IN VITRO ASSESSMENT OF N-PHENYL IMIDES IN THE MANAGEMENT OF *MELOIDOGYNE INCOGNITA*

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The infestation with root knot nematode *Meloidogyne* spp. is a key issue in agriculture. Conventional control methods are based on the use of synthetic nematicides, which comes with severe environmental problems. In this study, N-phenyl imide and N-phenyl phthalamic acid were synthesized and reacted independently with *Enantia chlorantha* crude extract–manganese chloride complex. The effects of the resulting organic compounds were appraised against the root knot nematode *Meloidogyne incognita* (Kofoid and White 1919) juveniles and eggs in two laboratory experiments. The most active compound was N-phenyl phthalamic acid (PN/TLMA) with 4% egg hatch over a 9-day observation after treatment as against distilled water which recorded 100% egg hatch at 9 days after treatment. N-Phenyl phthalamic acid showed 100% juvenile mortality at 10 days of observation compared to carbofuran dissolved in water (CBFN/water) and carbofuran dissolved in hydroxypropyl-β-cyclodextrin (CBFN/HPCD) while no outstanding ($P < 0.05$) difference was recorded between the effects of other organic compounds and carbofuran in both solvents. The different rates of treatment applications were not appreciably ($P<0.05$) dissimilar on percentage juvenile mortality and egg hatch. The nematicidal test results indicated that the synthesized imide compounds with manganese complex moiety are a promising basis for developing new nematicidal compounds with less environmental hazard.

carbofuran, *Enantia chlorantha*, imides, nematicides, manganese, pollution

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INTRODUCTION

Plant parasitic nematodes are present in all soils and as such they are notable pathogens in agriculture (Decraemer, Hunt, 2013). The major root knot nematode species recognized worldwide is *Meloidogyne incognita* (Kofoid & White 1919) (Xavier-Mis et al., 2017) posing a significant risk to food security also in Africa. *M. incognita* infestation gives rise to countless impairment of crop growth which is manifested by chlorosis, stunting, toppling, delayed maturity and reduction in yield and quality of crops (Onkendi, Moleleki, 2013; Fabiyi, 2021a). Chemical control approaches, either individually or in combination with other methods, have been adopted in the management of *M. incognita* (Norshie et al., 2011) leading to noteworthy progress in the limitation of its population. However, such approaches are associated with a snare of harmful residues in the food chain and development of resistance by the targeted nematode species (Strajnar, Sirca, 2011). Considerable amounts of pesticide residue have been detected in fruits, grains and vegetables, the long term consumption of these may result in grievous consequences. To safe guard the environment and mitigate the health risks brought about by the use of toxic synthetic nematicides, research into less toxic substances has intensified (Atolani et al., 2014a, b) with the ultimate goal of producing promising and environmentally friendly alternatives (Atolani, Fabiyi, 2020; Fabiyi et al., 2020; Fabiyi, 2021b). Phthalimides are organic substances containing the aromatic nucleus and 1,2-cyclic diamide.

Compounds with phthalimide moiety exhibit antibacterial and antiviral properties (Verschueren et al., 2005; Bhambi et al., 2009; Penta et al., 2013). Investigation of cyclic imides with monoacid and monoester functional groups blended with a natural product–metal complex is to the best of our knowledge yet to receive attention. The aim of this study is to synthesize a potent manganese complex nematicidal compound with minimal toxicity from phthalimides and anthraquinones from the family Anonaceae. *Enantia chlorantha* (Oliv.) is reported to have a wide spectrum of antimicrobial activity and antimalarial properties (Wafo et al., 1999; Adesokan et al., 2007; Gbadamosi et al., 2011), while the positive effect of manganese in boosting plant tolerance to microbes has also been described by Eskandari et al. (2018). It is expected that the synergistic effect of the secondary metabolites and manganese chloride may contribute to the potency of bionematicides.

MATERIAL AND METHODS

Chemicals

Aniline, phthalic anhydride, acetic anhydride, sodium acetate and manganese chloride were obtained from Sigma Aldrich, USA. All solvents used (dichloromethane (DCM), ethanol and methanol) were of analytical grade and were re-distilled before use. The manganese–*E. chlorantha* extract complex was prepared using 100 ml of *E. chlorantha* methanolic extract (0.235 g ml^{-1}) and 0.5 g manganese chloride.

Collection of plant materials

The bark of *Enantia chlorantha* known as Awopa in Yoruba language in Nigeria was collected within Ilorin metropolis. The *E. chlorantha* bark was shredded into tiny (2 cm) pieces with knife and left to dry for 3 days at room temperature in the lab.

Preparation of organic compounds

N-Phenyl phthalamic acid. Phthalic anhydride (3.5 g) was suspended in 50 ml of DCM. Aniline (3 ml) was added drop-wise to the suspension and stirred at room temperature for 90 min on a magnetic stirrer. The solvent (DCM) was decanted and the precipitate was re-crystallized in methanol.

Reaction of N-phenyl phthalamic acid with the manganese–*E. chlorantha* complex. N-phenyl phthalamic acid (1.0 g) was dissolved in methanol and reacted with 5 ml of the manganese complex solution of *E. chlorantha.* This was stirred on a magnetic stirrer at room temperature for an hour. The precipitate obtained was filtered. Thin layer chromatography (Merck, Darmstadt Germany) was carried out on the reaction product using n-hexane/DCM ratio 1: 2 as the solvent system and the product was partially characterized with infra-red spectroscopy (SHIMADZU 8400S FTIR spectrophotometer).

N-Phenyl phthalimide. Phthalic anhydride (3.5 g) was dissolved in 10 ml of DCM. Acetic anhydride was added to the solution at 3 ml and stirred using the magnetic stirrer at room temperature for 90 min. Sodium acetate was added at 0.5 g and stirred with heating for another 30 min. The homogeneous hot solution was filtered. The filtrate was concentrated and the spectroscopic properties of the precipitate formed were determined. N-Phenyl phthalimide (1.0 g) was dissolved in ethanol; 5 ml of the manganese-*E. chlorantha* complex solution was added and stirred for 2 h with a magnetic stirrer at room temperature, and a homogeneous solution was obtained. The solution was filtered, and the filtrate was concentrated. The product properties were analysed using infra-red spectroscopy spectral data. The various reaction products were coded as follows: (i) PNID (N-phenyl phthalimide), (ii) PN/TLMA (phenyl phthalamic acid), (iii) PNID/Mn/ENCT (phenyl phthalimide manganese complexed *E. chlorantha*), (iv) PN/TLMA/Mn/ENCT (phenyl phthalamic acid manganese complexed *E. chlorantha*).

Nematode eggs extraction. *Meloidogyne incognita* pure culture eggs sustained on *Celosia argentea* roots were picked up from the inoculum micro plots of the Department of Crop Protection and Environmental Biology, University of Ibadan. The roots were uprooted and washed to detach stones and debris. Then they were chopped into 1–2 cm long bits and put into a glass beaker of 1000 ml volume and 0.5 % of sodium hypochlorite was added. The mixture was vigorously shaken for 4 min. The solution containing chopped roots and water was poured through a stack of sieves of 73, 56 and 28 µm, respectively. The content in the last sieve (28 µm) was rinsed gently with sterile water into a small beaker.

Juveniles extraction. The juveniles were extracted using the pie-pan method (Whitehead, Hemming, 1965) from the egg–water suspension left in a beaker on the laboratory bench for five days. The water solution was poured into a sieve containing tissue with tray at the base and left at room temperature to collect the second stage juveniles. The freshly hatched juveniles were collected after decanting the water in the tray at 24 h (Coyne et al., 2007; Fabiyi et al., 2020).

Nematicidal assay. One millilitre of egg suspension containing approximately fifty eggs measured using a syringe was dispensed into the counting wells. A completely randomized design was used, with six treatments at three dosages of application and each was replicated three times. The four imides were solubilized in hydroxypropyl-β-cyclodextrin (HPCD). Carbofuran was dissolved in water and HPCD separately to serve as

Table 1. The effect of treatment and concentration levels on the percentage hatch of *Meloidogyne incognita* eggs at 0–9 days after treatment (DAT)

Treatment	Hatch of *Meloidogyne incognita* eggs (%)									
	DAT 0	DAT 1	DAT 2	DAT 3	DAT 4	DAT 5	DAT 6	DAT 7	DAT 8	DAT 9
PNID	0	2^{b}	6^{d}	8^{c}	12^{d}	15^{d}	21^{e}	27^{e}	35^{e}	44^{e}
PN/TLMA	0	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	4^{a}	4^{a}	4^{a}	4^{a}
PNID/Mn/ENCT	0	0^{a}	0^{a}	4^{b}	6^{b}	8^{b}	10^{b}	17^{b}	23^{b}	27^{b}
PN/TLMA/Mn/ENCT	0	2^{b}	2^{b}	8^{c}	10^{c}	13^{c}	15^{c}	21^{c}	29^{c}	48^{f}
CBFN (water)	0	2^{b}	6^{d}	8^{c}	12^{d}	19^{e}	23^{e}	25^{d}	29^{c}	42^{d}
CBFN (HPCD)	0	0^{a}	4^{c}	13^{d}	15^{e}	23^{f}	23^{e}	27^{e}	31^{d}	37^{c}
Water	0	2^{b}	17^{e}	75^{e}	79^{f}	81^{g}	88^{f}	98^{f}	100^{f}	100^{g}
Levels/dosage										
2.33 ml	0	0^{a}	4^{a}	6^{a}	6	10^{a}	15^{a}	19^{a}	25^{a}	35^{a}
1.75 ml	0	0^{a}	2^{b}	4^{b}	6	10^{a}	12^{b}	17^{b}	23^{b}	33^{b}
1.44 ml	0	2^{b}	2^{b}	4^{b}	6	8^{b}	12^{b}	13^{c}	19^{c}	25^{c}

DAT = days after treatment, PNID = N-phenyl phthalimide, PN/TLMA = phenyl phthalamic acid, PNID/Mn/ENCT = phenyl phthalimide manganese complexed *E. chlorantha,* PN/TLMA/Mn/ENCT = phenyl phthalamic acid manganese complexed *E. chlorantha*, CBFN = carbofuran, HPCD = hydroxy propyl-β-cyclodextrin

$^{a–g}$ means in a segment of a given column followed by the same superscript are not significantly different at $P < 0.05$ using the new Duncan's multiple range test

the positive control. Distilled water sufficed as negative control and all were examined in parallel to monitor any interference of HPCD with the assays. A total of 54 counting wells were used. The treatments were applied at 2.33 ml, 1.75 ml, and 1.4 ml into the counting wells. The experimental set up was monitored for nine days under an Olympus CX33 microscope. The number of hatched eggs was counted on daily basis. Similarly, approximately 50 juveniles were equally dispensed into each of the 54 counting wells for the juvenile mortality assessment. The treatments were applied at 2.33 ml, 1.75 ml, and 1.4 ml and the numbers of dead juveniles were counted daily for ten days. The experimental procedure was repeated twice.

RESULTS

Chromatography and spectroscopy

Thin layer chromatography (Merck, Darmstadt, Germany) was carried out on the recrystallized N-phenyl phthalamic acid using n-hexane/DCM (1:1) as the solvent system, and the chromatogram confirmed a pure product (R_f 0.56). The infrared spectroscopic analysis (SHIMADZU 8400S FTIR spectrophotometer) was conducted to ascertain the functional groups present in the synthesized N-phenyl phthalamic acid. The result indicates the presence of O-H vibration at 3376.25 cm^{-1}, while N-H stretch of primary amines was noted at 3394 cm^{-1}. The band at 2974.14 cm^{-1} indicates C-H stretch of secondary amines, the signal from benzene ring was observed at 2901.32 cm^{-1}. The band 1707.65 cm^{-1} represents the C=O of carboxylic acid, 1675.17 cm^{-1} the C=O of amide and 1500–500 cm^{-1} the C-O of carboxylic acid. This affirms that the N-phenyl phthalamic acid was synthesized.

Nematicidal assay

The effect of imides on the percentage egg hatch of *M. incognita* cumulatively for 9 days is presented in Table 1. Among the treatments, the phenyl phthalamic acid (PN/TLMA) was remarkably ($P < 0.05$) more effective than carbofuran, there was no egg hatch from the first day after treatment (DAT 1) to the fifth day after treatment (DAT 5), however 4% hatch was recorded at DAT 6 and this level remained constant till DAT 9. At 2–3 DAT, there was no appreciable difference in effectivity between N-phenyl imide (PNID) and carbofuran dissolved in water (CBFN/water). Egg hatch was strikingly inhibited in the N-phenyl imide manganese *E. chlorantha complex* (PNID/Mn/ENCT) exhibited a strong activity among the other treatments between DAT 1 and 2. At 3 DAT, egg hatch was remarkably low in the N-phenyl imide–manganese *E. chlorantha* complex (PNID/Mn/ENCT) as against the percentage egg hatch observed in carbofuran dissolved in hydroxypropyl-β-cyclodextrin (CBFN/HPCD), however CBFN/HPCD had the highest percentage egg hatch at 5–6 DAT. At 7 DAT, egg hatch was significantly low in PN/TLMA and PNID/Mn/ENCT. Egg hatch in distilled water cumulated to 100 % at 8 DAT. A notable ($P < 0.05$) difference was seen in the two highest concentrations (2.33 ml and 1.75 ml), as opposed to the lowest level (Table 1). The lowest

Table 2. The effect of treatment and concentration levels on the percentage mortality of *Meloidogyne incognita* juveniles at 0–10 days after treatment (DAT)

Treatment	Mortality of *Meloidogyne incognita* juveniles (%)										
	DAT 0	DAT 1	DAT 2	DAT 3	DAT 4	DAT 5	DAT 6	DAT 7	DAT 8	DAT 9	DAT 10
PNID	0	18^{d}	36^{c}	38^{c}	42^{c}	47^{c}	51^{c}	55^{bc}	60^{b}	66^{b}	74^{b}
PN/TLMA	0	89^{a}	98^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
PNID/Mn/ENCT	0	30^{b}	43^{b}	49^{b}	53^{b}	57^{b}	60^{b}	60^{b}	64^{b}	68^{b}	74^{b}
PN/TLMA/Mn/ENCT	0	23^{c}	28^{d}	32^{d}	38^{c}	40^{d}	42^{d}	43^{d}	47^{d}	51^{d}	55^{d}
CBFN (water)	0	17^{d}	26^{d}	34^{d}	38^{c}	42^{d}	47^{c}	51^{c}	53^{c}	62^{c}	72^{b}
CBFN (HPCD)	0	9^{e}	17^{e}	32^{d}	38^{c}	42^{d}	43^{d}	49^{cd}	51^{c}	57^{c}	64^{c}
Water	0	0^{f}	0^{f}	0^{e}	2^{d}	2^{e}	2^{e}	2^{e}	2^{e}	4^{e}	6^{e}
Levels/dosage											
2.33 ml	0	40	53	57	60	62	62	66	70^{a}	75^{a}	81^{a}
1.75 ml	0	42	53	57	57	60	62	64	68^{a}	70^{b}	75^{b}
1.44 ml	0	42	49	57	57	60	62	64	66^{ab}	70^{b}	75^{b}

PNID = N-phenyl phthalimide, PN/TLMA = phenyl phthalamic acid, PNID/Mn/ENCT = phenyl phthalimide manganese complexed *E. chlorantha,* PN/TLMA/Mn/ENCT = phenyl phthalamic acid manganese complexed *E. chlorantha*, CBFN = carbofuran, HPCD = hydroxy propyl-β-cyclodextrin
$^{a–f}$ means in a segment of a given column followed by the same superscript are not significantly different at $P < 0.05$ using the new Duncan's multiple range test

level/dosage of control (1.44 ml) was not as potent as the two higher dosages.

Juvenile mortality was 100% in N-phenyl phthalamic acid from 3 DAT till the end of observation (DAT 10) (Table 2). The N-phenyl imide–manganese *E. chlorantha* complex (PNID/Mn/ENCT) also showed notably higher percentage juvenile mortality. All other treatments had equally significant effect on percentage mortality; carbofuran in distilled water and HPCD was not as effective as PN/TLMA (Table 2). The variation in concentration (dosage) did not have a significant effect on percentage juvenile mortality. Mortality values were not dissimilar between 1.75 ml and 1.44 ml (dosages 2 and 3), although 2.33 ml (dosage 1) had significant values at DAT 9–10 of experiment (Table 2).

DISCUSSION

Cyclic imides are organic compounds with the general structure -CO-N(R)-CO-, The R stands for a hydrogen atom, aryl or alkyl group. This makes the imides biologically active (Prado et al., 2004; Jafari et al., 2017). Imide derivatives are an important group of bioactive compounds which show antibacterial, antifungal and antiviral properties. The hydrophobic nature of these compounds confers permeability into the membranes of microorganisms (Prado et al., 2004). Strong fungicidal effects of imides have been proved. Sortino et al. (2008) described a strong fungicidal activity of imides against clinical isolates of *Candida albicans* and *Candida* spp. Jafari et al. (2017) reported on the antibacterial activity of cyclic imide against three types of bacteria and a fungus; they established that phthalimide synthesized from benzylamine exhibited remarkable antimicrobial activity against *E. coli*. Imides are generally classified as compounds not expected to be toxic or harmful to humans and not conjectured to be persistent or bio accumulative in the environment. Hence, they are not surmised to be an environmental toxin. Furthermore, manganese salt, used as a ligand in this study, is known to inhibit the growth of *Gaeumannomyces graminis* var. *tritici* (Walker) on wheat (Brennan, 1992); the acuteness of infection was lessened with the application of manganese sulphate in two trial experimental sites. Fertilization of wheat genotypes with manganese is reported by Rengel et al. (1993) to reduce lesions of the take-all fungus (*Gaeumannomyces graminis*). Studies by Simoglou, Dordas (2006) revealed that the application of manganese as a foliar spray subdued the growth of *Drechslera tritici-repentis* (Died.) Shoem., the causal agent of the leaf tan spot disease of wheat. The resistance of cucumber to powdery mildew caused by *Podosphaera fuliginea* was enhanced with the foliar application of manganese, a significant reduction in the fungal disease was achieved (Eskandari, Sharifnabi, 2020). A salient fungal disease of tomato, the black mould caused by *Pseudocercospora fuligena,* was brought under control with manganese as root substrate (Heine et al., 2011). In addition, Perveen, Rehman (2000) observed an increase in fruit yield of sweet orange with foliar use of manganese. The cyclic imides–*E. chlorantha* manganese complex has shown appreciable nematicidal properties, which suggests promising application in the area of

plant parasitic nematode control. The complexation of metal salts such as manganese chloride with imides and incorporation of natural products containing alkaloid/anthraquinone may go a long way in reducing the environmental pollution characteristic of synthetic pesticides. Further studies are required to confirm the observed nematicidal properties in the field as well as testing of the toxicity to mammals.

CONCLUSION

The conclusion drawn from this research is that the imides complexation with manganese has effectively reduced egg hatch of *M. incognita,* while a considerably high percentage juvenile mortality was also achieved. The practical implication of this is that egg hatch inhibition will reduce the population of *M. incognita* on the field, while the population of existing juveniles will equally be brought down below the threshold level.

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