



Influence of biodegraded bark extracts of *Anacardium occidentale* on survival of *Meloidogyne incognita* infecting cabbage plants

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Abstract

Plant parasitic nematodes (PPNs) cause great damage to all agricultural crops; they infect all plant parts and cause reduction in yield. Synthetic nematicides have been used lately to combat the menace of PPNs on farmlands. The drawbacks of synthetic nematicides gave rise to interest in alternative methods of managing plant parasitic nematodes. In this regard, compounds from biodegraded as well as fresh stem bark of (cashew) *Anacardium occidentale* were extracted, isolated and partially characterized. The crude extracts and isolates were tested for their nematicidal activity in laboratory and field experiments. Significant ($p < 0.05$) differences were observed in the activity of the extracts and isolates. The isolated compounds from *A. occidentale* biodegraded stem bark extract (ANCO/BDG/CMP) exhibited higher juvenile mortality and reduced egg hatch in the laboratory while the activity of the isolated compound from fresh bark (ANCO/FRS/CMP) extract was significantly lower. The crude extracts ANCO/FRS/CRD and ANCO/BDG/CRD had weaker nematicidal activity. *Meloidogyne incognita* population was notably low after harvest in all treated cabbage plants on the field. Spectroscopic results revealed that microbial action during biodegradation of the stem bark produced a different compound having nematicidal properties.

Keywords: *Anacardium occidentale*, Cabbage, Cashew stem bark, *Meloidogyne incognita*, Nematicide

Introduction

Meloidogyne species are one of the well-known groups of phytonematodes which are arduous to control. They are parasites of more than 5,500 plant species (Goodey et al., 1965). The most damaging in order of importance are *Meloidogyne incognita*, *M. arenaria*, *M. hapla* and *M. javanica* (Moens, 2009; Wasemael et al., 2011; Fabiyi, 2020). *M. incognita* is the most pervasive; its infestation brings about terrible crop damage and heavy yield loss (Sasser, 1989; Ekanayak et al., 2000; Fabiyi, 2021) especially in vegetable production (Sikora and Fernandez, 2005). Broadly, an appraised loss of about \$100 billion is incurred worldwide on yearly basis owing to their habitat in plant tissues and excessive reproductive capacity (Oka et al., 2000). The huge amount of crop loss to plant

parasitic nematode infection has made the application of synthetic nematicides a key practice in agriculture. This exercise has continued because there is paucity of information in connection with the pernicious nature of pesticides. Lack of expertise in pesticide application by farmers has also contributed largely to environmental pollution (Jatto et al., 2012). Pesticide residues have been detected in fruits and vegetables due to the persistence of nematicides in the environment (Fabiyi and Olatunji, 2021). However, the problems arising from environmental pollution associated with the persistent use of synthetic nematicides are alarming (Siddiqui and Shaukat, 2003; Nico et al., 2004). Consequently, research on botanical extracts for the control of plant parasitic nematodes is in a momentous state (Atolani and Fabiyi, 2020). The exigency of biodegradable and bio-active plant

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extract was emphasized by Atolani et al. (2014a; 2014b). *Anacardium occidentale* L. is domesticated in the tropics with much relevance in customary medicine (Barbosa-Filho et al., 2015), which is assignable to the phytochemical and secondary metabolites that are accountable for the pharmacological, cytotoxicity, toxicological and biological properties of the plant (Barros et al., 2020). In this context, *A. occidentale* extracts have been employed in the treatment of diverse diseases (Taiwo et al., 2017; Baptista et al., 2018). The stem bark extracts of *A. occidentale* have been reported to possess a wide spectrum of anti-bacterial (bacteriostatic and bactericidal) and anti-fungal substances (Arekemase et al., 2011; Alia et al., 2016). This research was undertaken to evaluate the nematocidal potential of biodegraded and fresh stem bark extracts of cashew (*A. occidentale*).

Materials and Methods

Preparation of Materials

The stem bark of cashew was collected from the mother tree around G.R.A area in Ilorin, Nigeria. It was air dried, cubed into pieces and weighed. One kilogram was extracted cold in ethanol for five days; it was then decanted and concentrated using a rotary evaporator. Another 1 kg was buried underground at one-foot-deep to undergo a decay process. After a month it was exhumed and extracted cold in ethanol. The crude extracts were spotted on commercial TLC (Thin Layer Chromatographic) plates (Merck, Germany), and developed with n-hexane/dichloromethane (3:1) in a chromatographic tank. The chromatograms were viewed under the UV lamp at a wavelength of 366 nm. Two prominent compounds with distinct fluorescence were isolated using preparative thin layer chromatography (PTLC). The isolated compounds from the fresh bark, the decomposed (biodegraded) bark and the two crude extracts were coded ANCO/FRS/CRD (*A. occidentale* fresh crude extract), ANCO/BDG/CRD (*A. occidentale* biodegraded crude extract), ANCO/FRS/CMP (*A. occidentale* fresh isolated compound) ANCO/BDG/CMP (*A. occidentale*

biodegraded isolated compound). Carbofuran was maintained as standard check and coded CBFN.

Spectroscopic Measurement

The infrared spectra of the isolated compounds were recorded on Shimadzu 8400 (Shimadzu Corporation Kyoto Japan) Fourier Transform-Infrared (FT-IR) spectrophotometer using KBr pellets.

Nematicidal Assay

Pure culture of *M. incognita* eggs was extracted from infested roots of tomato plant (*Lycopersicon esculentum* Mill). Briefly, 500 ml of 0.4% sodium hypochlorite (NaOCl) solution was added to diced (1-2 cm) galled tomato roots in a 1000 ml beaker for the extraction of nematode eggs from the root galls following the Hussey and Baker (1973) method of extraction. The extracted eggs were washed through 73, 56 and 25 µm sieves to obtain free eggs for the experiment; some of the extracted eggs were incubated at 27°C for 48 hours to hatch out the second stage juveniles. Suspensions of approximately 35 juveniles/ml and 35 eggs/ml were used in the *in vitro* experiment. Both the larval and egg suspensions (2 ml) were transferred into counting dishes. The crude ethanol extract and isolated compounds of degraded and fresh bark of *A. occidentale* were dissolved in distilled water and various concentrations of 10, 20 and 30 mg/ml were prepared. A single concentration of 150 mg in 30 ml water was made for carbofuran. Distilled water represented the control. Each concentration was transferred separately into counting dishes containing eggs and juveniles independently and kept at room temperature. The experiment was set in a completely randomized design (CRD). There were three replicates each for the five treatments at four dosages. A total of sixty (60) counting dishes were used. Observations on juvenile mortality and egg hatching were made on daily basis for 6 days after treatment using a stereomicroscope at x100 magnification. Juveniles that did not react to probing were considered dead. Juvenile mortality and rate of egg hatch were determined by the following formula (Elasyed et al., 2021).

Juvenile mortality = $100 \times (\text{number of dead } J^2 / \text{total number of } J^2)$

Egg hatching rate = $100 \times (\text{number of } J^2 / (\text{number of eggs} + \text{number of } J^2))$

Field Experiment

The experimental site was the University of Ilorin Teaching and Research Farm, located within the guinea savannah vegetation. The area experiences a tropical climate. Average rainfall is estimated to be about 1185 mm, with an annual temperature of approximately 26°C . The experimental field size was 40 m x 45 m. This was ploughed and harrowed and was divided into beds of 1.6 m² each. The experimental layout was a randomised complete block design, (RCBD). Cabbage seedlings (Two weeks old) were transplanted from the nursery onto the beds at a spacing of 35 cm within the rows and 45 cm between rows. The seedlings were inoculated with an average of 450 *M. incognita* juveniles per ml a week after transplanting (Fabiyyi, 2019). Two weeks after inoculation, treatments were applied at 10, 20 and 30 mg/ml, while carbofuran was administered at 1.0, 1.5 and 2.0 kg a.i./ha. Each of the five treatments with four dosages was replicated three times. Every single cabbage plant was mulched after inoculation and treatment. Weeding was done by hand as the need arises. Data was taken on leaf diameter, number of leaves, head weight and nematode population in root and soil. The obtained data were evaluated by utilizing GenStat 5.32. Different means were separated with Duncan's multiple range test (DMRT) at alpha level <0.05.

Results and Discussion

The infra-red spectroscopic data of the compound isolated from the fresh ethanol bark extract of *A. occidentale* depicts N-H stretching vibration of amines at 3441 cm^{-1} , with a corresponding C-N stretching vibration at 1265 cm^{-1} . The C-H aliphatic stretching was observed at 2852 and 2924 cm^{-1} and C=O of aldehydes at 1734 cm^{-1} , while the C-H bending of alkanes was seen at 1458 and 1377 cm^{-1} . The spectroscopic data of the biodegraded extract

had O-H and C-O of alcohol at 3545 cm^{-1} and 1112 cm^{-1} respectively. An aliphatic C-H stretch was observed at 2928 cm^{-1} , while the C=C peak of an alkene appeared at 1622 cm^{-1} which was also supported by the =C-H bending frequency of alkene at 802.41 cm^{-1} . The isolated compound from the fresh ethanol extract is suspected to be a secondary aliphatic amine (Fig. 1) while the compound isolated from the biodegraded extract could be an unsaturated aliphatic alcohol (Fig. 2).

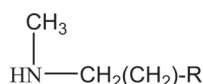


Figure 1. Secondary aliphatic amine

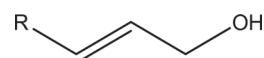


Figure 2. Unsaturated aliphatic alcohol

The effects of *A. occidentale* fresh and biodegraded ethanol stem bark extract and the compounds isolated from the fresh and biodegraded extracts on the juvenile mortality and egg hatch of *M. incognita* are presented in figures 3 & 4. Weak nematocidal activity was observed in all the treatments tested, but the compound isolated from the biodegraded ethanol stem bark (ANCO/BDG/CMP) was significantly ($p < 0.05$) better than all the other treatments with 56 percent juvenile mortality on the 6th day of exposure (Fig. 3). Carbofuran (CBFN) was however significantly more effective with 74.22 percent mortality (Fig. 3). The highest concentration of treatments was significantly more effective as opposed to all the other lower concentrations with higher percentage mortality of 39.10 percent. Egg

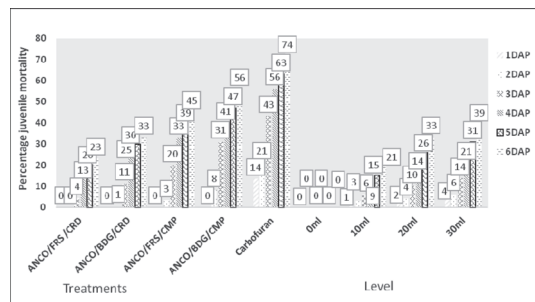


Figure 3: Effect of treatments on juvenile mortality of *M. incognita*.

DAP= Days after treatment application

hatch was delayed in all the treatments. A significantly ($p < 0.05$) higher percentage of egg hatch (11.12%) was seen in the fresh stem bark extract (ANCO/FRS/CRD), thus demonstrating its weak activity, while the isolated compound from the biodegraded extract exhibited a stronger activity with a significantly lower egg hatch percentage (1.14%). Carbofuran, the standard check was however significantly ($p < 0.05$) more effective (Fig. 4).

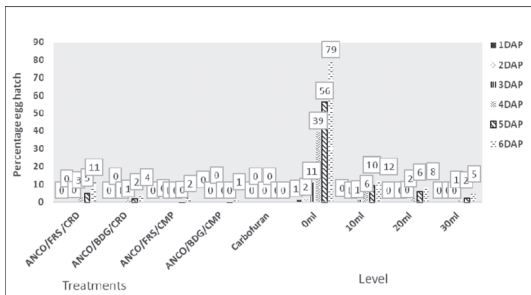


Figure 4: Effect of treatments on egg hatch of *M. incognita*

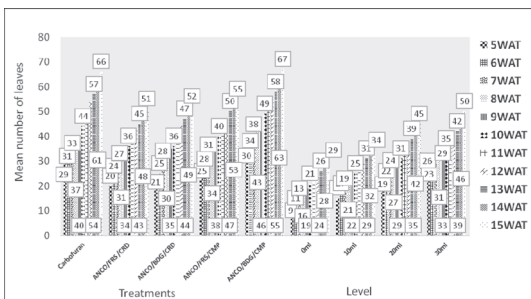


Figure 5: Effect of treatment on mean number of leaves (No/Plant) WAT= Weeks After Treatment

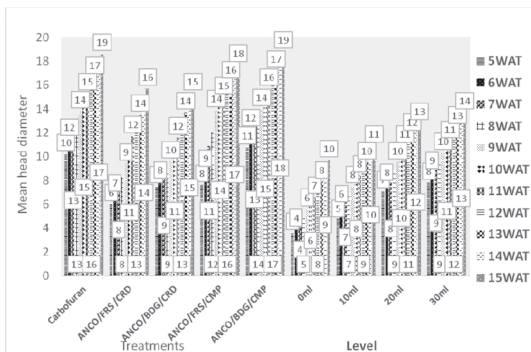


Figure 6: Effect of treatments on mean head diameter (cm) of cabbage plants

The outcome of application of extracts from *A. occidentale* as a measure of *M. incognita* management on the field is depicted in Figures 5-9. Variations were observed among treatments on their effect on the growth and yield of cabbage plants. From Figure 5, *A. occidentale* biodegraded compound (ANCO/BDG/CMP) gave the best outcome of the vegetative growth of cabbage plants. Head diameter was appreciably wider in cabbage plants treated with *A. occidentale* biodegraded compound over a period of eleven weeks (Fig. 6). Likewise, there was a corresponding significantly

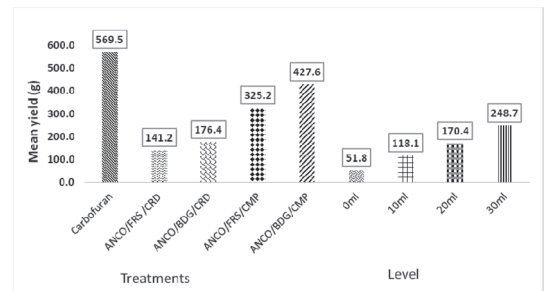


Figure 7: Effect of treatments on yield of cabbage plants at harvest

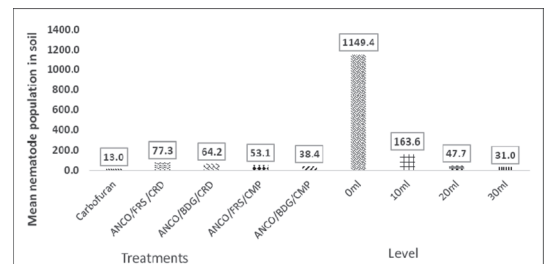


Figure 8: Effect of treatments on nematode population in soil of cabbage plants after harvest

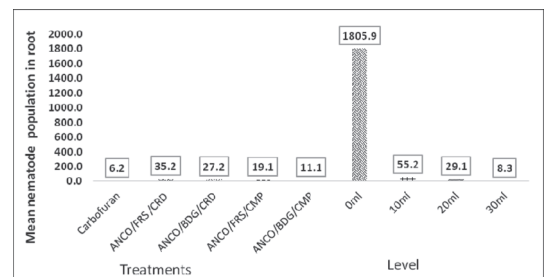


Figure 9: Effect of treatments on nematode population in root of cabbage plants after harvest

heavier head weight as opposed to what was obtained in cabbage plants treated with other materials from *A. occidentale*. Notwithstanding, carbofuran (CBFN) treated cabbage plants had noteworthy head weights and were remarkably heavier than what was achieved in biodegraded compound treated plants (Fig. 7), this is consequential from the mean number of leaves and head diameter of the cabbage plants over the period of observation. Population of recovered *M. incognita* from CBFN treated plants was notably lower than the populations in all *A. occidentale* extract treated plants, this result was however superior to what was attained in the control plants (Figs. 8 & 9).

Comprehensively, the variation in concentration was notably significant on the performance of the treatment materials. In the laboratory experiment, percentage mortality was directly proportional to concentration. Throughout the observation period, there was no mortality of juveniles in the control experiment, albeit 39.10 per cent juvenile mortality was observed in the highest concentration of 30 mg/ml on day 6. At higher concentration, egg hatch was totally inhibited in the first two days of the experiment, while few hatches were later recorded. On the 6th day of the experiment, untreated control with distilled water had 79.19 percentage egg hatch, while 5.11 per cent was documented in the highest concentration of 30 mg/ml (Figs. 3 and 4). More numbers of cabbage leaf and wider diameters were obtained in plants treated with the highest concentration as opposed to the untreated plants. Few nematodes were recovered from the soil and roots of cabbage plants administered with high concentration of materials. Equivalently, higher yield was also observed with high concentration of materials.

Differences were observed in the functional groups of compounds isolated from the fresh and biodegraded plant materials. Soil micro-organisms can initiate biotransformation of plant constituents when buried underground. Adetitun et al. (2016)

established the bio-degrading activities of some gram-negative bacilli in soil. Plant materials incorporated into the soil as soil amendments have been reported widely to be effective in the management of soil borne diseases (Mohilal and Dhanachand, 2003; Lopes et al., 2005; Fabiyi, 2021). Plant materials made into composts have been employed in the management of plant parasitic nematodes. Decomposed (decayed) plant materials have been found effective in the control of *Meloidogyne*, *Helicotylenchus* and *Xiphinema* species. Olabiyi et al. (2007) recorded 62.45, 43.52 and 59.42 percentage reductions in nematode population using decomposed wild sunflower, decomposed maize stover and decomposed cassava peel on the populations of *Meloidogyne*, *Helicotylenchus* and *Xiphinema* species respectively.

Similarly, crop residues integrated into soil are known to improve soil physicochemical properties and inhibit the reproduction of nematodes. This is attainable through slow release of secondary metabolites which are primary constituents of the residues (Kirkegaard et al., 1993; Zaynab et al., 2019; Cherr, 2006). Effectiveness of soil admix materials relies on biomass composition and class of secondary metabolites (Sandoval-Ruiz Grabau, 2023). Metabolites including flavone-C-glycosides, cyanides, triterpene glycosides and isothiocyanates are released from plant tissues applied as soil amendment (Soriano et al., 20004; Kamo et al., 2006; Mylona et al., 2008; Vig et al., 2009; Cressey and Reeve, 2019), and have been implicated in the inhibition of plant parasitic nematode invasion and reproduction in plant tissues with varying degrees of effectiveness (Soriano et al., 2004)

Comparably, *Eucalyptus globulus* applied as soil admix brought down the population of *Pratylenchus* spp infecting maize as against the population in untreated maize plants at harvest (Fabiyi et al., 2020). In this experiment, the extract obtained from the biodegraded stem bark of *A. occidentale* and

the isolated compound exhibited stronger nematicidal activity as opposed to the fresh stem bark material. This supports the fact that biodegradation occurs below ground whereby constituents of the decomposing plant materials bio transform to new organic compounds. Generally, nematode populations have been noted to reduce with amendment of soil using plant materials. Hence this method serves as a good way of combating plant parasitic nematodes while maintaining a healthy environment.

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