Research Article

Fractions from *Mangifera indica* as an Alternative in *Meloidogyne incognita* Management

Oluwatoyin Adenike Fabiyi

Department of Crop Protection, Faculty of Agriculture, University of Ilorin.

Abstract | Pesticide residues and metabolites are often found in fruits, vegetables, soil and underground water as contaminants. This necessitated the search for bio-nematicides, accordingly the nematicidal prospect of chromatographic fractions from *Mangifera indica* as a substitute to synthetic nematicides in the control of *Meloidogyne incognita* pests of tomato was evaluated. Crude extracts of *M. indica* bark was fractionated on silica-gel (120-150 mesh) using glass column. The resulting fractions were investigated in screenhouse and field trials. The fractions were equated with carbofuran a synthetic nematicide. Each experimental pot containing 40 kg of pasteurized soil was inoculated with 1000 eggs of *M. incognita* in the screenhouse, while 2500 eggs was introduced to the base of each tomato plant on the field. Significant ($p<0.05$) increase was noted in the vegetative growth of treated tomato plants. Fruit weight per plant and number of fruits per plant increased notably as opposed to the untreated tomato plants. Nematode population in root and soil of treated tomato plants also reduced significantly. Terpenes, esters, aldehydes, phenols and ketones were identified as the major constituents of the fractions with partial characterization. The reduction in nematode population in the screenhouse and field signifies that fractions from *M. indica* could be employed in place of the environmentally undependable synthetic nematicides, while encouraging a sustainable and safe environment in tomato cultivation.

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***Correspondence** | Oluwatoyin Adenike Fabiyi, Department of Crop Protection, Faculty of Agriculture, University of Ilorin; **Email:** fabiyitoyinike@hotmail.com
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Introduction

Tomato (*Lycopersicon esculentum*) is a key vegetable crop in Nigeria, after onions and pepper. It is a rich source of vitamins A, B, C, E, iron and phosphorus (Fawusi, 1978; Karen, 2007). Tomato plants are inclined to infestation by plant parasitic nematodes, particularly the *Meloidogyne* spp. which brings about general reduction in the yield of tomato fruits in western Nigeria (FAO, 2004; Fabiyi, 2018; Fabiyi and Olatunji, 2021a). *Meloidogyne incognita* invades the root of vegetable plants feed and reproduces, causing galls in the root tissue (Safdar *et al*., 2012; Fabiyi and Olatunji, 2021b; Fabiyi *et al*., 2022), which in turn reduces the movement of water and nutrients from the root to the shoot, thus

weakening the plant. In severe cases death of the plants and yield loss of about 30% has been recorded (Shakeel *et al*., 2012). Owing to the devastating effect of *M. incognita* infestation on vegetables, carbofuran (*2,3-dihydro-2,2-dimethyl-benzofuran7-yl-N-methylcarbamate*) is customarily employed for control. Carbofuran is relatively mobile in soil and surface runoff because of its high water solubility (351 ppm) and low adsorption coefficient (Lau *et al*., 2007), consequently contaminating lakes, streams and groundwater (Goad *et al*., 2004). Environmental issues have increasingly limited the use of carbofuran (Rich *et al*., 2004), while diverse options of control are put forward (Fabiyi *et al*., 2020; Atolani and Fabiyi, 2020). This study was initiated as a result of serious concern for the enormous damage resulting from yield reduction associated with *M. incognita* infestation of tomato plants. The environmental risk sequential upon extensive and indiscriminate use of synthetic nematicides presupposes the pursuit of an alternative plant derived nematicide (Atolani *et al*., 2014a, b; Fabiyi, 2021a, b, c). *Mangifera indica,* a member of the family Anacardiaceae is reported to be endowed with medicinal properties. The stem bark of *M. indica* is known to have antimicrobial and anti-amoebic properties (Das *et al*., 1989; Tona *et al*., 2000). The treatment of syphilis and diarrheal is equally associated with extracts from *M. indica* (Ross, 1999), while Munza *et al*. (1994) documented the activities of the stem bark extract in the treatment of skin diseases and mouth sores. Accordingly, in this experiment, the toxicity of organic compounds isolated from the stem bark of *M. indica* on *M. incognita* infesting tomato plants in screenhouse and field studies is assessed.

Materials and Methods

Collection of plant materials

The stem bark of *Mangifera indica* was scrapped from the mother tree, the large pieces were meticulously diced into small bits of about 2cm each and were dried at room temperature for four weeks (Fabiyi *et al*., 2012a). The initial weight was 33.34 Kg and final weight at ambient temperature drying was 29.08 Kg. The materials at equal weight of 9. 69 Kg each was subjected separately to cold extraction in methanol, ethyl acetate and n-hexane for five days. The crude extracts were poured off the jars, it was then filtered and concentrated with rotary evaporator (Buchi Rota Vapour R-300).

Fractionation and spectroscopy

A glass column was filled with silica gel 60 (80-200 mesh), each of the crude extracts was put through fractionation at 500g each for methanol, ethyl acetate and hexane extracts. The mobile phase was hexane, hexane/dichloromethane ratio 2:1, hexane/dichloromethane ratio 1:1, hexane/dichloromethane ratio 1:2, hexane/dichloromethane ratio 2:1, hexane/dichloromethane ratio 3:1, hexane/dichloromethane ratio 3:2. Similar fractions were merged after thin layer chromatography (TLC, silica gel GF254, 0.25mm Merck Germany plates) test of each of the fractions. The infrared of fractions was analysed on Buck 500M spectrophotometer with KBr pellets, while Gas Chromatography-Mass Spectroscopy was carried out with Agilent 7890A GC/MS equipped with a Quadrupole Mass Spectra Detector and an Auto-sampler.

Screenhouse experiment

Pasteurized loamy soil was apportioned into experimental pots at 40 kg each. The experimental design had 4 treatments, 5 replicates and 4 dosages of application. Two weeks' old tomato (cv Roma) seedlings were transplanted into each experimental pot at a seedling per pot. Fresh, newly hatched eggs of *M. incognita* was inoculated at the base of each seedling (approximately 1000 each) a week after transplanting (Fabiyi *et al*., 2019).

Field experiment

A piece of land measuring 55m by 40m was laid out, ploughed and harrowed. It was outlined and segmented into 80 beds of 1m by 7m (7.0m^2) in size with a passage way of 0.5m in between (Fabiyi and Olatunji, 2021a). On each bed, a spacing of 50 cm between plants and 75 cm between rows was used (Wageningen, 2005; Gudugi *et al*., 2012). Plantlets of tomato were later transplanted from the nursery to the field. Each tomato plant on the bed was inoculated with approximately 2500 freshly hatched eggs of *M. incognita* following the method of Fabiyi *et al*. (2019).

Treatment application and data collection

Carbofuran 3G, was applied on the field at 1.0, 1.5 and 2.0 kg a.i/ha while the chromatographic fractions were applied at 35 mg/kg soil, 41 mg/kg soil and 47 mg/kg soil. In the screenhouse, carbofuran was used as applied on the field, while fractions were applied at 15 mg/kg soil, 21 mg/kg soil and 27 mg/kg soil. Each quantity was dissolved in 300 mL distilled

water. 30 mL of a non-ionic surfactant emulsifier was introduced to attain complete solubility and to come up with a uniform solution of the fractions. Data on vine lenght of tomato plants, number of leaves and days to 50% flowering was taken in the screenhouse and field during the growth period of the tomato plants. Numbers of fruits per plant and fruit weight per plant was recorded progressively at maturity, while nematode population in root and soil in the screenhouse was evaluated by removing 250ml soil from the rhizosphere of each tomato plant. On the field, five core samples (0-25 cm deep) were gathered from the base of tomato plants on each bed, these were pulled together to constitute a single sample for each bed. The soil samples were taken to the laboratory and the nematode population was estimated using Whitehead and Hemming (1965) tray method of nematode extraction. Later, roots were graded for severity of galling using a scale of 0-5, as expressed by Taylor and Sasser (1978). Where 0 = no gall; 1 = 1-20% of the root system galled; 2 = 21-40% of the root system galled; 3 = 41-60% of the root system galled; 4 = 61-80% of the root system galled; and 5 = 81-100% of the root system galled. Subsequently, root samples were neatly washed under running tap water and cut into pieces of 2cm each and macerated in a warring blender for 30 seconds. The contents of the blender were emptied on to a two ply tissue paper in a sieve on a pie-pan. All data taken were then subjected to analysis of variance using GenStat 5.32 and separation of means done with Tukey's honest significant difference test (Fabiyi, 2020).

Results and Discussion

Several functional groups were seen in the result of the infrared analysis of chromatographic fractions. Absorption bands at 3420cm^{-1}, 3211cm^{-1}, 2954cm^{-1}, 2933cm^{-1}, 2855cm^{-1}, 1869cm^{-1}, 1737cm^{-1}, 1707cm^{-1}, 1627cm^{-1}, 1601cm^{-1}, 1450cm^{-1}, 1380cm^{-1}, 1375cm^{-1}, 1274cm^{-1}, 1176cm^{-1}, 1105cm^{-1}, and 1024cm^{-1} were noted in the spectra of fractions from methanol extract. These depicts the presence of O-H, secondary amines, aliphatic N-H stretch, C-H stretch aldehyde, C-O of carbonyl, C=O acid anhydride, N=O aliphatic nitro, C=C of aromatic ring, C=C alkene, C=C-N stretch, C-H of alkyl group, C-O phenol, C-O acid and a C-H vibration individually. These designates aliphatic chains, aldehydes, amines, amides, acids, esters, ketones and lactones. The ethyl acetate fractions are dominated by vibrations at 3413cm^{-1}, 2928cm^{-1}, 2856cm^{-1}, 1702cm^{-1}, 1646cm^{-1} and 1456cm^{-1} which signifies N-H stretch, C-H aliphatic, C-H aldehyde, C=O ketone, C=O amide and alpha CH_2 bending singly. From the n-hexane fraction, C-H stretch aliphatic and C-H stretch aldehyde is recurrent. A large number of compounds which ranges from esters, alcohols, terpenes, ketones, phenols, fatty acids, sesquiterpenes, carboxylic acids, terpenes, steroids, aldehydes, hydrocarbons and ethers were detected in the GCMS of the various fractions. Some of these include N-phenyl-1naphtalenamine, 9-beta campesterol, trans-caryophyllene, 3-pentadecylphenol, hexanoic acid, manglupenone, dodecanal, terpinene, 6-methyl-3-heptanol,

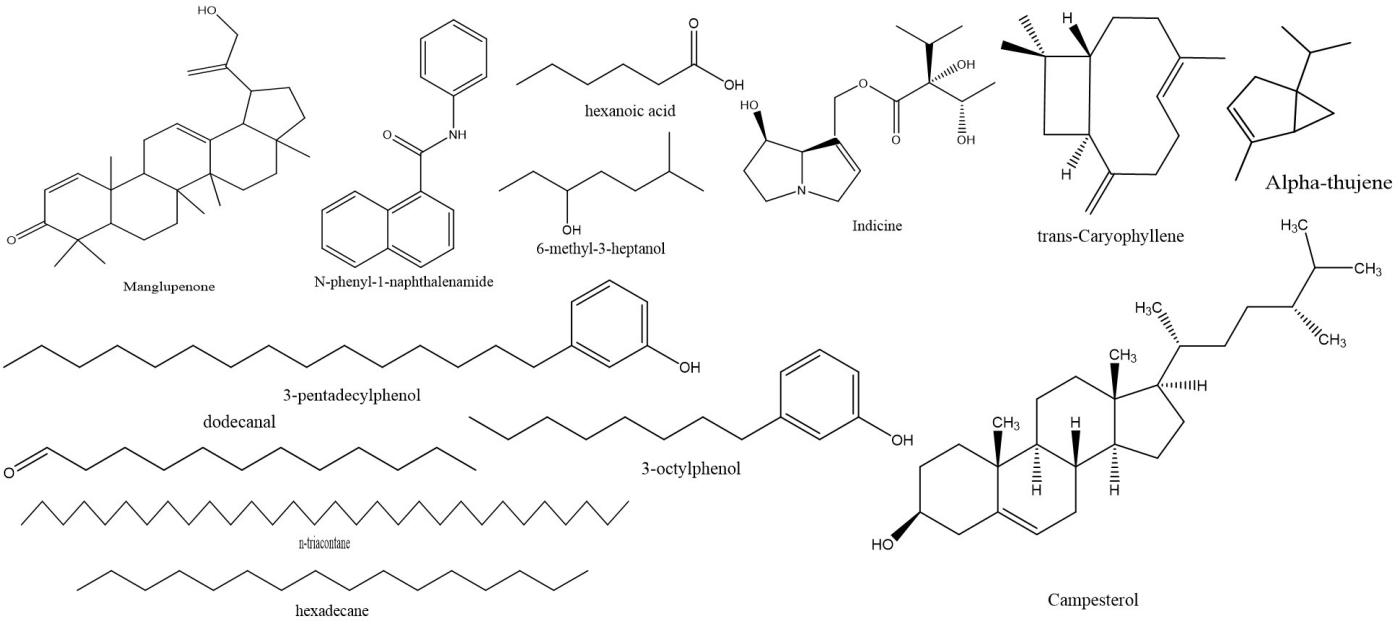

Figure 1: *Structure of compounds obtained from fractions of Mangifera indica.*

n-triacontane, 2, 5 dimethyl-4hydroxy-3(2H)-furanone, hexadecane, indicine, 3-octylphenol, mangiferolic acid methyl ester and alpha-thujene (Figure 1).

From the screenhouse, significant differences were not observed between the vine length of tomato plants treated with fractions from methanol extract (MANG/MeOH) and (CBFN) carbofuran (Figures 2, 3). Explains the effect of the quantity of treatments applied on the vine length of tomato plants. Longer vines were recorded in the highest quantity of treatments (27mg/ml), while the lengths of the untreated tomato plants were short. In like manner, observations from the field on vine lengths depicts that vine lengths were also longer in tomato plants

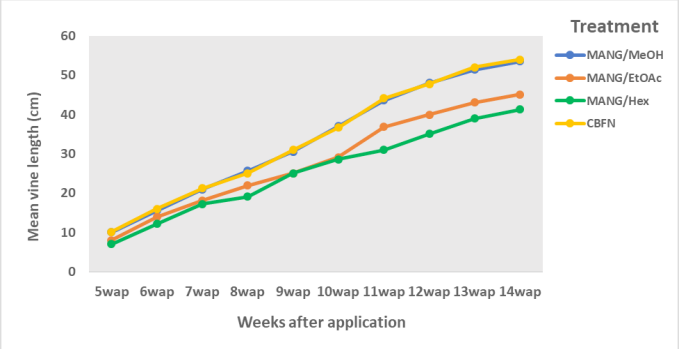

Figure 2: *Effect of treatment on vine length.*

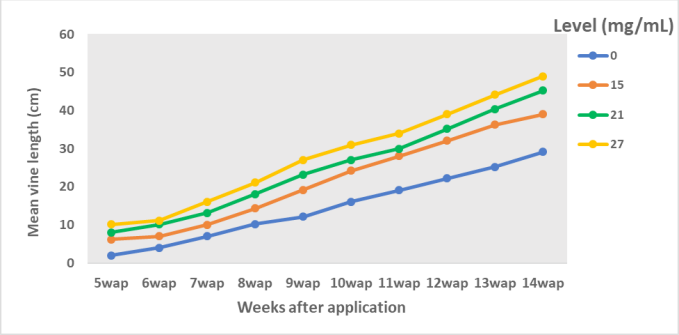

Figure 3: *Effect of level on vine length.*

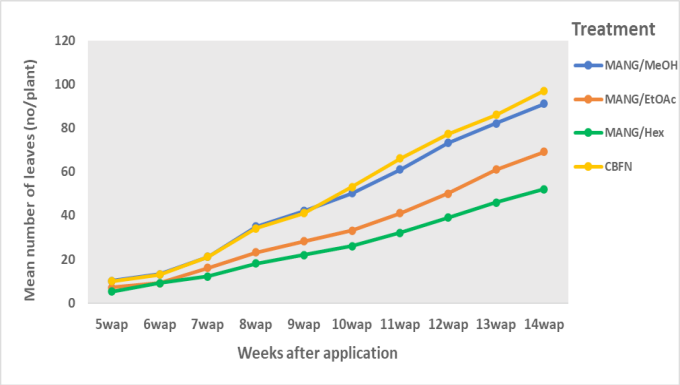

Figure 4: *Effect of treatment on number of leaves.*

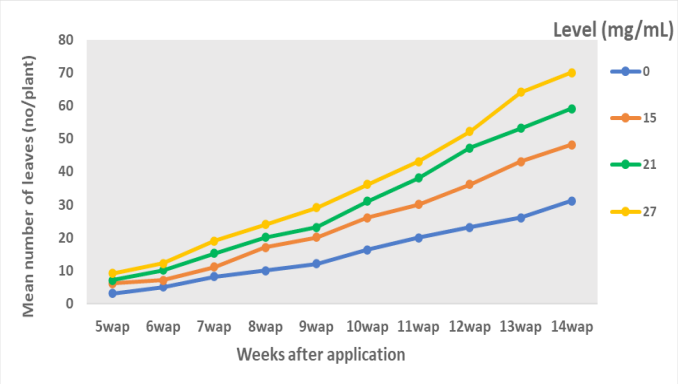

Figure 5: *Effect of level on number of leaves.*

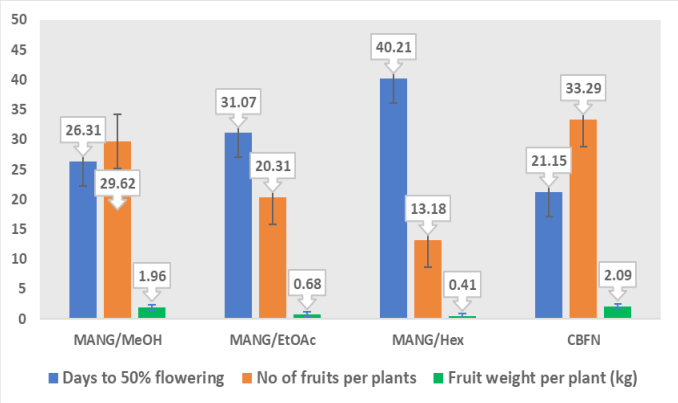

Figure 6: *Effect of treatment on yield attributes.*

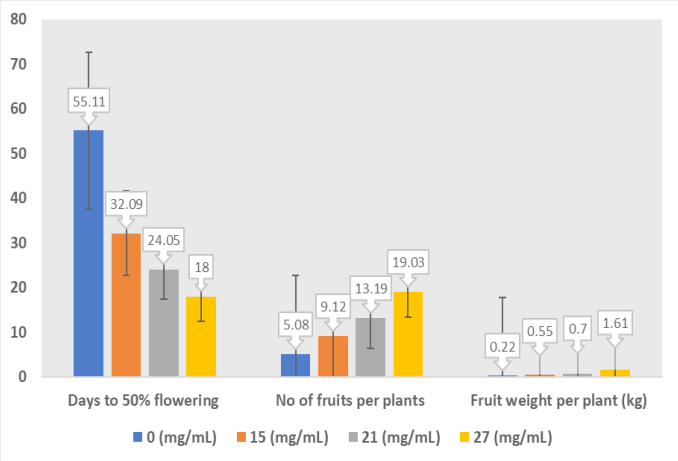

Figure 7: *Effect of level on yield attributes.*

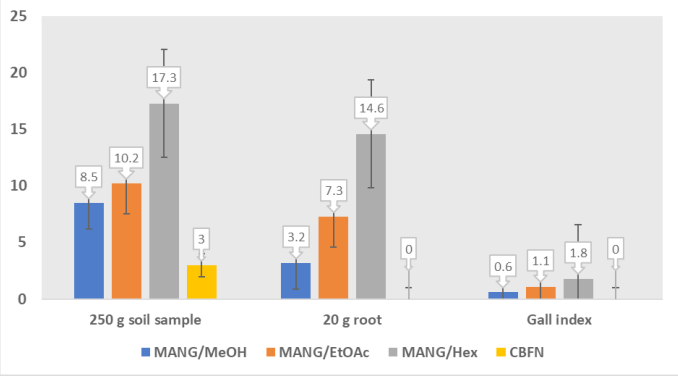

Figure 8: *Effect of treatment on nematode population.*

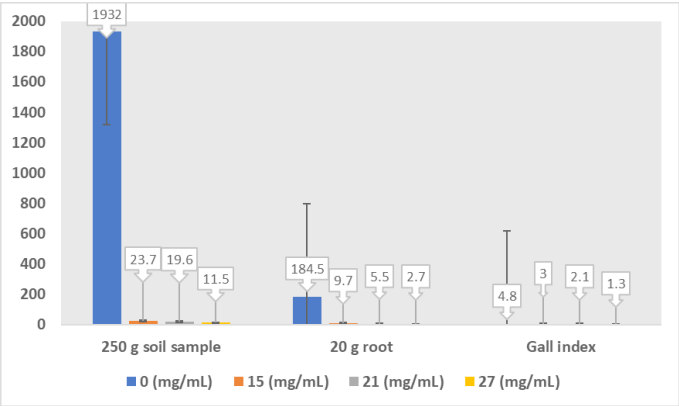

Figure 9: *Effect of level on nematode population.*

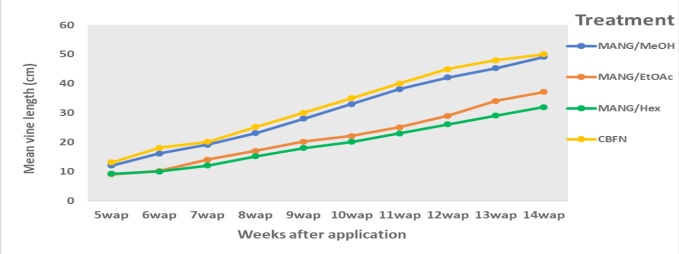

Figure 10: *Effect of treatment on vine length.*

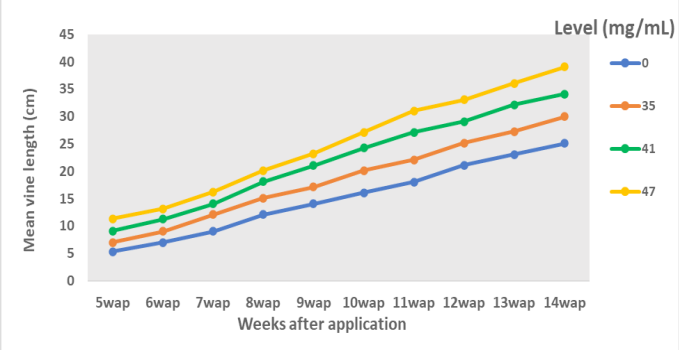

Figure 11: *Effect of level on vine length.*

treated with CBFN and MANG/MeOH, while there is no notable difference between the two treatments (Figure 10). The highest quantity of treatment materials applied on the field (47mg/ml) equally produced longer vines as opposed to the vine length recorded in untreated tomato plants and those treated with lower quantity of materials (Figure 11). From Figures 4 and 12, numbers of leaves of tomato plants were remarkably more in plants treated with MANG/MeOH and CBFN in the screenhouse and field. Fewer leaves were produced in plants treated with MANG/EtOAc and MANG/Hex (*Mangifera indica* ethyl acetate fractions and *Mangifera indica* n-hexane fractions). The effect of quantity of treatment application on numbers of leaves of tomato plants is shown in Figures 5 and 13. More leaves were recorded in treated plants as against untreated tomato plants. The influence of treatment materials on the yield attributes of tomato plants is presented in Figures 6 and 14. In the screenhouse and field trials plants treated with MANG/EtOAc and MANG/Hex flowered lately as contrasted with plants treated with MANG/MeOH and CBFN which flowered earlier. Correspondingly on the field, flowering was late in MANG/EtOAc and MANG/Hex, but early in MANG/MeOH and CBFN. Number of harvested fruits was significantly more in CBFN and MANG/MeOH treated plants in the screenhouse and field trials compared with all other treatments applied (Figures 6 and 14). In the same vein, heavier fruits were recorded in CBFN and MANG/MeOH treated plants in the screenhouse and field rather than the weights obtained in other treatments. The highest dosage of treatment materials in the screenhouse and field had a positive effect on the tomato plants with early flowering. Likewise, significantly more numbers of fruits and heavier fruits were recorded (Figures 7 and 15). Remarkably low soil nematode population was recorded at harvest in treated plants both in the screenhouse and field trials (Figures 8 and 16) in comparison with the population recorded in untreated plants (Figures 9 and 17). Identically, nematode population in 20 g of tomato roots was notably low with a corresponding low gall index (Figures 9 and 17). In general, the influence of *M. incognita* was more pronounced on the untreated tomato plants. The improvement in vegetative growth of tomato plants administered with fractions from *M. indica* is accredited to the fragments and integrant component of the various chromatographic fractions that were employed in the treatment of the infected tomato plants in screenhouse and field trials. Chromatographic fractions are acknowledged to be nematicidal (Fabiyi *et al.*, 2012). *M. indica* is admitted to contain diverse active principles and metabolites which are reported to be nematicidal (Aiyelaagbe and Osamudiamen, 2009; Shah *et al.*, 2010; Zasada, 2010; Joona *et al.*, 2013). Similarly, the bark extract of *M. indica* is proven to be nematicidal against nematodes parasitizing *Ananas comosus* (PIP, 2011). The functional groups identified in the fractions through infrared analysis is nearly almost connected with the functional groups of mangiferin a xanthone and isomangiferin which has been described as the vital constituents of *M. indica* and several other metabolites like polyphenols, flavonoids, triterpenoids, alpha-beta unsaturated ketones, acids, aldehydes, esters and lactones. Stoilova *et al.* (2005) and Perrucci *et al.* (2006) stated the anti-parasitic, antifungal and

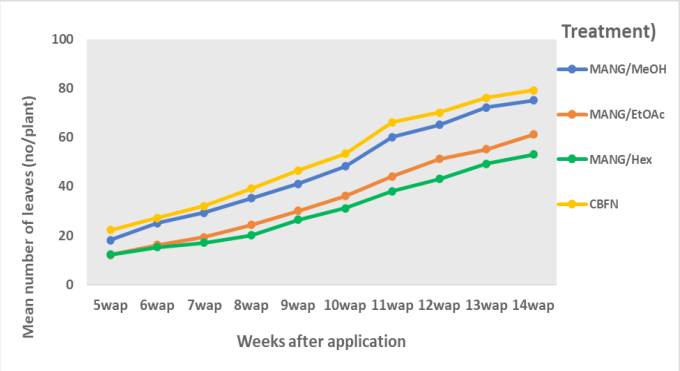

Figure 12: *Effect of treatment on number of leaves.*

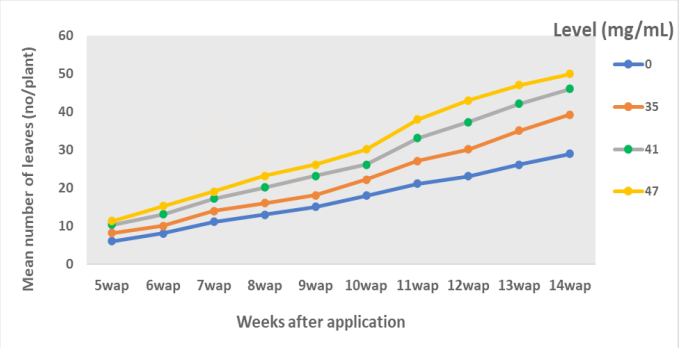

Figure 13: *Effect of level on number of leaves.*

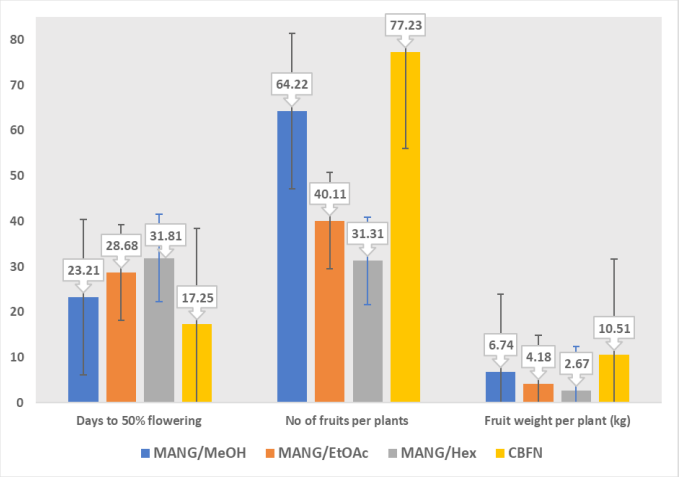

Figure 14: *Effect of treatment on yield attributes.*

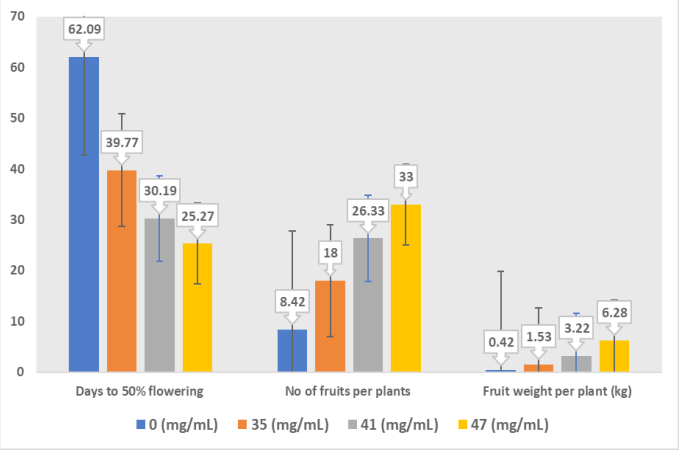

Figure 15: *Effect of level on yield attributes.*

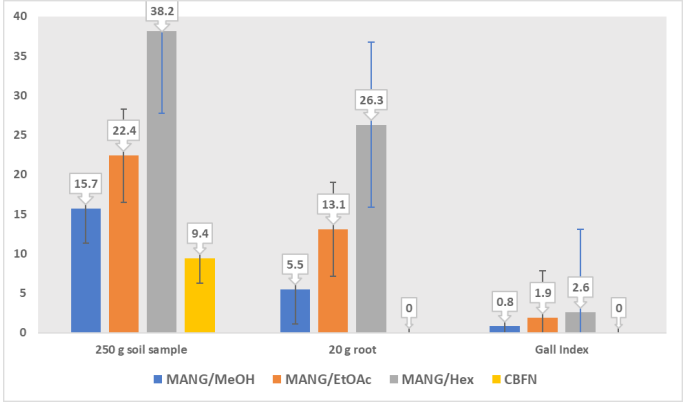

Figure 16: *Effect of treatment on nematode population.*

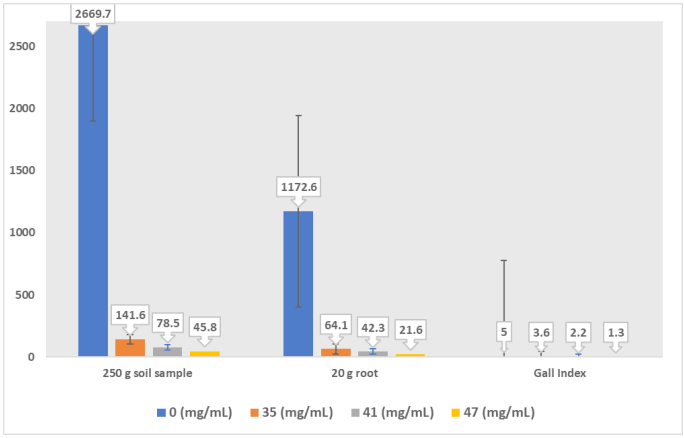

Figure 17: *Effect of level on nematode population.*

antibacterial action of mangiferin. Analogously, Guha *et al.* (1996), Engels *et al.* (2009, 2010, 2011), Ansari *et al.* (2000) and Sairam *et al.* (2003), additionally emphasized the anti-bacterial, anti-diarrhoeal, antimicrobial and anti-HIV of mangiferin and gallotannins from *M. indica*. The effectiveness of extract from *M. indica* leaves on malarial parasite was confirmed by Asase *et al.* (2010). This assertion was corroborated by Bidla *et al.* (2004), they confirm the action of *M. indica* extracts on *Plasmodium falciparum*. In like manner, Malann *et al.* (2013) gave insight into the effectiveness of the leaf extract on *P. berghei*, while inhibition of HSV-1 and HSV-2 herpes simplex virus replication was communicated by Zhu *et al.* (1993) and Zheng and Lu (1990). The stage reliant action of *M. indica* aqueous extract on animal nematode *Trichinella spiralis* was accentuated by Garcia *et al.* (2003) while, Gehad *et al.* (2013) reported the 100% multiplication inhibition of *Strongyloides stercoralis* larvae with aqueous extract of unripe *M. indica* fruit. Several phenolic compounds are documented to be nematicidal. Compounds such as methoxycinnamic acid, protocatechuic acid, juglone, 3-phenylphenol, 7-OH-coumarin, vanillic and syringic acid have been established to have high nematicidal action on

M. incognita (Mahanja *et al*., 1992). Ohri and Kaur (2010) equally reported the potential of polyphenolic compounds in the management of plant parasitic nematodes and this was reiterated by Leontopoulos *et al*. (2020). The wide range of plant metabolites in *M. indica* could be harnessed in the management of *M. incognita*.

Conclusions and Recommendations

Plant bioactive compounds are diverse in the class of phenolic, terpenoid and alkaloids. These could be consolidated in *M. incognita* management for a safer environment.

Novelty Statement

Metabolites from *Mangifera indica* could be employed as part of IPM program in the control of *Meloidogyne incognita* on tomato

Conflict of interest
The authors have declared no conflict of interest.

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