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IN VITRO TOXICITY OF EXTRACTS FROM *Hyptis suaveolens* (L.) POIT ON EGGS AND SECOND-STAGE JUVENILES OF *Heterodera sacchari*

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ABSTRACT

The replacement of synthetic nematicides with comparatively more bio-degradable, bio-active plant extracts is desirable. Studies on the nematicidal potential of different extracts of Hyptis suaveolens on the eggs and second stage juveniles of sugarcane nematode, Heterodera sacchari was conducted. Leaves of H. suaveolens were hydro distilled to extract the essential oil. A second set of the leaves were extracted separately in dichloromethane (DCM) and in water. Essential oil (EO) obtained was analyzed using Gas chromatography mass spectroscopy (GC/MS). The essential oil was tested in-vitro along with other extracts on the eggs and second stage juveniles of H. sacchari. The fractions were significantly ($p<0.05$) effective in inducing mortality. Bioactivity was highest at a concentration of 20mg/mL, which was significantly ($p<0.05$) different from all other concentrations. The essential oil (EO) compared well with the standard carbofuran (CBFN) at 65.58% and 66.06% mortality respectively, while the dichloromethane and aqueous extracts were not as effective. Mortality increased with increase in exposure time. There was total egg hatch inhibition with the essential oil and carbofuran, all the levels of concentration also inhibited egg hatch. The constituent of H. suaveolens as revealed by GC/MS include sabinene (29.5%), beta-caryophyllene (11.8%), terpinolene (9.8%) and 1, 8-cineole (7.3%). The extracts of the leaves of H. suaveolens are nematicidal and holds promise as a natural bio-degradable alternative crop protectant against Heterodera sacchari

Key words: *Heterodera sacchari*, dichloromethane, essential oil,*Hyptis suaveolens*

INTRODUCTION

Sugarcane cyst nematode, *Heterodera sacchari,* was originally reported from sugarcane in Congo Brazzaville (Luc and Merny, 1963) and later found parasitizing roots of rice in several African countries including Nigeria (Babatola, 1983). *H. sacchari* was reported to have a wide host range including the Cyperaceae and Graminaeae family indigenous to West African Savanna and humid lowlands (Odihirin, 1975). It causes major damage and severe yield reductions in sugarcane and rice. This has consequently placed it on the quarantine list of several countries of the world. Infected

rice plants are usually chlorotic with necrotic, blackened and twiggy root systems (Babatola, 1983). Rice growth and tillering were significantly reduced at 18, 36 and 72 cysts/l inoculum levels. Grain yields were also reduced by 24.8%, 58.9%, 64.3% and 75.2% at 9, 18, 36 and 72 cysts/l inoculum densities respectively (Babatola, 1983). Cyst nematodes, by their nature, have high survival and dissemination capacity thus, making their control rather difficult (Hassan *etal.,* 2013). Although, nematicides are efficient in nematode management they are expensive and not readily available when needed. It requires some expertise in application and is environmentally hazardous (Fatoki and Fawole, 1999; Adegbite, 2011; Hassan *et al*., 2013), some local famers don't understand instructions on pesticide labels, and this exposes them to pesticide poisoning (Jatto *et al.,* 2012). There is therefore the need to search for safer and inexpensive alternative control strategies. Several botanicals are effective and have favourable eco-toxicological properties (low mammalian toxicity, rapid degradation and reduced environmental pollution), which make them potentially suitable for use in nematode pest management (Rajapakse, 1990; Atungwu, 2009). Thus, the leaves of *Hyptis suaveolens* were investigated for their possible nematicidal activity. *Hyptis suaveolens,* though classified as a weed worldwide is an important aromatic plant which is employed in the treatment of respiratory, gastrointestinal infections, indigestion, colds, fever and skin diseases (Gonzalez Ayala, 1994; Weimann and Heinrich, 1997; Chukwujekwu, 2005). The antioxidant, antimicrobial, anti-diarrhoeal, anti-helmintic, anti-diabetic, anti-inflammatory and insecticidal activity of *H. suaveolens* have been reported (Ajaiyeoba *et al*., 2001; Shoba *et al*., 2001; Grassi *et al*., 2006; Nayak *et al*., 2013). In view of this and the economic importance of rice and sugarcane including their vulnerability to severe damage by the sugarcane cyst nematode, *H. sacchari*, this research was undertaken to investigate the toxicity of essential oil (EO) from *Hyptis suaveolens* to eggs and second-stage juveniles of *Heterodera sacchari.*

MATERIALS AND METHODS

Collection of Plant Materials and Preparation of Extracts

Hyptis suaveolens plants were collected at the flowering stage from the University of Ilorin campus. The plant samples were identified at the herbarium unit of the Department of Plant Biology, University of Ilorin. Essential oil (EO) was obtained by hydro-distillation (3 hours) of the fresh leaves. The volatile oil obtained was separated from the aqueous solution using DCM and was dried over

anhydrous sodium sulphate (Na_2SO_4). The oil was a pale yellow clear liquid which was acidic to litmus paper. Density and viscosity were about 0.90g/mL and 2.56 centipoises respectively. GC/MS was used for the analysis of the chemical constituents. The dichloromethane (DCM) and aqueous extracts were obtained by air drying the leaves at room temperature for three weeks. The leaves were pulverized using a laboratory mill. Five hundred grams (500g) each of the leaves were soaked separately in DCM and water for extraction. The extraction lasted five days. The organic solvent was decanted, filtered and concentrated under reduced pressure, while the aqueous extract was allowed to dry at 37^0C in Petri dishes

The Instrument

A Gas Chromatography-Mass Spectroscopy (GCMS-QP 2010) PLUS (Shimadzu Japan) system coupled with a finigan MAT ion trap detector was used with the column being an RTX5MS column packed with 100% grade dimethylpolysiloxane. The GC-MS was operated under the following conditions; column temperature was initially held at 60°C for 5 min with injection volume of 1 □L and then programmed to rise at the rate of 5°C per minute to 250°C. The injector temperature was set at 200°C while the detector (mass spectrophotometer) temperature was maintained at 250°C. Helium was used as the carrier gas at a linear velocity of 46.3 cm sec-1 and pressure of 100.2 Kpa. Ionization mode was electron impact (EI) at a voltage of 70 eV. Identification of the components was carried out using the peak enrichment technique of reference compounds and as final confirmation of the peak identification by GC-MS; their spectral were compared with those of NIST library mass spectra.

Nematicidal assay

Cysts from a population of *Heterodera sacchari* cultured on rice cultivar NERICA 1 at the International Institute of Tropical Agriculture (IITA), Ibadan were extracted using Fenwick can. They were collected, broken in a Petri dish and washed into a 200mL beaker. The egg suspension in the beaker was thoroughly mixed using a magnetic stirrer. The number of eggs in 1mL of suspension was counted in a counting dish under the stereo microscope (x100). The average of three counts was taken to estimate the egg population per mL of egg suspension. Some of the eggs were incubated at 27^0C to hatch out the second stage juveniles. An average of three counts was also used to estimate the juvenile population per mL of suspension. A total of one hundred and fifty (150) eggs and juveniles/mL were used in each counting dish for the experiment. The experimental

design for each assessment was a 4x4x3 factorial experiment conducted in Complete Randomized Design (CRD), containing four (4) treatments at four (4) levels and each replicated three (3) times. **A total of forty eight (48) Petri dishes were used. Essential oils, DCM, and aqueous extracts were applied at 10mg/mL, 15mg/mL and 20mg/mL.** 1mL of a non-ionic surfactant emulsifier (Tween, 80) was added to achieve total solubility and to provide homogeneous solution of the essential oil and the DCM extract. **Distilled water served as control (0mg/mL), while carbofuran a synthetic nematicide was used as a positive check.**

The Results

The constituents of *Hyptis suaveolens* as revealed by the GCMS result (Table 1) included Sabinene (29.5%), beta-caryophyllene (11.8%), terpinolene (9.8%) and 1, 8-cineole (7.3%). The result of the effects of the different concentrations of essential oil from *Hyptis suaveolens*on percentage mortality of *Heterodera sacchari* juvenilesis shown in Table 2. There were significant differences in the percentage mortality between the various treatments. In the 3rd and 5th hour of exposure, carbofuran (CBFN) was significantly ($p<0.05$) more effective than the essential oil (HPTS/EO), dichloromethane extract (HPTS/DCM) and the aqueous extract (HPTS/H_2O) with a percentage mortality of 21.09 and 33.15% respectively. From the 7th hour to the end of the observation at the 24th hour, there was no significant difference between carbofuran (66.06%) and the essential oil (65.58%). The aqueous extract was however the least effective among the extracts with 23.49% mortality at twenty four hours. The effect of the level of application of extracts and carbofuran was significant throughout the time of exposure. There was increase in percentage mortality with an increase in the level of application of treatments (Table 2). At 20mg/mL there was a gradual increase in mortality which cumulated in 41.10% at 24hours as opposed to 33.29% and 25.51% recorded in 15mg/mL and 10mg/mL respectively.The 0% treatment (control) recorded no(0.00%) mortality throughout the period of observation. From Table 3, the essential oil (EO) was effective in inhibiting egg hatch, it compared well with carbofuran while a few egg hatches were recorded in aqueous and dichloromethane extracts. All concentrations were significantly ($p<0.05$) effective. A cumulative egg-hatch of 11.08% was recorded in the control at the end of the

Table 1: **Chemical Composition (%) of Essential oil from *Hyptis suaveolens***

GC/MS analysis of *H. suaveolens* showed the presence of twenty three (23) constituents of which nineteen (19) were identified

GC Peak No	Compound	Rt (min)	Peak area (%)
1	4-terpineol	3.15	3.2
2	á-pinene	4.23	2.0
3	Sabinene	4.50	29.5
4	beta pinene	5.16	5.4
5	naphtalene	5.34	4.6
6	α-terpinene	5.56	1.5
7	1,8-cineole	6.03	7.3
8	Limonene	6.29	5.7
9	gama-terpinene	6.37	1.3
10	terpinolene	6.53	9.8
11	beta caryophyllene	9.18	11.18
12	Trans-α-bergamotol	9.34	2.1
13	a-terpinole	10.09	1.6
14	bicyclogermacrene	10.17	2.3
15	myrcene	11.03	2.5
16	3-octanal	11.45	1.3
17	a-humulene	13.27	2.9
18	cyclohexane	13.51	3.12
19	b-selinene	15.11	2.7

Table 2: The effects of extracts from *Hyptis suaveolens* on *H. sacchari* juvenilemortality

	Exposure Time				
Treatments	**3hrs**	**5hrs**	**7hrs**	**9hrs**	**24hrs**
HPTS/EO	18.13^{b}	25.19^{b}	39.61^{a}	47.02^{a}	65.58^{a}
HPTS/DCM	7.04^{c}	11.00^{c}	18.24^{b}	25.00^{b}	32.43^{b}
HPTS/H_2O					
CBFN	1.38^{d}	4.22^{d}	10.21^{c}	16.18^{c}	23.49^{c}
	21.09^{a}	33.15^{a}	40.05^{a}	46.79^{a}	66.06^{a}
S.E.M	0.10	0.13	0.18	0.10	0.16
Treatment level (mg/mL)					
0	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}
10	1.14^{c}	9.37^{c}	13.26^{c}	20.15^{c}	25.51^{c}
15	6.09^{b}	14.28^{b}	21.18^{b}	28.36^{b}	33.29^{b}
20	11.21^{a}	19.74^{a}	27.43^{a}	35.31^{a}	41.10^{a}
S.E.M.	0.03	0.02	0.05	0.10	0.11

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

Table 3: The effect of extracts from *Hyptis suaveolens* on egg hatch of *Heterodera sacchari*

	Time of Exposure				
Treatments	**Day1**	**Day2**	**Day3**	**Day4**	**Day5**
HPTS/EO	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
HPTS/DCM	0.00[a]	0.00[a]	0.00[a]	0.03[a]	0.09[a]
HPTS/H_2O					
CBFN	0.08[a]	0.10[b]	0.16[b]	0.20[b]	0.24[b]
	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
S.E.M	0.00	0.00	0.00	0.00	0.01
Treatment level (mg/mL)					
0	2.10[b]	4.00[b]	6.05[b]	8.21[b]	11.08[b]
	0.00[a]	0.00[a]	0.00[a]	0.03[a]	0.07[a]
10					
	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
15					
	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
20					
S.E.M	0.00	0. 00	0.00	0.01	0.01

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

DISCUSSION

The GC/MS analyses of the essential oil from *Hyptis suaveolens* confirmed specific compounds such as sabinene (29.5%), beta-caryophyllene (11.8%), terpinolene (9.8) and 1,8-cineole (7.3) as the main constituents. Peerzada (1997), confirmed beta-caryophyllene and 1, 8-cineole as the major constituent of *H. suaveolens,* whileAzevedo, *et al*., (2001);Witayapan *et al.,*(2007) and Eshilokun *et al.,* (2005) in their studies stated that the plant essential oils contains sabinene, 1,8-cineole, beta pinene, beta-caryophyllene, terpinolene, eugenol and 4-terpenol as the major constituents. The presence of sabinene, limonene, bicyclogermacrene, naphthalene, alpha-terpinene, 1, 8-cineole and gamaterpinene in *H. suaveolens* was also corroborated by Sidibe *et al.,* (2001). Babu and Sukul, (1990) established the nematicidal activity of essential oils of *H.*

suaveolens. They reported 100% mortality of *Meloidogyne incognita* larvae at 30minutes of exposure and stated the major constituents of the essential oils as D-limonene and menthol. The application of *H. suaveolens*as soil amendment was investigated by Christopher *et al.,* (2014). They observed a reduction in the pathogenicity of *M. javanica* and an improvement in the growth parameters of the test plant. The bio-efficacy of *Hyptis suaveolens* extracts against fish pathogens was demonstrated by Renisheya *et al.,* (2012). The ethanol and ethyl acetate extract inhibited the growth of bacteria at 100µg/mL and 75µg/mL respectively. Furthermore, antimicrobial activities against various bacteria and fungi were described for the essential oil of H. *suaveolens* (Jain *et al*., 1974; Iwu *et al.,* 1990; Singh *et al*., 1992; Pandey and Dubey, 1994; Kishore *et al.*, 1996). In several studies,*H. suaveolens* EO has shownuseful insecticidal properties against many cereal and stored product pests(Peerzada, 1997; Othira *et al*., 2009). *H. suaveolens* EOshowed toxic activity against *Plutella xylostella* (L.)(Lepidoptera Plutellidae) larvae and *Callosobruchusmaculatus* (F.)(Coleoptera Bruchidae) adults (Kéïta *etal*., 2006; Tripathi and Upadhyay, 2009). In recent studies, it was reported that *H. suaveolens* EO had a markedtoxic and repellent activity against adults of both *S. granarius* and *S. zeamais* (Motschulsky) (Coleoptera Dryophthoridae) (Conti *et al*., 2010; 2011). The *in-vitro* anti-helmintic activity of ethanol and aqueous extract of *H. suaveolens* were investigated by Nayak *et al.,* (2010) against the Indian earthworm *Pheretima posthuma* and *Ascardia galli.*Extracts *were* found to exhibit significant activity at the highest concentration of 100mg/ml. Yuji Oka *et al.,* (2000) studied the activity ofsome essential oils extracted from aromaticplants *in vitro* and in pots. It was found that essential oils of *Carum carvi, Foeniculum vulgare, Mentha rotundifolia*, *Mentha spicata Origanum vulgare, O. syriacum* and *Coridothymus capitatus* have some nematicidal potential against root knot nematodes. The observed nemato-toxic effects of *H. suaveolens* leaf essential oil (EO) and extracts can be attributed to the presence of some bioactive compounds that are nematicidal, some of which were established from the result of the GC/MS analyses of the test plants. Further studies on field experiments were recommended.

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