in ethanol extract of ginger reaching 17.8µg/ml. Moreover, **Rubila and Ranganathan, (2014)** found the methanolic extract of ginger showed antioxidant activity reaching 67.6%.

5. Laboratory Experiment:

5.1. Impact of cinnamon and ginger extracts on second juveniles moratlity of root-knot nematode *Meloidogyne incognita*

The efficacy of cinnamon and ginger using three solvents i.e. Hexane, Ethyl acetate and Methanol at two concentrations (50 and 10ul) on juveniles mortality of *M. incognita* compared to DMSO solvent and nematode alone is represented in table (6). Irrespective to concentrations and solvents used, all extracts were found to cause nematode mortality to various degrees. Ethyl acetate extracts gave better results than did methanol or hexane extracts. Moreover, ginger extracts gave promising results after 72hr of exposure compared to cinnamon ones. Hence, among ginger extracts the maximum mortality in nematode juveniles was achieved with ethyl acetate (68.0 %) followed by methanol (42.0%) and hexane extracts (30%) @ the concentration of 50 µl after 72 hours Similar trend was noticed with cinnamon extracts with percentage of mortality reached 48.0, 40.0 and 30.0% for ethyl acetate, methanol and hexane extracts respectively. These results are in accordance with the findings of **Asadi Sardari et al. (2015) and Salim et al. (2016).** Higher concentrations of aqueous ginger extract (100% concentration) suppressed *M. javanica* egg hatching and caused juveniles mortality **(Amer-Zareen, 2003).** However, the highest percentage of *Meloidogyne* spp. mortality was achieved by application of nerium extract (78.2%) followed by neem (43.4%), ginger (39.1%), garlic and eucalyptus (30.4%), castor bean (26.0%) and cinnamon (21.7%) in concentration 5% **(Salim et al., 2016).**

Table (6): Impact of cinnamon and ginger extracts on juveniles mortality of root-knot nematode *Meloidogyne incognita* under laboratory conditions.

Treatments	Extracts	Mortality %					
		Conc.					
		50 µl			10µl		
		Exposure period					
		24hr	48hr	72hr	24hr	48hr	72hr
Cinnamon	Hexane	11.0^{f}	18.0^{f}	30.0^{e}	11.0^{e}	16.0^{e}	29.0^{e}
	Ethyl acetate	24.0^{b}	32.0^{b}	48.0^{b}	18.0^{b}	20.0^{d}	37.0^{b}
	Methanol	16.0^{d}	30.0^{d}	40.0^{d}	14.0^{d}	24.0^{b}	31.0^{c}
Ginger	Hexane	13.0^{e}	24.0^{e}	30.0^{e}	11.0^{e}	20.0^{d}	28.0^{f}
	Ethyl acetate	33.0^{a}	41.0^{a}	68.0^{a}	21.0^{a}	37.0^{a}	50.0^{a}
	Methanol	21.0^{c}	31.0^{c}	42.0^{c}	16.0^{c}	21.0^{c}	30.0^{d}
Nematode alone		0.0^{g}	0.0^{g}	0.5^{f}	0.0^{f}	0.0^{f}	3.0^{g}
DMSO solvent		0.0^{g}	0.0^{g}	0.5^{f}	0.0^{f}	0.0^{f}	3.0^{g}

Each value presented the mean of six replicates.

Means in each column followed by the same letter(s) did not differ at P ≤ 0.05 according to Duncan`s multiple range test.

6. Greenhouse Experiments:

The influence of cinnamon and ginger ethyl acetate at three concentrations (100, 200, and 300 ml) on plant growth response of tomato plant cv. Castle Rock infected with *M. incognita* is shown in table (7). Results revealed that *M. incognita* infection caused a significant reduction in plant growth parameters (shoot and root length, shoot weight) with reduction percentage in total plant fresh weight reached 28.0%. Irrespective to tested concentrations, all treatments showed remarkable improvent in plant growth parameters with various degrees. A significant improvement in shoot length (108.33%), plant fresh weight (196.0 %) and shoot dry weight (260.0%) was recorded with ginger ethyl acetate @ 300 ppm concentration (10ml/plant). The increase in such plant parameters showed that tested extracts acted as plant growth promoters which is in accordance with those reported by **Farahat et al. (1993)**; **Ibrahim (2010& 2013)**; **Bawa et al. (2014) and El-Deriny (2016)** Oxamyl as a standard nematicide showed moderate improvement in previous criteria of tomato with % of increase reached 84.52, 28.0 and 80%, respectively.

Table (7): Impact of cinnamon and ginger ethyl acetate extracts on plant growth parameters of tomato cv. Castle Rock infected with *Meloidogyne incognita* under greenhouse conditions (27±5ºC).

Treatments	Conc./ ppm	*Plant growth response									
		Length (cm)				Plant fresh wt.(g)		Total	Inc. %	Shoot Dry wt. (g)	Inc. %
		Shoot	Inc. %	Root	Inc. %	Shoot	Root				
Cinnamon Ethyl acetate	100	18.5^{g}	10.1	6.0^{f}	13.2	2.4^{f}	0.2^{e}	2.6^{e}	4.0	0.6^{g}	20.0
	200	24.0^{e}	42.9	10.0^{d}	88.7	2.9^{d}	0.6^{c}	3.5^{d}	40.0	0.8^{e}	60.0
	300	28.5^{c}	69.6	12.0^{c}	126.4	3.1^{c}	0.9^{b}	4.0^{c}	60.0	1.0^{c}	100.0
Ginger Ethyl acetate	100	23.0^{f}	36.9	8.0^{e}	50.9	2.7^{e}	0.4^{d}	3.1d^{e}	24.0	0.7^{f}	40.0
	200	26.5^{d}	57.7	13.0^{b}	145.3	5.9^{b}	0.9^{b}	6.8^{b}	172.0	1.6^{b}	220.0
	300	35.0^{a}	108.3	16.0^{a}	201.9	6.2^{a}	1.2^{a}	7.4^{a}	196.0	1.8^{a}	260.0
Oxamyl		31.0 ^{b}	84.5	12.0 ^{c}	126.4	2.1^{g}	1.1^{a}	3.2^{de}	28.0	0.9^{d}	80.0
Untreated Uninoculated plants		17. 6^{h}	4.8	10.3^{d}	94.3	2.0^{g}	1.2^{a}	3.2^{de}	28.0	0.7^{f}	40.0
N alone		16.8 ^{i}	0.0	5.3^{g}	0.0	1.9^{h}	0.6^{c}	2.5^{ef}	0.0	0.5^{h}	0.0

*Each value presented the mean of five replicates.

Means in each column followed by the same letter(s) did not differ at $P \leq 0.05$ according to Duncan`s multiple range test.

Table (8): Impact of cinnamon and ginger extracts on population densities and reproduction of *Meloidogyne incognita* infecting tomato cv. Castle Rock under greenhouse conditions (27±5ºC).

Treatments	Conc.	Nematode population in *			Final population	** Rf	Red. %	No. of galls	Red. %	No. f Egg masses	Red. %
		Soil	Root								
			Developmental stages	Females							
Cinnamon Ethyl acetate	100	3478.0^{b}	49.0^{b}	38.0^{b}	3565.0^{b}	3.6	40.4	35.0^{b}	25.5	29.0^{b}	17.1
	200	2600.0^{c}	38.0^{c}	35.0^{c}	2673.0^{c}	2.7	55.3	35.0^{b}	25.5	24.0^{c}	31.4
	300	2322.0^{d}	32.0^{e}	30.0^{f}	2384.0^{d}	2.4	60.1	33.0^{c}	29.8	22.0^{e}	37.1
Ginger Ethyl acetate	100	2150.0^{e}	33.0^{d}	35.0^{c}	2218.0^{e}	2.2	63.0	26.0^{d}	44.7	23.0^{d}	34.3
	200	1880.0^{f}	28.0^{f}	28.0^{e}	1936.0^{f}	2.0	67.7	23.0^{e}	51.1	20.0^{f}	42.9
	300	1470.0^{g}	22.0^{g}	24.0^{g}	1516.0^{g}	1.5	74.7	20.0^{f}	57.4	20.0^{f}	42.9
Oxamyl		1100.0 ^{h}	19.0^{h}	33.0^{d}	1152.0^{h}	1.2	80.8	19.0 ^{g}	4.0	10.0 ^{g}	71.4
N alone		5850.0 ^{a}	58.0^{a}	49.0^{a}	5957.0^{a}	6.0	0.0	47.0 ^{a}	4.0	35.0 ^{a}	0.0

*Each value presented the mean of five replicates. N = *M. incognita* (1000 J_2/ plant)

** Rf= $\frac{\text{nematode population in soil + No. of developmental stages + No. of females + No. of egg masses}}{\text{No. of eggs inoucula}}$

Means in each column followed by the same letter(s) did not differ at P ≤ 0.05 according to Duncan's multiple range test.

Cinnamon and ginger ethyl acetate at tested concentrations showed nematicidal properties against root-knot nematode, *M. incognita* infecting tomato plants (Table 8). Total nematode population was significantly suppressed with all tested treatments with reproduction factor (Rf) ranged from 1.2 to 2.7. Ginger ethyl acetate (300 ppm) performed the best and significantly suppressed total nematode population (74.7%), root galling (57.4%), and number of egg masses (42.9%). However, cinnamon ethyl acetate at the concentration of 300 ppm exhibited moderate reduction in nematode population (60.1%), root galling (29.8%) and number of egg masses (37.1%). On the other hand, total nematode population was significantly suppressed with oxamyl (80.8%) relative to control plants.

The current study revealed the nematicidal activity of ginger as well as cinnamon ethyl acetate against *M. incognita* infecting tomato plant, that could be attributed to the presence of saponins, alkaloids, flavonoids, phenols, terpenoids, glycosides and tannins in ginger ethyl acetate (300 ppm). **Patel et al. (1993)** noticed that plant extracts of neem, chili pepper, ginger and garlic on the microplots suppressed the population of *M. incognita* and *M. javanica* in tomato. **Singh et al. (2011)** stated that ginger and curcuma caused anthelminthic effect which may be due to the synergistic effect of active phyto-constituents including alkaloids, saponins, flavonoids, terpenes, steroids etc.. present in the extracts. **Youssef et al. (2015)** showed that ginger applied as soil drench at concentrations of 10, 5 and 2.5 % decreased nematode criteria including number of galls and egg masses and hatched juveniles in roots and soil of eggplant infected with *M.incognita***.** The potential of cinnamon ethyl acetate for the control of *M.incognita* infecting tomato could be related to the presence of alkaloids, flavonoids, phenols, steroids, terpenoids, glycosides and tannins.

Phenol content

Total phenol evaluated in whole plant of tomato infected with *M. incognita* revealed a significant increase in total phenol compared to untreated uninoculated plants (Table 9). However, the highest reduction in total phenol was recorded with ginger ethyl acetate (20.6%) at 300 ppm concentration. These results are in line with the findings of **Ibrahim (2010&2013); Youssef et al. (2015); El-Deriny (2016).**

Defense related proteins

Data presented in (Table 9) revealed that cinnamon and ginger ethyl acetate at the three tested concentrations and oxamyl as well differed in their ability to stimulate peroxidase (PO) and polyphenol oxidase (PPO) activities in tomato plant inoculated with *M.incognita*. In such treatments increased PO activity was more pronounced in ginger then cinnamon ethyl acetate at lower concentration (100 ppm) compared to untreated inoculated plants. Conversely, PPO activity was increased in cinnamon ethyl acetate at the concentrations of 100 ppm and 300 ppm then ginger ethyl acetate (100 ppm) these results agreed with **Ibrahim (2013) and El-**

Deriny (2016) who reported that PO and PPO have been related to defense mechanism against *M. incognita* infecting sugar-beet and cucurbits respectively.

Table (9): Impact of cinnamon and ginger extracts on peroxidase (PO), polyphenol oxidase (PPO) activities and phenolic compounds in whole plant of tomato cv. Castle Rock infecting of *Meloidogyne incognita* under greenhouse conditions (27±5ºC).

Treatments	Conc.	Enzyme activities (Units / mg protein)		Total phenol
		PO	PPO	
Cinnamon Ethyl acetate	100	0.315 ^{c}	0.438 ^{b}	0.58^{b}
	200	0.307 ^{e}	0.426 ^{f}	0.57^{c}
	300	0.298 ^{f}	0.437 ^{c}	0.55^{d}
Ginger Ethyl acetate	100	0.321 ^{b}	0.432 ^{e}	0.53^{e}
	200	0.311 ^{d}	0.423 ^{g}	0.51^{f}
	300	0.295 ^{g}	0.415 ^{h}	0.50^{g}
Oxamyl		0.266 ^{h}	0.433 ^{d}	0.58^{b}
Untreated Uninoculated plants		0.256^{i}	0.403^{i}	0.44^{h}
N alone		0.398 ^{a}	0.458 ^{a}	0.63^{a}

Each value presented the mean of five replicates. N = *M. incognita* (2000 eggs/ plant)

Means in each column followed by the same letter (s) did not differ at P ≤ 0.05 according to Duncan's multiple range test.

Total chlorophyll

Infected plants of tomato exhibited significant reduction in photosynthetic pigment contents (chlorophyll *a*, and *b*) compared to non-infected ones with percentage of reduction in total chlorophyll reached 16.7 % (Table 10). A significant induction in total chlorophyll (8.57%) was recorded with pots receiving the application of ginger ethyl acetate at the concentration of 300 ppm. Results are not far to those found in the literature: **Ibrahim (2010&2013); Youssef et al. (2015) and El-Deriny (2016).**

Biochemical activities

Nitrogen, phosphorus and potassium contents.

Untreated tomato infected with *M. incognita* exhibited significant reduction in N, P and K contents as compared with untreated uninoculated plants (Table 10). A remarkable induction in nitrogen (240.0%) phosphorus (153.3%) and potassium (105.9%) contents was recorded with ginger ethyl acetate (300 ppm). These results are in accordance with those reported by **Ibrahim (2010); El-Nagdi et al. (2010) and El-Deriny (2016)** Enhanced nutrient status may be due to faster absorption of the nutrients via roots.

Table (10): Impact of cinnamon and ginger extracts on NPK and total chlorophyll in leaves of tomato var. Castle Rock infected with *Meloidogyne incognita* under greenhouse conditions at 27±3 °C.

Treatments	Conc.	N	P	K	Chlorophyll Mg/g F.W		
					Chlo. *a*	Chlo. *b*	*a+b*
Cinnamon Ethyl acetate	100	2.6^{f}	0.32^{e}	2.4^{f}	0.41^{e}	0.24^{e}	0.65^{f}
	200	2.8^{e}	0.33^{d}	2.6^{e}	0.43^{d}	0.24^{e}	0.67^{e}
	300	2.9^{d}	0.35^{c}	2.8^{d}	0.44^{c}	0.25^{d}	0.69^{d}
Ginger Ethyl acetate	100	3.1^{c}	0.35^{c}	3.0^{c}	0.46^{b}	0.26^{c}	0.72^{c}
	200	3.2^{b}	0.37^{b}	3.2^{b}	0.47^{a}	0.27^{b}	0.73^{b}
	300	3.4^{a}	0.38^{a}	3.5^{a}	0.47^{a}	0.28^{a}	0.76^{a}
Oxamyl		1.5^{g}	0.24^{j}	2.0^{i}	0.39^{g}	0.19^{h}	0.70^{g}
Untreated Uninoculated plants		2.6^{f}	0.29^{i}	2.4^{f}	0.39^{g}	0.24^{e}	0.70^{g}
N alone		1.0^{h}	0.15^{k}	1.7^{j}	0.34^{h}	0.16^{i}	0.60^{j}

*Each value presented the mean of five replicates.

Means in each column followed by the same letter(s) did not differ at P ≤ 0.05 according to Duncan's multiple range test.

In conclusion ginger and cinnamon ethyl acetate extracts induced significant improvement in plant growth criteria of tomato and showed nematicidal and antioxidant activities against root-knot nematode *M.incognita* under greenhouse conditions. Such extracts increased chemical constituents and defense relative proteins. However, further studies are needed to identify and isolate the most potent active ingredients of ginger rhizome and cinnamon bark extracts and screen such ingredients under laboratory, greenhouse and field conditions.

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الملخص العربي

التقييم الكيماوي والنشاط الإبادي لمستخلصات القرفة والزنجبيل ضد نيماتودا تعقد الجذور (*ميلودوجين إنكوجنيتا*) التي تصيب الطماطم

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** قسم بحوث المبيدات الفطرية والبكتيرية والنيماتودية – المعمل المركزي للمبيدات – مركز البحوث الزراعية.

هدفت الدراسة إلى قياس التأثير الإبادي لمستخلص الزنجبيل والقرفة باستخدام ثلاث مذيبات (ايثيل أسيتات، ميثانول ون–هكسان) تجاه نيماتودا تعقد الجذور (ميلودوجين إنكوجنيتا) التي تصيب الطماطم. كما أجريت اختبارات كيماوية لهذه المستخلصات.

أسفر التحليل الكيماوي للمستخلصات المستخدمة عن الآتي:

- وجود نسبة كبيرة من الفينولات والفلافينويد في مستخلصات الإيثيل أسيتات والميثانول بالمقارنة بالهكسان للزنجبيل والقرفة.
- بلغت أعلى نسبة للفينولات والفلافينويد في الايثيل اسيتات للزنجبيل والقرفة بمعدل ١٠٥.٦، ٩٣.٦ ملجم فيوليك است)، (٥٤.٢، ٤٥.١ ملجم كورستيرين /جم من وزن الجاف) على التوالي.
- بلغ النشاط المضاد للأكسدة أقصاه في مستخلص الزنجبيل باستخدام مذيب الإيثيل أسيتات والميثانول بنسبة ٩٠.٥، ٨٩.١% على التوالي.

أسفرت دراسة فعالية كل من مستخلص القرفة والزنجبيل باستخدام ثلاث مذيبات بتركيزين (٥٠ و١٠ ميكروليتر) على نسبة موت اليرقات لنيماتودا تعقد الجذور بعد مرور فترات زمنية مختلفة عن النتائج الآتية:

- أعطت المعاملة بمستخلص الإيثيل أسيتات نتائج أفضل من مستخلصات الميثانول أو الهكسان.
- أعطت معاملات الزنجبيل باستخدام المذيبات السابقة نتائج واعدة بعد مرور ٧٢ساعة من التعرض مقارنة مع تلك المعاملة بالقرفة.
- بلغ الحد الأقصى لنسبة موت اليرقات لنيماتودا تعقد الجذور باستخدام مذيب الإيثيل أسيتات (٦٨.٠٪) يليه الميثانول (٤٢.٠٪) ومستخلصات الهكسان (٣٠٪) لنبات الزنجبيل عند تركيز ٥٠ ميكروليتر بعد مرور٧٢ ساعة من المعاملة.

أسفرت دراسة فاعلية كلا من القرفة والزنجبيل باستخدام مذيب إيثيل أسيتات بثلاث تركيزات (١٠٠، ٢٠٠ و٣٠٠ جزء في المليون) على نيماتودا تعقد الجذور التي تصيب الطماطم تحت ظروف الصوبة السلكية عن الآتي:

- جاءت المعاملة بإيثيل أسيتات الزنجبيل (٣٠٠ جزء في المليون) الأفضل في زيادة الوزن الكلى للمجموع الخضري الطازج بنسبة بلغت ١٩٦.٠٪ مقارنة بمعاملة الكنترول. كما أدت هذه المعاملة إلى خفض معنوي في أعداد النيماتودا في التربة، أعداد العقد الجذرية وعدد كتل البيض.
- أثرت المعاملات المستخدمة على المحتوى الكيماوي لأوراق نباتات الطماطم من عناصر النيتروجين والفوسفور والبوتاسيوم والكلورفيل الكلي والبروتينات والفينولات.
- ازداد النشاط الإنزيمى لبيروكسيد أوكسيديز في جذور الطماطم المصابة بنيماتودا تعقد الجذور عند المعاملة بالإيثيل أسيتات للزنجبيل عند التركيز الأقل (١٠٠ جزء في المليون). على العكس من ذلك ازداد النشاط الإنزيمى لبولي فينول أوكسيديز عند المعاملة بالإيثيل أسيتات للقرفة (١٠٠ جزء في المليون).

ونستنتج من هذه الدراسة أن مستخلصات القرفة والزنجبيل لهما فعالية عالية كمواد مضادة للأكسدة ومكافحة النيماتودا وهذه التأثيرات البيولوجية قد ترجع إلى احتواء النباتات علي المركبات الهدم الثانوية.