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Full Length Research Paper

Organic Waste Extracts as Hatching Inhibitors or Promoters for Meloidogyne incognita Eggs

Osei K.[1]*, Addico R.[2], Nafeo A.[2], Edu-Kwarteng A.[1], Agyemang A.[1], Danso Y.[1] and Sackey-Asante J.[1]

[1]Crops Research Institute, Box 3785, Kumasi, Ghana.

[2]West Africa Fair Fruit, PMB KD 11, Kanda, Accra, Ghana.

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Plant parasitic nematodes significantly hinder food production particularly in the developing world where peasant farmers have little knowledge of these pests. Currently, synthetic pesticides which are highly detrimental to man and the environment are the principal means of nematode control. However, plant products might provide a sustainable control option as nematicidal properties have been identified in many higher plants. An *in vitro* experiment involving five organic waste extracts; citrus waste, cocoa bean testa, compost, poultry manure and oil palm bunch waste were therefore evaluated for their hatching inhibitory potential to the southern root-knot nematode, *Meloidogyne incognita* eggs. Three concentration levels (w/v) that is 4, 8 and 10% of cold aqueous extracts of the organic waste compounds were filtered into 9 cm Petri dishes and infested with 100 eggs each. Hatching of eggs was monitored over a three time period; 24, 48 and 72 h after infestation of the eggs into the cold aqueous extracts of the five candidates in Petri dishes. The best result was obtained with citrus waste extract in which 5 eggs hatched while 73 eggs hatched in the distilled water (the control treatment) representing a hatching reduction of 93% at the highest time and concentration of 72 h and 10% respectively.

Key words: Hatching, *Meloidogyne incognita*, organic waste extracts, plant parasitic nematodes.

INTRODUCTION

Plant parasitic nematodes are major limiting factor affecting plant growth and yield. The root-knot nematode, *Meloidogyne* species are the most devastating (Williams–Woodward and Davis, 2001). Globally, over 90 species of the genus, *Meloidogyne* have been described (Sikora and Fernandez, 2005). However, three *M. arenaria, M. javanica*, and *M. incognita* are of the greatest agronomic importance, being responsible for at least 90 % of all damage caused by root-knot nematodes (Castagnone-Sereno, 2002). These poikilothermic pests which produce conspicuous galls on plant roots and tubers are among the top five major plant pathogens (Bharadwaj and Sharma, 2007). The southern root-knot nematode, *M. incognita* is polyphagous, infecting about 232 genera of plant species (Swarup et al., 1989). The menace of root-knot nematodes in Ghana and the West African sub region has been well documented. In Ghana, Addoh (1971) reported 33% crop loss on tomato in one season due to this pest. A "*Meloidogyne-javanica-incognita-arenaria* species complex" was responsible for severe galling of winged bean in the Côte d'Ivoire (Fortuner et al., 1979) and root-knot nematode species caused severe growth reduction, unfilled spikelets, wilting and poor yield of rice in Nigeria (Babatola, 1984). Root-knot nematodes are difficult crop pests to control Chitwood (2002), partly because they have high reproduction rates Ananhirunsalee et al. (1995), are sedentary endoparasites and therefore protected in plant tissues EPPO (2009), overwinter in weed species Quénéhervé et al. (1995) and have extensive host range (Mitkowski and Abawi, 2003).

Currently, synthetic pesticides are the most effective means of control, but they are both expensive and environmentally unfriendly. For sustainability of agriculture therefore, farmers should divorce the synthetic pesticides strategy and marry the phytochemicals option which is non-toxic to man and the environment, biodegradable and affordable to the peasant farmer in the developing world. The "evils" of synthetic pesticides have

*Corresponding author. E-mail: kosei7392@gmail.com.

been a major concern to environmentalists. Current research efforts have therefore been shifted towards the use of plant extracts as alternative to synthetic compounds (Papachristos and Stamopoulos, 2002).

In the management of plant parasitic nematodes, OWC have only been used as soil amendments with varying degrees of successes. For instance, refuse dump, applied at 45 t/ha significantly reduced root-knot nematodes population compared with the control treatment, resulting in significant increase in the yield of tomato by 17-100% (Hassan et al., 2010). Also, Agu (2008) demonstrated that poultry and farm yard manures applied at 2.5 t/ha significantly reduced root-gall nematode damage on African yam bean and also improved growth and yield of the plant. Previous efforts have never been with extracts of organic waste compounds (OWC). The prospect of utilizing plant extracts is promising as nematicidal properties have been found in many higher plants (Chitwood, 2002). Nematicides of plant origin include; isothiocyanates, thiophenics, glucosides, alkaloids, phenolics and fatty acids (Gommers, 1973). The present study was conducted to determine the effect of cold water extracts of some OWC on the hatching of *M. incognita*.

MATERIALS AND METHODS

Five OWC; citrus (cv. Late Valencia) waste, cocoa bean testa, compost (rice husk parent material), poultry manure and oil palm bunch waste were evaluated for nematicidal activity in a field experiment. As a sequel to the former study, an *in vitro* experiment involving the five candidates on hatching of *M. incognita* eggs was investigated in the current study. The experiment was conducted in the laboratory of the Nematology section of the Crops Research Institute, Kumasi, Ghana. The laboratory experienced an average temperature of 25°C, 12 h of photoperiod and a relative humidity of 87.79% during the period of experimentation.

Chemical properties (pH, N, OC, OM, P, K, and Mg%) of the five OWC were analyzed. Soil reaction (pH) was determined by using the glass electrode pH meter (Radiometer Analytical, Lyon, France) in water at 1:1 (soil: water) ratio. Organic carbon (C%) was determined by the dichromate-oxidation method of Walkley-Black (Walkley and Black, 1934). From these values, the organic matter (OM%) content of samples was computed using a correction factor of 1.72 (Nelson and Sommers, 1996). Total nitrogen (N%) was determined by the Kjeldahl method (Pereira et al., 2006). Phosphorus was determined colorimetrically by the Molybdenum blue method (Bray and Kurtz, 1945). Potassium was determined with a flame photometer (Corning EEL model 100) and Magnesium was determined with Hitachi model 207 atomic absorption spectrophotometer.

One egg mass of *M. incognita*, identified through perineal patterns (Jepson, 1987) was collected from plant house cultures using a pair of forceps and extracted from tomato (*Solanum lycopersicum* L.) roots by shaking for 3 min in 0.05% NaOCl solution and rinsing for 2 min under running tap water (Stanton and O'Donnel, 1994). The eggs were cultured on the dwarf determinate tomato cv. Tiny Tim in the plant house at 25°C. Six weeks after inoculation (WAI), when two generations had occurred under these optimum conditions (McLeod et al., 2001), eggs were extracted from the tomato roots as described above and used in the experiment. Eggs were counted under a stereo microscope x 100.

The moisture level of the five OWC samples was reduced in a convection air oven at 60°C for 24 h. Dried samples were then milled into powder with a Kenwood dry mill. Fifty grams each of the OWC was isolated for 60 min in 750 mL^{-1} of distilled water. The cold aqueous suspensions were maintained at room temperature for 16 h with intermittent stirring. Suspensions were then filtered through cheesecloth to obtain an extract of 10% concentration (w/v). Serial dilutions were then performed to obtain concentrations of 8 and 4%. Distilled water was the control treatment. Two milliliters each of the aqueous suspensions were pipetted into 9 cm Petri dishes and one hundred freshly extracted *M. incognita* eggs in two milliliter suspension were poured into the aqueous extracts and properly labeled. The experiment was arranged in a randomized complete block design and replicated three times (Figure 1).

Hatching was monitored over a three time period; 24, 48 and 72 h respectively. Data was analyzed using Genstat 8.1. Means were separated using Duncan's Multiple Range Test (DMRT).

RESULTS

The organic waste compounds possessed different chemical properties. Cocoa bean testa and palm bunch waste recorded the highest organic carbon (OC%) and organic matter (OM%) contents (43.85 and 44.80) and (87.70 and 89.60) respectively. The least (OC%) and (OM%) of (9.50 and 19.00) were recorded by poultry manure. Higher OC% and OM% contents were associated with lower pH levels (6.10) recorded by cocoa bean testa and palm bunch waste (Table 1).

Generally, hatching inhibition was significant at the highest concentration level (10%) and time period of 72 h. Cocoa bean testa, compost, citrus waste, palm bunch waste and poultry manure recorded approximately (7.0, 18.0, 5.0, 34.0, and 20.0) hatching respectively, out of a total of 100, while the distilled water, the control treatment recorded 73 hatching (Table 2). Citrus waste was the most effective candidate in which significantly lower number of eggs, 5 hatched. However, it was not significantly different ($P < 0.05$) from cocoa bean testa in which 7 eggs hatched. Similarly, compost and poultry manure were the same in ability to inhibit hatching. Palm bunch waste was the least effective candidate in which 34 out of the 100 eggs hatched.

DISCUSSION

Several plant extracts are known to possess nematotoxic properties (Sosamma and Jayasree, 2002). In the current study, all the selected OWC inhibited the hatching of *M. incognita*. The effect of different concentration levels and exposure time on hatching of eggs varied with the treatments. At the highest concentration level (10%), percentage hatch in the control at 24 h was 29% and increased to 73% at 72 h, while citrus waste was 0 % and 5% and palm bunch waste was 3.7 and 34% respectively. The organic carbon and organic matter contents of palm bunch waste was not different from cocoa bean testa. However, the inhibition potential of cocoa bean testa was

Figure 1. Set up of aqueous extracts of organic waste compounds. Cold aqueous extracts (compost, poultry manure, cocoa bean testa, citrus waste, palm bunch waste and distilled water-control). Four concentration levels of extracts (0%-distilled water, 4, 8 and 10%).

Table 1. Chemical properties of organic compounds.

Treatment	pH	N%	OC%	OM%	P%	K%	Mg%
Compost	7.00b	1.98a	17.85b	35.70b	0.40a	0.48a	0.72b
Poultry manure	7.50c	2.07a	9.50a	19.00a	1.37b	0.66a	0.13a
Cocoa bean testa	6.10a	3.42b	43.85d	87.70d	0.44a	1.16c	1.01c
Citrus waste	7.80d	3.05b	36.40c	72.80c	0.46a	0.80b	0.53b
Palm bunch waste	6.10a	1.75a	44.80d	89.60d	0.13a	0.29a	0.40b
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.02	<0.001
SED	0.11	0.20	2.65	2.12	0.15	0.21	0.06
CV (%)	2.3	3.3	2.7	4.3	17.9	8.9	9.4

significantly higher than palm bunch waste. Thus, hatching inhibition potential of the OWC was not dependent on their chemical properties. The active ingredients of the treatments therefore might be responsible for hatching inhibition. Compared with the control; cocoa bean testa, compost, citrus waste, palm bunch waste and poultry manure inhibited hatching by (90, 75, 93, 53 and 73%) respectively. It was observed from the study that, the higher the concentration, the lower the percentage hatch and vice versa. Thus, higher concentrations of extracts were inhibitory in action against egg hatching of the nematode.

Table 2. Effect of cold water extracts of organic waste compounds on hatching of *Meloidogyne incognita* eggs

Treatments	Concentration (%)	Hatching (%)		
		24 h	**48 h**	**72 h**
Cocoa bean testa	4	6.0 ab	7.0 a	13.3 a
	8	6.3 ab	7.0 a	10.7 a
	10	0 a	4.0 a	7.0 a
Compost	4	9.7 ab	22.3 bc	29.0 bc
	8	7.0 ab	13.3 ab	23.7 b
	10	0 a	10.7 ab	17.7ab
Citrus waste	4	0 a	3.7 a	6.7a
	8	0 a	3.0 a	5.3 a
	10	0 a	3.0 a	5.0 a
Palm bunch waste	4	10.0 ab	42.7 cd	53.0 d
	8	7.0 ab	22.3 bc	42.0 c
	10	3.7 ab	34.0 c	34.0 c
Poultry manure	4	15.7 b	33.0 c	40.0 c
	8	13.3 b	36.3 c	43.0 c
	10	0 a	13.7 a	19.7ab
Distilled water		29.0 c	52.7 d	73.0 e
P		<0.001	<0.001	<0.001
SED		5.3	6.4	6.6
CV (%)		18.9	11.3	8.4

Results from the current study corroborated the findings of previous workers. Bharadwaj and Sharma (2007) observed that higher concentration levels (13.3, 16.6 and 20.0%) of *Carica papaya* inhibited hatching of *M. incognita*. Similarly, Ibrahim et al. (2006), reported that Clove extracts (1 mg liter^{-1}) of *Allium sativum* significantly reduced hatching activity of *M. incognita* to below 8% while the flower extracts of *Foeniculum vulgare* reduced hatching to below 25%. Also, root exudates of *Gaillardia pulchella* were lethal to J2 of *M. incognita* and inhibitory to hatch of eggs at the concentration of 250 ppm or higher (Tsay et al., 2004). Moreover, plant extracts of stored pulpified peels of lemon, orange and grapefruit demonstrated significant nematicidal activity against *M. incognita* second stage juveniles after 48 h of exposure (Tsai, 2008).

It has been sufficiently demonstrated in this study that water extracts of OWC may be beneficial for nematode control. The advantages of the strategy include; cost effectiveness, environmental acceptability and availability of the organic materials. However, further study on the identification of active ingredients of the organic compounds and other botanicals as biopesticides would reduce the over reliance on synthetic pesticides.

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