EVALUATION OF *TERMINALIA GLAUDESCENS* CHROMATOGRAPHIC FRACTIONS ON *MELOIDOGYNE INCOGNITA* INFECTING CHILI PEPPER (*CAPSICUM CHINENSE*)

Short communication

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ABSTRACT

Nematodes are widespread organisms and pose a serious problem in vegetable production. The use of synthetic nematicides is a common method of bringing down the nematode population to increase crop yield. This has, however, brought about serious pollution to the environment. Leaves and twigs of *Terminalia glaucescens* were extracted in organic solvents and then chromatographed on a silica gel column. Chromatographic fractions obtained from the methanol and acetone crude extracts were characterized with UV-visible, FTIR, and GCMS and tested for their potential nematode suppressing activity in field micro plots. Spectroscopic analysis of the fractions revealed the presence of compounds such as aldehydes, acids, ketones, phenols, terpenes, hydrocarbons, and fatty acid esters. The extract fractions of *T. glaucescens* were applied at 100, 75, and 50 mg·ml⁻¹ at 100 ml each and carbofuran at 0.5, 1.0, and 1.5 kg a.i.·ha⁻¹. The vegetative growth of pepper plants as well as the number and weight of fruits increased significantly in plants treated with the above-mentioned extract fractions. The protective role of fractions compared favorably with the synthetic nematicide (carbofuran). The most effective in reduction of the nematode population, and plant growth and yield was the highest concentration of 100 mg·ml⁻¹. Thus, organic solvent extract fractions from *T. glaucescens* can serve as a biopesticide and an alternative to the environmentally hazardous synthetic nematicide.

Key words: carbofuran, *Meloidogyne incognita* control, *Terminalia glaucescens*, methanol and acetone fractions

INTRODUCTION

Capsicum chinense Jacq., also known as chili pepper, belongs to the family Solanaceae. It is an erect herbaceous plant growing up to about 60 cm tall (Grubben & El Tahir 2004). Peppers are a good source of nutrients such as vitamins A, B6, C, K, calcium, iron, zinc, and fiber. The nutrient and other phytochemical composition of peppers vary with genotype and maturity stage (Deepa et al. 2007). Nigeria is one of the major producers of pepper in the world, producing about 50% of all other African countries (Erinle 1989). Estimated yield of pepper is about 9 t·ha⁻¹ compared to 15 t·ha⁻¹ obtained in Europe.

This low yield is attributed mainly to diseases and pests including plant-parasitic nematodes (Jaliya & Sani 2000; Cerkauskas 2004). Successful pepper production is hindered by *Meloidogyne incognita* infestation (El-Sherif et al. 2007; Yap 2013; Bom-malinga et al. 2013; Agaba & Fawole 2015), which affects pepper roots (Khan et al. 2006; Haougui et al. 2013). To reduce nematode population and increase yield, synthetic nematicides are used by farmers. Though effective, but have resulted in adverse effect on non-target organisms, costly environmental pollutions and pest resistance (Fabiyyi et al. 2020a). The increasing concern over the harmful effects of synthetic nematicide necessitates the need

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for alternative methods of control (Ornat et al. 2001; Fabiyi & Olatunji 2018; Fabiyi et al. 2020b). This study examined the possible nematocidal activity of *Terminalia glaucescens* (Planch. Ex Benth), a common tree in the savannah regions that belongs to the Combretaceae family, native to West Africa (Arbonnier 2002). It is widely distributed in the tropical regions of the world. In southwestern Nigeria, ethnomedicine practitioners use *T. glaucescens* as an anti-malaria medicine, to treat diarrhea, and its twigs are recommended as chewing sticks against tooth decay (Rotimi et al. 1988; Ndukwe et al. 2005). The antimicrobial activity of *T. glaucescens* has been widely reported and it is found to show impressive activity against a broad spectrum of organisms (Taiwo et al. 1999). The potential of the aqueous extract of the stem bark has been extensively investigated (Ojo et al. 2006) and such extract is used as a “wonder-cure” concoction (Epa-ijebu in South West Nigeria) and for the treatment of tuberculosis (Adeleye et al. 2008). These bioactivities documented on *T. glaucescens* prompted the investigation into the possible nematocidal activity.

MATERIALS AND METHODS

Preparation of plant extracts

The leaves and twigs of *Terminalia glaucescens* were collected from Tanke area of Ilorin, Nigeria, and deposited at the herbarium of Plant Biology Department, University of Ilorin, Nigeria, as sample number HI1812. They were chopped into tiny pieces and air dried in the laboratory for two weeks. The weight of leaves and twigs after drying was 15.68 kg and this was divided into three equal parts of 5.23 kg each and extracted cold in 25 l each of methanol (MeOH), acetone (Me₂CO) and water (H₂O). Each extraction lasted five days. The organic solvents were decanted, filtered, and concentrated under reduced pressure (Fabiyi et al. 2020c), while the aqueous extract was allowed to evaporate to dryness at 37 °C. The crude extracts of *T. glaucescens* were coded TEMG/MeOH, TEMG/Me₂CO, and TEMG/H₂O from methanol, acetone and aqueous, respectively.

Column chromatography

The concentrated methanol and acetone crude extracts (250 g) were eluted over silica gel column (70–220 mesh) with n-hexane as the eluent. Nineteen fractions of 2000 ml were collected. Polarity of eluting solvents was increased to hexane/dichloromethane 10 : 3 (500 ml) and later dichloromethane (800 ml) alone. The individual fractions were tested for similarities in R_f on precoated TLC plates (G_{F254}, 0.25 mm Merck Germany). They were developed with appropriate solvent system and viewed under UV lamp at 366 and 254 nm. Fractions with identical R_f were combined accordingly, giving rise to twelve fractions in all. Further purification of the fractions over aluminum oxide (alumina) afforded two clean fractions each from methanol and acetone crude extracts.

Phytochemical screening of fractions

The fractions were screened for the presence of important plant metabolites such as carbohydrates, terpenoids, steroids, alkaloids, polyphenols (tannins), flavonoids, anthraquinones, saponins and reducing sugars following standard methods (Furniss et al. 1989; Trease & Evans 1989; Harborne 1976; Reid & Sarker 2006). All reagents used were of analytical grade and were not further purified. Vanillin spray colour reactions with heat (60 °C) on precoated TLC plates (G_{F254}, 0.25 mm Merck Germany) were also used to identify and characterize the phytochemicals.

Spectroscopic instruments

Ultra violet-visible (UV-Vis) of fractions were taken on Du 730 Life Science UV/Vis spectrophotometer Beckman Coulter. Infrared (IR) spectra were recorded on SHIMADZU 8400s FTIR (Fourier Transform) spectrophotometer and gas chromatography-mass spectrometry was carried out with Agilent 7890A GC/MS equipped with a quadrupole mass spectra detector and an auto sampler. The GC-MS system settings are as follows: 2000 °C, interfaced temperature, 2500 °C, solvent cut time; 2.50 min; relative detector mode, ACQ mode; Scan; start time–end time, 3.00–56.00 min; event time, 0.50 s; scan speed 1428. Then, the GC system was equilibrated to a stable temperature of 2500 °C, the isolates were diluted with acetone and put into micro sample vials.

***M. incognita* isolation**

Pure culture of *M. incognita* race 2 raised on *Celosia argentea* were used as a source of inoculum in the micro plots. The roots were washed under running tap water to remove soil debris, then chopped with a sharp knife into 1–2 cm pieces and packed in a container with 600 ml of 0.6% NaOCl (Hussey & Baker 1973). The contents were agitated constantly for 4 minutes. The solution was sieved to separate the eggs from root particles by passing through 73, 56, and finally, 25 μm aperture sieves. Eggs were rinsed thoroughly from the 25 μm sieve into a clean beaker (Makete et al. 2012). This was left in the laboratory for the eggs to hatch into juveniles. The modified Baermann (1917) technique was used to separate the eggs from hatched juveniles (Fabiyyi et al. 2020a). The juveniles were transferred into Doncaster's (1962) counting dish for enumeration under the microscope ($\times 40$), and 1 ml was found to contain approximately 315 of *M. incognita* juveniles.

Micro plot experiment

A portion of land of 35 m by 25 m was mapped out, ploughed, and harrowed. This was divided into 48 micro plots of 1 m by 5 m (5.0 m^2) in size with an alley way of 0.5 m between them (Fabiyyi et al. 2012). The selected site had been planted with tomatoes for three consecutive seasons. Soil samples were taken from four points on each of the micro plots using systemic sampling method (Coyne et al. 2007), to identify the native nematode genera in the soil and their population. 192 points were sampled in all to represent the entire plot. These were taken to the laboratory and the nematode population was estimated using Whitehead and Hemming (1965) tray method of nematode extraction. A sub sample of 250 cm^3 soil was taken from 800 cm^3 of each composite sample for extraction. Pepper seedlings (*Cap-sicum chinense*) local cultivar were transplanted from the nursery on to each micro plots with a spacing of 50 cm between the rows and 20 cm in the rows, thus making a total of 10 plants per plot. Two weeks after transplanting, each pepper plant was inoculated with approximately 315 juveniles per ml following the method of Fabiyyi et al. (2019). After completion of the experiments, the population of nematodes in soil and roots of pepper was examined.

Root nematodes were extracted for enumeration using the blending and sieving method. Briefly, the roots of harvested pepper plants were washed, cut, dried, and weighed. The roots were placed in a blender with some water to cover the roots, and blending was done at low speed for 15 seconds. Blended roots were transferred to pie-pan tray set up; this was left for 24 hours and nematodes migrated to the water in the pan. The water was decanted and nematodes were transferred to a vial until counting under the microscope. Each soil sample was sieved to get out lumps and debris, and was measured out at a quantity of 100 ml from each sample. The soil was poured in a sieve lined with two ply tissue paper placed on a tray. Water was added in the gap between tray and sieve. The sieve and tray was carefully removed and water in the tray was poured into a beaker. The samples were left overnight and later decanted into vials. This procedure was carried out twice for each soil sample. Thus, 200 ml soil was extracted for nematodes from each sample and the average nematode count was documented.

Treatment application

Carbofuran 3G traded as worm force (2,3-dihydro-2,2-dimethylbenzofuran-7-yl-N-methylcarbamate) was applied at 0.5, 1.0, and 1.5 kg a.i. ha^{-1} . The chromatographic fractions of *T. glaucescens* in concentrations 100, 75, and 50 $\text{mg}\cdot\text{ml}^{-1}$ at 100 ml each were dissolved in 200 ml of nonionic surfactant to achieve total solubility and provide homogeneous solution of the fractions. They were then applied in a small 3 cm deep trench made at the base of each plant at 300 ml per plant.

Statistical design and analysis

There were four main treatments in the experiment: *T. glaucescens* methanol extract fraction (TEMG/MeOH), *T. glaucescens* acetone extract fraction (TEMG/Me₂CO), *T. glaucescens* aqueous extract (TEMG/H₂O), and carbofuran – a standard synthetic nematicide (CBFN). Each treatment had three concentrations (0, 100, 75, and 50 $\text{mg}\cdot\text{ml}^{-1}$) and for carbofuran 0, 0.5, 1.0, and 1.5 kg a.i. ha^{-1} was applied. All the treatments were replicated three times in a randomized complete block design (RCBD).

The experiment was conducted over a two-year period. Altogether, there were 48 micro plots in both years. The following data were collected: population of *M. incognita* in 200 ml soil and 10 g root sample, number of days to 50% flowering, number of fruits per plant, fruit weight per plant, height of pepper plants, numbers of leaves and branches. A two-way analysis of variance (Anova) was used to evaluate the data obtained using GenStat 5.32. Duncan's multiple range test was employed to establish different means at alpha level <0.05.

RESULTS

Phytochemical characteristics

Phytochemical screening of the chromatographic fractions has revealed that they contained alkaloids, flavonoids, terpenoids, saponins, anthraquinones, phenols, and carbohydrates in the methanol fraction, while anthraquinone was absent in acetone fraction. Colour reaction with vanillin spray and heating also confirmed the presence of terpenes and alkaloids in all the *T. glaucescens* fractions. The UV-visible spectra of the fractions in dichloromethane showed that they contained a wide range of compounds, which absorbed in different ultraviolet regions, while GCMS analysis of the fractions revealed twenty-six compounds belonging to triterpenes, terpene hydrocarbons and fatty acid esters of which (E)-3-penten-2-one, pyridine, isovaleric acid, p-cymene, 2-ethylfuran, 2-propylfuran, 2-methyl-tetrahydrofuran-3-one, α -phellandrene, α -terpinene and salicylaldehyde are more than 5%. From the infrared analysis, the fractions exhibited, hydroxyl, aliphatic hydrocarbons, aldehydes, phenol, carboxylic acid, ester, and alkyl functional groups.

Evaluation of nematode population

The initial native nematode population in the soil samples before inoculation was as follow: *Meloidogyne* spp. 534, *Pratylenchus* spp 105, *Scutellonema* spp. 22 and *Helicotylenchus* 9. *M. incognita* population in 200 ml soil samples at harvest was at the

similar level in treatments of CBFN, TEMG Me₂CO, and TEMG MeOH (161–170) and significantly higher when water extract of TEMG was applied (234). In the nontreated soil, the number of *M. incognita* was 656. The effect of the treatments depended on the concentration of *T. glaucescens* extracts lowering from 656 in nontreated to 36 at 50 mg·l⁻¹ to 17 at 100 mg·l⁻¹. The populations of *M. incognita* scored in roots was three times higher in the nontreated roots than in the treated ones, but it did not differ between CBFN and different TEMG extracts. The lowest number of nematodes was found at the highest concentration of extracts (Table 1).

Influence of treatments on growth and productivity of pepper

Vegetative growth and yielding attributes depended on the applied protective treatments against *M. incognita*. An earlier flowering was recorded in pepper plants protected with CBFN and Terminalia methanol and acetone extracts, especially with the highest concentration. The 100 mg·ml⁻¹ treated plants needed an average of 44 days to 50% of flowering, while an average of 77 days was recorded in the untreated pepper plants and 52 days for plants protected with CBFN. The flowering was delayed (63 days) in plants treated with aqueous extracts. The numbers and weights of harvested pepper fruits had similar values distribution – more and heavier fruits in protected plants with methanol and acetone extracts and CBFN, especially at the highest concentration, except water extract that gave similar results as was recorded for unprotected plants. The most detrimental effect caused by a lack of protection was low number and low fruit weight resulting from a weak vegetative growth. Likewise, the growth of the plants treated with the methanol and acetone extracts and carbofuran fractions improved gradually and consistently; the plants were significantly taller, had more leaves and side shoots compared to the plants treated with the aqueous extract (Table 1), which were characterized by growth inhibition and fewer leaves and branches.

Table 1. Effect of *T. glaucescens* extracts on *M. incognita* population and on pepper plants parameters

Treatments	<i>Meloidogyne incognita</i> population		Days to 50% flowering	Plant height (cm)	Number of leaves	Number of branches	Number of fruits per plant	Mean fruit weight (kg)
	soil	roots						
CBFN	170±22 ^b	70±11 ^{ab}	52±03 ^a	35.8±02 ^a	104±21 ^a	18±00 ^a	7±01 ^a	0.344±01 ^a
TEMG/H ₂ O	234±36 ^a	86±16 ^{bc}	63±05 ^b	25.0±01 ^b	53±03 ^b	10±01 ^b	2±00 ^b	0.010±00 ^d
TEMG/Me ₂ CO	161±18 ^a	59±09 ^a	52±03 ^a	35.4±00 ^a	104±21 ^a	18±00 ^a	7±01 ^a	0.294±03 ^c
TEMG/MeOH	170±22 ^b	64±05 ^a	52±03 ^a	35.8±04 ^a	104±21 ^a	18±00 ^a	7±01 ^a	0.325±04 ^b
LSD (p < 0.05)	33.38	ns	0.81	0.63	0.99	0.18	1.34	0.03
Concentration of extracts (mg·l⁻¹)								
0	656±29 ^d	245±33 ^c	77±06 ^c	19.8±00 ^d	42±05 ^c	8±00 ^c	2±00 ^c	0.001±00 ^d
50	36±00 ^c	17±01 ^b	49±01 ^b	31.1±01 ^c	103±11 ^b	16±03 ^b	5±00 ^b	0.169±01 ^c
75	26±05 ^b	12±00 ^b	49±01 ^b	36.3±00 ^b	104±09 ^b	16±03 ^b	5±00 ^b	0.178±01 ^b
100	17±01 ^a	5±00 ^a	44±00 ^a	41.2±03 ^a	116±17 ^a	24±01 ^a	12±01 ^a	0.619±03 ^a
LSD (p < 0.05)	52.1	48.3	0.81	0.66	1.80	0.22	1.88	0.03

Values with different letters show significant differences at p < 0.05

DISCUSSION

The activity of *T. glaucescens* extracts can be attributed to secondary metabolites such as alkaloids, terpenoids, flavonoids, tannins, saponins, phenols, and anthraquinone present in the chromatographic fractions of *T. glaucescens*. Adeleye et al. (2008) and Mann (2012) reported saponins, steroids, tannins, terpenes, and anthraquinones as some of the secondary metabolites present in *T. avicennioides*. The presence of flavonoids, terpenoids and tannins in *Terminalia* was also reported by Dongmo et al. (2006). Tannins were reported to have antiviral and antimicrobial activities. Steroids possess insecticidal and antimicrobial properties, while terpenes are found to be nematocidal (Callow & Young 1936, Dewick 2002; Fabiyi et al. 2020c). The methanol extract of *T. arjuna* bark exhibited nematocidal activity on *Haemonchus contortus* (Bachaya et al. 2009), while the antihelminthic activity of *T. glaucescens* on gastrointestinal nematodes of sheep was documented by Mbafor et al. (2014).

Vegetative growth of *C. chinense* depended on the applied nematicides. The methanol and acetone fractions were not significantly different from carbofuran in their activity against *M. incognita*, while the aqueous extract exhibited significantly (p < 0.05) weaker nematocidal activity in most parameters measured.

Similarly, Zia-ul-Haq et al. (2010) reported the toxicity of the ethanol extracts of *Terminalia chebula* fruit to *M. incognita* and *Cephalobus littoralis*. Furthermore, Nguyen et al. (2013) isolated 3,4-dihydroxybenzoic acid from the methanol extract of *T. nigrovenulosa* bark. They observed 94.2% of *M. incognita* J2 mortality and 85.0% egg hatch inhibition in a 1.0 mg·ml⁻¹ concentration of the above compound. Ethyl acetate extract of *T. glaucescens* roots showed the antifungal activity against *Aspergillus flavus* and *A. fumigatus* (Turaki et al. 2020). Terminalin has been reported to show a wide spectrum of antibacterial activity against periodontopathic bacteria, and also a toxicity to ruminants (Oelrichs et al. 1994; Mann et al. 2011).

The antiplasmodial, cytotoxicity, antifungal and antiviral activity of the methanol and ethanol extracts of *T. avicennioides* was also found by Mann et al. (2011) and Mann (2012). The presence of triterpenes in the extracts corroborates the findings of DerMarderosian and Beutler (2002), who stated that *Terminalia* species are rich in triterpenes that are associated with antiviral and antifungal activities. The chromatographic fractions from the acetone and methanol extracts of *Terminalia glaucescens* was comparable with carbofuran activity in limitation of *M. incognita* which was reflected in the growth and fruiting of the pepper.

CONCLUSION

Bioactive compounds from *Terminalia glaucescens* leaves and twigs could be used in *M. incognita* control on pepper plantations. Further experiments should be targeted at precise formulation and technology of use of *Terminalia* products as a highly efficient bionematicide, while granted that probable fraction residues in soil and pepper products are also investigated.

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