Nematicidal activity of *Citrullus colocynthis* extracts against root-knot nematodes

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Abstract

The nematicidal activity of *Citrullus colocynthis* seed, pulp, fruit and creeper extracts were evaluated against root-knot nematodes (*Meloidogyne* spp.) at 28 ± 2 °C in laboratory. Gas chromatograph interfaced to a Mass spectrometer (GC-MS) results indicated that *C. colocynthis* contains various bioactive compounds. The mentholic extracts of seed had the highest mortality (100%) at highest exposure time (72 hrs) while, the pulp and fruit extracts showed 60 and 80% mortality, respectively. Among various extracts of creeper hexane extract (HH-CC) showed 100% mortality after 72 hrs at all concentrations as compared with standard carbofuran. Whereas, HE-CC and HW-CC showed 100% mortality only at highest concentrations after 72 hrs. Ethyl acetate fraction was most active at highest concentrations causing 60% nematode mortality after 72 hrs. However, aqueous fraction (1 and 0.5) showed 40% mortality after 72 hrs exposure.

Cucurbitaceae or cucurbit family commonly referred as the cucumber, gourd, melon or pumpkin family, primarily found in the warmer regions of the world. *Citrullus colocynthis* is biochemically, economically important (Badifu & Ogunsua, 1991) and found in Northern Tropical Africa, Atlantic Islands, Australia, North-West India and Pakistan. The different extracts of *C. colocynthis* showed antibacterial, antioxidant, free radical scavenging and larvicidal activity. Methanolic and aqueous extracts of leaves showed significant anti-diabetic activity and also suspected to therapeutic value against breast cancer cells, while only fruit extract reported against *Meloidogyne incognita* (Muniasamy *et al*., 2010).

Plant parasitic nematodes are recognized as the causes of serious yield losses on a wide range of crops (Javed *et al*., 2007). Root-knot nematodes are plant-parasitic nematodes from the genus *Meloidogyne* Goeldi, 1887. *Meloidogyne* is Greek word which means apple-shaped female. Root-knot nematodes (*Meloidogyne* spp.) are among the most destructive nematodes in agriculture, causing an estimated yearly crop loss of $100 billion worldwide (Oka *et al*., 2000). Nematodes are difficult to control because of their wide host range and high rate of reproduction, with females capable of producing up to thousand eggs/female (Natarajan *et al*., 2006). In this study, different extracts of *C. colocynthis* were used for the screening of differed constituents by GC-MS analysis.

Materials and Methods

Survey was conducted to collect samples of plants infested with root-knot nematodes from different localities of Karachi district. Plants showing disease symptoms were carefully uprooted. Soil samples along with plant roots were placed in the polythene bags, labeled, stored in ice chest box and brought to laboratory for processing. The second stage juveniles (J_2) of root-knot nematode hatched from a single egg-mass collected from tomato root served as the initial inoculum to the seeding of brinjal *Solanum melongena* (L.) in earthen pots containing sterilize soil-manure mixture. The nematode species was identified by studying the perineal patterns of the mature female (Eisenback & Triantaphyllou, 1991).

Fruit extracts of *Citrullus colocynthis*: The air-dried and powder of fruit (fruit without seeds {pulp} 3.5 kg, seed 4 kg and fruit with seed 1.5 kg) were extracted with 80% methanol in water at room temperature for 10 days (3 times). After evaporation of the solvent methanolic extracts were obtained. Methanolic extracts of pulp, seed and fruit (seed + pulp) were evaluated for

nematicidal activity against *Meloidogyne* spp., at 28 ± 2 °C in laboratory. Fresh egg-masses collected from stock culture and inoculated in brinjal was used for egg hatching. The larvae emerged within 48 hrs were used in tests for larval mortality. Aqueous extracts and compounds were dissolved in 5 mg/ml distilled water and then made different concentrations.

To determine the nematicidal activity 100 root-knot nematode larvae were added in different concentrations of the extract and compounds. Cavity blocks without any crude extract and compounds served as control. Mortality of root-knot larvae was recorded after ½, 1, 2, 3, 4, 24, 48 and 72 hrs under stereoscopic microscope. Mortality was confirmed by touching the larvae movement with fine needle (Rizvi & Shameel 2006; Atta-ur-Rahman *et al*., 1997).

Creeper extracts of *Citrullus colocynthis*: The air-dried and powdered plant material (creeper part, 5 kg) was extracted with 80% methanol at room temperature for 10 days (3 times). After evaporation of the solvent crude methanolic extract (301.4 g) was suspended in distilled water and partitioned between n-hexane, chloroform, ethyl acetate and aqueous fraction. For getting the polar fraction from the hexane fraction was repeated again but the partitioned mannered was inverse. For this purpose hexane fraction suspended in distilled water and partitioned between ethyl acetate, chloroform and n-hexane as presented in Scheme 1.

In route 1, MeOH in 2 mg/2ml solution of each fraction was prepared from gummy residue poured in glass cavity blocks and kept open for 48 hrs to evaporate the methanol and then added 2 ml distill water while in route 2, 2 mg/2ml solution in cavity block after four different doses of each fraction were prepared (Table 2-3). To determine the nematicidal activity, 100 root-knot nematodes larvae were added in different concentrations of the extract and placed at room temperature. Cavity blocks without any crude extract served as control. The number of dead root-knot nematodes was recorded after ½, 1, 2, 3, 4, 24, 48 and 72 hrs under stereoscopic microscope. Mortality was confirmed by touching the larvae with fine needle (Rizvi & Shameel, 2006; Atta-ur-Rahman *et al*., 1997). In route 2, fractions directly dissolve in water and screened for nematicidal activity against *Meloidogyne* spp., and same concentrations of conventional nematicide Carbofuran (furadan) was taken for standard comparison.

GC-MS analysis: GC-MS was taken with an agilent 6890N (USA) JMS 600H (JEOL, Tokyo, Japan [EI mode; ionizing potential, 70 ev; capillary column ZB-5 (30 m × 0.32 mm × 0.22 µm film) (Zebron, Phenomenex); oven temperature, 50-250 ºC (rate of temperature increase = 5 ºC/min) Carrier gas, He; flow, 1.8 ml/min; split ratio, 30]. Interpretation on mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST; database (http://webbook. nist.gov/chemistry) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

GC-MS analysis of the creeper extract of *C. colocynthis* was performed using a Perkin-Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 ev. Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 µl was employed (a split ratio of 10:1). The injector temperature was maintained at 250 °C; ion-source temperature 200 °C, the oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min to 200 °C, then 5 °C/min to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 ev; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent

delay was 0 to 2 min and the total GC-MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

Statistical analysis: The experiment was laid down in completely randomized block design. Treatment effects were analyzed by multifactor analysis of variance (ANOVA); if the ANOVA was significant, differences in treatments were separated through Duncan's Multiple Range Test ($P < 0.05$) using SPSS statistical software. Percentage data were arcsine of square root transformed. LC_{50} values were analyzed with probit analysis by using the PROC PROBIT routine of SAS, 2000.

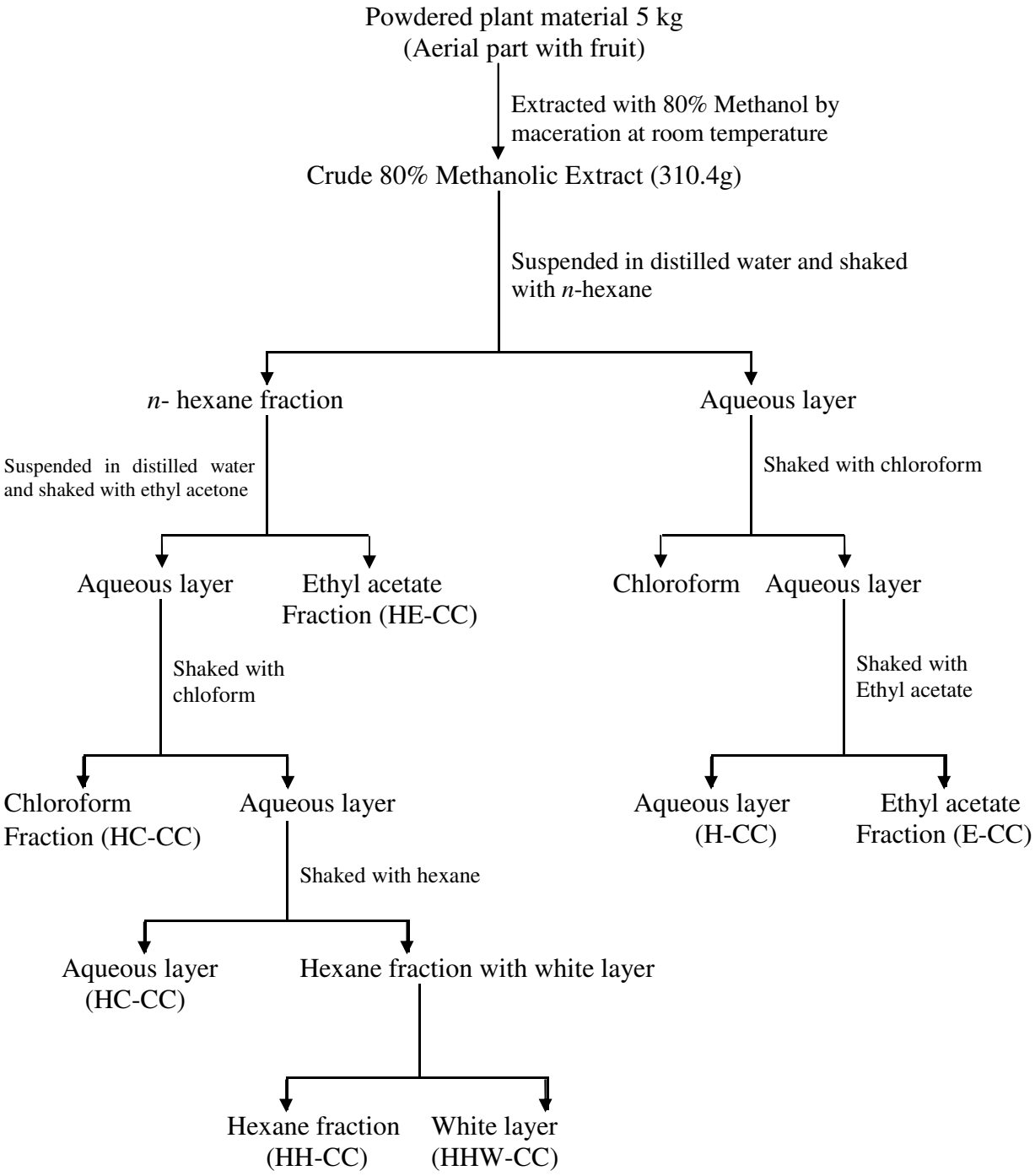
Results and Discussions

A total of 50 samples were collected from two major vegetable growing areas of Malir, Karachi.

Nematicidal activity of fruit and creeper extracts: Methanolic extracts of seeds, pulp and fruit screened for nematicidal activity against *Meloidogyne* spp. Seed extracts showed 100% mortality of the nematode at highest concentration after 72 hrs of exposure, while the pulp and fruit extract showed 60 and 80% mortality, respectively. However, 100% mortality was observed at all the levels of carbofuran after 24 and 48 hrs (Table 1). While, various extracts of creeper tested for nematicidal activity against *Meloidogyne* species through two routes. Route 1 was adopted to screen to less polar fraction for nematicidal activity. In this route ethyl acetate extract appeared to be the most active at highest concentration (1.0), as it caused 60% mortality of the nematode after 72 hrs of exposure to its extract while the water and ethyl acetate fraction showed 40% mortality (Table 3). In route 2, hexane sub-fraction (HH-CC) showed 100% mortality of nematode after 72 hrs and no significant difference found among all concentrations while HE-CC and HW-CC showed 100% mortality only at highest concentration (Table 4).

The nematicidal results of same fraction are different due to solvent. The fractions purely dissolved in the water showed good nematicidal effect rather than the extract dissolved in MeOH and then in distill water. Seed extract showed 10-20% mortality after ½ h of exposure while pulp and fruit extract showed no nematicidal activity. As concentration increased nematicidal activity also increased. The results from the different extracts of *Citrullus colocynthis* are shown in Table 1, 3 and 4. LC_{50} values calculated from probit analysis are given in Table 2, 5 and 6. In control treatment all nematodes remained alive.

GC-MS analysis of creeper extracts: GC-MS chromatogram analysis of the ethyl acetate fractions *C. colocynthis* creeper extract showed eight peaks which indicating the presence of eight phytochemical constituents in which three exist as major constituents while in hexane fractions (HW-CC, HHCC) indicating the 13 and 14 phytochemicals in which 4 and 5 exist as major constituents, respectively. Mass spectra of the constituents compared with the NIST library, all phytocompounds were characterized and identified (Table 7). The mass spectra of all the phytochemicals identified in *C. colocynthis* creeper extract were presented in Fig. 1-3. In all the identified compounds, the major phytochemical constituents of ethyl acetate extract (E-CC) and hexane extracts (HWCC, HHCC) were as E-CC = 1-Hydroperethylhomooctatasilsesquioxane (61%); Phenol, 2-methoxy 6-(3,7,11,15,19,23,27,31,35-nonamethyl-2,6,10,14,18,22,26,30,34-hexatriaco-ntanonaenyl)-(8.85%), 9,9-Dimethoxybicyclo [3. 3.1]nona-2,4-dione (6.36%); HW-CC = N-Methoxy-aziridine-2-carboxamide,N-(2-amino ethyl) (19.51%), Unknown (15.75%), Ethyl a'-d-glycopyranoside (12.05%), N-(Pentafluorosulfanyl) urea (9.47%) and HH-CC = Carbamodithioic acid, dibutyl-,methyl ester (10.35%), 5-Diacetylmethyl-2,3,7,8,12,13,17,18-octaethyl-21H, 23H-porphine copper (II) (9.23%), Oleic acid (8.72%), Benzene, (1-methyldodecyl)- (8.53%), 1-Hydroperethy lhomooctatasilse-squioxane (6.89%), Zinc, α4-oxohexakis[α-(propanoato-O:O')] tetra- (6.83%), respectively.



Scheme 1. Extraction of *Citrullus colocynthis*.

Table 1. Mortality (%) of *Meloidogyne* spp. against fruit extracts.

Treatments	Mortality (%) intervals (hrs)								
	Dose mg/ml	½	1	2	3	4	24	48	72
Seed	1	22 a	40 a	65 a	77 a	85 a	92 a	97 a	100 a
	0.5	12 b	35 b	62 b	65 b	67 c	75 c	87 b	90 b
	0.25	10 bc	30 c	60 c	62 c	70 b	77 b	85 c	90 b
	0.125	10 bc	30 c	60 c	62 c	67 c	72 d	80 d	90 b
Pulp	1	0	32 a	35 a	40 a	52 a	55 a	57 a	60 a
	0.5	0	30 ab	32 b	37 b	40 c	40 c	55 b	60 a
	0.25	0	22 c	30 bc	32 cd	42 b	42 b	47 c	50 b
	0.125	0	17 d	25d	35 c	40 c	42 b	45 d	50 b
Fruit (Seed + Pulp)	1	0	30 a	40 a	52 a	60 a	75 a	77 a	80 a
	0.5	0	27 b	35 c	47 b	55 b	62 b	72 b	77 b
	0.25	0	20 c	37 b	42 c	50 c	55 c	67 c	70 c
	0.125	0	20 c	35 c	42 c	47 cd	52 d	55 d	60 d
Carbofuran (Furadan)	1	0	0	0	0	0	100 a	-	-
	0.5	0	0	0	0	0	100 a	-	-
	0.25	0	0	0	0	0	80 b	100 a	-
	0.125	0	0	0	0	0	40 c	100 a	-

Means followed by the same letters are not significant from each other at P = 0.005.

Table 2. Median lethal concentrations (LC_{50}) of fruit extracts against *Meloidogyne* spp.

Time (hrs)	Seed	Pulp	Fruit (Seed + Pulp)
½	0.1738 (1.861-2.548)	-	-
1	0.339 (1.076-2.145)	0.225 (1.360-2.252)	0.288 (1.704-2.845)
2	0.659 (0.038-2.786)	0.399 (1.495-3.074)	0.903 (1.390-4.957)
3	0.217 (0.037-0.820)	0.577 (1.147-3.429)	0.119 (0.003-0.468)
4	0.184 (0.901-0.172)	0.570 (0.012-2.149)	0.157 (0.257-0.364)
24	0.198 (1.162-1.946)	0.320 (0.016-1.280)	0.208 (1.162-1.946)
48	0.112 (0.823-0.378)	0.266 (0.058-0.993)	0.112 (0.835-0.010)
72	0.107 (0.599-1.026)	0.3507 (0.617-0.767)	0.181 (0.129-0.845)

LC_{50} expressed as mg/ml
Confidence limit, CL, are given in parenthesis

Table 3. Mortality (%) of *Meloidogyne* spp. against creeper extracts (Route 1).

Treatments	Dose mg/ml	Mortality (%) intervals (hrs)							
		½	1	2	3	4	24	48	72
HH-CC	1	0	0	0	0	7 a	12 a	15 a	20 a
	0.5	0	0	0	0	0 b	0 b	7 b	10 b
	0.25	0	0	0	0	0 b	0 b	0 c	0 c
	0.125	0	0	0	0	0 b	0 b	0 c	0 c
HE-CC	1	0	0	0	0	5 a	10 a	15 a	20 a
	0.5	0	0	0	0	0 b	0 b	10 b	10 b
	0.25	0	0	0	0	0 b	0 b	0 c	0 c
	0.125	0	0	0	0	0 b	0 b	0 c	0 c
HW-CC	1	0	0	0	0	0	5 a	7 a	10 a
	0.5	0	0	0	0	0	0 b	0 b	10 a
	0.25	0	0	0	0	0	0 b	0 b	10 a
	0.125	0	0	0	0	0	0 b	0 b	0 b
E-CC	1	0	0	22 a	35 a	47 a	55 a	57 a	60 a
	0.5	0	0	0 b	20 b	27 b	32 b	35 c	40 b
	0.25	0	0	0 b	0 c	18 d	22 d	37 b	40 b
	0.125	0	0	0 b	0 c	20 cd	27 c	32 cd	40 b
W-CC	1	0	0	0	18 a	27 a	30 a	35 b	40 a
	0.5	0	0	0	0 b	25 b	30 a	37 a	40 a
	0.25	0	0	0	0 b	0 c	15 b	22 c	25 b
	0.125	0	0	0	0 b	0 c	0 c	15 c	20 c
C-CC	1	0	0	22 a	25 a	30 a	35 a	42 a	50 a
	0.5	0	0	0 b	0 b	18 b	20 c	27 b	30 b
	0.25	0	0	0 b	0 b	0 c	25 b	27 b	20 c
	0.125	0	0	0 b	0 b	0 c	0 d	10 c	30 b

Means followed by the same letters are not significant from each other at p = 0.005.

Table 4. Mortality (%) of *Meloidogyne* spp. against creeper extracts (Route 2).

Treatments	Dose mg/ml	Mortality (%) intervals (hrs)							
		½	1	2	3	4	24	48	72
HH-CC	1	0	30 a	42 a	57 a	72 a	87 a	95 a	100a
	0.5	0	25 b	35 b	52 b	67 b	80 b	92 b	100a
	0.25	0	22 c	27 c	42 c	62 d	77 c	87 c	100a
	0.125	0	20 cd	25 cd	37 d	65 bc	80 b	85 cd	100a
HE-CC	1	17 a	25 a	37 a	45 ab	67 a	87 a	92 a	100a
	0.5	0 b	22 b	35 ab	47 a	65 ab	72 b	77 b	80 b
	0.25	0 b	0 c	0 c	27 c	35 c	42 c	47 d	50 c
	0.125	0 b	0 c	0 c	10 d	10 d	30 d	50 c	50 c
HW-CC	1	30 a	42 a	57 a	62 a	77 a	87 a	95 a	100a
	0.5	0 b	37 b	47 b	57 b	70 b	77 b	85 b	90b
	0.25	0 b	0 c	30 c	42 c	57 c	62 c	67 d	70c
	0.125	0 b	0 c	0 d	30 d	42 d	57 d	72 c	80
E-CC	1	22 a	37 a	42 a	57 a	62 a	70 a	82 a	90 a
	0.5	0 b	0 b	27 b	30 b	42 b	57 b	72 b	80 b
	0.25	0 b	0 b	15 c	25 c	30 c	32 c	37 c	40 c
	0.125	0 b	0 b	0 d	20 d	27 cd	30 cd	35 bc	40 c
W-CC	1	0	0	7 a	15 a	20 a	25 a	27 a	35a
	0.5	0	0	0 b	0 b	0 c	0 c	15 c	20 b
	0.25	0	0	0 b	0 b	0 c	0 c	12 d	20 b
	0.125	0	0	0 b	0 b	7 b	12 b	17 b	20 b

Means followed by the same letters are not significant from each other at p = 0.005.

Table 5. Median lethal concentrations (LC_{50}) of creeper extracts against *Meloidogyne* spp.

Duration (hrs)	HH-CC	HE-CC	HW-CC	E-CC	W-CC	C-CC
½	-	-	-	-	-	0.200 (1.687-2.478)
1	-	-	-	-	-	0.149 (0.995-1.584)
2	-	-	-	-	-	0.155 (0.638-1.298)
3	-	-	-	-	0.110 (1.266-1.712)	0.139 (0.250-0.799)
4	1.807 (13.42-20.724)	-	0.118 (1.339-1.817)	0.118 (1.339-1.817)	0.102 (1.078-1.487)	0.120 (0.075-0.319)
24	1.039 (6.38-10.591)	-	0.138 (1.848-2.399)	0.138 (1.848-2.399)	0.119 (0.990-1.463)	0.151 (0.990-1.463)
48	0.156 (1.620-2.068)	0.118 (1.339-1.817)	0.133 (1.840-2.399)	0.133 (1.840-2.399)	0.1743 (1.073-1.762)	0.193 (1.073-1.762)
72	0.413 (2.322-3.967)	0.088 (1.244-1.598)	0.250 (2.786 -3.778)	0.250 (2.786-3.778)	0.1220 (1.012-0.531)	0.144 (0.369-0.991)

Table 6. Median lethal concentrations (LC_{50}) of creeper extracts against *Meloidogyne* spp.

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Duration (hrs)	HH-CC	HE-CC	HW-CC	E-CC	W-CC
½	-	-	-	-	-
1	0.325 (1.860-3..147)	-	-	-	-
2	0.202 (0.902 -1.700)	-	-	-	-
3	0.199 (1.875 -0.973)	-	-	-	0.110 (1.266-1.712)
4	0.387 (13.42-20.724)	-	0.118 (1.339-1.817)	0.118 (1.339-1.817)	0.102 (1.078-1.487)
24	1.039 (6.38-10.591)	-	0.138 (1.848-2.399)	0.138 (1.848-2.399)	0.119 (0.990-1.463)
48	0.156 (1.620-2.068)	0.118 (1.339-1.817)	0.133 (1.840-2.399)	0.133 (1.840-2.399)	0.1743 (1.073-1.762)
72	0.413 (2.322-3.967)	0.088 (1.244-1.598)	0.250 (2.786 -3.778)	0.250 (2.786 -3.778)	0.1220 (0.531-1.012)

LC_{50} expressed as mg/ml.
Confidence limit, CL, are given in parenthesis.

Table 7. *Citrullus colocynthis* creeper extracts identified by GC-MS.

S. No.	Fractions	Retention time	Name of the compounds	Mol. formula/Mol. weight	Basis of identification or mass fragments of its mass	Peak area %
1	E-CC	15.17	1-Nitrosoadamentane	C10H15NO /165.23	MS	0.39
2	E-CC	28.73	1-Hydroperethylhomooctatasilsesquioxane	C18H46O13Si9 / 723.32	MS	61.02
3	E-CC	30.32	Phenol, 2-methoxy 6-(3,7,11,15,19,23,27,31,35- nonamethyl-2,6,10,14,18,22,26,30,34-hexatriacontanonaenyl)-	C52H80O2 / 737.19	MS	8.85
4	E-CC	32.24	1,6:3,4-Dianhydro-2-deoxy-a'-d-lyxo-hexopyranose	C6H8O3 / 128.13	MS	3.79
5	E-CC	34.06	Benzo [β] furan-5-carboxylic acid, 2,3-dihydro-, 4-chlorophenyl ester	C15H11ClO3 / 274.70	MS	2.05
6	E-CC	36.91	Cis-Vaccenic acid	C18H34O2 / 282.46	MS	0.57
7	E-CC	38.99	9,9-Dimethoxybicyclo [3.3.1]nona-2,4-dione	C11H16O4 / 212.24	MS	6.36
8	E-CC	44.81	Erucic acid	C22H42O2 / 338.57	MS	4.09
9	HW-CC	11.48	Benzofuran, 2,3-dihydro-	C8H8O / 120.15	MS	2.31
10	HW-CC	12.07	Unknown	-	120.1,19.1,65.1, 44.0	1.38
11	HW-CC	16.83	N-(Pentafluorosulfanyl)urea	CH3F5N2OS / 186.11	MS	9.47
12	HW-CC	19.72	1,2,3,4-Cyclopentanetetrol,(1a', 2a',3a',4a')	C5H10O4 / 134.13	MS	0.75

13	HW-CC	21.81	Ethyl a'-d-glycopyranoside	C8H16O6 / 208.21	MS	12.05
14	HW-CC	23.59	Benz[α]azulene	C14H10 / 178.23	MS	7.6
15	HW-CC	24.34	Unknown	-	178.1, 152.1, 125.0, 109.1, 89.0, 76.0, 63.0, 44.0	15.75
16	HW-CC	26.93	Fluorene-9-methanol	C14H12O / 196.24	MS	4.47
17	HW-CC	30.39	Benzo[α]furan-5-carboxylic acid, 2,3-dihydro-,4-chlorophenyl ester	C15H11ClO3 / 274.70	MS	2.28
18	HW-CC	30.90	Unknown	-	256.3, 236.3, 227.2, 213.2, 199.2, 194.2, 185.1, 181.1, 171.1, 157.1, 152.1, 129.1, 115.1,73.0, 55.0, 41.0	0.73
19	HW-CC	33.56	Unknown	-	280.3, 264.3, 222.2, 207.2, 165.1, 147.1, 124.1, 111.1, 97.1, 83.1, 69.1, 55.1, 41.0	4.57
20	HW-CC	36.81	N-Methoxy-aziridine-2-carboxamide,N-(2-aminoethyl)	C6H13N3O2 / 159.19	MS	19.51
21	HW-CC	40.89	Cyclohexane, 1,4-dimethyl-2-octadecyl-	C26H52 / 364.69	MS	7.81
22	HH-CC	23.83	Tetraethyl 7, 8-epoxy-2,7,12,17-tetramethyl-21H, 23H-prophine-3,8,13,18-tetrapropionate	C44H54N4O9 / 782.92	MS	1.33
23	HH-CC	24.46	3a-Hydroxy-9b-lanosta-7,24-dien-26,23-olide	C30H46O3 / 454.68	MS	2.59
24	HH-CC	30.43	6-Oxa-15,20,24,27-	C28H36N4O4 /	MS	4.73

			tetraazatetracyclo[13.9.6.2(2,5).1(7,11)]tritriaconta-2,4,7,9,11(31),12,32-heptaene-14,26-dione,8-hydroxy-,[s-(Z)]-	492.61		
25	HH-CC	30.91	Benzene, (1-methyldodecyl)-	C19H32 / 260.46	MS	8.53
26	HH-CC	31.87	Carbamodithioic acid, dibutyl-,methyl ester	C10H21NS2 / 219.41	MS	10.35
27	HH-CC	32.81	Bicyclo[5.3.1]undecan-11-one	C11H18O / 166.26	MS	5.71
28	HH-CC	33.15	1-Hydroperethylhomooctatasilsesquioxane	C18H46O13Si9 / 723.32	MS	6.89
29	HH-CC	33.69	Unknown	695.0, 675.0, 659.0, 631.0, 596.0, 556.0, 532.0, 485.0, 449.0, 394.0	MS	4.75
30	HH-CC	34.37	Zinc,α4-oxohexakis[α-(propanoato-O:O')]tetra-		MS	6.83
31	HH-CC	35.40	Oleic acid	C18H34O2 / 282.46	MS	8.72
32	HH-CC	37.27	5-Diacetylmethyl-2,3,7,8,12,13,17,18-octaethyl-21H,23H-porphine copper (II)	C41H50CuN4O2 / 694.41	MS	9.23
33	HH-CC	39.43	Glycine, N-pentadecafluorooctanoyl-, pentyl ester	C15H14F15NO3 / 541.25	MS	3.61
34	HH-CC	40.48	Cyclohexanol, 4 –ethyl-methyl-3-(1-methylethenyl)-, (1a',3a',4a',)	C12H22O / 182.30	MS	0.98
35	HH-CC	44.21	Erucic acid	C22H42O2 / 338.57	MS	7.16

*MS, identification by comparing EI mass spectrum with NIST mass spectral database; E-CC, ethyl acetate fraction, HW-CC & HH-CC, hexane fractions.

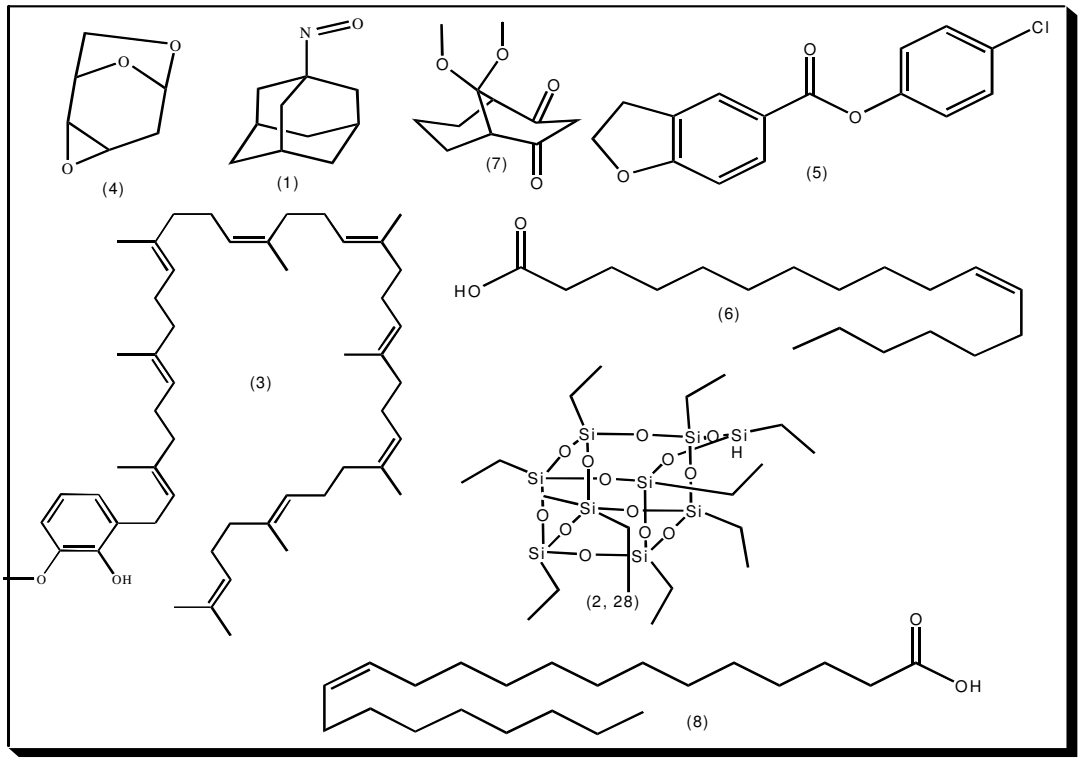

Fig. 1. Structure of phytocomponents identified by GC-MS in the creeper extracts (E-CC) of *Citrullus colocynthis*.

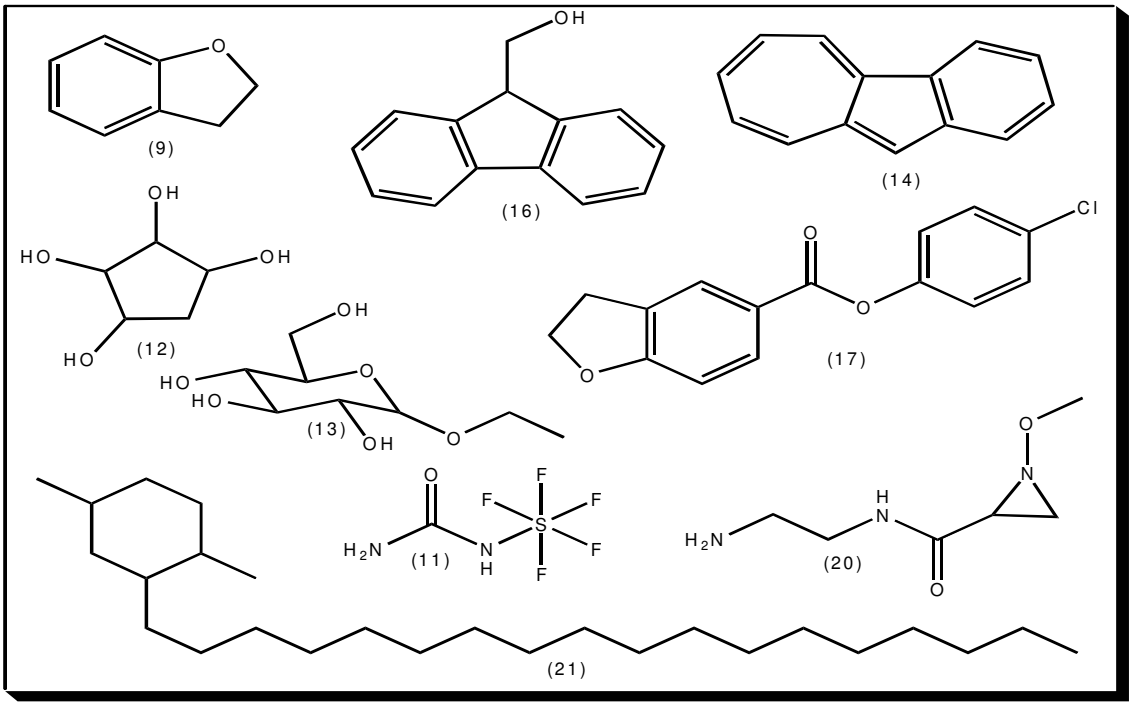

Fig. 2. Mass spectrum and structure of phytocomponents identified by GC-MS in the creeper extracts (HW-CC) of *Citrullus colocynthis*.

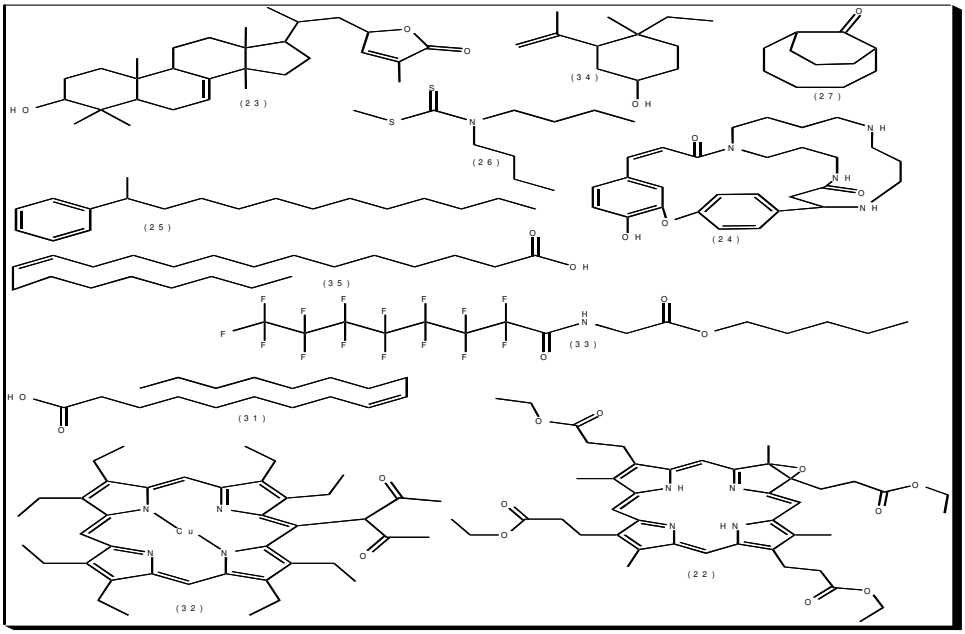

Fig.3. Structure of phytocomponents identified by GC-MS in the creeper extracts (HH-CC) of *Citrullus colocynthis*.

Conclusion

It is concluded that the seed and creeper extracts of hexane and ethyl acetate show most active response against the nematicidal activity. GC-MS results indicated that *Citrullus colocynthis* contains various bioactive compounds and is suggested as a plant phytochemical.

References

Atta-ur-Rahman, Khan, A.M., Shabbir, M., Abid, M., Chaudhary, M.I., Nasreen, A., Maqbool, M.A., Shameel, M. & Sualeh, R. 1997. Nematicidal activity of marine organisms. *Pakistan Journal of Nematology* 15, 95-100.

Badifu, G.I.O. & Ogunsua, A.O. 1991. Chemical composition of kernels from some species of *Cucurbitaceae* grown in Nigeria. *Plants Food for Human Nutrition* 41, 35-44.

Eisenback, J.D. & Triantaphyllou, H.H. 1991. Root-knot nematodes: *Meloidogyne* species and races. In: Nickle, W.R. (Ed.). *Mannual of Agricultural Nematology*, Marcel Dekker, Inc. New York, 191- 274 pp.

Goeldi, E.A. 1887. Relatorio sobre a molestia do cafeeiro na provincia do Rio de Janeiro. Apparently and advanced separate of Arch. mus. nac. *Rio de Janeiro* 8, 7-121.

Javed, N., Gowen, S.R., Haq, M.I., Abdullah, K. & Shahina, F. 2007. Systematic and persistent effect of neem (*Azadirachta indica*) formulations against root-knot nematodes, *Meloidogyne javanica* and their storage life. *Crop Protection* 26, 911-916.

Rizvi, M.A. & Shameel, M. 2006. *In vitro* nematicidal activities of seaweed extracts from Karachi coast. *Pakistan Journal of Botany* 38, 1245-1248.

Muniasamy, S., Pavaraj, M. & Rajan, M.K. 2010. Efficacy of the fruit extract of *Citrullus colocynthis* (L.) on the root-knot nematode *Meloidogyne incognita* infecting *Vigna ungiculata* (L.). *Journal of Biopesticides* 3, 309-312.

Nataragan, N., Cork, A., Boomathi, N., Pandi, R., Velavan, S. & Dhaskshanamoorthy, G. 2006. Cold aqueous extracts of African marigold, *Tagetes erecta* for control of tomato root-knot nematode, *Meloidogyne incognita*. *Crop Protection* 25, 1210-1213.

Oka, Y., Koltai, H., Bar-Eyal, M., Mor, M., Sharon, E., Chet, I. & Spiegel, Y. 2000. New strategies for the control of plant parasitic nematodes. *Pest Management Science* 56, 983-988.

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