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RESEARCH ARTICLE

REACTION OF *Meloidogyne incognita* EGGS TO HATCHING IN AQUEOUS PLANT EXTRACTS

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ABSTRACT

The southern root-knot nematode, *Meloidogyne incognita* is a major limiting biotic factor affecting plant growth and yield in the tropical and sub-tropical worlds. Synthetic pesticides constitute the principal means to control the menace of the pest. Natural pesticides derived from plant parts offer alternative control strategy due to the identification of nematicidal properties in many flowering plants. A preliminary *in vitro* experiment evaluated the potential of five plant extracts (pawpaw root, leaf, seed and false yam tuber and leaf) to inhibit the hatching of eggs of *M. incognita*. Three concentration levels (w/v) that is 2, 4, and 8% of cold aqueous extracts of the plant parts were filtered into 6 cm Petri dishes and Petri dish, infested with 100 *M. incognita* eggs. Hatching of eggs was monitored over a three time period; 24, 48 and 72 h after infestation of the eggs in the respective aqueous extracts. The best result was obtained with false yam tuber extract in which 0, 3 and 5 eggs hatched while 26, 44 and 76 eggs hatched in distilled water (the control treatment) representing a hatching inhibition of 100, 93 and 93% at the three exposure time and concentration levels respectively. The current study which documents the first attempt at using false yam, *Icacina senegalensis* to control plant parasitic nematodes is environmentally friendly and cost effective. The formulation of the active ingredients of these botanicals as bio-pesticides would reduce the over-dependence on synthetic pesticides which have deleterious effects on man and the environment.

INTRODUCTION

Successful production of crops especially vegetables has been hampered to some extent by nematode pests, particularly, the southern root-knot nematode, *Meloidogyne incognita* (Agu and Ogbuji, 2001). *Meloidogyne* species which excite conspicuous galls on plant roots and tubers are among the top five major plant pathogens known to man (Bharadwaj and Sharma, 2007). Three species of the genus *Meloidogyne*; *M. arenaria, M. incognita* and *M. javanica* predominate in Ghana (Addoh, 1971). Root-knot nematodes, *Meloidogyne* species have extensive host ranges infecting a host of plant species (Trudgill and Blok, 2001). More importantly, root-knot nematodes cause significant economic losses in cultivated crops (Webster and Davis, 2007; Starr and Morgan, 2002). The economic significance of the southern root-knot nematode, *M. incognita* in the West African sub-region has been well documented. In Ghana for instance, *M. incognita* has been implicated as the most damaging nematode pest associated with vegetable production (Hemeng, 1980). Tomato yield losses ranging from 28-68% due to *M. incognita* has been reported in Nigeria (Adesiyan *et al.,* 1990) and in the Ivory Coast, Fargette and Braaksma (1990) observed that *M. incognita* populations overcame the resistance of the high yielding tomato cv. Rossol. Root-knot nematodes are difficult crop pests to control (Gowen *et al.,* 2005). In Ghana, synthetic chemicals usage has been the most effective strategy farmers employ to manage nematodes. This management strategy has been criticized by environmentalists in recent times due to the demerits of the strategy including; prohibitive prices and increase in toxicity in soil system (Vawdrey and Stirling, 1997), phytotoxicity, environmental pollution and nematode resistance (Blackman, 1997). Current research efforts have therefore been directed towards the use of plant extracts as alternative to synthetic compounds (Papachristos and Stamopoulos, 2002).

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Currently, over 2000 species of plants are known to possess some insecticidal activity (Kabaru and Gichia, 2009; Jacobson, 1989). Similarly, nematicidal properties have been reported in a wide variety of flowering plants (Bharadwaj and Sharma, 2007; Adegbite and Adesiyan, 2005; Onifade and Egunjobi, 1994). There was the need therefore to develop naturally occurring nematicides, which may be less toxic to man, animals and the environment but as effective against nematodes as synthetic products (Adegbite and Adesiyan, 2005). The current research effort therefore, aims at evaluating the effect of cold aqueous extracts of pawpaw, *Carica papaya* L. and false yam, *Icacina senegalensis* A. Juss on hatching of *M. incognita* eggs. The selection of the plants was informed by availability. Pawpaw is commercially cultivated while false yam grows abundantly in the wild in Ghana.

MATERIALS AND METHODS

An *in vitro* experiment was conducted to evaluate the potential of five aqueous extracts procured from two plants; pawpaw and false yam on hatching of *M. incognita* eggs. The candidates were pawpaw root, fresh leaves and dried seed and false yam tuber and fresh leaves. The false yam was obtained from an experimental field of the Crops Research Institute at Ejura (Fig.1a) while the pawpaw tree was found in a garden at Ejisu near Kumasi (Fig.1b). 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^{o}C, 12 h of photoperiod and a relative humidity of 87.79% during the period of experimentation.

Inoculum preparation

One egg mass of *M. incognita*, identified through perineal pattern as described by (Taylor and Netscher, 1974) was collected from plant house cultures and extracted from tomato (*Solanum lycopersicum* L.) roots by blending in a Waring Laboratory Blender® (Christison

Scientific Equipment Ltd. UK) and shaking for 3 min in distilled water and rinsing for 2 min under running tap water. The eggs were cultured on the dwarf determinate tomato cv. Tiny Tim in a plant house at the Crops Research Institute, Kumasi with a minimum temperature of 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.

Figure 1. The two plants used in the experiment A, False yam (*Icacina senegalensis*) and B, Pawpaw (*Carica papaya*).

Plant extracts preparation

The moisture level of the five plant 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 samples 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 8% concentration (w/v). Serial dilutions were then performed to obtain concentrations of 4% and 2%. Distilled water was the control treatment. One milliliter each of the aqueous suspensions was pipetted into 6-cm Petri dishes and one hundred freshly extracted *M. incognita* eggs in one milliliter suspension were poured into the aqueous extracts and properly labeled. The experiment was arranged in a randomized complete block design, repeated twice and replicated three times each time (Fig. 2). Hatching was monitored over a three time period; 24, 48 and 72 h respectively. Data was subjected to the Analysis of variance (ANOVA) using the mixed models (REML) approach in GenStat 8.1 statistical package. Means were separated using Fisher's protected least significant difference (LSD).

Figure 2. Set up of aqueous extracts of the plant parts. Cold aqueous extracts (pawpaw root, leaf, seed, false yam tuber and leaf) and distilled water-control. Three concentration levels of extracts; 2%, 4, 8 and 0%-distilled water.

RESULTS

The effect of different concentration levels of extracts on hatching of eggs varied with material and exposure time (Table 1). At the highest concentration level (8%) and time period of 72 h, pawpaw root, seed, leaf, false yam leaf and tuber recorded approximately (17, 7, 8, 13 and 5%) hatching respectively while distilled water, the control treatment recorded 76%. In the control treatment, hatching at 24 h was significantly ($P<0.05$) high (26%) compared with no hatch (0) at the highest concentration of false yam tuber (FYT). The percentage hatch increased from 26 at 24 h to 76 at72 h in the control treatment

Table 1. Number of *M. incognita* eggs hatched in cold water extracts of plants at three time periods

Treatments	Concentrations (%)	Hatching (%)		
		24 h	48 h	72 h
Control		26e[z]	44h	76h
Pawpaw root	2	7cd	25gh	47gh
Pawpaw root	4	3abc	13cdef	21de
Pawpaw root	8	1ab	6abc	17cd
Pawpaw seed	2	7cd	15def	18cd
Pawpaw seed	4	2ab	8abcd	13bc
Pawpaw seed	8	2ab	4ab	7ab
Pawpaw leaf	2	5bc	21fgh	35g
Pawpaw leaf	4	2ab	12bcde	17cd
Pawpaw leaf	8	1ab	3a	8ab
False yam leaf	2	11d	28gh	30fg
False yam leaf	4	7cd	17efg	26ef
False yam leaf	8	4bc	9abcd	13bc
False yam root	2	4bc	9abcd	12abc
False yam root	4	1ab	5abc	7ab
False yam root	8	0a	3a	5a
Grand mean		5.2	13.9	22.0
Lsd (0.05)		3.6	8.2	7.5

Data are means of three replicates
[z]Means with the same letter are not significantly different

while FYT increased from zero (0) to a significantly low hatch (5%) at the same exposure time. With the exception of pawpaw root (2% and 8% and pawpaw seed 8%) concentration levels, hatching of eggs reduced after 48 h exposure time for all extract candidates (Table 1). At the highest exposure time 72 h, the three concentration levels (2, 4 and 8%) of FYT recorded (12, 7 and 5%) hatching which were superior to false yam leaf (FYL) performance of (30, 26 and 13%). Similarly, pawpaw seed at the highest concentration level, significantly inhibited hatching (7%) compared with pawpaw root (17%) but not different from pawpaw leaf (8%).

DISCUSSION

Extracts of all the selected candidates inhibited hatching of eggs of *M. incognita*. At the three extract concentration levels, *C. papaya* seed extract inhibited hatching by 76, 83 and 91% respectively. The hatching inhibition could be due to ovicidal activity of *C. papaya*. The present result corroborates the findings of Wabo *et al.* (2011) who reported that aqueous extract of *C. papaya* seed was more potent on eggs than larvae of *Heligmosomoides bakeri*. At 2.75 mg/mL, only 8% of eggs embryonnated and 50% hatched to L_1 vs 57% embryonic development and 79% hatching occurred in ethanolic extract. Similarly, Kovendan *et al.* (2011) observed that leaf extract of *C. papaya* showed larvicidal and pupicidal effects on *Aedes aegypti* after 24 h of exposure. The tuber of *I. senegalensis* was found to be the most effective plant extract in inhibiting hatching of eggs of *M. incognita*. At the three concentration levels, *I. senegalensis* tuber extract inhibited hatching by 100, 93 and 93% respectively compared with the leaf extract performance of 95, 88 and 83% respectively. From the results, the hatching inhibition potential of *I. senegalensis* decreased with time while that of the control increased with time. The reaction of *M. incognita* to *I. senegalensis* has never been reported. However, in an ethnopharmacological survey conducted on plants used in traditional malaria treatment in Senegal, *I. senegalensis* was found to possess anti-plasmodial activity (IC_{50} < 5 µg/mL) without host cell toxicity. The dichlorothane fraction showed stronger anti-plasmodial activity than the total extract (Sarr *et al.,* 2011). At the highest concentration level and exposure time, *I. senegalensis* tuber was superior to the leaf extract while *C. papaya* seed was outstanding than the root extract. Thus, it could be inferred from the current study that different parts of a plant possess different active ingredients or different concentrations of the same active ingredients (Osei *et al.,* 2010). Prior to the current findings, Krishna *et al.* (2008) reported that the leaf of *C. papaya* contains Alkaloids carpain while the root contains carposide and myrosin. Similarly, Das (1980) found caricacin and oleanolic glycoside which causes sterility in male rats in the seed of *C. papaya*. The time of the experiment did not permit the inclusion of false yam seed as a candidate for evaluation which was a weakness in the experiment. We are therefore unable to assess false yam seed vis a vis false yam tuber which turned out as the best candidate. The formulation of the active ingredients of these botanicals as bio-pesticides would reduce the over-dependence on synthetic pesticides which have deleterious effects on man and the environment. The prospect of adoption of bio-pesticides by farmers is high for environmental friendliness.

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