Waterscout SM 100 (Spectrum instruments Inc.) connected micro stations (1525). The weather variables temperature and RH were measured in each growth chamber at 15 min interval throughout the growth period using a combined temperature and humidity meter. Final biomass harvest was done at 43 days after sowing. The experiment was run once only with the assumption that the well-controlled experiment is repeatable, and the fact that this type of controlled experiments are very costly. Wherever parameters were measured on all the plants, means were used for statistical analysis.

RESULTS

Results of the study showed that leaves area per plant, chlorophyll content, leaves DW, roots DW and total DW, root to shoot ratio N uptake and leaf chlorophyll fluorescence were significantly influenced by temperature stress (Table 1). These were greater in optimum temperature at 27^{o}C day and 18^{o}C at night. Further these were greatly reduced at high temperature 35/18^{o}C and 20/18^{o}C at day and night temperature respectively. Whereas leaves per plant, leaf thickness were not influenced by day and night temperature variation. Most of the canopy parameters were found non-significant by drought stress and well watered treatments except total Dry weight and N uptake. Similarly rate of N application was significant impact of canopy characters at early stage of canola except, leaf area, total dry weight and N uptake per plant. Total dry weight per plant was most significantly affected by interaction effect of temperature, water and N application. Results confirmed that canola is highly sensitive for high temperature, water and N stress during early part of the crop season.

Table 1. Canopy characteristics of canola influenced by drought stress, elevated temperature and nitrogen under controlled environment

	Leaves/ plant	Leaf area/ Plant	Total Chl Mg/g of leaf	Leaf thickness (mm)	Leaves DW (g)	Root DW (g)	Total DW (g)	Root: shoot	N uptake (g plant^{-1})	Fv/Fm
Temperature gradient (T)										
HT	5.05	345	0.92	6.63	3.31	15.87	05.83	0.137	0.33	0.72
MT	3.89	1,289	0.77	6.22	2.52	6.93	22.79	0.183	0.99	0.74
LT	4.72	728	0.43	5.64	6.91	4.60	11.52	0.210	0.66	0.67
CD@ 5%	NS	279	0.18	NS	2.74	6.69	2.11	0.046	0.10	0.04
Water stress (W)										
WW	4.52	767	0.68	6.46	4.50	9.50	11.43	0.187	0.60	0.71
DS	4.59	808	0.74	5.88	4.00	8.77	15.32	0.166	0.72	0.72
CD@ $%	NS	NS	NS	NS	NS	NS	1.72	NS	0.08	NS
Nitrogen rate (N)										
N120	4.34	879	0.84	6.09	4.47	9.48	14.00	0.162	0.90	0.69
N0	4.77	695	0.57	6.24	4.02	8.79	12.76	0.192	0.42	0.74
CD@5%	NS	395	0.14	NS	NS	NS	0.56	NS	0.08	0.03
Interaction										
T x W	NS	NS	NS	NS	NS	NS	2.99	0.065	0.14	NS
T x N	NS	NS	NS	NS	NS	NS	2.99	NS	0.14	0.05
W x N	NS	NS	NS	NS	NS	NS	4.22	NS	NS	NS
T x W x N	NS			NS					0.20	NS

HT- High temperature (35/18); MT- Medium temperature (27/18); LT- Low temperature (20/18)
WW- Well watered; DS- Drought stress; N120- nitrogen at 120 kg/ha: N0- Nitrogen free control

Phytochemical profiling and nematicidal activity of leaf extracts of *Tinospora cordifolia* against reniform nematode (*Rotylenchulus reniformis*)

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ABSTRACT

Reniform nematode, *Rotylenchulus reniformis* is an important nematode associated with castor influencing the productivity of the crop. Efficacy of the leaf extract of *Tinospora cordifolia* at different concentrations *viz*., 0.1, 0.5, 1.0, 2.0, 5.0, 10.0 and 20% against the nematode juveniles were evaluated *in vitro*. The extract, at 20%

concentration was recorded to be effective in the reduction of nematode juveniles to 93.8 per cent. The more active non-polar and medium polar compounds which are having melting point less than 200° C were analysed by GC-MS. Eighteen compounds of the total extract were identified of which six compounds were reported to possess nematicidal properties.

Keywords: Nematicidal activity, Phytochemicals, *Tinospora cordifolia*, *Rotylenchulus reniformis*

Reniform nematode (*Rotylenchulus reniformis*) are the most important nematode species associated with the castor crop, which are one of the factors influencing the yield reduction of the crop. With increased awareness of possible deleterious effects of chemical pesticides, there is an increasing interest in non-chemical nematode management strategies. One of the possible alternatives is the use of biopesticides from plant origin. Current study was designed to evaluate the nematicidal effects of *Tinospora cordifolia* (leaves) in controlling reniform nematode (*R. reniformis*) and to assess the chemical composition of methanolic leaf extract of the plant possessing nematicidal properties.

MATERIALS AND METHODS

The leaves of *Tinospora cordifolia* were collected during the month of July from ICAR-IIOR farm, Rajendranagar, Hyderabad. The leaves were dried under shade and powdered into fine particles with equal quantity of methanol in Pestle and mortar. Then the leaves were kept on a rotary shaker for 24h at 120 rpm. The solution was filtered through muslin cloth and then through Whatmann no. 1 filter paper. The filtrate was evaporated at 40°c in water bath to obtain the organic extracts of the leaf.

For maintenance and multiplication of pure culture of reniform nematode, *Rotylenchulus reniformis*, the soil was autoclaved at 15 psi pressure at 121° C for 30 min. The autoclaved soil was filled in the pots and castor seeds were sown in the pots. To the 30 days old seedlings, 4nd stage juveniles of nematodes were inoculated and the egg mass collected from the plant roots after 20-25 days of inoculation was allowed for hatching. From the hatched out juveniles, fourth stage juvenile was used for the mortality assay studies.

From the crude extract of *Tinospora cordifolia*, different concentrations *viz*., 0.1, 0.5, 1.0, 2.0, 5.0, 10.0 and 20% prepared by diluting with distilled water. Fourth stage juveniles of the nematode were suspended in sterile distilled water. This suspension was adjusted to contain the nematode juveniles of about 100 / 0.2 ml suspension. For mortality studies, 0.2 ml of nematode suspension was poured into 5 cm diameter Petri dishes containing 2 ml of diluted plant extract of each concentration. A control was maintained with only nematode juveniles. All the treatments were replicated four times in CRD. Dead juveniles were count under stereoscopic microscope after 12, 24 and 48 h exposure period and per cent mortality was assessed.

Methanolic extract of leaves of *T. cordifolia* were analysed for the presence of different compounds by GC-MS technique. GC-MS analysis of some of the potent volatile constituents present in the extracts was performed at The South India Textile Research Association (SITRA), Coimbatore (Tamil Nadu), India. GC analysis of the extracts was performed using a GCMS (Make: Agilent Model :CH-GCMSMS02, 8890 GC System, 7000 GC/TQ) equipped with a DB-5MS fused silica capillary column (30 m length × outside diameter 0.25 mm × internal diameter 0.25µm) and gas chromatograph interfaced to a Mass Selective Detector (MS-DSQ-II) with Mass Hunter software. For GC-MS detection, an electron ionization system with ionization energy of -70eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1ml/min and the sample injected was 2µl; Injector temperature 250°C; Ion source temperature 200°C. The oven temperature was programmed from 50 °C at 1 min hold time Run time : 1 min; 5 °C / min 120 °C hold 1 min Run time 16 mins; 10 °C / min 210 °C hold 1 min Run time 26 mins ; 10 °C / min 280 °C hold 5 mins Run time 38 mins . Total GC run time was 38 min. The relative percentage of the each extract constituents was expressed as percentage with peak area normalization. The identity of the components in the extract was assigned by comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. Per cent mortality data was subjected to statistical analysis using the factorial completely randomized design statistical package. The critical differences in main effects *i.e.* compounds, concentrations and days as well as in their interactions were tested at P = 0.05 %.

RESULTS AND DISCUSSION

The results, after 48 h of exposure, revealed that all the concentrations of the leaf extract caused significant mortality of *R. reniformis* juveniles when compared to control. The percentage of mortality ranged between 29.8 and 93.8 %. Among the different concentrations tested, 20% concentration exhibited highest per cent mortality (93.8) followed by 10% (85.0) and 5% (76.0) as compared to control (Table 1). The present report was in accordance with the results of Meena *et al.* (2010), Khan (2019) and Das *et al*. (2011) where they explored the efficacy of different botanicals against nematodes and reported the mortality of nematodes with the botanical extracts and powder against nematodes *in vitro*.

Mortality recorded by *T. cordifolia* leaf extracts are in accordance with the potential secretion of their metabolites as represented in Fig. 1. In GC-MS analysis, 18 compounds were identified of which 6 were reported with nematicidal properties (1, 2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester; Hexadecanoic acid, methyl ester; Dibutyl phthalate; Methyl stearate; Phthalic acid and Octadecadienoic acid) (Table 2) (Hooks *et al*., 2010; Kumar *et al*., 2017).

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Table 1 Effect of *T. cordifolia* leaf extracts on the juvenile (J_4) mortality of *R. reniformis*

Concentration	% Mortality (48 HAT)
0.1	29.8
0.5	34.0
1	44.5
2	49.3
5	76.0
10	85.0
20	93.8
Control	2.0
C.D. ($p< 0.05$)	6.73

Table 2 Chemical composition of *T.cordiofolia* leaves with nematicidal properties

Compound name	Retention Time (min)	Area %
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	7.54	4.52
Hexadecanoic acid, methyl ester	10.31	2.47
Dibutyl phthalate	11.74	2.39
Methyl stearate	22.76	9.55
Phthalic acid	28.57	38.26
Octadecadienoic acid	13.98	5.47

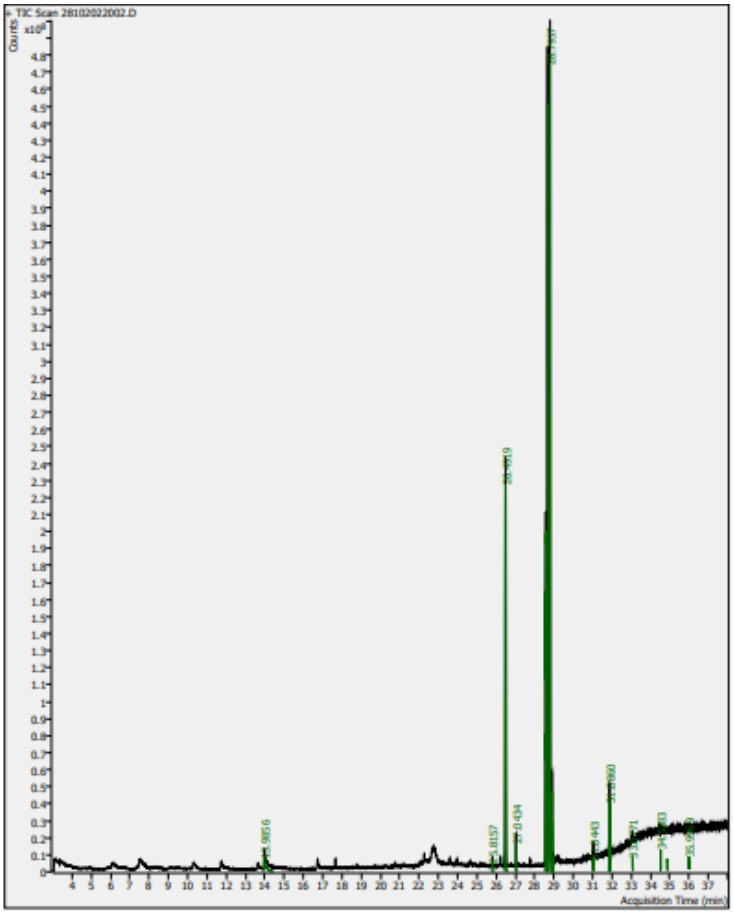

Fig. 1. GC-MS chromatogram of active methanol extract