AGRICULTURAL SCIENCE AND TECHNOLOGY, VOL. 14, No 1, pp 60-70, 2022
Published by Faculty of Agriculture, Trakia University, Bulgaria
ISSN 1313-8820 (print)
ISSN 1314-412X (online)
http://www.agriscitech.eu
DOI: 10.15547/ast.2022.01.009

Agriculture and Environment

Reduced egg hatch and increased juvenile mortality of potato cyst nematode *(Globodera spp.)* post *in-vitro* treatment with plant extracts

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(Manuscript received 25 March 2021; accepted for publication 28 January 2022)

Abstract. *In-vitro assays to determine the effect of plant extracts on egg viability and mortality of J2s of potato cyst nematode were evaluated. Methanol, ethyl acetate, hexane and water were used as solvents. Eggs and J2s were exposed to plant extracts for 24, 48 and 72 hrs. Treatments were arranged in a completely randomized design with three replications. Loss of egg viability and mortality of J2s significantly increased with an increase in the time of exposure to the extracts. Hexane extracts had a significantly higher loss of egg viability. Mexican sunflower extracts had a significantly higher loss of egg viability, having 93 and 89.2% non-viable eggs/cyst in experiments 1 and 2, respectively, compared to other plant extracts. This was followed by garlic, which had 89.5% and 86.3%, and then ginger, 86.8% and 85.9% non-viable eggs/cyst in experiments 1 and 2, respectively. Garlic, Mexican sunflower and ginger after 72 hrs of treatment exposure had significantly ($P<0.05$) high juvenile mortalities of 64.5%, 64.9% and 70.2%. Mexican sunflower, ginger and garlic extracts were effective in inducing loss of egg viability and mortality of J2s of PCN.*

Keywords: potatoes, plant extracts, cyst nematodes, nematicidal activity, alternative control

Introduction

Potato cyst nematodes (PCN), *Globodera pallida* and *Globodera rostochiensis*, are among the most important pests of potato (*Solanum tuberosum*) that cause severe losses in many potato growing areas worldwide. The losses due to PCN depend on the population density of the nematodes at planting, the potato cultivar, the weather and the soil type (Van Oijen et al.,1995; Trudgill et al., 2014). Annual yield losses of about 9-12% have been reported by Bates et al. (2002). However, yield losses can reach 70% in heavy infestations and often result in reduced numbers and sizes of tubers (Brodie, 1998; Ozarslandan, 2019).

The potato cyst nematode is a quarantine pest in over 100 countries (EPPO Bull, 2017) and was first reported in Kenya in 2015 (Mwangi et al., 2015). PCN spread has increased steadily to become a limiting factor in potato production (Haukeland, 2016). This spread has been aided by conducive agro-ecological conditions coupled with continuous potato growing in Kenya's infested fields (Mburu et al., 2020).

Although synthetic pesticides have been successfully used to control PCN, concerns about their effect on the environment and high cost make them unaffordable to the smallholder farmers and limit their use (El-Nagdi et al., 2014). The use of plant extracts for the control of PCN is becoming popular because plant extracts are cheap, easy to apply, produce no pollutants and have the capacity to structurally and nutritionally improve soil health (El-Nagdi et al., 2014). Natural plant products seem to provide a viable solution to the environmental problems caused by synthetic pesticides. They produce secondary metabolites (phytochemicals) that have potential use as botanical pesticides to control. These phytochemicals suppress pathogens through allelopathic interactions

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to inhibit reproduction, growth and biological activity (Bhattacharyya, 2017). Botanical pesticides are non-persistent since they are converted through oxidation, light and micro-organisms into less toxic products (Taniwiryono et al., 2009).

Plants extracts belonging to about 57 families including *Meliaceae*, *Asteraceae*, *Myrtaceae*, *Amaryllidaceae*, *Theaceae*, *Zingiberaceae*, and *Alliaceae* are reported to possess either nematicidal or insecticidal activity (Boulogne et al., 2012; Okwute, 2012). The majority of plants belonging to these families contain phytochemicals such as alkaloids, fatty acids, phenols, polyacetylenes, sesquiterpenes, glucosinolates thienyls, isothiocyanates and diterpenes (Khan et al., 2019; Ansari et al., 2020). These metabolites confer fungicidal, nematicidal, insecticidal, acaricidal and bactericidal properties to these extracts. Previous research has shown that plant extracts from Mexican marigold (Bhattacharyya, 2017), ginger (Amer-Zareen et al., 2003), *Eucalyptus spp* and onion bulb (Cetintas and Qadir, 2014), spring onions (Salifu et al., 2019) and sodom apple (Waweru et al., 2017) have nematicidal effects against root knot nematodes. Sultana and Khan (2018) found that plant extracts from tea were effective in controlling the plant parasitic nematodes *Helicotylenchus indicus, Xiphinema americanum* and *Xiphinema index,* whereas crude garlic extracts were effective against *Globodera pallida* in potato fields (Danquah, 2011).

Solvents play an important role in efficiency and efficacy of bioactive compounds of plants extracts. In addition, solubility of the phytochemicals varies with the phytochemical constituent's polarity in a plant and this can only be extracted with a suitable solvent (Gurnani et al., 2016). Despite the potential of plant extracts in controlling nematodes, there is paucity of information on their effectiveness in management of PCN under agro-ecological conditions characterized by continuous cultivation of potato in Kenya.

The aim of the study was to investigate the nematicidal potential of plant extracts from 10 locally available plant species obtained using different solvents against eggs and second stage juveniles (J2s) of the potato cyst nematodes in *in-vitro* conditions.

Material and methods

Collection of plant materials

The plant materials categorised as extract 1, 2, 3, 4, 5, 7, 8, 9 and 10 (Table 1) were used for the experiments. The plants were collected from Kenya Agricultural and Livestock Research Organization (KALRO) Tigoni, Limuru market, and farms around Juja in Kenya. The plants were selected on the basis that they possess nematicidal activity against *M. incognita* (Bhattacharyya, 2017; Cetintas and Qadir, 2014; Waweru et al., 2017). Neem (*Azadirachtaindica*, *Meliaceae*) extract was used as the standard whereas potato root diffusate was included as the negative control.

Table 1. Plant extracts and checks evaluated for nematicidal activity against eggs and second stage juveniles (J2s) of the potato cyst nematodes

Extract id.	Test plant extracts / product	Scientific name	Family	Part extracted	Previous work
1	Mexican marigold	*Tagetes minuta*	Asteraceae	Leaves	(Bhattacharyya, 2017)
2	Mexican sunflower	*Tithonia diversifolia*	Asteraceae	leaves	(Odeyemi and Adewale, 2011)
3	Garlic	*Allium sativum*	Amaryllidaceae	Bulb	(Danquah, 2011)
4	Eucalyptus leaves	*Eucalyptus grandis*	Myrtaceae	Leaves	(Cetintas and Qadir, 2014).
5	Eucalyptus bark	*Eucalyptus grandis*	Myrtaceae	Bark	(Cetintas and Qadir, 2014).
6	Spring onion	*Allium fistulosum*	Amaryllidaceae	Leaves	(Salifu et al., 2019)
7	Sodom apple	*Solanum incanum*	Solanaceae	Fruit	(Waweru et al., 2017)
8	Green tea leaves	*Camellia sinensis*	Theaceae	Leaves	(Sultana and Khan, 2018)
9	Ginger	*Zingiber officinale*	Zingiberaceae	Corm	(Amer-Zareen et al., 2003)
10	Onion bulb	*Allium cepa*	Amaryllidaceae	Bulb	(Cetintas and Qadir, 2014).
11	Neem (Positive control)	Commercial neem extract	Meliaceae	Azadirachtin 0.15 % w/w	(Trifonova and Atanasov, 2011)
12	Potato root diffusate (Negative Control)		*Solanum tuberosum*	Roots	(EPPO Bull, 2017)

Preparation of plant extracts using four solvents

The plant extraction procedure of the plant materials was performed according to the technique described by Chang et al. (1977). Plant materials were separately washed thoroughly using water to remove dust and then spread on a polythene sheet to air dry for ten days on a bench in a shaded glasshouse at KALRO-Tigoni. Two hundred grams (200 g) of the air-dried material from a sample of each plant species were then macerated into a fine powder using a Phillips kitchen blender. The powder obtained was then preserved in an airtight container at room temperature (25 ± 2°C) until needed (Chang et al., 1977). To prepare the extracts, 10 g powder of each sample was soaked in 200 ml of 4 solvents (water, methanol, ethyl acetate and hexane) separately in a 500 ml beaker and left for 12 hrs at room temperature (Chang et al., 1977; Dane et al., 2015). The concentration of the three organic solvents was 100%. The extracts were then filtered using a muslin cloth and the filtrates were subjected to evaporation under a laminar hood for 24 hrs. This process was repeated until a yield 10 g of pellet for each of the plant extracts was achieved. The pellets were then stored in an airtight container in a refrigerator at 10ºC. The 10 g pellets were dissolved in 200 ml of water resulting in a concentration of 50 mg/ml before use (Babaali et al., 2017).

Preparation of potato cyst nematode (PCN) inoculum

Potato cyst nematodes were collected from soil of a heavily infested field in Nyandarua County and extracted using Fenwick can floatation method (Fenwick, 1940) at KALRO Tigoni PCN extraction room. Cysts were then picked from the extracted samples under a binocular microscope at International Centre of Insect Physiology and Ecology (*icipe*) Nematology Laboratory.

To prepare second stage juveniles (J2s), potato root diffusate (PRD) was used as a hatching medium (EPPO Bull, 2017). The PRD diffusate was obtained by pouring 1000 ml of tap water in a 3-week-old potted potato plant growing in soil. The obtained PRD was sieved using muslin cloth, 5 ml of which was put into a 10 well hatching plate (EPPO Bull, 2017). Five hundred cysts were then added in the 5 ml PRD ensuring all cysts were completely submerged. The plate was then kept in the dark for seven days and observed for juveniles to emerge. The juvenile suspension obtained in the hatching vessel was then put in a 200 ml beaker and homogenized using an MRC magnetic stirrer for 3 minutes. From the juvenile suspension, a 0.5 ml PRD containing 100 infective juveniles was prepared for each treatment including the control in a 24 well plate. Counting of the juveniles was done under a Leica stereoscopic microscope at 40x magnification.

In vitro assays

The *in-vitro* assays were conducted at *icipe* Nematology Laboratory (25 ± 5°C). Both experiments were arranged in a Completely Randomized Design (CRD) with 3 replications per treatment and incubated for 24, 48 and 72 hrs. The experiments were repeated once.

Experiment 1. Determining PCN egg hatchability and viability when exposed to plant extracts at different time intervals

On average three cysts were recovered from 100 g of soil. Therefore, for the egg hatchability and viability test, 3 cysts of PCN were picked from already extracted samples and transferred into each of the 96 well plates. Aliquots of 250 µl of each of the 10 plant extracts from each solvent (methanol, ethyl acetate, hexane and water), negative control (PRD) and the positive control - Achook (neem based extract) from Organix Limited was prepared following manufacturer's recommendations, were dispensed into each well ensuring all the 3 cysts were well immersed. Each treatment had 3 cysts and was replicated three times. All the 96 well plates were covered with aluminium foil to avoid evaporation and were kept in the dark inside laboratory cabinets at 25 ± 5°C. After the designated time (24, 48 and 72 hrs) of exposure elapsed, one cyst was picked from each experimental unit in each treatment, after which the cysts were incubated in 0.1% Nile blue stain for 48 hrs (Faggian et al., 2012; Kroesf et al., 2011).

Experiment 2. Determining PCN second stage juvenile mortality when exposed to different plant extracts at different time intervals

For the juvenile mortality test, 0.5 ml PRD having 100 freshly hatched J2s (Asif et al., 2015; Fatemy, 2018) of PCN were filled in each of the 24 well plates containing 1.5 ml of the 10 plant extracts, the negative control (PRD) and the positive control Achook (neem based extract) from Organix Limited. The positive control was prepared according to manufacturer's recommendations. Each treatment had three experimental units and was replicated three times. The well plates were wrapped with aluminium foil and then incubated at 25°C for 24, 48 and 72 hrs. The experiment was done twice.

After incubation, the aliquots were homogenized in each well and 2 ml was drawn and put on a counting dish and the number of both live and dead J2s counted under a Leica stereoscopic microscope after 24, 48 and 72 hrs. J2s showing any mobility (active) were considered living and those showing no movement were considered dead, they were probed with a surgical needle to ascertain their status (Cayrol et al., 1989).

Data analysis

Normality of all the data set was tested by the Shapiro-Wilk test. Data on loss of egg viability (experiment 1) was log (x+1) transformed before analysis to maintain homogeneity of variance. Analysis of variance was made to assess the effect of plant extracts, exposure time and solvents on PCN eggs and juveniles using the Genstat 12.1 statistical package. Means for all data were compared using Fisher's protected least significant differences (LSD) test at the $p<0.05$ level. Percent viable PCN eggs were computed using the following formula:

% Viable eggs = {viable eggs / (viable eggs + non-viable eggs)} X 100 (Ryan and Devine, 2005)

On juvenile mortality, data collected on live and dead J2s were used to compute the mean percentage juvenile mortality using the formula:

% viable juvenile mortality = number of non-viable juveniles / number of viable juveniles + non-viable juveniles (Fatemy, 2018)

Results

Experiment 1. Determination of the hatchability and viability of PCN eggs when exposed to plant extracts at different times

Effect of the plant extracts on the PCN egg viability

The microscopic images of the eggs observed with Nile blue stain indicated that the eggs were non-viable eggs, the unstained eggs were recorded as viable eggs and empty shells were recorded as hatched eggs and also disintegrated cysts were also observed (Figure 1).

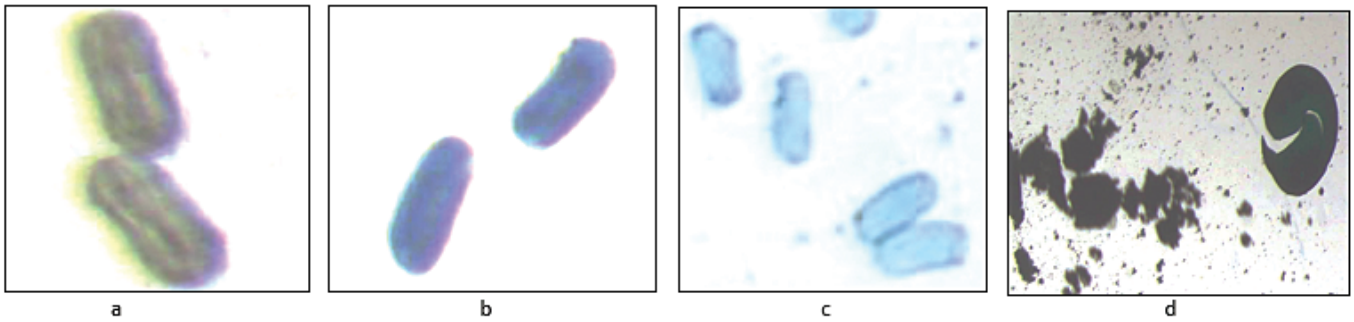

Figure 1. Microscopic images of potato cyst nematode eggs; (a) Viable eggs (unstained eggs), (b) Non-viable eggs (Nile blue stained eggs), and (c) Hatched eggs (egg shells) d) Disintegrated eggs

Plant extracts, solvents and time of exposure were highly significant (Table 2). All the plant extracts tested were found to exhibit some level of inhibition towards egg hatch and viability of the potato cyst nematode. The percentage number of non-viable and viable eggs for each plant extract after 72 hrs after treatment is presented in Table 2. In both experiments, all the plant extracts exhibited a low percentage of viable eggs (11.8% to 39.1%) compared to potato root diffusate which had 72.4% to 78.1% viable eggs (Table 3). Exposure of PCN eggs to plant extracts led to a significant ($P<0.05$) decrease of viable eggs/cyst ranging from 18.3 to 43.7 compared to PRD which had 79.4 and 108.1 viable eggs/cyst in experiment 1 and 2, respectively (Table 3).

Among the ten plant extracts evaluated, only 3 (ginger, Mexican sunflower, and garlic) showed a high and consistent number of non-viable egg counts. Ginger had 112.2 non-viable eggs/cyst in experiment 1 and 120.2 non-viable eggs/cyst in experiment 2. Mexican sunflower had 151.9 non-viable eggs/cyst in experiment 1 and 144.9 non-viable eggs/cyst in experiment 2. Garlic had 138.1 non-viable eggs/cyst in experiment 1 and 134.0 non-viable eggs/cyst in experiment 2. Both garlic and Mexican sunflower had a high number of non-viable eggs compared to the Neem (positive control). The lowest loss of egg viability per cyst was recorded in the PRD treatment, which had 30.3 and 30.2 non-viable eggs/cyst for experiment 1 and 2, respectively (Table 3).

Table 2. The main effects of plant extracts, exposure time and solvent on potato cyst nematode non-viable eggs

Sources of variation	df	Experiment 1.			Experiment 2		
		Sum of squares	F	P	Sum of squares	F	P
Treatments	11	343838	4.66	<.001	315032	4.05	<.001
Hours of incubation	2	7086	0.58	<.001	14139	1.09	<.001
Solvent	3	377744	20.50	<.001	342728	17.62	<.001

Table 3. Effect of different plant extracts on potato cyst nematode egg viability

Plant extracts	Experiment 1			Experiment 2		
	Mean number			Mean number		
	Non-viable eggs/cyst	Viable eggs/cyst	% Viable eggs	Non-viable eggs/cyst	Viable eggs/cyst	% Viable eggs
Mexican sunflower	151.9(2.1efg)	25.8 (1.2de)	14.5	144.9(2.1de)	29.9 (1.3d)	17.1
Garlic	138.1 (2.0e)	18.5 (1.1f)	11.8	134.0(2.0cd)	28.8 (1.3d)	17.7
Neem (Positive control)	137.6 (2.0ef)	23.6(1.0def)	14.6	124.6(2.0cd)	29.2 (1.3d)	19.0
Ginger	112.2 (1.9de)	22.8(1.2def)	16.9	120.2(2.0cd)	25.4 (1.3d)	17.4
Mexican marigold	86.2 (1.8cd)	27.0(1.3d)	23.9	85.9 (1.9c)	43.7 (1.5)	33.7
Spring onion	68.1 (1.7bc)	26.5 (1.2d)	28.0	71.1 (1.7b)	30.6 (1.3d)	30.1
Sodom apple	67.3 (1.7bc)	37.3 (1.2c)	35.7	64.09 (1.7b)	25.3 (1.2d)	28.3
Eucalyptus leaves	66.9 (1.7bc)	18.8 (1.1f)	21.9	67.9 (1.7b)	20.2 (1.2d)	22.9
Eucalyptus bark	64.4 (1.6b)	31.2 (1.4d)	32.6	64.8 (1.6b)	28.9 (1.2d)	30.8
Green tea leaves	62.5 (1.6b)	20.5(1.1def)	24.7	59.3 (1.6b)	38.0 (1.4c)	39.1
Onion bulb	59.9 (1.6b)	19.9 (1.1ef)	24.9	59.4 (1.6b)	18.3 (1.1e)	23.6
[1]PRD (Negative control)	30.3 (1.4a)	79.4 (1.7a)	72.4	30.3 (1.4a)	108.1(2.0a)	78.1
$LSD_{0.05}$	21.0 (0.2)	6.7 (0.2)	7.79	22.6(0.15)	7.8(0.08)	7.79

1PRD-Potato root diffusate. Figures in parenthesis represent transformed data; all data are means of three replicates; Means followed by the same letter within the columns are not significantly different at $p<0.05$ according to Fisher's protected least significant difference test

Effect of exposure time of the different plant extracts on loss of egg viability

The *in-vitro* assay showed that all plant extracts were effective in reducing potato cyst nematode eggs viability as compared to PRD when subjected to the three-time regimes post treatment (Figure 2a, b). Generally, the loss of egg viability increased with increase in exposure time. Treatment exposure after 72 hrs on potato cyst nematode had the highest loss of egg viability. Mexican sunflower, garlic and ginger extracts were highly significant ($P<0.05$) in inducing loss of egg viability compared to the other plant extracts and potato root diffusate at all the three exposure times. At 72 hrs, Mexican sunflower extracts resulted in 85.1% and 82.3% non-viable eggs/cyst in experiment 1 and 2, respectively. While garlic extracts at 72 hrs had 80% and 88% non-viable egg/cyst in experiment 1 and 2, respectively, whereas those with ginger had 85.1% and 86.6% of non-viable egg/cyst in experiment 1 and 2, respectively at 72 hrs (Figure 2a, b). Amongst the plant extracts, Mexican sunflower activity led to 80.2% and 73.4% non-viable eggs/cyst in experiment 1 and 2, respectively at 48 hrs. At 48 hrs, garlic induced percentage loss of egg viability per cyst of 79.5% and 68.3% in experiment 1 and 2, respectively. Ginger activity was associated with 79.5% and 64.4% non-viable eggs/cyst in experiment 1 and 2, respectively at 48 hrs (Figure 2a, b). After 24 hrs of exposure of potato cyst nematode eggs to plant extracts, a higher nematicidal activity was recorded in Mexican sunflower having percentage loss of egg viability per cyst of 75.2% and 60% in experiment 1 and 2 respectively, followed by garlic having 69.9 and 64.9% non-viable eggs/cyst in experiment 1 and 2, respectively. Ginger had 69.6% and 61.5% loss of egg viability per cyst in both experiments, respectively (Figure 2a, b).

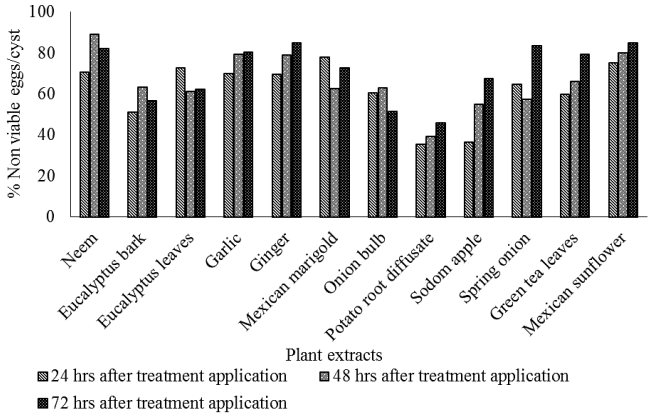

Figure 2a. Effect of incubation period of the plant extracts on loss of egg viability of the potato cyst nematodes in experiment 1

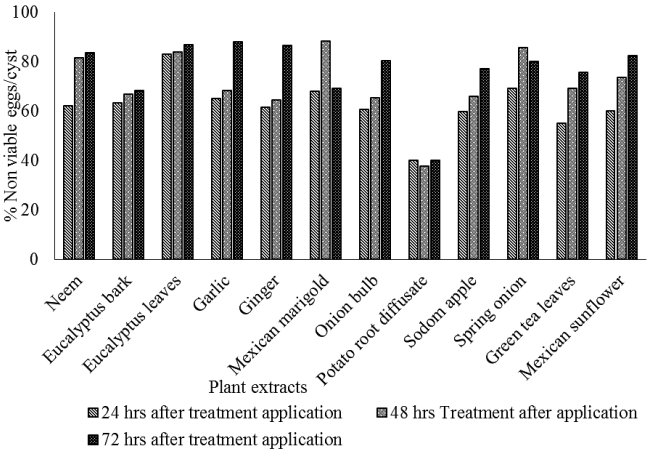

Figure 2b. Effect of incubation period of the plant extracts on loss of egg viability of the potato cyst nematodes in experiment 2

Effect of exposure time of the different plant extracts on percentage egg hatch

The results showed that when PCN eggs were exposed to plant extracts at different times (24, 48 and 72 hrs), egg hatch of infective juveniles occurred (Figure 3a, b). After all the three exposure times PCN egg hatch in the PRD control was significantly higher ($P<0.05$) than in all other treatments (Figure 3a, b). In experiment 1, among the plant extracts, Sodom apple had the least percentage of PCN egg hatch/cyst (3.0 %) after 24 hrs of exposure, while at 48 and 72 hrs of treatment exposure, garlic had the least percentage of egg hatch/cyst of 5.0% and 2.5%, respectively (Figure 3a). In experiment 2, the lowest percentage of egg hatch inhibition/cyst (4.5%) was observed in the spring onion extract after 24 hrs of exposure (Figure 3b). After 48 and 72 hrs of treatment exposure, garlic had the least percentage of egg hatch of 3.3% and 2.4%, respectively (Figure 3b).

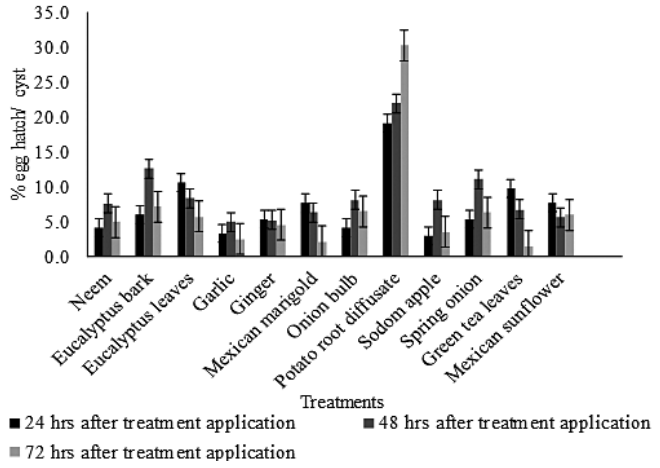

Figure 3a. Effect of plant extracts on percentage of egg hatch of the potato cyst nematodes after 24, 48 and 72 hrs of exposure in experiment 1

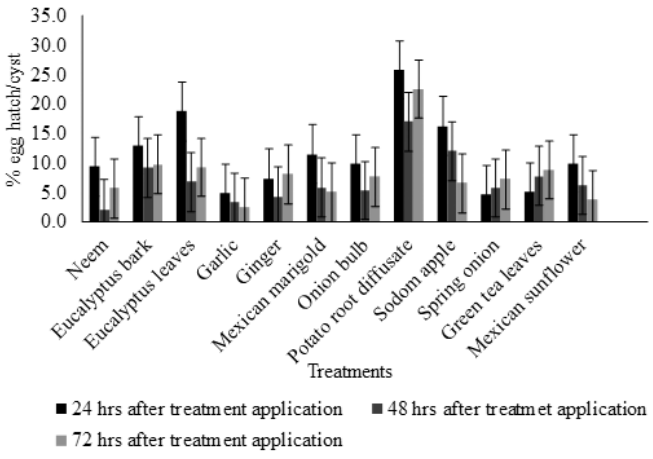

Figure 3b. Effects of plant extracts on percentage of egg hatch of the potato cyst nematode after 24, 48 and 72 hrs of exposure in experiment 2

Effect of different solvent extracts on loss of egg viability

Three of the four solvents (ethyl acetate, hexane and methanol) used to extract phytochemicals from the plant extracts caused higher loss of egg viability compared to that of water extracts (Figure 4a, b). However, hexane extracts had significantly higher and consistent mean loss of egg viability in the two experiments compared to other solvent extracts (Figure 4a, b). Hexane extracts of Mexican sunflower resulted in a significantly higher loss of egg viability having 93% and 89.2% non-viable eggs/cyst in experiment 1 and 2, respectively, compared to other plant extracts. This was followed by garlic, which had 89.5% and 86.3%, and then ginger 86.8% and 85.9% non-viable eggs/ cyst in experiment 1 and 2, respectively (Figure 4a, b).

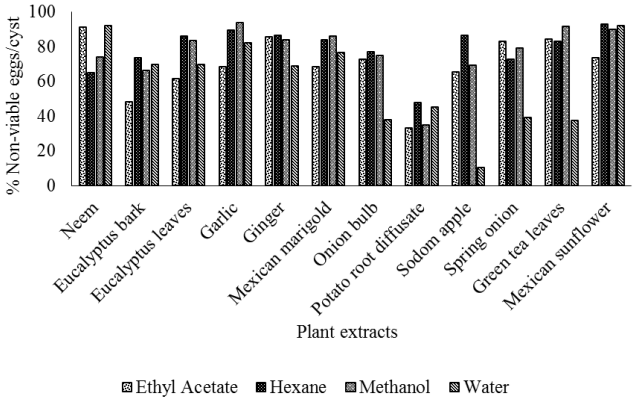

Figure 4a. Effect of solvent extracts on loss of egg viability of potato cyst nematodes in experiment 1

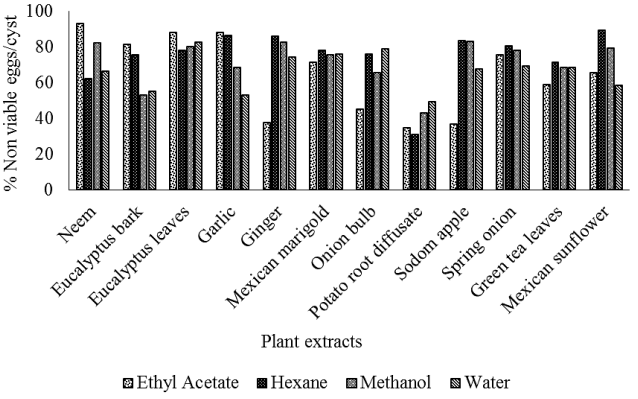

Figure 4b. Effect of solvent extracts on loss of egg viability of potato cyst nematodes in experiment 2

Experiment 2. Determining PCN second stage juvenile mortality when exposed to different plant extracts at different time intervals

Effect of solvents and plant extracts on PCN second stage juvenile mortality of potato cyst nematodes J2s

In the juvenile mortality assay, all the four solvents (ethyl acetate, hexane, methanol and water) had no significant ($P>0.05$) influence on juvenile mortality (data

not shown). All the 10 plant extracts induced juvenile mortality. Ginger induced the highest juvenile mortality of 68.9% and 63.3% in experiment 1 and 2 respectively, followed by treatments with Mexican sunflower with 67.9% and 60.1% dead juveniles, then garlic with 64.3% and 60.8% dead juveniles in experiment 1 and 2, respectively (Table 4). Mexican sunflower, garlic and ginger were highly significant (P<0.05) compared to potato root diffusate. Water alone had a few J2s of potato cyst nematodes (25.7% and 25.2%) compared to the plant extracts (Table 4). Potato root diffusate had the least juvenile mortality in both experiments (15.5% and 17.7%) (Table 4). An illustration of live juveniles is when J2s were subjected to control checks and dead juveniles when J2s were subjected to plant extracts treatments is as shown in Figure 5.

Table 4. Effect of different plant extracts on mortality of second stage juveniles of potato cyst nematodes

Treatments	Experiment 1		Experiment2	
	Dead Juveniles/2ml (%)	Active juveniles/2ml (%)	Dead juveniles/2ml (%)	Active juveniles/2ml (%)
Neem (Positive control)	69.4e	30.6a	65.1d	34.9a
Ginger	68.9e	31.1a	63.3d	36.7a
Mexican sunflower	67.9e	32.1a	60.1d	39.9a
Garlic	64.3e	35.7a	60.8d	39.2a
Onion bulb	47.4d	52.6b	43.6c	56.4b
Spring onion	44.1cd	55.9bc	43.3c	56.7b
Eucalyptus leaves	43.3cd	56.7bc	43.4c	56.6b
Mexican Marigold	42.8cd	57.7bc	44.5c	55.5b
Sodom apple	41.9cd	58.1bc	42.4c	57.6b
Eucalyptus Bark	41.7cd	58.3bc	41.3c	58.7b
Green tea leaves	39.5c	60.5c	42.3c	57.7b
Water (negative control)	25.72b	74.28d	25.3b	74.75c
[1]PRD (negative control)	15.5a	84.5e	17.7a	82.3d
$LSD_{0.05}$	6.51	6.518	6.37	6.37

[1]PRD-Potato root diffusate

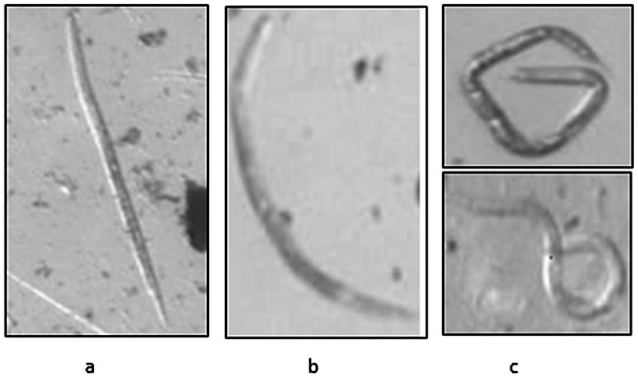

Figure 5. Characteristic shapes of dead juveniles: **a**. straight (I-shape), **b.** Slightly bent (banana-shape), and **c**. curled (∞ -shape)

Determination of second stage juvenile mortality when exposed to different plant extracts at different time intervals

The bioassay showed that all the plant extracts positively influenced the mortality of the potato cyst nematode J2s when applied at 24, 48 and 72 hrs (Figure 6a, b). Juvenile mortality rate was significantly influenced (P<0.05) by exposure time positively. In both experiments, when juveniles were exposed to the plant extracts, juvenile mortality increased significantly (P<0.05) from 36.0% to 73.0%, in all the three time regimes compared to the untreated check PRD. In experiment 1, among the 10 plant extracts tested, garlic, Mexican sunflower and ginger had significantly high (P<0.05) juvenile mortalities (68.8%, 72.6% and 73.0%, respectively) after 72 hrs of treatment exposure compared to other plant extracts (Figure 6a, b). In experiment 2, after 72 hrs of treatment exposure garlic, Mexican sunflower and ginger had significantly high (P<0.05) juvenile mortalities of 64.5%, 64.9% and 70.2%. These plant extracts (garlic, Mexican sunflower and ginger) showed a similar efficacy after 24 and 48 hrs of treatment exposure in both experiments. The PRD treatment had the lowest juvenile mortality in all the three time intervals in both experiments.

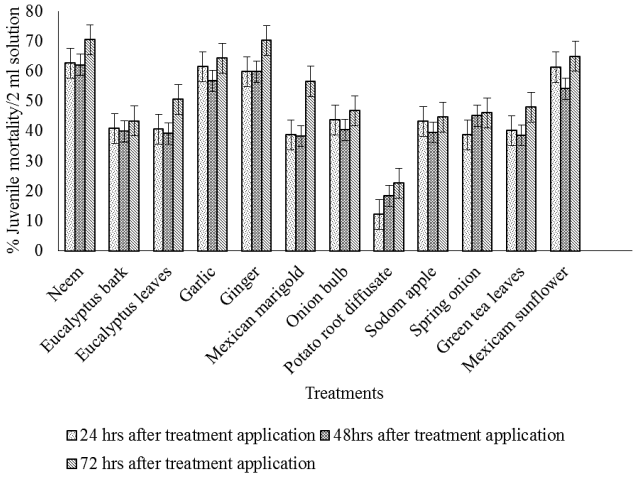

Figure 6a. Percentage mortality of juveniles in plant extracts at different exposure times in experiment 1

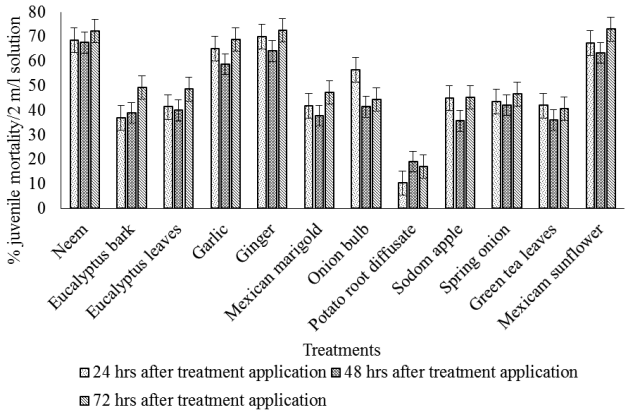

Figure 6b. Percentage mortality of juveniles in plant extracts at different exposure times in experiment 2

Discussion

In the present study, we evaluated the nematicidal efficacy of 10 plant extracts of selected plant species available in Kenya against potato cyst nematode eggs and infective juveniles. Potato cyst nematode is not an old pest in Kenya, although first reported in 2015 in the country, it is currently a dominant pest in many potato farms in Kenya (Haukeland, 2016; Mburu et al., 2020)

The results of the bio-assays revealed that three of the 10 plant extracts garlic, Mexican sunflower and ginger showed a higher potency in loss of egg viability and juvenile mortality of PCN compared to the other extracts. We observed that after treatment of potato cyst nematode eggs and infective juveniles with garlic, Mexican sunflower and ginger extracts the internal organs were gradually disintegrated and became indistinct. Our study is the first to report on effects of these plant extracts on PCN. Similar observations were made on J2s and eggs of the root knot nematode *Meloidogyne javanica* on intestine damage after treatment with Camellia seed cake (Yang et al., 2015). Additionally, Khan et al. (2019) observed that the cytoplasmic membrane of *Meloidogyne incognita* was dissolved when exposed to plant extracts. We observed that the dead J2s had 4 body shapes - straight, slightly bent, curved or sigmoid shape. The changes in body shape might be apportioned to the toxic effects of phytochemicals that affect the nervous system (Taniwiryono et al., 2009). This is attributed to substances such as alkaloids which are reported to affect the nervous system causing paralysis and saponins which cause disintegration of internal organs and membrane alteration (Correia, 2014). Therefore, it can be assumed that the immobility and morphological change observed are related to the effect of the extracts assayed.

The nematicidal effect of the three extracts on potato cyst nematode J2s and eggs could also be linked to the presence of bioactive compounds such as sulphuric acid compounds (allicin and diallylsulphuric), pyruvic and ammonia obtained from garlic (Auger et al., 2004; Gupta and Sharma, 1993), saponins and alkaloids from Mexican sunflower (Tona et al., 2000), alkaloids, saponins, flavonoids, glycosides, tannins, terpenoids and phenols present in ginger (Hussein, 2017) all of which have been reported to confer resistance to nematodes. Our findings are in concurrence with Gupta and Sharma (1993) who indicated that garlic extracts when used as a drench caused 87-100% J2 mortality of *M. incognita,* while Mexican sunflower extracts were reported to significantly reduce the number of eggs, number of juveniles and galls of *M. incognita* (Odeyemi and Adewale, 2011; Tsay et al., 2004). Ginger extracts, on the other hand, were reported to suppress reproduction of *M. incognita* in tomato (Hussein, 2017).

There are several botanical pesticides which have been formulated and commercialized. These include Achook a neem based nematicide from organic Kenya LTD and NEMguard a garlic based nematicide from Dudutech LTD.

Contrary to *Solanaceae* crops being hosts of potato cyst nematodes enhancing egg hatch and maintaining egg viability, this study found that Sodom apple fruit extracts induced loss of PCN egg viability, reduced egg hatch and increased mortality of the infective juveniles under *in-vitro* conditions. This finding is probably because we used fruits and not the roots as the source of our extracts. In *Solanaceae* crops it is the root exudates that induce hatching. The finding could be attributed to the complex interactions between hatching factors and other chemicals such as solanine present in Sodom apple which can cause both inhibition and stimulation of hatching factors depending on the solanine concentration. In support of this speculation, a high concentration of solanine in Sodom apple fruit extract was reported by Byrne et al. (1998) to inhibit hatching activity of the potato cyst nematode eggs in potato root leachate.

Plant species, plant part and type of solvent (water or alcoholic), and nematode species play important roles in achieving different nematicidal effects. In this study, plants extracts were obtained from natural sources using different solvents. The results demonstrated that water extracts of all the tested plant extracts showed the least nematicidal effect on loss of egg viability of potato cyst nematodes compared to methanol, ethyl acetate, hexane extracts. This suggests that compounds that are nematostatic in nature were not water soluble or they were low in quantity or of lower quality. Seenivasan (2019) made a similar observation where water extracts of all tested plant species extracts did not show any inhibitory effect on the banana nematode (*Radopholus similis)* compared to ethyl acetate, hexane and ethyl acetate which caused immobility on *R. similis.* According to Gupta et al. (2013) alcoholic extracts of *Datura stramonium* contain more chemical components than aqueous extracts. In this investigation, hexane extracts were found to have the strongest nematicidal activity against PCN eggs in comparison to ethyl acetate and methanol plant extracts which exhibited moderate activity. It is probable

that hexane extracts were richer in the levels of bioactive compounds, thus the superior performance of its extracts relative to the other extracts. This finding is supported by Siti et al. (2019) who found out that when hexane was used as solvent, the extraction efficiency of bioactive compounds of leaf extracts of *Vernonia amygdalina,* like isiothiocynates, thiophenics, glycosides, alkaloids, phenolics and fatty acids considered nematicidal in nature were enhanced. We observed that there were differences in performance of the plant extracts on potato cyst nematodes mortality of J2s, egg hatch inhibition and egg loss of viability. This could be attributed to the differences in polarities of the four solvents used in this study which have different solubility levels of phytochemicals leading to a wide variation in the level of bioactive compounds in the extracts used in this study (Seenivasan, 2019).

In the present study, we also found that loss of egg viability of potato cyst nematodes increased with the exposure time to the plant extracts. Ginger, Mexican sunflower and garlic extracts exhibited the highest nematicidal activity of both eggs and juveniles of potato cyst nematodes at 72 hrs of the bioassay period. As the time increased from 24 hr to 72 hrs, loss of egg viability of potato cyst nematodes and reduction in egg hatching increased in a progressive manner suggesting that if exposure time is prolonged, the plant extracts will exhibit stronger nematostatic effects. In this study, Eucalyptus bark and leaves extracts had similar results where loss of egg viability of potato cyst nematodes increased with the exposure time to the plant extracts. This finding is in agreement with previous studies on nematicidal effects of *Eucalyptus* spp extracts and *Camellia* seed cake on *Meloidogyne javanica* which observed an increase in loss of egg viability with increased exposure time (Dawar et al., 2007; Yang et al., 2015). On the other hand, potato cyst nematode J2s mortality was not consistent with increase in exposure time. This differs from an earlier report that showed plant extracts caused increased juveniles mortality of *M. incognita* with increase of exposure time (Abdalla et al., 2008). This inconsistency of the juveniles' mortality rate could be as a result of larvicidal action value of the extracts which vary from one plant to the other. The assumption by this study was that the solvents used were volatile and were allowed to evaporate during extraction process, therefore they had noncontact nematicidal activity against potato cyst nematodes.

None of the previous investigations have studied the direct bio-activity of garlic, Mexican sunflower and ginger against potato cyst nematode eggs and juveniles under *in vitro* conditions. Therefore, this study will open new avenues for control of potato cyst nematodes using plant extracts. Mexican sunflower is a plant occurring naturally in the fields in Kenya and is freely accessible. In addition, the use of Mexican marigold, ginger and garlic as nematicides have good prospects as they are environmentally friendly and can be produced locally. Besides the direct nematicidal effects of these test extracts, there is a possibility that they might be taken up by host roots, thus modifying host recognition by the nematode and they may also change the rhizosphere of the host crop thus protecting the roots from infective juveniles. Studies conducted by Mwamba (2016) illustrated that volatile organic compounds of *Tagetes* spp. influenced host seeking behavior by repelling of *Meloidogyne incognita* around the root rhizosphere and when taken systemically by the plant.

The extracts used in the present study have not been tested for nematicidal activity against potato cyst nematodes in the field and how they compare to commercial nematicides. The complex interactions that occur in the soil, combined with the effect of agricultural practices such as tillage, fertilization and irrigation on soil community structures which greatly influence the biological and chemical activities in soil can also interfere with the effectiveness, degradation and reactions of these plant extracts. There is, therefore, need to complement *in vitro* tests with field experiments so as to determine the effectiveness of plant extracts.

Conclusion

The findings of this study demonstrated that all the 10 plants extracts evaluated were effective on PCN egg hatch inhibition, inducing loss of egg viability and increasing J2s mortality although the effectiveness varied depending on the plant extract. The study showed that most effective extracts were Mexican sunflower*,* garlic and ginger. This is an important finding in the quest for identification of alternative approaches in management of PCN.

Acknowledgements

We are grateful to Kenya Climate Smart Agriculture Project (KCSAP) for funding the study and Director General, Kenya Agricultural and Livestock Research Organization (KALRO) for providing facilitation and support during the study period. We thank *icipe* management for providing necessary facilities during the experiment.

Conflict of Interest

The authors declare no conflict of interest in the publication of this manuscript

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