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Nematicidal potential of some indigenous plant extracts against root-knot nematode on cacao

Samuel Bukola Orisajo* and Lelia Nkechiyere Dongo

Plant Pathology Division, Cocoa Research Institute of Nigeria, P.M.B. 5244, Ibadan, Nigeria

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ABSTRACT: The effect extract of *Ocimum gratisimum* L., *Carica papaya, Azadirachta indica* A. Juss, *Vernonia amygdalina* and *Bixa orellana* L. on pathogenicity and reproduction of *Meloidogyne incognita* race 3, the causal organism of the root-knot disease of cacao was investigated in the greenhouse. Leaf extracts were prepared by blending and filtration of 25g of chopped leaves in 100ml of distilled water. Each cocoa seedling in a naturally infested soil received 40ml water extract of the leaves. Nematode populations were reduced by the application of the extracts in a descending order of *A. indica, B. orellana, O. gratissimum, C. papaya* and *V. amygdalina*. Plant vigour was enhanced based on the treatments compared to the control. The significance of these findings is discussed in relation to the non-synthetic chemical means of disease control on cacao and use by the resource-poor farmers in Nigeria.

Key words: Plant extracts, *Meloidogyne incognita*, Cacao seedlings, Pest control.

Introduction

Cocoa continues to play an important role in the agricultural sector of the economy of Nigeria. Nearly 90% of the cocoa fields are 20 hectares or less in extension, most not larger than 5-hectares, showing that cocoa is still a small holder crop. Cocoa farmers are aware of the need to improve the productivity of cocoa and upgrade the socio-economic values of the crop, but pests have been a constraint. There has been a downward trend of cocoa beans production in Nigeria from 180,000 tonnes in 2002 to 165,000 tonnes in 2003 (ICCO, 2003).

Nematodes such as *Dolichodorus* and *Meloidogyne* species, especially *M. incognita* and *M. javanica* have caused losses in cocoa around the world including yield decrease, sudden death of trees and growth retardation of seedlings in the nursery (Campos *et al*., 1990). In Nigeria, the ropot-knot nematode, *Meloidogyne incognita* has been implicated as being pathogenic on cacao seedlings resulting in failure of seedlings to establish on the field. The damage could be die-back, wilt, chlorotic symptoms, stunted shoot, galling of the root or complete death of the seedlings. Evidence exists that fresh cacao plot can be contaminated with phytonematodes from the nursery (Afolami and Caveness, 1983).

*To whom correspondence should be addressed.
E-mail: sambukki@yahoo.com

Control strategies have been on the use of chemicals, which is highly effective and reliable resulting in healthy seedling growth (Afolami, 1993). A prime dilemma associated with nematicidal use, however, is how to realize the benefit mentioned above without causing further deterioration of the environment. Furthermore, the chemicals are expensive and not assessable to the resource-poor farmers.

Plant extracts have shown to be alternatives to the use of nematicides in effective management of these nematodes resulting in plant health (Akhtar and Alam, 1990; Dias *et al*., 2000); Salgado *et al*., 2003). However, this growing and promising method of disease control has not been attempted on the root-knot disease of cacao.

This work, therefore, examines the nematicidal effects of pawpaw leaf (*Carica papaya*), scent leaf (*Ocimum gratissimum* L.); neem leaf (*Azardirachta indica* A. Juss); Bitter leaf (*Vernonia amygdalina*) and 'Aje' leaf (*Bixa orellana*) on *M. incognita* in cacao seedlings.

Materials and Methods

Preparation of Leaf Extracts

Fresh leaves of *C. papaya, O. gratissimum, A. indica, V. amygdalina* and *B. orellana* were thoroughly washed under running tap water and sterile water. Leaf extracts of each plant were prepared by blending 25g of chopped leaves in 100ml-distilled water with a waring blender. Thereafter, the suspension was filtered through sterile muslin cloth.

Pathogenicity Test

Sandy-loam top soil (sand 60.8%, silt 34%, clay 5.2%, organic matter 6.8%) normally used for raising cocoa seedlings was taken in bulk from the field, thoroughly mixed and stored in sealed containers at 10°C until use. To establish initial population densities of nematodes, five replicate 175cm^3 sub-samples were extracted for five days on trays (Whitehead and Hemming, 1965) just before starting the experiments. The soil contained 897±25.1 (standard error of mean, SE) second stage juveniles (J2) of *M. incognita* 175cm^3 of soil, 8.6±2.6 and 4.1±0.8 of *Hemicycliophora saueri* and *Scutellonema brachyurum* respectively. Soil was dispensed into 185mm-diameter pots – the usual one for raising cacao seedlings commercially (2.5dm^3 soil/pot).

All pots were planted each with three seeds of *Theobroma cacao* cv. F_3 Amazon, which was later thinned to one per pot a week after emergence. Each pot received 40ml of the leaf extracts immediately after thinning by pouring the aqueous solution of the extracts into four holes, 4cm deep, around the seedling in each pot. An untreated treatment, without the plant extract, served as control. Five replicate pots per treatment were arranged in a completely randomised design on a screenhouse bvench and were watered regularly. Ambient temperature during the experiment was in the range of 26-28°C.

Effects of leaf extracts on the populations of .M. incognita and plant health

After sowing, regular observations were made to record data on phytotoxic effects of any treatment on plants and disease symptoms. The experiment was terminated 26 weeks after planting. The growth parameters viz; plant height, stem girth and number of leaves was taken. Plant areas were recorded with the aid of electronic lead meter. The roots were carefully freed of soil, washed under a gentle stream of tap water, mopped and galls counted using a hand lens at 3-5 times magnification. Root galling was assessed by using the 0-5 gall index (Sasser *et al*., 1984). Nematodes were extracted from 5g of roots using sodium hypochlorite method (NaOCl) of Hussey and barker (1973) and all the developmental stages of the nematode were counted collectively.

An aliquot of 175cm^3 soil from each pot was assayed for juveniles of *M. incognita* using Whitehead and Hemming (1965) tray modification of the Baermann technique. Nematode suspensions were concentrated to a 20ml of water and a 2-ml aliquot counted.

Statistical Analysis

Before statistical analysis, nematode densities were subjected to logarithm transformations. Analyses of variance (ANOVA) were carried out and the treatment means compared with the LSD test (P = 0.05) using GENSTAT package.

Results and Discussion

The leaf extract of all the plants enhanced the growth of cacao seedlings significantly in the presence of the nematode compared to the control (P = 0.05). However, there was a reduction in the height of plants treated with leaf extract of *V. amygdalina* compared with the rest (Table 1). There were significant differences in stem girth, leaf area, dry shoot and root weights between the seedlings treated and the control. Soil treatment with leaf extracts did not result in significant differences in the number of leaves (Table 1).

None of the leaf extracts have phytotoxic effects on the cacao seedlings, however, chlorotic symptoms and shoot stunning were observed on the seedlings treated with *V. amygdalina* but more pronounced with the untreated seedlings. Small galls were observed on all the roots of the seedlings, more abundant on the untreated control. The gall index were 2.1±0.2, 2.1±0.3, 2.0±0.2, 2.3±0.2 and 2.0±0.3 for cacao seedlings treated with leaf extracts of *O. gratissimum*, *C. papaya*, *A. indica*, *V. amygdalina* and *B. orellana*, respectively; while the gall index for the control was 3.4±0.4.

All soil treatments reduced densities of *M. incognita* J2/175cm^3 of soil (Figure 1); all treatments except leaf extract of *V. amygdalina* alone reduced densities of *M. incognita* J2/5g root (Figure 2). Densities of *H. sauieri* J2/175cm^3 of soil were not significantly different between untreated soils and soils with leaf extracts (15.3±5.2 and 13±4.2 SE), respectively.

Leaf extracts of neem, *A. indica* suppressed *M. incognita* multiplication on cacao seedlings in this experiment. The triterpenois (azadirachtin, nimbin and salannin) present in crude extracts of neem have been reported to inhibit penetration and induce mortality of juveniles of *M. incognita* in the roots of mungbean seedlings (Mojumder *et al*., 2002). Neem leaves have also been shown to contain tannins, which are lethal to the nematode (Fatoki and Fawole, 2000). The potential of neem extract in managing root-knot nematode disease of cacao in the nursery was reported in this experiment.

The antifungal effects of *O. gratissimum* leaf extract have been reported (Okigbo and Emoghene, 2003). The essential oil of *O. gratissimum* inhbited the growth of a protozoan (trypanosamatid), *Herpetomonas ssamuelpessoai* (Holtez *et al*., 2003). The nematicidal effect of the leaf extract was reported in this experiment with the marked reduction of *M. incognita* population both in the roots of cacao seedlings and the soil.

Leaf extracts of *B. orellana* effectively inhibited the multiplication of *M. incognita* thereby allowing the cacao seedlings to grow effectively. However, *M. exigua* population in coffee was reportedly reduced by the leaf extract application of *B. orellana* without any increase in plant height (Salgado and Campos, 2003). There is need for more work to be carried out in order to determine the chemicals in the leaf extract that is responsible for its nematicidal activity.

The leaf extract of *C. papaya* contain papain as well as the alkaloid carpine, which have been shown to be effective against *M. incognita* in *Phaseolus vulgaris* (Stoll, 2000). A high efficacy of *C. papaya* latex against animal-parasitic nematode, *Heligmosomoides polygyrus* has been demonstrated in mice (Satrija *et al*., 1995). However, water extract of *C. papaya* inhibited the multiplication of the nematode in this experiment. Isothiocyanates has been demonstrated to be responsible for the toxicity of seed extracts of *C. papaya* to *M. incognita* (Nagesh, 2002).

Although water leaf extract of *V. amygdalina* expressed low nematicidal activity in this experiment, the ethanolic leaf extract has been shown to be toxic against the infective larval stage of *Strongyloides papillosus*, a highly pathogenic animal-parasitic nematode (Musongong *et al*., 2004).

Nigeria is endowed with many indigenous leaf vegetables species, which spread across the estimated cultivable land area of 71.2 million hectares, some of which are use for herbal medicine (Opabode and Adeboye, 2005). The outcome of this study revealed the nematicidal potential of five leaf extracts out of these plants. The use of leaf extract is suggested as a good substitute for nematicide use in the management of root-knot nematode disease of cacao seedlings. This will reduce the current level of frustration faced by the resource-poor farmers in establishment of cacao seedlings on the field.

Table 1: Effect of leaf extract of *O. gratisdsimum, C. papaya, A. indica, V. amygdalina* and *B. orellana* on the growth of cacao cv F_3 Amazon seedlings infested with *M. incognita.*

Treatments	Plant height* (cm)	Sterm girth* (cm)	Leaves/plant* (number)	Leaf area* (cm^2)	Dry shoot weight* (g)	Dry root weight* (g)
O. gratissimum	59.12a	0.97a	20.70a	98.76a	19.51a	3.11a
C. papaya	59.13a	0.97a	20.71a	98.77a	19.52a	3.12a
A. indica	59.14a	0.97a	20.71a	98.77a	19.53a	3.13a
V. amygdalina	49.82b	0.96a	20.69a	91.10b	16.01b	2.97b
B. orellana	59.10a	0.98a	20.70a	98.76a	19.51a	3.13a
Untreated (control)	39.67c	0.69b	20.68a	84.20c	15.90b	2.63c

*Means of five replications.

[1]Means followed by the same letter in the same column are not significantly different (P = 0.05).

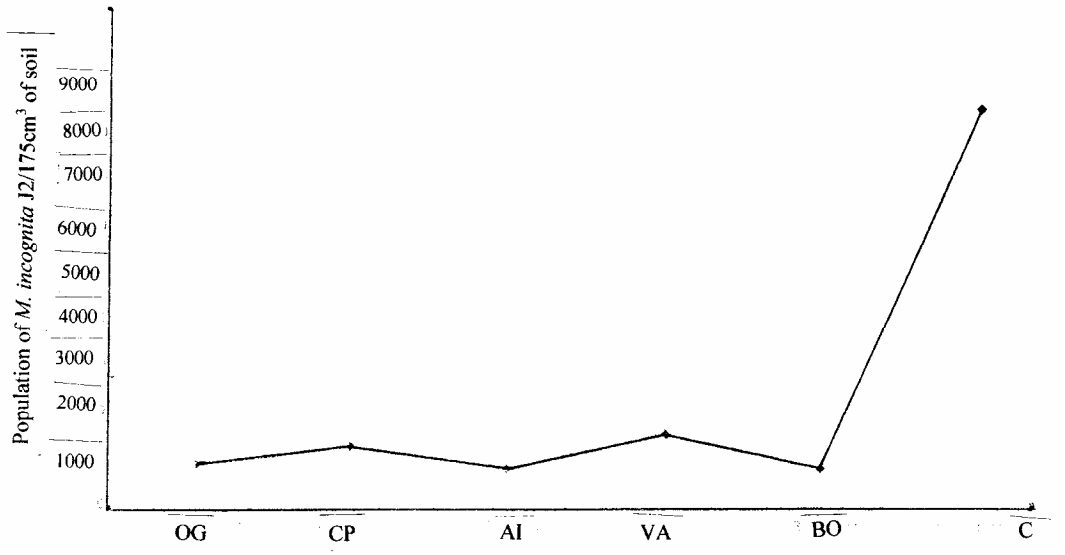

Fig. 1: Effect of leaf extracts treatment on the populations of *M. incognita* J2/175 cm^3 on caocao seedlings. (Transformed data [ln (x+1)] used in statistical analysis; back – converted means of J2/175cm^3 of soil shown).

[OG = *O. gratissimum*; CP = *C. papaya*; AI = *A. indica*; VA = *V. amygdalina*; BO = *B. oreliana*; C = Untreated (Control)]

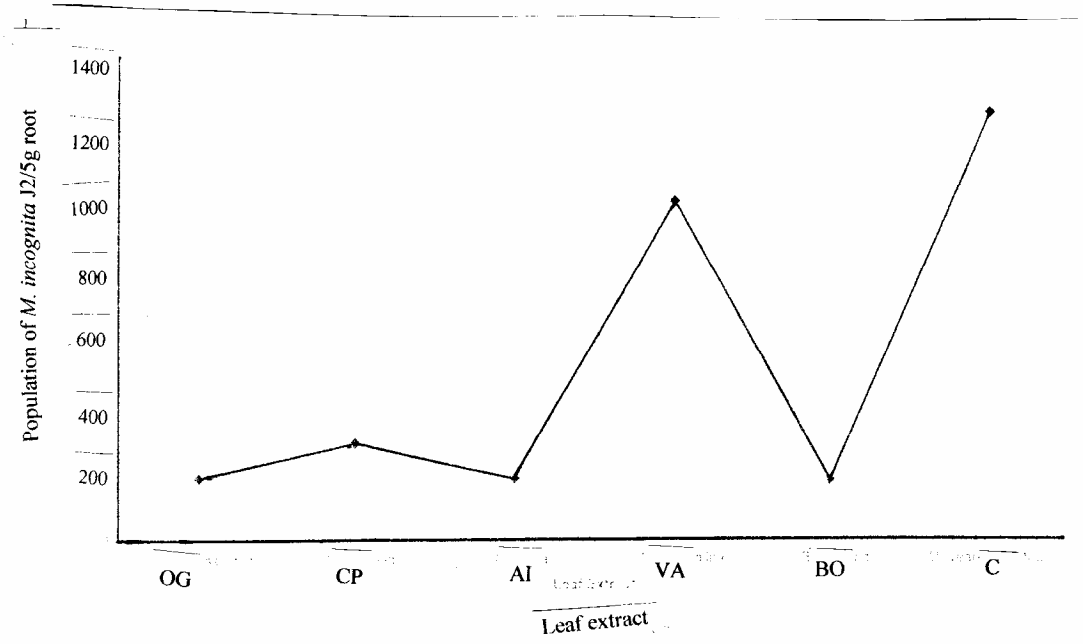

Fig. 2: Effect of leaf extracts treatment on the populations of *M. incognita* J2/5g root on caocao seedlings. (Transformed data [ln (x+1)] used in statistical analysis; back – converted means of J2/5g root shown).

Further research will be conducted on the field so as to ascertain the nematicidal stability of the extracts in the soil over time period.

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