Ceylon Journal of Science 47(3) 2018: 235-239
DOI: http://doi.org/10.4038/cjs.v47i3.7539

RESEARCH ARTICLE

Effect of thermal degraded products of *Cymbopogon citratus* on the *In vitro* survival of *Meloidogyne incognita* eggs and juveniles

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Received:27/12/2017; Accepted:01/07/2018

Abstract: The products of thermally-degraded *Cymbopogon citratus* were evaluated for nematicidal activity as an alternative of toxic synthetics. The products exhibited moderate nematicidal activity, but not as significantly ($p<0.05$) effective as carbofuran, a synthetic nematicide. Thermally degraded products of fresh *Cymbopogon citratus* (CMGC/th/fresh) was the most promising at 90 mg/mL and could be used in place of the toxic synthetic nematicide.

Keywords: *Meloidogyne incognita,* environmental pollution, carbofuran, spectroscopy, *Cymbopogon citratus,* nematodes.

INTRODUCTION

Sedentary endoparasitic nematodes, such as *Meloidogyne* species (root-knot nematodes RKN), induce morphological and physiological changes within the plant root system. They induce galls in infected plants, causing stunted growth, wilting and reduction in yield (Fortnum *et al.,* 1991). Consequently they have become a major constraint in the production of many economically important crops (Sasser, 1980, 1990) and therefore are of great economic importance. In Nigeria, *Melodogyne incognita* is considered as a parasite of many crops. Babatola (1984) reported that *M. incognita* could reduce rice yield substantially and Jute Mallow by 30 % in western Nigeria as reported by Fabiyi *et al*. (2012).

Control of root knot-nematode (RKN) has been achieved by the use of chemical nematicides, this call for reappraisal owing to the prevalence of side effects leading to restriction of use or outright ban of some nematicides. The crop productivity can be enhanced with the use of bio-nematicides in place of the environmentally unsafe synthetic nematicides employed in plant parasitic nematode control (Martin, 2003). Several plant species with essential oils have been found to exhibit nematicidal properties (Ibrahim *et al*., 2009; Abo-Elyousr *et al*., 2010). *Cymbopogon citratus* (DC) stapf, which belongs to the family Poaceae is a tufted perennial grass with citrus flavour, commonly called lemon grass (Weiss, 1997, Kumar *et al.,* 2000) and was evaluated for its potential nematicidal action. *Cymbopogon citratus* is commonly used in treating sore throat, catarrh, rheumatism and cholera (Rao and Jamir, 1982; Carbajal *et al.,* 1989; Filipoy, 1994). The essential oils contain in *C. citratus* has bactericidal, fungicidal, anti-amoebic and anti-malarial properties (Onawunmia *et al*., 1984; Kimbi and Fagbenro-Beyioku, 1996; Wannissorn *et al*., 1996; Sved *et al*., 1990; Tchoumbougnang *et al*., 2005; Wannissorn *et al*., 2005). This study investigated and compared the nematicidal effect of thermally degraded products and ethanol extracts of *C. citratus* leaves on *Meloidogyne incognita* eggs and juveniles under *in vitro* conditions.

MATERIALS AND METHODS

Cymbopogon citratus leaves were collected from Tanke Oke odo area located in Ilorin Kwara state in Nigeria and identified by a taxonomist at the Department of Plant Biology, University of Ilorin. The leaves were cut into tiny pieces of about 2cm and air dried under the shade. The final weight of air dried leaf material was 1,816 g from which 700 g was taken for extraction in distilled ethanol. After five days the crude ethanol extract was concentrated in a rotary evaporator under reduced pressure, then stored in a small laboratory sample bottle. A second set of the leaves of *Cymbopogon citratus* was collected from the same habitat and were used as fresh material without air drying.

Thermal Degradation of Plant materials

Degradation of fresh and air-dried C. citratus

Two hundred grams (200 g) of fresh *C. citratus* leaves were weighed and packed in a boiling tube with a quick fit mouth to which a condenser was connected and lowered into a sand bath. The tube was wrapped with aluminium foil. The temperature of the sand bath was monitored and kept constant at 250 ^{0}C. The distillate obtained following the thermal degradation was collected and kept separate. The tube used for the thermal degradation was cooled, and the plant residue inside was extracted with 200 ml of distilled ethanol. The extract was concentrated using a rotary evaporator, weighed and stored. The same procedure was followed for thermal degradation of air-dried leaves of *C. citratus*.

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Silica gel mediated thermal degradation of dried C. citratus

Fifty grams of silica gel was mixed with powdered 200 g of air-dried *C. citratus* in a porcelain mortar. The mixture was packed in a boiling tube and inserted into the sand bath. The distillate obtained following the thermal degradation was collected. It was later concentrated and stored.

Thin Layer Chromatography and Spectroscopic analysis

The distillates collected from the thermal degradation and the concentrated crude ethanol extract from plant residues were spotted on thin layer chromatographic plates using n-hexane and dichloromethane (1:6) as the solvent system. The prominent spots observed on the TLC plates guided the isolation of the products on PTLC plates (Si gel). After loading, each plate was developed in a chromatographic tank (n-hexane/ DCM 1:6) and the isolated chemical constituents coded CMGC/EtOH, CMGC/th/fresh, CMGC/th/dry, and CMGC/th/dry/si, were subjected to infrared spectroscopic analysis on SHIMADZU 8400s FTIR (Fourier Transform) spectrophotometer using KBr pellets.

Treatment Key

CMGC/EtOH----------*Cymbopogon citratus* ethanol extract

CMGC/th/fresh--------*Cymbopogon citratus* thermal degraded fresh leaves

CMGC/th/dry----------*Cymbopogon citratus* thermal degraded dry leaves

CMGC/th/dry/si--------*Cymbopogon citratus* thermal degraded dry leaves plus silica gel

CBFN----------Carbofuran

***In-vitro* Nematicidal Assay**

The eggs of the root-knot nematode *Meloidogyne incongita* (Tylenchida: Heteroderidae) were isolated from a pure culture of *M. incognita* raised on infested roots of tomato plants (*Lycopersicon esculentum* Mill). Sodium hypochlorite (NaOCl) solution at 0.6 % was used for the isolation of nematode eggs from root galls following the Hussey and Baker (1973) method of extraction. The extracted eggs were washed over 200 mm sieve nested over 400 mm mesh sieve to obtain free eggs for the experiment. Some extracted eggs were incubated at 27 ^{0}C for 48 hours to hatch the fresh second stage juveniles. A total of 215 larvae/ml and 104 eggs/ml was used in the experiment. Two millilitres of larvae and egg suspensions were distributed separately into glass vials. The crude extracts and products of degradation were diluted into three different concentrations of 50 mg/ml, 70 mg/ml and 90 mg/ml, using a non-ionic surfactant as an emulsifier to increase aqueous solubility. Each concentration was transferred separately into counting dish and kept at room temperature. The experiment was laid in a completely randomised design (CRD). There were three replicates each for the five treatments at four levels. Eggs and juveniles in ordinary distilled water served as the control. A total of sixty (60) counting dishes were used. Juvenile mortality and egg hatch was recorded at 4, 6, 8, 10 and 24 hours of treatment using a stereomicroscope at x100 magnification. Juveniles that did not react to probing were considered as dead. Juvenile mortality and rate of egg hatch were determined using the following formula (Sun *et al*., 2006)

Juvenile mortality = 100 × dead juveniles/total number of juveniles

Percentage egg hatch = 100 × juveniles/(eggs + juveniles)

The experiment was repeated twice. Data collected was subjected to two way analysis of variance and separation of means was done using Duncan's multiple range tests.

RESULTS

The effect of thermal degradation products of *C. citratus* on the % of Juvenile Mortality of *Meloidogyne incognita* over a period of 4 days is shown in Table 1 and the effect of thermal degradation products of *C. citratus* on % egg hatch of *Meloidogyne incognita* over a period of 8 days is shown in Table 2.

Table 1: Effect of Thermal Degradation Products of *C. citratus* on the % of Juvenile Mortality of *Meloidogyne incognita* over a period of 4 days.

	Hours				Days			
Treatments	**2**	**4**	**6**	**8**	**1**	**2**	**3**	**4**
CMGC/EtOH	0.00^{b}	1.11^{d}	2.72^{d}	7.15^{d}	15.00^{d}	19.07^{d}	22.65^{d}	27.40^{d}
CMGC/th/fresh	0.00^{b}	6.00^{b}	16.18^{b}	22.07^{b}	36.25^{b}	41.18^{b}	48.12^{b}	56.02^{b}
CMGC/th/dry	0.00^{b}	3.22^{c}	6.58^{c}	12.05^{c}	20.19^{c}	25.12^{c}	30.00^{c}	34.09^{c}
CMGC/th/dry/si	0.00^{b}	3.41^{c}	7.05^{c}	11.66^{c}	19.53^{c}	24.60^{c}	29.36^{c}	33.68^{c}
CBFN	3.25^{a}	11.15^{a}	23.29^{a}	31.02^{a}	45.62^{a}	59.17^{a}	66.27^{a}	73.00^{a}
Treatment level (mg/mL)								
0	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.18^{d}	1.23^{d}	2.07^{d}	3.21^{d}
50	0.14^{c}	1.31^{c}	4.31^{c}	7.00^{c}	11.51^{c}	20.27^{c}	25.28^{c}	34.70^{c}
70	1.03^{b}	3.28^{b}	8.08^{b}	13.06^{b}	18.66^{b}	26.39^{b}	33.41^{b}	42.23^{b}
90	4.16^{a}	7.09^{a}	11.12^{a}	19.34^{a}	25.13^{a}	32.11^{a}	44.00^{a}	51.18^{a}

Means in a segment of a given column followed by the same letter are not significantly different at p<0.05 using the new DMRT. DMRT=Duncan's multiple range test. Each value is a mean of three replicates and an average of data taken twice.

Table 2: Effect of thermal degradation products of *C. citratus* on % Egg Hatch of *Meloidogyne incognita* over a period of 8 days.

	Days							
Treatments	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
CMGC/EtOH	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
CMGC/th/fresh	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
CMGC/th/dry	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
CMGC/th/dry/si	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
CBFN	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
Treatment level (mg/mL)								
0	10.21[b]	13.14[b]	18.32[b]	21.17	24.51[b]	31.26	38.43[c]	45.13[b]
50	0.00[a]	0.00[a]	0.02[a]	0.00[a]	0.07[a]	0.09[a]	0.13[b]	0.18[b]
70	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
90	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]

Means in a segment of a given column followed by the same letter are not significantly different at p<0.05 using the new DMRT. DMRT=Duncan's multiple range test. Each value is a mean of three replicates and an average of data taken twice.

Spectroscopy

Diagnostic bands were seen in the region between 3,500 cm-[1] and 1,500 cm-[1], while characteristic fingerprint bands were observed between 1,500 and 500 cm-[1]. The infrared spectrum of the isolated chemical constituent from the ethanol extract of *C. citratus* displayed a strong absorption band at 2,849 cm-[1] which is attributed to the C-H stretching of an aldehyde. A carbonyl functional group (C=O) of aldehyde was equally seen at 1,725 cm-[1]. The signal at 1,378 cm-[1] indicates the C-H stretch of an alkyl group. The result of the thermal degradation product of fresh *C. citratus* had the C-H stretching vibration of an alkyl group at 2,853 cm-[1]. A sharp carbonyl signal was seen at 1,738 cm-[1], which denotes the presence of an ester. The absorption bands 1,463 cm-[1] and 1,075 cm-[1] represents a C-H bending vibration of alkyl groups and C-O of phenols respectively. The products of thermal degradation of dry *C. citratus* had a characteristic broad band of hydroxyl functional group at 3,415 cm-[1], the peaks at 2,849 and 1,317 cm-[1] are C-H vibrations of alkyl groups, while the band at 1,040 cm-[1] represents C-O vibration of ethers. The product of degradation of dry *C. citratus* incorporated with silica gel had a C-H stretching vibration of aldehyde at 2854cm-[1], the carbonyl stretching of a ketone at 1,727 cm-[1] and C-H of alkyl group at 1,375 cm-[1].

Nematicidal Effect

There was consistent increase in percentage mortality of *M. incognita* juveniles with the increased in exposure time. The thermal degradation product of fresh *C. citratus* (CMGC/th/fresh) was the most effective ($p < 0.05$) among the products of degradation with 36.25 % mortality after day one in compared to the degradation product of dried plant material and dried plant material mixed with silica gel (CMGC/th/dry and CMGC/th/dry/si). This was the trend throughout the duration of the experiment. Carbofuran (CBFN) a synthetic nematicide however had significantly higher percentage mortality than all the degradation products with 73 % mortality after four days (Table 1). The 90 mg/ml rate of application was significantly ($p < 0.05$) better in all the treatments tested. It was also observed that all treatments inhibited egg hatch from day 1 to day 8. Few hatches were observed at 50 mg/ml rate of application which is the lowest rate of application (Table 2).

DISCUSSION

The results of the nematicidal assay depicts that the products of thermal degradation and isolate from the ethanol extract have nematicidal activity although at various degrees. The constituents of *C. citratus* could be partly responsible for the nematicidal effect. The essential oils of *C. citratus* have been established to contain mainly aldehydes and other compounds such as hydrocarbons, terpenes, alcohols, ketones and esters (Abegaz *et al*., 1983; Trease, 1996). Ming *et al*., (1996) stated that citral which is a mixture of stereo isomeric monoterpene aldehydes, geranial (40-60%) and neral (25-38%) is the major constituent of *C. citratus.* Two triterpenoids were isolated by Hanson *et al*., (1976) namely cymbopogon and cymbopogonal while Miean *et al.,* (2001), isolated flavonoids, quercetin apigenin and kaempferol from the aerial parts. Phenolic compounds such as caffeic acid, chlorogenic acid, catecol, elimicin and hydroquinone have also been reported to be present in the plant (Faruq, 1994). The essential oil of *C. citratus* is reported to be ascaricidal and 50% concentration of extract resulted in more than 91% mortality of mites (Azima *et al.,* 2011). Different concentration of lemongrass oil in liquid paraffin has also been reported to have repellent activity against the adults of *Aedes egypti* (Oyedele *et al.,* 2002) and this repellent activity was attributed to citral. Minami *et al.,* (2003) reported the antiviral activity of *C. citratus* oil at 0.1% concentration; viral replication was completely inhibited for 24 hours at 4 ^{0}C direct interaction with the virions. Pedroso *et al*., (2006), also demonstrated the anti-protozoan effect of *C. citratus* on two strains of *Crithida deanei.* Reports by Park *et al.,* (2005) and Barbosa *et al.,* (2010), demonstrated the nematicidal activity of essential oil from *C. citratus* against the pinewood nematode, *Bursaphelenchus xylophilus*. Similarly Macedo *et al.,* (2015) highlighted the nematicidal activity of essential oils of *C. citratus* on *Haemonchus contortus* population, recording a population reduction of 38.5 % at 0.62 mg/ml.

CONCLUSION

The current findings justify that *Cymbopogon citratus* has nematicidal properties. The extracts or thermal degradation

products of *C. citratus* could serve as a substitute to the toxic synthetic nematicide. The findings may help developing environmental friendly nematicide formulations for the farmers at a low cost of production. Further research is recommended to assess the nematicidal activity of thermal degradation products of *C. citratus* under field conditions.

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