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MANAGEMENT OF *Meloidogyne incognita* DAMAGING LETTUCE: POTENTIAL OF CHROMATOGRAPHIC FRACTIONS AND EXTRACTS FROM *Lawsonia inermis*

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ABSTRACT

Plant parasitic nematodes (PPNs) are acknowledged as a paramount factor which limits the production of staple crops and vegetables. Preferred standard control method had been the use of synthetic nematicides. However, owing to the undesirable consequences of the residual effect of nematicides in the environment, the need for alternative approaches becomes pertinent and this has prompted investigation into the nematicidal potential of extracts from *Lawsonia inermis* for practicable application on lettuce plants infected with *Meloidogyne incognita* in field experiments. The leaves of *L. inermis* were collected and divided into four parts for separate extraction and these were compared with the standard nematicide carbofuran individually. The essential oil (EO) was significantly more potent than the other extracts from *L. inermis*. There was no significant difference between plants treated with carbofuran and EO. The fractions were significantly better than crude methanol and ethanol extracts of *L. inermis.* Reproduction of *M. incognita* on lettuce plant roots was considerably reduced by utilization of *L. inermis* extracts. Notably higher vegetative growth was observed in treated lettuce plants. The IR, ^{1}H-NMR and ^{13}C-NMR spectral data analysis confirmed the presence of sesquiterpenes in the chromatographic fraction. The GC-MS profile indicated phytol as the major constituent of the EO. The results obtained from this study indicates that extracts from *L. inermis* could be a viable option in the management of *M. incognita* damaging lettuce in dependable vegetable production.

Keywords: bio-pesticide, carbofuran, chromatography, essential oil, nematode, pollution, vegetable.

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INTRODUCTION

Plant parasitic nematodes (PPNs) infection bring about adverse consequences on nearly all crops (Fabiyi *et al.,* 2018; Fabiyi, 2020; Fabiyi *et al.,* 2020a). PPNs induce a sizeable damage in agriculture (El-Eslamboly *et al.,* 2019; Fabiyi *et al.,* 2020b; Fabiyi, 2021), worldwide losses of about US$78 billion in a year have been reported (Similey, 2005). Root-knot nematodes (RKNs) *Meloidogyne* spp. are the principal PPNs which affects yield in several crops (Taniwiryonoc *et al.,* 2009; Fabiyi, 2019; Fabiyi and Olatunji, 2021a). Largely, amidst the specie *Meloidogyne incognita* is a foremost pest of vegetables (Fabiyi and Olatunji, 2021b). Lettuce (*Lactuca sativa*), a leafy vegetable in the family Asteraceae grown for its leaves which are used in salad preparations is prone to *M. incognita* infection (William, 2012; de Souza Alonso *et al.,* 2022; Fabiyi, 2022a). Productivity of lettuce is limited by several pests and diseases. Infection by the root knot nematode *M. incognita* is a key factor which limits yield extensively in lettuce plants (Oliveira *et al.,* 2015; Koleva and Mitova, 2021).

Considerably high economic losses are attributed to *M. incognita* in lettuce production. Several lettuce cultivars are known to be highly susceptible to *M. incognita,* with production losses of about 100% (Widmer and Abawi, 2000; Wilcken *et al.,* 2005; Rodrigues *et al.,* 2012). Symptoms exhibited by infected crops include chlorosis, stunting, withered head and root galling (Siddiqi *et al.,* 2001; Pinheiro *et al.,* 2013; de Souza Alonso *et al.,* 2022). Conventionally, the control of nematode pest of lettuce relies mostly on the use of synthetic nematicide like carbofuran. This is, however, unsafe particularly in a country like Nigeria where there are no implementable stringent regulations on pesticide residue limits in crops at harvest and farmers hardly read pesticide labels (Jatto *et al.,* 2012). Hence the probability of applying below or above the manufacturers recommended dosage for crops is very high.

The concern for public health and the environment has spurred interest in safer alternative methods of *M. incognita* management (Atolani *et al.,* 2014a; Atolani *et al.,* 2014b; Fabiyi *et al.,* 2020). There are quite a number of researches on plant extracts and purified compounds regarding their potential in the control of various pests and diseases (Javaid *et al.,* 2018; Khan *et al.,* 2020; Fabiyi, 2021b; Fabiyi, 2022b). Reports by Viaene and Abawi (1998) specified that amending soil with Sudan grass notably reduced *M. hapla* reproduction on lettuce resulting in increase of lettuce head weight at harvest. Countless leading-edge studies have shown that extracts of various plant species such as *Chenopodium quinoa* (Khan and Javaid, 2020), *Euphorbia prostrata* (Ferdosi *et al.,* 2021a), *Bergenia ciliata* (Ferdosi *et al.,* 2021b), *Eucalyptus officinalis* (Fabiyi, 2021c), *Khaya senegalensis* (Fabiyi, 2022c) and diverse purified active principles contain potent nematicidal compounds (Atolani and Fabiyi, 2020).

Lawsonia inermis is widely known for its cosmetic uses, the anti-bacterial activity against clinical bacteria isolates was documented by Arun *et al.* (2010) and Gull *et al.* (2013), the anti-fungal activity was also reported by Sharma and Sharma, (2011). Reports on the application of organic extracts and isolates from *L. inermis* in the control of PPNs particularly *M. incognita* is however scanty in literature, hence the necessity for this research. Consequently, this research aimed at evaluating isolated compounds and different solvent extracts from *L. inermis* as a possible option in the management of *M. incognita* pest of lettuce.

MATERIALS AND METHODS

Collection and preparation of plant materials: *Lawsonia inermis* leaves were collected in large quantity from Sentu village, which is about 8 km north east of Ilorin town and were air dried for three weeks at ambient temperature in the laboratory. The leave samples were identified at the University of Ilorin herbarium unit. The dry leaves were divided into four parts of 1 kg each and were milled into tiny pieces to increase the surface area. One kilogram of the dry leaves was hydro-distilled for three hours (3 hrs). The yellow volatile oil yielded was separated from the aqueous layer with dichloromethane, the solution was then dried with anhydrous sodium sulphate (Na_2SO_4). Two parts were packed separately in aspirator and extracted with ethanol and methanol, the last part was used directly as soil amendment.

Column chromatography: The ethanol and methanol extracts were decanted, filtered and concentrated using a rotary evaporator. A part of the crude extract was chromatographed over silica gel 100-120 mesh grade (Fabiyi *et al.,* 2012) in a glass column with an initial mobile phase of 100% n-hexane. The polarity was increased to n-hexane/dichloromethane ratio 2:1, n-hexane/ dichloromethane ratio 1:1, 100% dichloromethane, then finally dichloromethane/ ethanol ratio 2:1. The 100% dichloromethane elution afforded 21 fractions of 200 ml each. The fractions each were spotted on Thin Layer Chromatographic Plates (Al_2O_3, G_F-254 0.2mm, Merck, Germany). The spots were visualized using UV light (254 and 366 nm). All fractions which gave the same retention factor (R_f) on the thin layer chromatographic plate (TLC), were then combined, concentrated and subjected to IR, H^1-NMR and ^{13}C-NMR analysis. The EO obtained from the hydro distillation procedure was analysed by Gas Chromatography Mass Spectroscopy (GC-MS).

Instruments: IR spectra were recorded on 8400s Fourier Transform Infrared (FTIR). Nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) was evaluated with JEOL 400 MHz. The chemical shifts were documented in ppm relative to TMS, while the coupling constants are in Hz. Gas Chromatography-Mass Spectroscopy was conducted on GCMS-QP 2010 PLUS (Shimadzu Japan) attached to finigan. The column is an RTX5MS with a MAT ion trap detector, which was packed with dimethylpolysiloxane at 100% grade. The condition of the GC-MS is as follows. Initial column temperature was 60℃ and was held for 5 min, the injection volume was 1L. Temperature was programmed to rise at 5℃ per minute up to 250℃, for injection the temperature was set to 200℃, while maintaining detector (mass spectrophotometer) temperature at 250℃. The carrier gas was helium operating at 46.3 cm/s linear velocity and 100.2 kPa pressure. Electron impact (EI) was the ionization mode set at 70 eV voltage. The peak enrichment technique for reference compounds was used for component identification, which finally confirmed the peaks identified by GC-MS. NIST library mass spectra was compared with the spectral data obtained.

Field experiments: Field experiments were conducted in 2017 and 2018 cropping seasons at the Teaching and Research Farm University of Ilorin, Nigeria (Lat 8^o 29^1 N of the Equator; Long: 4^o 40^1 E of the Greenwich Meridian). The experimental field was 30 m x 25 m in size, this was harrowed and seventy-two beds of 1.5 m^2 raised to a height of 15 cm each were made. The experiment was a randomized complete block design (RCBD). There were four dosages of application, treatments were six in number. Each treatment had three replicates. *L. sativa* 'Mindelo' seedlings raised from seeds were transplanted to the field at eighteen days after emergence from the nursery. Spacing was 20 cm apart and 50 cm between the rows. Pure culture of *M. incognita* (Kofoid and White, 1919) Chitwood, 1949 raised on *Celosia argentea* was extracted using the method of Hussey and Baker (1973), the extracted eggs and juveniles served as source of inoculums. Approximately 1000 eggs and juveniles per ml of *M. incognita* were inoculated around the base of each lettuce plant (Fabiyi *et al.,* 2019) at four days after transplanting. The crude extracts, fractions and essential oil were applied a week after inoculation at 200 ml in variants of 50, 75 and 100 mg/ml, while the dry powder leaves used as soil amendment was applied at 150, 175 and 200 g. The reference standard check was carbofuran (a synthetic nematicide) applied at 0.5, 1.0 and 1.5 kg/ai/ha. Fertilizer was applied at 30 kg/ ha-1 a month after transplanting. Head diameter and numbers of leaves were recorded on weekly basis. Head weight, gall index, nematode population in the roots and rhizosphere soil of lettuce plants were evaluated after harvest. The gall index was evaluated on a scale of 0-4 (Hussey and Janssen, 2002), where 0 = no galls (root healthy), 1 = 1-5 small galls (galls on 1-25% of root system), 2 = 2-15 small galls (galls on 26-50% of root system), 3 = 16-25 galls, many part of roots functioning (galls on 56-75% of root system), 4 = >26 galls (galls on >76% of root system). All data were subjected to Analysis of variance using GenStat 5.32. Means were separated were necessary at 5% level of probability using the new Duncan's multiple range test.

RESULTS

The infra-red spectrum of the dichloromethane fraction exhibited diagnostic bands at 2958 and 2950 cm^{-1} which is attributed to C-H stretch of aliphatic compounds. The presence of an aldehyde was observed at 2850 cm^{-1}, while 1734 cm^{-1} depicted the C=O of an ester. C-H of aromatics in the finger print region was noted at 828 and 730 cm^{-1} The GCMS result of the essential oil depicted 11 compounds (Fig.1) and eight were more than 5%, phytol (21.02 %) had the highest percentage composition, this was closely followed by trans pulegol (15.03%), sabinene (13.05%), terpinolene (12.14%), terpinen-4-ol (11.05%) and limonene (9.49%), α-humulene (8.01%), gamma-eudesmol (5.03%), beta bisabolene (2.07%), caryophyllene (2.01%) and δ-cadinene (1.10%). The ^1H-NMR (400MH_z $CDCl_3$) results depicted three protons in the olefinic region δ 5.0-5.1, four tertiary methyl groups were observed at δ 1.60 -δ 1.72. A singlet at 1.60 is attributed to three methyl groups; a multiplex peak at δ 1.95-2.14 is associated to nine protons. From the ^{13}C-NMR spectrum, olefinic carbons were observed from δ 123.2-137.8, another chemical shift was seen at δ 15.0-38.6 which is for the aliphatic group, and these data are in consonance with that expected for sesquiterpenes**.** The activity of extracts and dosages of utilization of *L. inermis* and carbofuran on leaf number of lettuce is depicted in Fig 2, lettuce plants treated with essential oil and carbofuran (LSNI/EO and CBFN) had significantly ($p<0.05$) more numbers of leaf, leaf diameters were equally significantly wider with a corresponding heavier head weight at harvest (Figs. 3 &

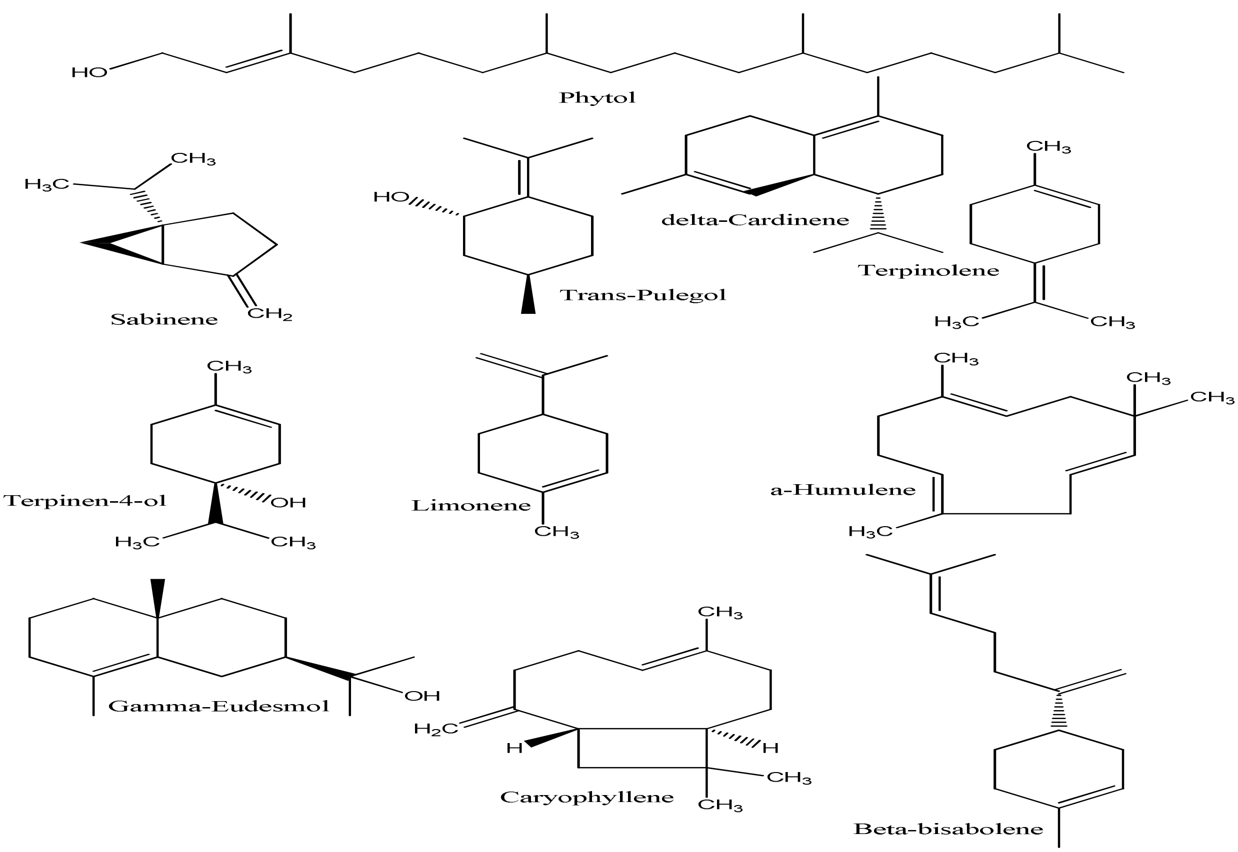

Figure 1: Structures of compounds obtained from the essential oil of *Lawsonia inermis*.

4). Plants treated with powdered materials as soil amendments and crude extracts from ethanol and methanol extracts (LSNI/ODR, LSNI/EtOH and LSNI/MeOH) produced significantly fewer numbers of leaves, smaller head diameter and lighter head weight as opposed to the essential oil and carbofuran treated plants (Figs. 2, 3 & 4). The untreated control plants were not as healthy as the treated plants. Lettuce plants in beds amended with plant materials (LSNI/ODR) were significantly better in vegetative growth relative to the untreated control (0 mg/ml) plants. Nematode count in root and rhizosphere soil of untreated control plants were significantly (p< 0.0.5) more compared to all treated plants (Figs. 5 & 6). A higher gall index was also recorded in the untreated control plants (Fig. 7). The variance in the quantity of extracts applied brought about a remarkable effect on the parameters evaluated. Vegetative growth of lettuce was directly proportional to the quantity of extracts and fractions applied. The highest (100 mg/ml) dosage of treatment produced more leaves with a wider diameter and heavier head weight, similarly nematode population was lower in soil and roots of plants treated with 100 mg/ml as opposed to the untreated control plants (Figs. 2, 3, 4, 5, 6 & 7).

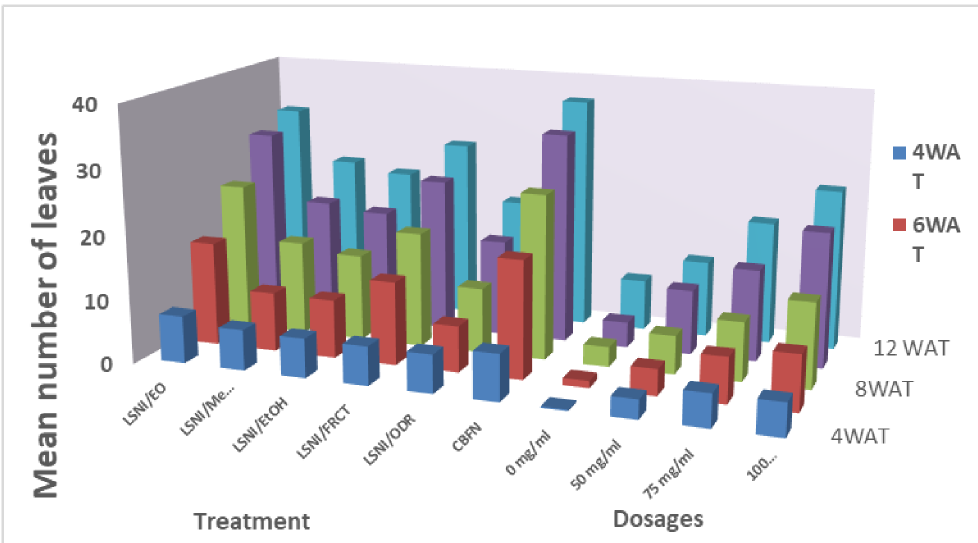

Figure 2: Effect of different treatments and dosages of *Lawsonia inermis* extracts on mean leaf number of lettuce plants

Key: LSNI/EO (*Lawsonia inermis* essential oil), LSNI/EtOH (*Lawsonia inermis* ethanol extract), LSNI/MeOH (*Lawsonia inermis* methanol extract), LSNI/FRCT (*Lawsonia inernis* Fraction) and CBFN (carbofuran).

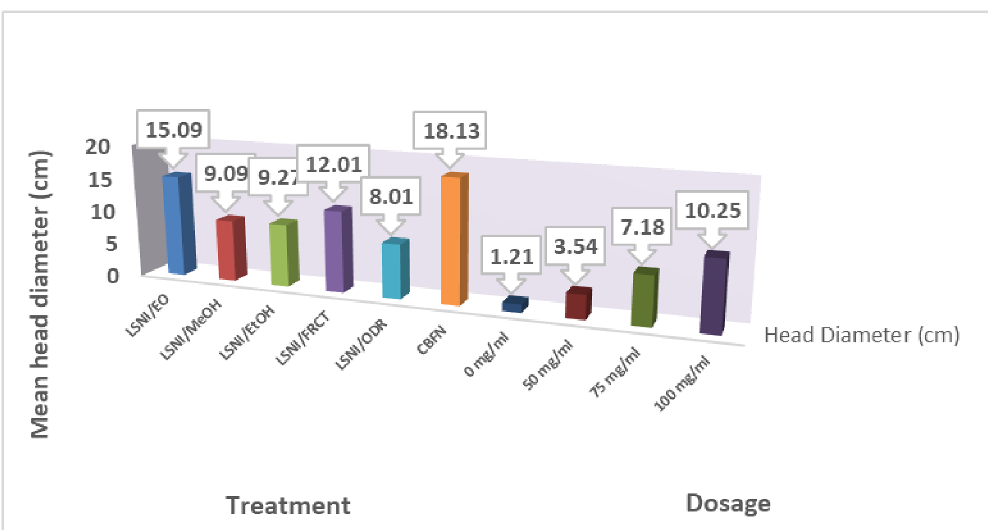

Figure 3: Effect of different treatments and dosages of *Lawsonia inermis* extracts on mean head diameter of lettuce plants.

Key: LSNI/EO (*Lawsonia inermis* essential oil), LSNI/EtOH (*Lawsonia inermis* ethanol extract), LSNI/MeOH (*Lawsonia inermis* methanol extract), LSNI/FRCT (*Lawsonia inernis* Fraction) and CBFN (carbofuran).

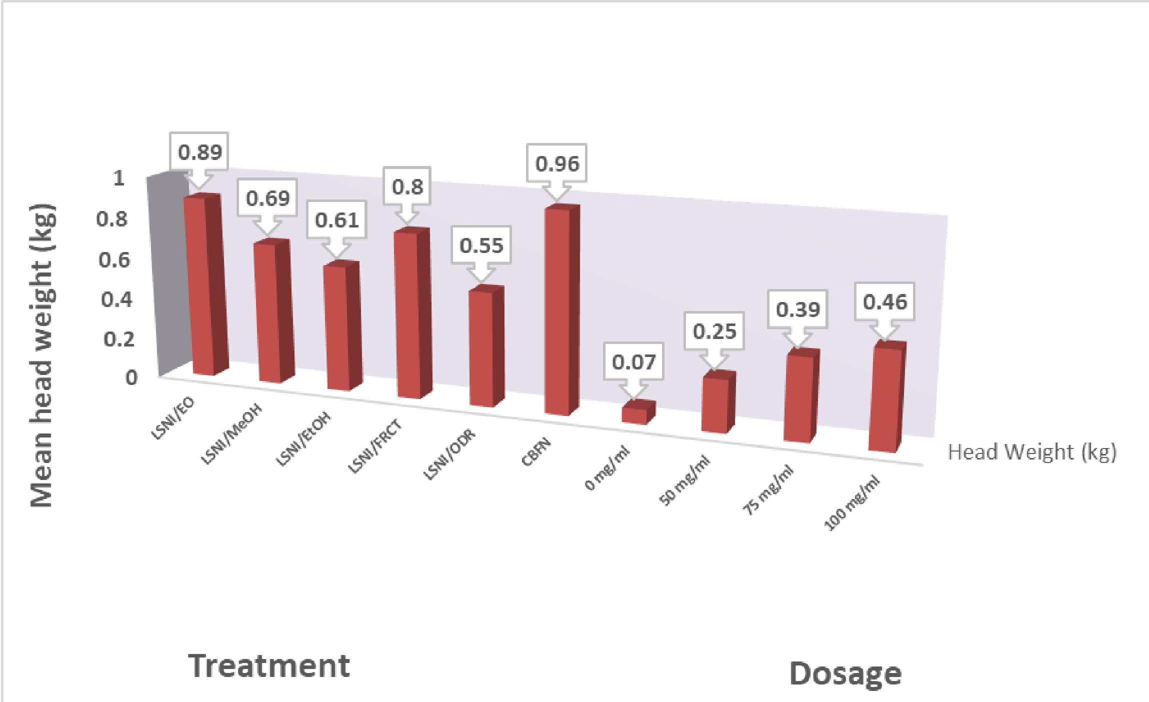

Figure 4: Effect of different treatments and dosages of *Lawsonia inermis* extracts on mean head weight of lettuce plants.

Key: LSNI/EO (*Lawsonia inermis* essential oil), LSNI/EtOH (*Lawsonia inermis* ethanol extract), LSNI/MeOH (*Lawsonia inermis* methanol extract), LSNI/FRCT (*Lawsonia inernis* Fraction) and CBFN (carbofuran).

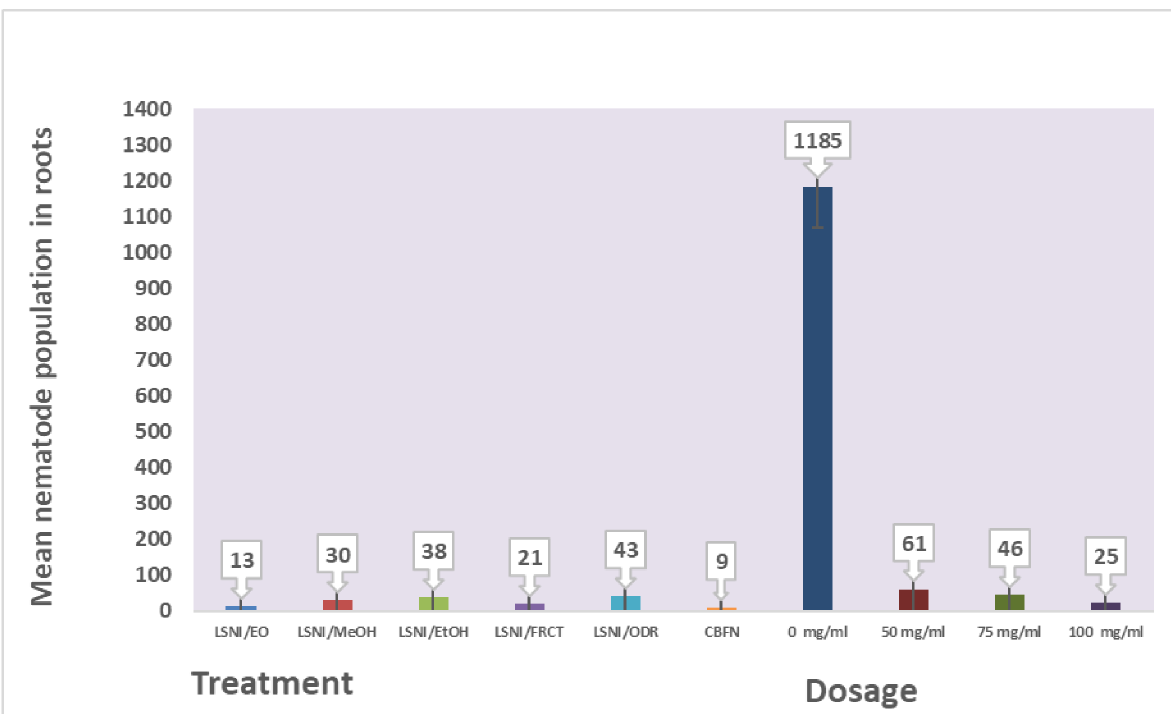

Figure 5: Effect of different treatments and dosages of *Lawsonia inermis* extracts on mean nematode population in roots of lettuce plants

Key: LSNI/EO (*Lawsonia inermis* essential oil), LSNI/EtOH (*Lawsonia inermis* ethanol extract), LSNI/MeOH (*Lawsonia inermis* methanol extract), LSNI/FRCT (*Lawsonia inernis* Fraction) and CBFN (carbofuran).

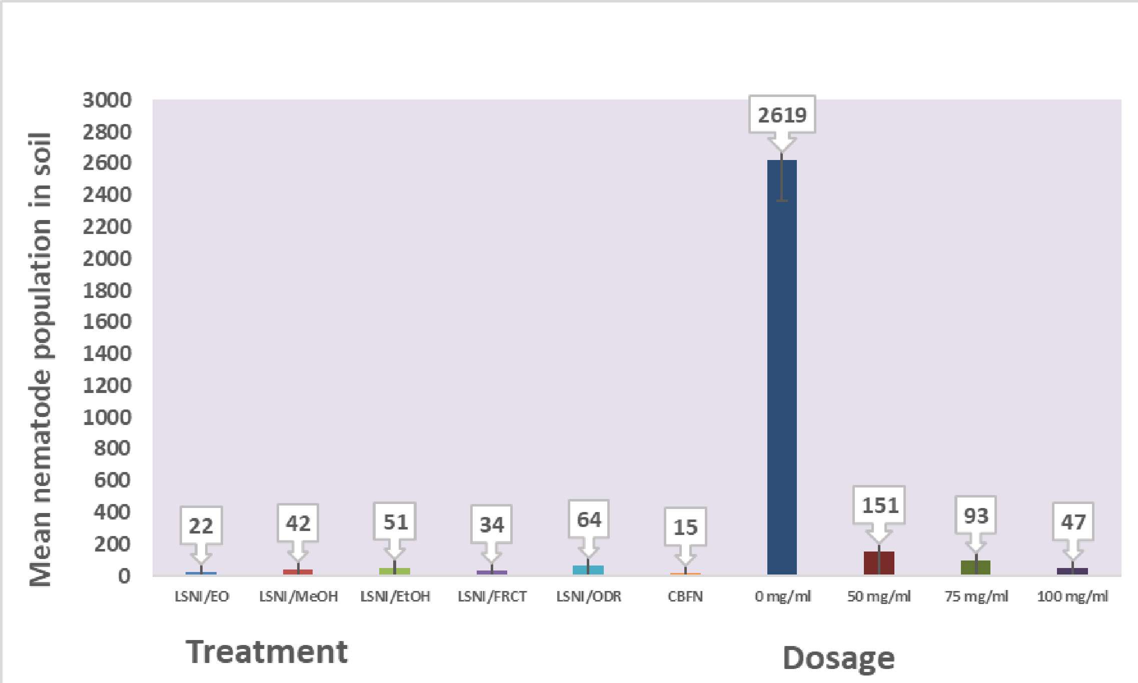

Figure 6: Effect of different treatments and dosages of *Lawsonia inermis* extracts on nematode population in rhizoshere soil of lettuce plants

Key: LSNI/EO (*Lawsonia inermis* essential oil), LSNI/EtOH (*Lawsonia inermis* ethanol extract), LSNI/MeOH (*Lawsonia inermis* methanol extract), LSNI/FRCT (*Lawsonia inernis* Fraction) and CBFN (carbofuran).

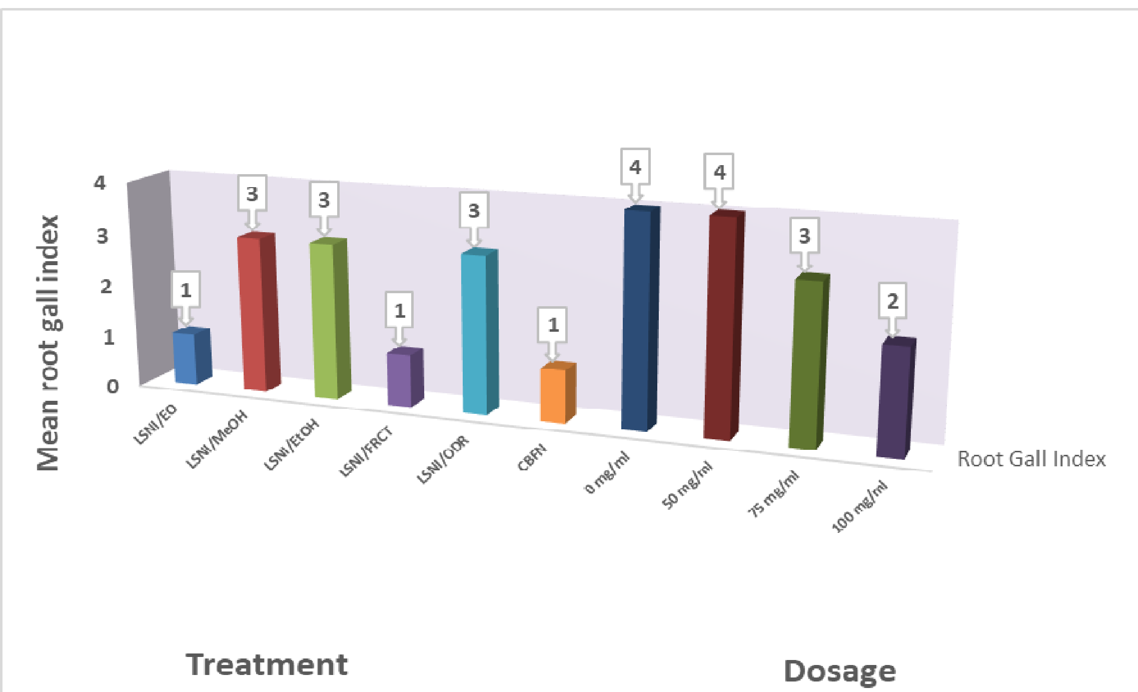

Figure 7: Effect of different treatments and dosages of *Lawsonia inermis* extracts on root gall index of lettuce plants

Key: LSNI/EO (*Lawsonia inermis* essential oil), LSNI/EtOH (*Lawsonia inermis* ethanol extract), LSNI/MeOH (*Lawsonia inermis* methanol extract), LSNI/FRCT (*Lawsonia inernis* Fraction) and CBFN (carbofuran).

DISCUSSION

The issue of environmental pollution brought about by pesticide use calls for caution in agricultural pest management. Several plant materials have been indicated as alternatives in the control option of plant parasitic nematodes (Chitwood, 2002; Fabiyi, 2021c) with positive outcomes. In this research the extracts from *L. inermis* has proven effective in the control of *M. incognita* damaging lettuce. The nematicidal action observed in this study could be attributed to the secondary metabolites contained in *L. inermis.* The constituents identified in this research aligns with the reports of Asmah *et al.* (2006) who also reported phytol as one of the constituents of EO from *L. inermis,* while the presence of sabinene, terpinolene and terpinen-4-ol was established by Najar and Pistelli (2017), as major composition of *L. inermis* EO. The nematicidal activity displayed by *L. inermis* essential oil (EO) was comparable to that of a synthetic nematicide. Results from ^{1}H-NMR and ^{13}C-NMR indicates that the fractions contain sesquiterpenes. The accounts of El-Habashy *et al.* (2020) confirmed the efficacy of sesquiterpenes in *M. javanica* management in laboratory and greenhouse tests. Strong nematicidal activity was displayed with remarkable reduction in number of second stage juveniles of *M. javanica* in soil, egg masses per plant and number of galls at 500 mg/l. The vegetative parameters of eggplant were also increased in parallel with oxamyl a reference standard. In the laboratory, notable reduction in egg hatch of *M. javanica* was observed simultaneously with high juvenile mortality. *L. inermis* is known to be a highly potent plant and has been documented to be antiviral, antibacterial and antifungal (Najar and Pistelli, 2017). The tuberculostatic activity of *L inermis* was reported by Sharma (1990), 6 µg/ml of extract inhibited $H_{37}Rv$ *in vitro* and *L. inermis* extract at 5 mg/kg body weight of mice led to significant cure of mice infected with *M. tuberculosis* $H_{37}Rv$. Owing to the potency of *L. inermis* extracts, it has been indicated in the treatment of trypanosomiasis in livestock (Atawodi *et al.,* 2002). Identically, Okpekon (2004), reported that *L. inermis* was particularly effective among all plants evaluated for antihelminthic and trypanocidal activity. The ability of *L. inermis* to control *Tinea pedis* in humans was demonstrated by Sherifa *et al.* (2015). Fabiyi and Atolani (2011), reported the *in vitro* and screenhouse evaluation of aqueous extract and powdered material of *L. inermis.* Significant reduction in egg hatch of *M. incognita* was achieved with 15% crude extract concentration. Increased vegetative growth of *Corchorus olitorius* and nematode population reduction was also observed in the screenhouse at 15% concentration. The EO from *L. inermis* was exceptionally effective in comparison with other extracts of *L. inermis.* A handful of study have confirmed the potency of essential oils in the management of *Meloidogyne* sp. Ozdemir and Gozel (2018) substantiated the findings in this study, they found essential oil from different plants to reduce the number of eggs masses and gall formation on tomato plant roots at 3 and 5% level of application. Comparably, Jardim *et al.* (2020) corroborated the potency of EO on *M. incognita* juveniles and eggs. They found 63 µg/ml of garlic essential oil significantly more active than 173 µg/ml of carbofuran on eggs and second stage juveniles of *M. incognita.* The reproduction and infectivity of *M. incognita* on tomato plants was remarkably reduced with application of 0.2 ml of garlic EO per litre of substrate. The effectiveness of garlic EO was statistically the same as 0.25 g of dazomet. Likewise, Pardavella *et al.* (2020a) evaluated essential oil extracted from *Cuminum cyminum* (cumin) seeds on survival, hatching and motility of second stage juveniles and eggs of *M. incognita* and *javanica.* At 62.5 µl/l concentration, the J2s were paralyzed, while hatching of eggs decreased with increase in EO concentration. In the same vein, Pardavella *et al.* (2020b) recorded significant reduction in nematode population in infested soil and roots of tomato plants after application of EO from *Satureja hellenica* at 4000µl/l, while 100% paralysis of *M. incognita* and *javanica* J2s was noted after 96 hours of exposure. They confirmed that the EO of *S. hellenica* contains 4-terpineol (3.65%), γ-terpinene (4.63%), carvacrol methylether (6.77%), carvacrol (23.25%), borneol (6.79%) and p-cymene (27.46%) as active principles. The use of EO from *L. inermis* promoted vegetative growth in lettuce; hence it can be employed in the control of *M. incognita* in lettuce, thus reducing the unnecessary use of pesticides in lettuce production.

Conclusion: In this study, the leaves of *L. inermis* was extracted in different ways whereby, the EO was found to be significantly more potent than other extractives**.** The active chemical compounds in the EO could be isolated individually and considered for proper formulation into commercial use to minimise the challenges associated with *M. incognita* management. Dry leaves of *L. inermis* can also be incorporated into the soil to bring down *M. incognita* population in lettuce cultivation.

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