NEMATICIDAL EFFECT OF ORGANIC EXTRACT METAL COMPLEX ON *MELOIDOGYNE INCOGNITA* INFECTING GROUNDNUTS (*ARACHIS HYPOGEA*)

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The control of plant parasitic nematodes is constantly associated with environmental pollution problems which emanates from the ability of synthetic nematicides to bind strongly to different soil types owing to their hydrophilic nature. This research attempted to determine the nematicidal potential of copper, iron and zinc salts complexed with methanolic extract of *Enantia chloranta* on *Meloidogyne incognita* infecting groundnut plants on the field. Carbofuran, a synthetic nematicide was used as a reference standard. The experiment was a randomised complete block design which was conducted in two raining seasons (2017 & 2018). Two grams (2 g) of each of the metal salts were reacted with 300 ml of *E. chloranta* methanolic extract. Good nematicidal activity was displayed by *E. chloranta* crude extract copper complex (ENCT/$CuSO_4$) close to the value obtained with carbofuran (CBFN), iron and zinc salt complexes (ENCT/$FeSO_4$ and ENCT/$ZnSO_4$) were also significantly ($p<0.05$) effective. Toxicity of the metal salt complex to *M. incognita* was dose dependent. Vegetative growth and yield was significantly ($p<0.05$) higher in plants treated with the highest concentration of the metal complex solution. Significantly lower nematode population was seen at harvest in the roots of treated plants as opposed to the untreated plants. This research has demonstrated that the *E. chloranta* metal complex is a promising nematicidal substance.

metals, nematodes, pesticides, environmental pollution, carbofuran, complexation

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INTRODUCTION

Groundnut *Arachis hypogaea* (L.) of the family Fabaceae is widely grown in Africa as a subsistence and food crop (Tanzubil, 2016). It is highly nutritious when eaten boiled or roasted, a hundred grams contains 15% protein, 30% carbohydrate, 3% lipid and 17% minerals (Janila et al., 2013). In Africa groundnut based meals has been employed in the treatment of malnourished children (UNICEF, 2007). Nigeria accounted for 51% of groundnut production in West Africa through the year 1956 to 1979; however the sudden decline in production is attributed to infestation by pest and diseases, most especially plant parasitic nematodes (PPN) Ajeigbe et al., (2014). A wide range of crops are attacked by PPN annually with an estimated worldwide annual loss of $125 billion, about seventy percent of this is attributed to *Meloidogyne* spp (Pandey et al., 2010; Pandey, 2011). In groundnut production systems the root knot nematodes *Meloidogyne incognita* are pests of economic importance (Tanzubil, 2016). Synthetic nematicides have been used in the management of nematodes; though with impressing results but pesticide residues have been detected in groundwater, fruits and vegetables most especially in developing economies, such environmental hazard calls for caution where human and animal health are involved (Xaki, Harris, 1982; Fabiyi et al., 2016). This disadvantage in pesticide usage has brought about interest in alternative methods of managing PPN. In this regard numerous bio nematicides have been developed in order to redeem the environment from pollution which is likely to escalate from continuous use of synthetic nematicides. Substantial improvement in the control of PPN has been achieved in this context, but there is the need to develop highly potent substances to combat the menace of PPN and *Meloidogyne incognita*

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Table 1. Effect of treatments and level on plant height (cm)

Treatments	6th WAP	7th WAP	8th WAP	9th WAP	10th WAP	11th WAP	12th WAP
CBFN	19.75	21.42	26.50	28.60	27.40	31.76	32.75[a]
ENCT/$FeSO_4$	19.12	23.42	25.77	27.73	28.73	29.75	29.80[a]
ENCT/$ZnSO_4$	19.92	23.29	26.01	29.48	29.88	30.46	30.80[b]
ENCT/$CuSO_4$	19.99	23.67	26.61	29.98	29.98	30.99	31.60[a]
Levels (ml)	NS	NS	NS	NS	NS	NS	
0	16.82[b]	19.50[bc]	21.25[c]	22.09[c]	24.63[c]	25.97[c]	26.30[c]
10	19.04[a]	21.25[b]	24.83[b]	26.12[b]	27.79[b]	28.10[ab]	28.40[b]
15	19.58[a]	23.33[a]	27.09[a]	28.91[a]	30.04[a]	30.88[a]	30.40[a]
20	20.79[a]	24.33[a]	28.71[a]	30.11[a]	31.14[a]	31.75[a]	31.88[a]

WAP = week after planting, CBFN = Carbofuran, ENCT = *Enantia chloranta*, NS = not significant

[a–c]means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using new Duncan's multiple range test; each value is a mean of three replicates and an average of data taken over a two year period

in particular. The use of metal ion complexes in the production of nematicides is a new area of research. Many metal complexes are known to have superior activity than the starting mother compound (Wasi et al., 1987; Bahl et al., 2010; Gasser, Metzler-Nolte, 2011). *E. chloranta* is a valuable tree with medicinal and therapeutic properties (Iwu, 1993; Bassey, Ettebong, 2017). It is native to the dense forest of tropical Africa; the bark is known to contain mostly alkaloids, flavonoids and anthraquinone. Plant secondary metabolites are known to possess significant anti fungal and nematicidal activities (Tripathi et al., 2014; Nyong et al., 2015). The complexation of metals with plant secondary metabolites is expected to enhance the established antimicrobial activity of the metabolites. This study seeks to evaluate the potency of copper, iron and zinc sulphate complex with *Enantia chloranta* bark methanolic extract.

MATERIALS AND METHODS

Collection of plant materials

Enantia chlorantha bark was collected from the mother tree in Ilorin metropolis, it was cut into small pieces of 1-2 cm and left to dry for three weeks in the laboratory. The dried plant materials were packed in to glass aspirator for extraction in methanol. After five days the extract was decanted and filtered. All solvents used were of analytical grade.

Metal complexation

2 g each of Copper, Iron and Zinc sulphates purchased from Adfolak Chemicals Nigeria were used to react separately with 300 ml of the methanolic extract of *Enantia chloranta* and stirred for 24 h on the magnetic stirrer. During stirring, colour changed from light green to dark green in $CuSO_4$ and $ZnSO_4$. The colour change was also observed in $FeSO_4$ - from light green to brown. The $FeSO_4$ and $ZnSO_4$ had residues which were filtered off and the clear solution of each complex was stored until use.

Field experiment

A field experiment was conducted in two raining seasons (2017 & 2018) at the University of Ilorin Teaching and Research farm (8°29′N, 4°40′E). The experiment was a Randomised Complete Block Design (RCBD) with four treatments, at four dosages of application and each was replicated three times. This gave a total of forty eight experimental plots; the land was ploughed and harrowed prior to division into plots. Each experimental plot is made up of six 10 m long ridges spaced at 0.5 m apart. The soil is a well drained sandy loam with pH 6.5. The initial soil nematode population was evaluated prior to planting. Two groundnuts seeds were sown per hole at a depth of 4 cm and a spacing of 20 cm on the ridge. The groundnut plants were thinned down to a single plant each at two weeks after emergence and were inoculated to augment the field nematode population with approximately 500 eggs of *Meloidogyne incognita* at the base of each plant following the method of Iheukwumere et al., (1995). A week after inoculation treatments were added at 10, 15 and 20 ml, while carbofuran was applied at 0.3, 0.5, and 1.0 kg ai/ha^{-1} (ai/ha^{-1} :active ingredient per hectare) Plants used for data taken were tagged accordingly on each plot. At harvest, soil cores were taken from the depth of 15 cm to enumerate nematode population at the base of each groundnut plant. A diagonal transect method of sampling was used. The soil samples were thoroughly mixed to give a composite sample for each plot, and total number of nematodes at harvest was determined using the modified Baermann sieve method (Coyne

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Table 2. Effect of treatment and level on number of leaves

Treatments	6th WAP	7th WAP	8th WAP	9th WAP	10th WAP	11th WAP	12th WAP
CBFN	133[a]	138[a]	234[b]	261[a]	270[a]	281[a]	288[a]
ENCT/$FeSO_4$	108[c]	119[c]	175[d]	203[c]	220[c]	231[c]	243[c]
ENCT/$ZnSO_4$	126[b]	131[b]	199[c]	254[ab]	261[b]	270[b]	276[b]
ENCT/$CuSO_4$	135[a]	141[a]	246[a]	258[a]	265[ab]	277[a]	283[a]
Levels (ml)							
0	67[c]	81[d]	90[d]	97[d]	112[d]	119[d]	126[d]
10	95[b]	102[c]	118[c]	125[c]	131[c]	137[c]	142[c]
15	95[b]	116[b]	129[b]	137[b]	146[b]	154[b]	166[b]
20	111[a]	128[a]	141[a]	157[a]	154[a]	168[a]	176[a]

WAP = week after planting, CBFN = Carbofuran, ENCT = *Enantia chloranta*

[a–d]means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using new Duncan's multiple range test; each value is a mean of three replicates and an average of data taken over a two year period

Table 3. Effect of treatments and dosages of application on root nematode population

Treatments	Root nematode
CBFN	61[a]
ENCT/$FeSO_4$	106[d]
ENCT/$ZnSO_4$	89[c]
ENCT/$CuSO_4$	75[b]
Levels (ml)	
0	2238[d]
10	601[c]
15	218[b]
20	130[a]

CBFN = Carbofuran, ENCT = *Enantia chloranta*

[a–d]means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using new Duncan's multiple range test; each value is a mean of three replicates and an average of data taken over a two year period

et al., 2007), number of nematodes per unit volume was estimated by taking aliquots of one ml three times and counting under a stereomicroscope using Doncaster's (1962) counting dishes. Data was collected on plant height and number of leaves on weekly basis on the field. Groundnut yield kg/ha^{-1}, nematode population in 250 g soil and nematode population in 20 gram root sample were determined in the laboratory after harvest. All parameters were subjected to analysis of variance (ANOVA).

RESULTS

Table 1 shows the effect of *Enantia chloranta* metal complex on groundnut plants. There was no significant difference among all the treatments and carbofuran from the 6th to 11th week after planting. Plants treated with *E. chloranta* copper complex (ENCT/$CuSO_4$) were significantly higher, matching the value shown by the carbofuran reference standard throughout the period under observation. Vegetative growth was dose dependent. The highest dosage of application produced significantly taller plants as opposed to the untreated control plants (Table 1). Leaf production was significantly ($p<0.05$) greater in plants treated with ENCT/CuSO4 throughout the period under observation. The untreated groundnut plants had significantly ($p<0.05$) less leaves; while plants treated with the third dosage of application (20 ml) produced more numbers of leaves (Table 2). There was a significant ($p<0.05$) reduction

Table 4. Effect of treatments and dosages of application on soil nematode population

Treatments	Soil nematode
CBFN	81[a]
ENCT/$FeSO_4$	116[d]
ENCT/$ZnSO_4$	103[c]
ENCT/$CuSO_4$	90[b]
Levels (ml)	
0	3107[d]
10	175[c]
15	89[b]
20	55[a]

CBFN = Carbofuran, ENCT = *Enantia chloranta*

[a–d]means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using new Duncan's multiple range test; each value is a mean of three replicates and an average of data taken over a two year period

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Table 5. Effect of treatments and dosages of application on groundnut yield

Treatments	Yield (kg ha^{-1})
CBFN	25.9^{a}
ENCT/$FeSO_4$	18.4^{cd}
ENCT/$ZnSO_4$	19.2^{c}
ENCT/$CuSO_4$	23.8^{b}
Levels (ml)	
0	5.2^{d}
10	9.8^{c}
15	11.4^{b}
20	18.2^{a}

CBFN = Carbofuran, ENCT = *Enantia chloranta*

$^{a–d}$means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using new Duncan's multiple range test; each value is a mean of three replicates and an average of data taken over a two year period

in the number of plant parasitic nematodes in the roots of treated plants as opposed to the untreated (control) plants after harvest. Plants treated with *Enantia chlorantha* copper complex had significantly ($p<0.05$) fewer nematodes among the metal salts. Higher population of nematode was recovered from the roots of untreated control plants (Table 3). Similarly the *E. chlorantha* metal complex effectively reduced the population of nematodes in the soil of treated plant. The *Enantia chlorantha* copper complex was significantly more effective in reducing nematode population than all the other treatments (Table 4). A corresponding higher yield was recorded in plants treated with *E. chlorantha* copper complex as opposed to plants treated with other metal complexes. Yield was also higher in plants treated with the highest dosage of application (Table 5). The results of initial field nematode count before planting in the first year revealed that the field nematode population was augmented by inoculation (Table 6).

DISCUSSION

There has been increasing interest in the application of metal complexes in biological sciences and drug production for the control of many diseases (Hubin et al., 2014). Metal complexes are known to exhibit anti microbial activity, they enhance the efficacy of existing drugs. The activity of gold complex with N-heterocyclic carbenes was reported by Hemmert et al., (2016). The cationic gold (I) complexes showed high potency against *Plasmodium falciparum.* Also catechin copper (II) complexes were found to damage the cytoplasmic membrane of *Escherichia coli* by Hoshino et al., (1999). *Escherichia coli* strain ATCC11775 cells were destroyed by (-)-epigallocatechin and (-)-epicatechin, with depletion in the ATP (adenosine triphosphate) and potassium pools of the cells. In vitro toxicity of Mn(II), Pt(II) Co(II) and Cu(II) complexes with anti malarial drugs were tested on *Trypanosoma cruzi, Leshmania donovani*, *T.b. rhodesiense* and chloroquine resistant strains of *Plasmodium falciparum* by Ajibade, Kolawole, (2008), they confirmed that enhanced anti-protozoa activity was displayed by the Pt(II) complex. Sánchez-Delgado et al., (1998) synthesized metal complex against the causative organism of Chagas disease, *Trypanosoma cruzi,* and the metal complexes were found to be active against the organism. The antiplasmodia activity of iron and zinc metal complexes was reported by Navarro et al., (2014) and Rice et al., (2017), the iron complex was clearly effective without toxicity while the zinc complex also exhibited significant activity against three strains of *Plasmodium falciparum.* Iron complex have been found to be a highly potent antimicrobial substance, strong activity was exhibited against *E. coli*, *A. niger*, *A. flavus* and *S. aureus* with a large zone of growth inhibition (Obaleye et al., 2006; Adediji et al., 2009). The growth stimulating activity of copper sulphate was recorded by Jiang et al., (2001). In this research, copper *E. chloranta* crude extract copper complex displayed impressive nematicidal activity, with increase in vegetative growth, yield and reduction in *Meloidogyne incognita population.* Kobamoto, Izumi (1984), confirmed that copper ion is highly nematicidal, they observed a rapid increase in percentage mortality and at three hours of exposure there was a hundred percent mortality of the pine wilt nematode *Bursaphelenchus xylophilus*. The nematicidal activity of copper sulphate to *Bursaphelenchus xylophilus* was reported by Tan et al., (2013). At 50 mg/l concentration mortality was 100 % after 72 hours of exposure. Similarly, Hu et al., (2008), found Iron sulphate to be toxic to *Caenorhabditis elegan*s wild type N2 (L4-larva stage), the exposed nematode was severely impaired with multiple biological defects. The toxicity of copper

Table 6. Field nematode population count

Nematode	Population
Meloidogyne incognita	345
Meloidogyne javanica	129
Pratylenchus spp	75
Scutellonema bradys	5
Longidorous spp	18
Tylenchus spp	23
Hemicyclophora spp	33
Helicotylenchus spp	12
Tylenchus spp	41
Paralongidorous spp	28

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at an effective concentration (EC_{50}) of 0.68 mg/L to nematodes was established by Jiang et al., (2016) using *Caenorhabditis elegans* as a model organism. They observed that the acetylcholine esterase enzyme (AChE) in nematodes was more sensitive to copper than zinc and cadmium. Similarly, Jaworska, Gorczyca (2002), reported the toxicity of copper sulphate at 7.5 $mg \cdot dm^{-3}$ to *Steinernematidae feltiae*, high mortality and reduced rate of reproduction of the nematode was observed after 96 hours of contact. In the same vein Korthals et al., (2000) found increasing concentration of copper in agricultural soils to be toxic to some nematode taxa which include *Thonus*, *Alaimus* and *Aporcelaimellus*. At concentrations exceeding 50 mg kg^{-1} the nematodes disappeared from the soil. The toxic effect of copper to soil microcosm community was evaluated by Salamun et al., (2015), they reported that the nematodes exhibited stress responses to copper at 40 $mg.kg^{-1}$ concentration. *E. chlorantha* crude extract iron sulphate complex significantly reduced nematode population in the roots and soil of groundnut plants in this research. A significant 42 % reduction in number of galls on cucumber (cv Negin) roots by iron sequestrene (Fe-EDDHA) when added to soil as micro nutrient was reported by Mansourabad et al., (2016). Generally, copper based substances are employed as effective anti microbial in agriculture. This research has established clearly the nematicidal effect of *E. chlorantha* methanolic extract metal complex, which could serve as a building block for environmentally safe nematicides.

CONCLUSION

The application of metal complexes in the control of plant parasitic nematodes offers new opportunities to produce novel compounds containing metals which invariably can become a good candidate in nematode management in agricultural ecosystems. Structural diversity can be developed for the manufacture of new nematicides.

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