

RESPONSE OF *Pratylenchus* spp INFECTED TOMATO (*Lycopersicon esculentum* Mill) TO ORGANIC COMPOUNDS FROM *Mangifera indica*

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Abstract

The need to reduce the negative impact of synthetic nematicides on the environment necessitated the search for bio-pesticides. This study was conducted to evaluate the nematocidal potential of chromatographic fractions from *Mangifera indica* on tomato in the screenhouse and field. *M. indica* bark was extracted with ethanol (EtOH) and dichloromethane (DCM). The crude extracts were chromatographed and fractionated on silica-gel (100-120mesh) column. Fractions were tested at 0.2, 0.5 and 0.8mg in the screenhouse and 12, 18 and 24mg on the field. Vegetative growth was significantly ($p<0.05$) higher in plants treated with the highest concentration of fractions. There was a significant increase in number of fruits per plant and fruit weight per plant. Nematode population in soil and root also reduced significantly. The fractions were partially characterised and were found to contain fatty acid esters, hydrocarbons, terpenoids, flavonoids, alkaloids, acids, esters, aldehydes and ketones. Observed reduction in nematode population is a clear indication that *M. indica* bark extract is an effective agent against nematode infestation.

Key Words: *Lycopersicon esculentum*; *Mangifera indica*; *Pratylenchus* spp; ethanol; dichloromethane

Introduction

Tomato (*Lycopersicon esculentum*) is an important vegetable crop grown all over Nigeria. It is the world's largest vegetable crop after potato and sweet potato. In Nigeria, tomato is regarded as the most important vegetable after onions and pepper (Fawusi, 1978). Tomatoes are planted on an estimated 85% of the gardens each year. If well managed, it is highly productive. It is an important source of vitamin A, B, C, E, iron, phosphorus and minerals which reduces cholesterol level in the blood and minimizes the risk of prostate and lung cancer (Karen, 2007). Quantity and quality are the main

objectives of tomato production. Yield of tomatoes in the tropics is usually low compared to what is obtained in the temperate region (Muhammad and Singh, 2007). In the western part of Nigeria, tomato yield is generally low, this is attributed to several diseases and pests among which are plant parasitic nematodes. Many different species are known to damage tomato among these are the *Pratylenchus* spp (root lesion nematode). This nematode invades roots, feed and reproduce in the cortex causing necrosis of the root cells. As a result of this, browning, irregular longitudinal lesions appear on the infected roots (Ravichandra, 2014). The

root lesion nematode *Pratylenchus penetrans* has been reported to cause stunting of tomato plants due root injury resulting in significant crop losses (Reynolds *et al.*, 1992). Yield loss of about 30.2% has been associated with *P. penetrans* on vegetable fields (Safdar *et al.*, 2012), while Shakeel *et al.* (2012) observed 32.5% yield loss in vegetables due to *Pratylenchus* spp. Delay in fruit ripening, reduction in total weight of marketable tomatoes and numbers of large sized fruit was observed at higher population of *Pratylenchus penetrans* on the field (Potter and Olthof, 1977). Several methods have been employed in the control of nematode pest of tomato, some of which involves the use of synthetic nematicides, which has increased yield, but resulting in human poisoning, environmental pollution and adverse effect on non target organisms in the ecosystem (Yudelma, *et al.*, 1998). Thus, there is the need for an alternative method of control. In view of this, the bark of *Mangifera indica* was investigated for its possible nematicidal potential. The stem bark of *M. indica* has been stated to have antimicrobial and anti-amoebic properties (Das *et al.*, 1989; Tona *et al.*, 2000). Ross, (1999) also established the use of *M. indica* stem bark in the treatment of syphilis and diarrheal. In the same vein, Munza *et al.*, (1994) reported the use of stem bark in the treatment of skin diseases and mouth sores. Extensive literature review revealed that the stem bark of *M. indica* has not been employed in the control of plant parasitic nematode. In this study we report the toxicity of flavonoids and phenols from *M. indica* on *Pratylenchus* spp.

Materials and Methods

Preparation of Test Plant

The bark of *Mangifera indica* was collected from the mother tree at the back of the Faculty of Agriculture University of

Ilorin, Ilorin, Nigeria. The materials were chopped into tiny pieces and air dried in the laboratory for three weeks. The weight of the air dried bark after drying was 6.62 Kg, and was divided into three equal parts and extracted cold separately with 5.25litres each of ethanol (EtOH), dichloromethane (DCM) and water (H₂O). Each extraction lasted five days. The organic solvent extracts were decanted, filtered and concentrated under reduced pressure, while the aqueous (water) extract was allowed to evaporate to dryness at 37°C.

Preliminary Phytochemical screening of crude extracts

The crude extracts were screened for the presence of some plant metabolites such as carbohydrates, terpenoids, steroids, alkaloids, polyphenols (tannins), flavonoids, anthraquinones, saponins and reducing sugars following standard methods (Brian *et al.*, 1989; Trease and Evans, 1989; Harbone, 1973; Raymond and Sarker, 2006). All reagents used were of analytical grade and were used without further purification.

Chromatography

Gravity column chromatography of the ethanol and dichloromethane crude extract (150 gram each) was carried out on silica gel (100-120mesh) using n-hexane as the eluting solvent. This was followed by an increasing gradient of n-hexane/DCM (3:1; 3:2) and finally dichloromethane alone. The elution afforded twelve and nine fractions from the ethanol and dichloromethane extracts respectively. Fractions with the same retention factors as revealed on the thin layer chromatographic plate (Silica gel G_{F254}, 0.25mm Merck Germany plates) were combined accordingly. Column chromatography was repeated to increase the quantity of eluted fractions.

Spectroscopic analysis/Instruments

Ultra violet-visible (UV-Vis) spectra of fractions were taken on Du 730 Life

Science UV-Vis spectrophotometer Beckman Coulter, at the Chemistry Department University of Ilorin. Infra-red (IR) spectra was recorded on SHIMADZU 8400s FTIR (Fourier Transform) spectrophotometer and Gas Chromatography-Mass Spectroscopy was carried out on GCMS-QP 2010 PLUS (Shimadzu Japan) system coupled with a finigan MAT ion trap detector with an RTX5MS column packed with 100% grade dimethylpolysiloxane.

Pot Experiment

Forty eight (48) plastic buckets, each of eleven litre capacity were filled with 7kg of unpasteurized soil. This was done to have the same soil condition as on the field. Infected maize roots were incorporated into the buckets to build up the population of *Pratylenchus* spp for the experiment. Soil samples were taken from each bucket to identify the nematode genera present in the soil and to count the number of nematodes in each bucket; this was done before and after inoculation. Tomato seedlings were then transplanted from the nursery into the pots after nematode population had built up.

Initial/Final Nematode Population Count

Soil samples were taken from the field using systemic sampling method to identify the native nematode genera in the soil and their population. Two core (0-25cm deep) samples were taken from each vegetable bed on the field, this was bulked together to represent each bed. These were taken to the laboratory and the nematode population was estimated using Whitehead and Hemming, (1965) tray method of nematode extraction. This was also done after the experiment to check nematode population level.

Field Experiment

A portion of land measuring 30m by 20m was mapped out, ploughed and harrowed. This was divided into 48beds of

1m by 5m (5.0m²) in size with an alley way of 0.5m between them. On each bed a spacing of 50 cm between plants and 75 - 100 cm between rows was used (Wageningen, 2005). Each of the beds was inoculated with maize roots infected with *Pratylenchus* spp. Soil samples were taken before and after inoculation. Tomato seedlings were later transplanted from the nursery to the field.

Treatment Application

Carbofuran 3G, bought in Ilorin metropolis was applied on the field at 0.3, 0.6 and 1.0 kg a.i/ha while fractions were applied at 12mg, 18mg and 24mg which is equivalent to 0.6; 0.9 and 1.2mg/mL. In the screenhouse, carbofuran was used as applied on the field, while plant extracts were applied at 0.8mg, 0.5mg and 0.2mg (0.04, 0.025, and 0.01 mg/mL). Each quantity was dissolved in 20mL distilled water. 20mL of a non-ionic surfactant emulsifier was added to achieve total solubility and to provide homogeneous solution of the fractions.

Results

The various phytochemical tests revealed the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, saponins, glycosides, carbohydrates, anthraquinone, steroid and phenols in the ethanol extract. The DCM extract however had similar secondary metabolites with the ethanol extract but steroid was not present. The UV visible spectrum of the fractions in dichloromethane showed that they contain a wide range of compounds which absorbed in different ultraviolet regions; however intense absorption bands were seen at λ_{max} 296, λ_{max} 314 and λ_{max} 360nm. The infra-red showed a variety of functional groups. Generally fractions from ethanol extract showed the presence of an O-H functional group by the absorption band at

3421 cm^{-1} . The signal at 3203 cm^{-1} was attributed to secondary amines. The aliphatic C-H stretch in the fraction was expressed by the signals at 2956 cm^{-1} and 2922 cm^{-1} , while the C-H stretch of aldehyde was depicted by the stretching signal at 2854 cm^{-1} . The carbonyl stretching came up at 1735 cm^{-1} indicating an ester. The band at 1456 cm^{-1} is the C=C of an aromatic ring. 1377 cm^{-1} and 1172 cm^{-1} represent C-H stretch of an alkyl group and C-H bending vibration respectively. An O-H vibration is depicted in dichloromethane fractions with the vibration at 3412 cm^{-1} . The presence of an aliphatic C-H stretching is represented by the signal at 2926 cm^{-1} and a corresponding C-H stretching of an aldehyde was at 2854 cm^{-1} . The C=O stretching of conjugated ester is depicted by the band at 1707 cm^{-1} . The frequency at 1600 cm^{-1} , 1647 cm^{-1} and 1458 cm^{-1} represent C=O of an aromatic ring. The presence of an alkyl group is shown at 1379 cm^{-1} . The band at 1274 cm^{-1} and 1024 cm^{-1} represents the C-O of acid and phenol respectively. The GCMS of the fractions also revealed several terpenoids and fatty acid esters. From the screenhouse and field experiments, comparative effectiveness of the various treatments revealed that plants treated with fractions from *M. indica* ethanol extract (MANG/EtOH) were significantly better in plant height and number of leaves (Tables 1, 2, 5 and 6). All treatments differ significantly. The least number of leaves was observed in plants treated with the aqueous extract in the screenhouse and on the field. Days to 50% flowering was earlier in carbofuran and ethanol fraction treated plants, with a corresponding heavier fruit weight per plant. Similarly number of fruits per plant was significantly ($p < 0.05$) more in these plants (Tables 3 and 7). Nematodes were significantly reduced in 200g soil and 10g root sample of plants

treated with ethanol and DCM fractions, reduced population was observed in the aqueous extract treatment (Tables 4 and 8). Increased nematicidal activity corresponded with the rate of treatment application. The highest concentration of 0.8mg and 24mg was significantly more effective in the screenhouse and field respectively. Other dilutions, though significantly less effective, depicts that toxicity of the fractions decreases with dilution. However plants in the control experiment (0mg) had lower plant height, fewer number of leaves, fewer number of fruits per plant, reduced fruit weight and increased nematode population.

Discussion

The increased vegetative growth, *vis a vis* higher yield observed in the treated plants might be due to the phyto constituents present in *M. indica*. Preliminary phytochemical analysis revealed that *Mangifera indica* ethanol and DCM extracts contain some secondary metabolites such as flavonoids, terpenoids, saponins, phenols, anthraquinone, steroids alkaloids and glycosides. The report of Joon *et al.*, (2013) corroborates this. They reported the presence of flavonoids, terpenoids, saponins, tannins and glycosides in *M. indica*. Alkaloids and phenols have been proved to be toxic to nematodes (Khan, 1973; Hasan and Saxena, 1974). The results of Aiyelaagbe and Osamudiamen (2009), also supported the presence of saponins, steroids, tannin, flavonoid, reducing sugars, cardiac glycosides and anthraquinone in *M. indica*, while Shah *et al.* (2010), in their review reported polyphenols, flavonoids and triterpenoids as some of the constituents of *M. Indica*. Soap bark saponins from *Quillaja saponaria* have been established to moderately control root lesion nematode (Zasada, 2010), while mango bark extracts

are known to be active against nematode pests of *Ananas comosus* (PIP, 2011). The λ max values obtained for the fractions are 296, 314 and 360nm, which are closely related to what was reported for mangiferin a polyphenol- the major constituent of *M. indica*. The antibacterial, antifungal and antiparasitic (Stoilova *et al.*, 2005; Perrucci *et al.*, 2006) activity of mangiferin has been reported. Mangiferin has also been indicated in the inhibition of HSV-1 virus replication in cells and in antagonizing the cytopathic effects of HIV (Zheng *et al.*, 1990; Guha *et al.*, 1996). Garcia *et al.* (2003) highlighted the modest and stage dependent anti-helmintic effect of vimang (aqueous extract of *Mangifera indica*) and mangiferin on animal nematode *Trichinella spiralis*. The various bands exhibited in the infrared spectra agree with the main functional groups of compounds such as phenols, esters, aldehydes, acids and alpha beta unsaturated ketones. All these classes of compounds have been reported to be

toxic to nematodes (Fabiya *et al.*, 2012). Indicine, n-triacontane, alpha-thujene, palmitoleic acid, terpinene, manglupenone,, mangiferolic acid methyl ester, cyclohexanone, hexanoic acid, camphene and 2,5dimethyl-4hydrxy-3(2H)-furanone are the major constituents of the GCMS analysis of the fractions. These hydrocarbons, triglyceride, esters and terpenoids are in line with the reports of Idsteom and Schreier, (1985); Khan *et al.*, (1993); Pino *et al.*, 2005 and Shah *et al.*, (2010). Significant nematode population reduction observed in this study can be attributed to any of the compounds, or the synergistic effect of all the organic compounds present in the fractions. Thus *Mangifera indica* stem bark can be subjected to large scale industrial isolation of the active principles to replace toxic synthetic nematicides. The nematicidal activity of fraction from *M. indica* on plant parasitic nematodes is being reported for the first time.

Pot Experiments

Table 1: Effect of fractions from *M. indica* and carbofuran on plant height of *Lycopersicon esculentum* in the screen house

Treatments	4th Wap	5th Wap	6th Wap	7th Wap	8th Wap	9th Wap	10th Wap	11th Wap	12th Wap	13th Wap	14th Wap
MANG/EtOH	18.64 ^a	25.9 ^a	32.80 ^a	45.21 ^a	51.09 ^a	61.79 ^a	72.18 ^a	81.3 ^a	93.00 ^a	102.4 ^a	112.8 ^a
MANG/DCM	13.16 ^b	20.3 ^b	27.19 ^b	33.25 ^b	39.43 ^b	46.29 ^b	53.08 ^b	66.1 ^b	72.11 ^b	81.21 ^b	92.13 ^b
MANG/H ₂ O	5.32 ^c	9.15 ^c	14.27 ^c	19.12 ^c	24.00 ^c	31.33 ^c	38.25 ^c	47.1 ^c	54.29 ^c	62.33 ^c	74.39 ^c
CBFN	19.33 ^a	26.7 ^a	33.21 ^a	44.78 ^a	50.67 ^a	61.83 ^a	72.43 ^a	80.5 ^a	93.17 ^a	102.5 ^a	113.1 ^a
S.E.M	0.11	0.16	0.19	0.21	0.19	1.07	1.12	0.23	1.08	1.14	1.03
Level (mg/mL)											
0	4.07 ^d	6.28 ^d	9.03 ^d	11.22 ^d	16.33 ^d	20.07 ^d	25.00 ^d	31.3 ^d	37.18 ^d	43.68 ^d	49.00 ^d
0.2	8.21 ^c	10.1 ^c	14.10 ^c	18.00 ^c	23.05 ^c	28.11 ^c	34.14 ^c	42.1 ^c	49.14 ^c	54.06 ^c	60.29 ^c
0.5	11.09 ^b	15.3 ^b	19.17 ^b	24.12 ^b	31.15 ^b	37.25 ^b	43.21 ^b	51.9 ^b	58.12 ^b	66.18 ^b	71.59 ^b
0.8	16.25 ^a	21.8 ^a	26.00 ^a	31.21 ^a	39.61 ^a	47.12 ^a	56.00 ^a	65.2 ^a	71.00 ^a	78.31 ^a	86.72 ^a
S.E.M	0.03	0.07	0.11	0.07	0.09	0.10	0.20	0.16	0.13	1.09	1.06

Means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using the 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 2: Effect of fractions from *M. indica* and carbofuran on number of leaves of *Lycopersicon esculentum* in the screen house

Treatments	4th Wap	5th Wap	6th Wap	7th Wap	8th Wap	9th Wap	10th Wap	11th Wap	12th Wap	13th Wap	14th Wap
MANG/EtOH	48.69 ^a	57.71 ^a	69.83 ^a	78.91 ^a	91.65 ^a	115.8 ^a	139.4 ^a	153.0 ^a	185.2 ^a	207.08 ^a	216.3 ^a
MANG/DCM	39.04 ^b	45.13 ^b	56.06 ^b	63.22 ^b	74.18 ^b	87.01 ^b	103.2 ^b	121.0 ^b	136.7 ^b	143.31 ^b	156.1 ^b
MANG/H ₂ O	13.11 ^c	20.41 ^c	28.16 ^c	34.18 ^c	41.00 ^c	48.12 ^c	57.33 ^c	66.29 ^c	76.22 ^c	87.01 ^c	98.25 ^c
CBFN	49.08 ^a	58.26 ^a	70.00 ^a	79.06 ^a	92.11 ^a	116.2 ^a	139.3 ^a	152.7 ^a	184.9 ^a	206.70 ^a	216.4 ^a
S.E.M	0.13	0.11	0.16	0.12	0.18	1.10	1.15	0.28	0.31	0.36	1.18
Level (mg/mL)											
0	5.32 ^d	8.18 ^d	12.44 ^d	18.22 ^d	23.00 ^d	29.40 ^d	36.12 ^d	43.11 ^d	49.25 ^d	55.13 ^d	61.07 ^d
0.2	11.06 ^c	17.14 ^c	26.03 ^c	30.16 ^c	36.08 ^c	42.61 ^c	51.22 ^c	57.20 ^c	64.11 ^c	70.46 ^c	79.00 ^c
0.5	19.24 ^b	25.33 ^b	32.19 ^b	39.45 ^b	44.18 ^b	51.29 ^b	59.68 ^b	65.72 ^b	73.09 ^b	82.51 ^b	90.62 ^b
0.8	26.13 ^a	38.27 ^a	46.63 ^a	57.21 ^a	65.27 ^a	73.11 ^a	84.12 ^a	93.16 ^a	112.4 ^a	123.11 ^a	134.6 ^a
S.E.M	0.05	0.09	0.12	0.16	0.11	0.13	0.21	0.15	0.17	0.20	0.26

Means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using the 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 fractions from *M. indica* and carbofuran on yield attributes of *Lycopersicon esculentum* in the screen house

Treatments	Days to 50% flowering	No of fruits per plants	Fruit weight per plant (kg)
MANG/EtOH	21.27 ^a	33.81 ^a	1.71 ^a
MANG/DCM	35.11 ^b	22.64 ^b	0.98 ^b
MANG/H ₂ O	53.39 ^c	14.06 ^c	0.21 ^c
CBFN	21.08 ^a	34.16 ^a	1.64 ^a
S.E.M	1.05	0.11	0.01
Level (mg/mL)			
0	66.29 ^d	5.11 ^d	0.61 ^d
0.2	29.48 ^c	11.43 ^c	0.85 ^c
0.5	24.10 ^b	16.24 ^b	1.00 ^b
0.8	18.61 ^a	20.31 ^a	1.13 ^a
S.E.M	0.16	0.09	0.03

Means in a segment of a given column followed by the same letter are not significantly different at p<0.05 using the 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 4: Effect of fractions from *M. indica* and carbofuran on nematode population of *Lycopersicon esculentum* in the screenhouse

Treatments	Nematode population in 200g soil sample	Nematode population in 10g root
MANG/EtOH	0.40 ^a	1.06 ^a
MANG/DCM	0.43 ^a	1.08 ^a
MANG/H ₂ O	13.14 ^b	3.19 ^b
CBFN	0.41 ^a	1.04 ^a
S.E.M	0.01	0.00
Level (mg/mL)		
0	348.15 ^c	61.38 ^c
0.2	19.10 ^b	14.31 ^b
0.5	0.11 ^a	0.06 ^a
0.8	0.10 ^a	0.08 ^a
S.E.M	1.13	0.19

Means in a segment of a given column followed by the same letter are not significantly different at p<0.05 using the 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.

Field Experiments

Table 5: Effect of fractions from *M. indica* and carbofuran on plant height of *Lycopersicon esculentum* on the field

Treatments	5th wap	6th wap	7th wap	8th wap	9th wap	10th wap	11th wap	12th wap	13th wap	14th wap
MANG/EtOH	10.74 ^a	15.85 ^a	21.10 ^a	26.73 ^a	34.92 ^a	40.25 ^a	49.25 ^a	54.83 ^a	63.33 ^a	72.33 ^a
MANG/DCM	8.42 ^b	14.14 ^b	18.18 ^b	23.11 ^b	28.33 ^b	32.33 ^b	44.83 ^b	50.25 ^b	58.78 ^b	65.63 ^b
MANG/H ₂ O	7.14 ^c	12.33 ^c	15.16 ^c	19.80 ^c	25.33 ^c	28.67 ^c	39.38 ^c	46.87 ^c	53.58 ^c	62.50 ^c
CBFN	10.37 ^a	16.05 ^a	21.24 ^a	26.78 ^a	33.92 ^a	39.67 ^a	49.00 ^a	54.58 ^a	64.25 ^a	72.58 ^a
S.E.M	0.08	0.06	0.06	0.07	0.20	0.34	0.15	0.18	0.22	0.15
Level (mg/mL)										
0	6.92 ^c	8.93 ^d	12.18 ^d	16.98 ^d	22.58 ^d	26.33 ^d	35.67 ^d	40.75 ^d	46.86 ^d	53.75 ^d
12	9.04 ^b	12.03 ^c	16.73 ^c	21.81 ^c	27.83 ^c	32.58 ^c	42.67 ^c	48.08 ^c	57.42 ^c	66.42 ^c
18	9.60 ^b	15.98 ^b	20.63 ^b	26.03 ^b	33.25 ^b	38.33 ^b	48.55 ^b	54.08 ^b	65.67 ^b	73.22 ^b
24	11.11 ^a	21.43 ^a	26.84 ^a	32.20 ^a	38.83 ^a	43.67 ^a	55.58 ^a	63.62 ^a	70.00 ^a	79.67 ^a
S.E.M	0.06	0.04	0.03	0.06	0.15	0.26	0.17	0.14	0.11	0.12

Means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using the 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 6: Effect of fractions from *M. indica* and carbofuran on number of leaves of *Lycopersicon esculentum* on the field

Treatments	5th Wap	6th Wap	7th Wap	8th Wap	9th Wap	10th Wap	11th Wap	12th Wap	13th Wap	14th Wap
MANG/EtOH	12.33 ^a	17.33 ^a	41.00 ^a	62.10 ^a	75.17 ^a	98.75 ^a	116.08 ^a	146.75 ^a	171.67 ^a	193.83 ^a
MANG/DCM	7.75 ^b	11.50 ^b	24.75 ^b	41.08 ^b	50.67 ^b	69.08 ^b	84.25 ^b	102.67 ^b	124.33 ^b	140.17 ^b
MANG/H ₂ O	5.12 ^c	8.83 ^c	17.08 ^c	31.33 ^c	41.00 ^c	61.33 ^c	71.33 ^c	85.08 ^c	104.83 ^c	114.17 ^c
CBFN	12.03 ^a	17.42 ^a	41.42 ^a	61.67 ^a	74.50 ^a	98.25 ^a	115.50 ^a	147.00 ^a	171.42 ^a	194.08 ^a
S.E.M	0.18	0.15	0.21	0.27	0.30	0.25	0.28	0.27	0.32	0.41
Level (mg/mL)										
0	5.05 ^d	8.17 ^d	11.00 ^d	20.33 ^d	30.33 ^d	43.25 ^d	53.83 ^d	70.17 ^d	91.75 ^d	99.25 ^d
12	9.17 ^c	11.75 ^c	30.42 ^c	50.67 ^c	62.00 ^c	80.17 ^c	95.25 ^c	125.83 ^c	147.67 ^c	162.83 ^c
18	10.12 ^b	15.17 ^b	38.00 ^b	58.92 ^b	70.92 ^b	95.92 ^b	110.75 ^b	137.08 ^b	156.42 ^b	176.50 ^b
24	12.67 ^a	20.00 ^a	44.83 ^a	66.67 ^a	78.08 ^a	108.08 ^a	127.33 ^a	148.42 ^a	176.42 ^a	203.67 ^a
S.E.M	0.11	0.17	0.19	0.21	0.25	0.22	0.21	0.19	0.20	0.36

Means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using the 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 7: Effect of fractions from *M. indica* and carbofuran on yield attributes of *Lycopersicon esculentum* on the field

Treatments	Days to 50% flowering	No of Fruits per plant	Fruit weight per plant (kg)
MANG/EtOH	36.00 ^a	49.10 ^a	2.25 ^a
MANG/DCM	42.75 ^b	38.15 ^b	1.83 ^b
MANG/H ₂ O	48.75 ^c	25.01 ^c	0.98 ^c
CBFN	36.17 ^a	51.00 ^a	2.27 ^a
S.E.M	0.18	0.21	0.09
Level (mg/mL)			
0	56.58 ^d	10.15 ^d	0.59 ^d
12	44.92 ^c	17.21 ^c	1.62 ^c
18	37.08 ^b	30.07 ^b	2.00 ^b
24	30.08 ^a	41.33 ^a	3.83 ^a
S.E.M	0.16	0.16	0.07

Means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using the 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 8: Effect of fractions from *M. indica* and carbofuran on nematode population of *Lycopersicon esculentum* on the field

Treatments	Nematode population in 200g soil sample	Nematode population in 10g root
MANG/EtOH	10.10 ^a	8.03 ^a
MANG/DCM	16.75 ^b	12.83 ^b
MANG/H ₂ O	48.75 ^c	20.98 ^c
CBFN	9.71 ^a	7.82 ^a
S.E.M	0.32	0.08
Level (mg/mL)		
0	213.16 ^d	59.21 ^d
12	22.92 ^c	8.62 ^c
18	5.31 ^b	2.14 ^b
24	1.03 ^a	0.73 ^a
S.E.M	0.12	0.05

Means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using the 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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