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ORIGINAL ARTICLE

Genotoxicity assessment of two common curing weeds: *Hyptis suaveolens* (L.) Poir. and *Leucas indica* (L.) R. Br.

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Abstract *Hyptis suaveolens* and *Leucas indica*, two common weeds were selected for the present study, to reveal their probable cytotoxic potential. The meristematic root tips of *Allium cepa* were used for testing the cytotoxic property of the aqueous leaf extracts containing both polar and non-polar compounds, and that containing polar compounds alone, at different concentrations (0.125, 0.25, 0.5, 1 and 2 %) and at different time durations, using distilled water as negative control. Mitotic squash preparations were made using a standard protocol. The mitotic index of the treated root tip cells was found to be decreasing and the abnormality percentage was found to be increasing with increase in extract concentration when compared with the control. Maximum cytotoxicity was observed in the extract containing both polar and non-polar compounds. Both the tested plants were found to be cytotoxic. The abnormalities noticed were of both clastogenic (nuclear lesions, nuclear fragmentation, etc.) and non-clastogenic (aberrant cell wall formation at cytokinesis, ball metaphase, etc.) types. Both plant extracts were found to significantly ($P < 0.05$) inhibit root growth of *Allium cepa* with an EC_{50} value of 1.92 % ($R^2 = 0.594$) for *Hyptis* and 1.58 % ($R^2 = 0.757$) for *Leucas*. The results were also analysed statistically by using analysis of variance followed by appropriate post hoc tests. These two weeds are aromatic plants comprising of essential oils that are volatile, natural complex compounds characterized by a strong odour and formed as secondary metabolites. In nature, essential oils play an important role in the protection of the plants as insecticides by reducing their appetite for such plants. When specifically targeted the concept of effectively exploiting these weeds for the formulation of herbal insecticides/pesticides may be possible in the near future.

Keywords *Hyptis suaveolens* · *Leucas indica* · *Allium cepa* assay · Chromosomal aberrations · Mitotic index · EC_{50}

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Introduction

Agricultural crops are under constant assault by insect pests, making insecticides essential to reduce losses. Synthetic insecticides such as organophosphates are important, and effective tools in modern crop management. However, they pose serious threats to the environment and to people.

Several pressures have accelerated the search for more environmentally and toxicologically safe and more selective and efficacious pesticides (Kamau Mwangi et al. 2012). The increasing incidence of

pesticide resistance is also fuelling the need for new pesticides. Furthermore, most synthetic chemicals that have been commercialized as herbicides are halogenated hydrocarbons with relatively long environmental half-lives and more toxicological properties than most natural compounds. Natural pesticides are biodegradable, barely leave residues in the soil and are less likely to harm humans or animals. In addition, they are cheaper and more accessible in less developed countries especially if the sources of materials are abundant plants e.g. common weeds, prolific herbs, shrubs and trees having a wide and rich distribution (Agrawal et al. 2010). Thus, natural compounds have increasingly become the focus of those interested in the discovery of insecticides.

Tens of thousands of secondary products of plants have been identified and there are estimates that hundreds of thousands of these compounds exist. There is growing evidence that most of these compounds are involved in the interaction of plants with other species, primarily involved in the defence of the plant from plant pests. Thus, these secondary compounds represent a large reservoir of chemical structures with biological activity. This vast resource is largely untapped for use as pesticides (Kiran et al. 2011).

Bioassays have been carried out using different organisms in order to identify and evaluate the harmful effects of various agents at their different concentrations and exposure periods (De-Serres 1992). *Allium* test is a sensitive test that has often been used for the determination of cytotoxic and/or genotoxic effects of various substances (Timothy et al. 2014). *Allium cepa* root tip meristems have been widely used for the evaluation of cytotoxicity, anti-mitotic activity, and genotoxicity, by employing the growing roots of *A. cepa*.

The *Allium* L. test has important advantages (Turkoglu 2007a, b; Kuras et al. 2006; Rank 2003a, b) and has been used for many years in investigating physical and chemical mutagenesis, polluting agents, plant extracts, and cytogenetic effects of similar active material in mitotic cell division. *Allium* assay has been shown to have correlation with tests in other living systems and serve as an indicator of toxicity of the tested material (De Rainho et al. 2010). Several authors have demonstrated the efficiency of the analysis of chromosomal aberrations (CA) in *A. cepa* as to be more advantageous to investigate the action mechanism of testing agents on DNA, which enables a better understanding of the effects promoted by such agents (Leme et al. 2008; Matsumoto et al. 2006). Positive results of *Allium* test should be seen as a signal of warning and also an indication that the tested chemical may constitute a potential health risk (Fiskesjo 1985).

Genotoxicity studies are designed to determine chemicals that can perturb genetic material causing gene or chromosomal mutations. Chromosome aberrations have been considered as reliable indicators of mutagenic activity (Mohandas and Grant 1972), and there have been evidences for a correlation between chromosomal damage and toxic effects of extracts of different plants.

The use of plant tissues, primarily root tip for studying the induction of CA is one of the oldest, simplest, most reliable and inexpensive method (Auti et al. 2010). Onions are easy to store and to handle, and also macroscopic and microscopic parameters can be observed easily. Moreover this system correlates well with the data obtained from eukaryotic and prokaryotic systems (Özkara et al. 2014). The mitotic index (MI) could be used as a reliable parameter for evaluating the cytotoxicity of various agents (González et al. 2011).

Numerous crops and weeds have been investigated for their allelopathic characteristics. Weed is the plant that grows and reproduces aggressively outside its native habitat (Janick 1979). While the term "weed" generally has a negative connotation, many plants known as weeds can have beneficial properties. A number of weeds are known for their potent biochemical and pharmacological activities. According to Sathish and Maneemegalai (2008), the leaf and flower extracts of the weed *Lantana camara* and *Parthenium hysterophorous* exhibited larvicidal activity. *Achyranthes aspera* (Amaranthaceae) is a common weed that finds therapeutic use in rheumatism, bronchitis, skin diseases, rabies and malaria. Many plants including weeds have developed protection mechanisms, such as repellents and even insecticidal effects, to defend themselves against insect pest attacks. Thus extracts obtained from their different botanical parts having sap can be wisely used in suppressing insect pests (Ahmad et al. 2011). Earlier studies reported different plant extracts as an effective botanical agent against Red pumpkin beetle and fruit flies (Khan et al. 2012). The alkaloid mimosine in the extracts of *Mimosa*

pudica (Mimosaceae) are shown to possess antiproferative and apoptosis inducing activities. In Ayurveda and Unani system of medicine the plant is used for treatment of jaundice, leprosy, ulcers and small pox. *Sida cordifolia* (Malvaceae), an invasive weed is also known for its medicinal property in Ayurveda. It is used in the folk medicine for the treatment of oral mucosal inflammation and asthmatic bronchitis (Namita and Mukesh 2012).

Root extract and protein fraction from the latex of *Calotropis procera* has been found to produce a strong cytotoxic effect on COLO-320 (colon), Huh-7 (hepatoma), COS-1 (Kidney), and MCF-7 (breast) cell lines. The cytotoxic activity of latex is due to the partial inhibition of DNA synthesis which triggers apoptosis (Sharma et al. 2012a, b). Oleanonic acid isolated from *L. camara* exhibit potent cytotoxicity against human melanoma (A375) cell lines. Methanolic extract of leaves are reported to exhibit cytotoxicity against laryngeal (HEp-2) and lung (NCI-H292) cancer cell lines. Kumar et al. (2013) reported cytotoxic activity of leaf ethanolic extract against breast and leukemia cell lines.

The plants viz., *Leucas indica* and *Hyptis suaveolens* are aromatic medicinal weeds of the family Lamiaceae. *L. indica* is a perennial herb found in waste lands and road sides (Chandrashekar and Rav 2013). *Leucas* is reported to have antifungal, prostaglandin inhibitory, antioxidant, antimicrobial, antinociceptive and cytotoxic activities (Prajapati et al. 2010). In folk medicine, *Leucas* is used as an antipyretic in villages in southern India. The juice of leaves is used externally for skin eruptions and swellings. The flowers of some species are used orally to treat typhoid fever (Saha et al. 1997). In Bengal, flowers are given with honey for coughs and cold to children (Khare 2004). Several researchers reported the insecticidal property of *Leucas* (Srinivasan 2011; Vinayagam et al. 2008; Sakthivadivel and Daniel 2008).

Hyptis suaveolens is commonly used by the traditional population in several parts of the world to treat inflammation, gastric ulcer and infection and is used as a crude drug to relieve symptoms related with gastric ulcer or gastritis in northeastern and central region of Brazil (Jesus et al. 2013). In India the leaves of *H. suaveolens* have been utilized as a stimulant, carminative, sudorific, galactogogue and as a cure for parasitic cutaneous diseases (Mandal et al. 2007). Crude leaf extract is also used as a relief to colic and stomach ache. Leaves and twigs are considered to be antispasmodic and used in antirheumatic, antisudorific baths, as antinflammatory, and antifertility agents (Kirtikar and Basu 1991), and are also applied as an antiseptic in burns, wounds, and various skin complaints. The decoction of the roots is highly valued as appetizer and is reported to contain ursolic acid, a natural HIV-integrase inhibitor (Chatterjee and Pakrashi 1997). Fumes of the dried leaves are also used to repel mosquitoes and control insect pests of stored grains. Perusal of literature revealed the repellent and larvicidal activity of *Hyptis* (Palsson and Jaenson 1999; Amusan et al. 2005; Abagli and Alavo 2011).

Despite the widespread occurrence of *H. suaveolens* and *L. indica* and its use in the popular medicine, there is no study, to the best of our knowledge that has addressed its cytotoxic properties. Negligible investigation is available to document the safety or health risks of low-dose repeated exposures to chemical mixtures. Weed plants due to their inbuilt toxic nature have also pesticidal and even medicinal properties also (Chansang et al. 2005). In the light of this, the present study was conceived with the aim of evaluating the cytotoxic effects of the aqueous leaf extracts of the above said species using *Allium cepa* assay.

Materials and methods

Test organism/growth conditions

Equal-sized bulbs (25–30 mm in diameter, without any treatment) of a commercial variety of *A. cepa* L. ($2n = 16$) were used in the *Allium* test. The onions were kept cool and dry until the experiment. Just before use, the outer scales of the bulbs were carefully removed and the brownish bottom plates were scraped away without destroying the root primordia. The roots were protected from direct sunlight in order to minimize fluctuation of the rate of cell division (Elena and Paula 2011). The experiment was carried out under laboratory conditions.

Collection of plant materials

The plants were collected from the Calicut district of Kerala. The identification and verification of the plants were done by Dr A. K. Pradeep, Assistant Professor, Department of Botany, University of Calicut. Voucher

specimens were deposited at Calicut University Herbarium (CALI 59336, CALI 59337).

Preparation of leaf extracts

The fresh leaves were collected and washed thoroughly with tap water and air dried at room temperature. They were then powdered and 10 g powder was extracted with 100 ml hot water by boiling for 30 min to get the aqueous extract, which contains both polar and non-polar chemical principles. This was then filtered to remove particulate matter. Distilled water was used as medium for dilution. The non-polar components were selectively removed using diethyl ether so as to get extract containing polar components alone. Distilled water was used as negative control and 0.01 % methyl parathion was taken as positive control.

Allium cepa assay

The cytotoxic activity of the test plant extracts were screened using *Allium cepa* root tip meristematic cells. The bulbs were germinated over water before being transferred to each of the test plant extracts. When the roots were about 5 mm long, the bulbs were placed on beakers containing the leaf extracts of five different concentrations (0.125, 0.25, 0.5, 1.0, 2 %), such that the roots alone were immersed in the extracts. The duration of treatment of each extract was 3, 2, 1 h and 30 min. The sprouted roots were also treated with distilled water and 0.01 % methyl parathion, which served as negative and positive control, respectively. The experimental set up had five replicates.

Staining and microscopic parameters

The root tips were harvested after the treatment and fixed in Carnoy's fluid (1 part of glacial acetic acid: 2 parts of absolute alcohol) for 1 h and stored in 70 % alcohol. Mitotic squash preparations were conducted with the help of improved techniques (Sharma and Sharma 1990). The slides were scanned under a Leica DM 1000 trinocular research microscope (Leitz, Oberkochen, Germany) and photographs were taken.

The numbers of cells, dividing and non-dividing, were recorded. Incidence of chromosome aberrations was calculated by expressing the number of aberrant cells as a percentage of total dividing cells for each treatment. For MI, the different stages of mitosis were counted in at least 1000 cells/slide per concentration, and expressed as percentage. MI was calculated by expressing the number of dividing cells as a percentage of total cells counted for each of the treatments and control.

$$\text{Mitotic index} = \frac{\text{Number of dividing cell}}{\text{total number of cells}} \times 100$$

Macroscopic assessment of the root

The root initiation and growth assessment was done using the procedure of modified *Allium* test (Rank and Nielson 1993). The bulbs were planted directly on the extracts and their dilutions without initial rooting for 24, 48, 72 and 96 h. The experimental set up had five replicates. For macroscopic study, the root length was measured to the nearest millimeter using a graduated ruler every day for 96 h. The root form was studied to check for the presence of twists (crocket hooks), presence of swellings (c-tumors), broken root tips and color of root tips (white, pale and dark brown or black). Onion bulbs were allowed to germinate in distilled water for 24, 48, 72 and 96 h, which were used as negative control and 0.01 % methyl parathion was taken as positive control.

EC_{50} value and cytotoxicity test

EC_{50} is defined as the concentration that produces 50 % root growth inhibition. The *Allium* root growth inhibition test was carried out for determining the EC_{50} value of *Hyptis* and *Leucas* extracts. *A. cepa* bulbs were germinated in distilled water at room temperature (25 ± 5 °C) with a 15 h light/9 h dark photoperiod for 24 h. After the roots were homogeneously grown, 6 bulbs per treatment were exposed to different concentrations (0.125, 0.25, 0.5, 1 and 2 %) of *Hyptis* and *Leucas* aqueous extracts for 96 h. Test solutions and distilled water were changed every 24 h. The best developed 10 roots of each onion in each group were measured and mean root length calculated (negative and positive control as well as treatment group). Taking mean root length of control as 100 %. Length of different treatment groups was plotted against test concentrations. The point on the graph which showed 50 % growth was designated as EC_{50} concentration.

Statistical analysis

The data were subjected to statistical analysis using analysis of variance followed by appropriate post hoc tests. The means, with 95 % confidence limits and the standard errors for results of the root inhibition and chromosome aberrations of each concentration of the extracts were calculated. Data were expressed as mean ± standard error of mean (SEM). $P < 0.05$ was considered to be statistically significant. All statistical analyses were carried out using SPSS 22.0 statistical package.

Results

Macroscopic studies

Root-growth inhibition

The effect of aqueous extracts of *Hyptis* and *Leucas* on root elongation of *A. cepa* bulbs is summarized in Table 1. At the concentrations tested, a significant and dose-dependent reduction in length was observed after treatments with extracts, with an estimated effective concentration (EC_{50}) of 1.92 and 1.58 % for *Hyptis* and *Leucas*, respectively, whereas the negative control influenced the root growth only a little bit. This inhibition was significant in all concentrations from the 2nd day to the 4th day of treatment. The root bundles in the control sets had an average length of 5.92 ± 0.69 cm, after 96 h of hydroponic culture and the length of the roots treated with *Hyptis* extract was 1.96 ± 0.07 (2 %) and for *Leucas* extract it was 1.83 ± 0.17 (2 %). Macroscopic changes such as aberrant root forms were not observed in growing roots under any experimental condition.

The root growth was found to decrease with increase in the treatment concentration ($P < 0.05$). Reduction in root length was observed in both *Hyptis* and *Leucas* treatments. In *Hyptis* treatment, the mean root lengths observed at lowest and highest concentration at 24 h were 1.03 ± 0.03 cm (0.125 %) and 0.43 ± 0.07 cm (2 %) whereas for 96 h it was 3.33 ± 0.19 cm (0.125 %) and 1.96 ± 0.07 cm (2 %). For the same time period the mean root length shown by *Leucas* treatments at 24 h were 0.97 ± 0.03 cm (0.125 %) and 0.40 ± 0.07 cm (2 %). However for the 96 h treatment it was 2.8 ± 0.15 cm (0.125 %) and 1.83 ± 0.17 cm (2 %) (Table 1). Dose–response curves obtained between the concentrations of treatments and *Allium* root growth determined the effective concentration (EC_{50}) value which retards 50 % root growth as 1.92 % in *H. suaveolens* and 1.58 % in *L. indica*.

Table 1 Mean root length of *A. cepa* L. germinated in different concentrations of *H. suaveolens* and *L. indica* aqueous extracts

Treatment	Time duration (h)	*Hyptis* mean root length ± SE	*Leucas* mean root length ± SE
C^{+ve}	24	0.29 ± 0.02[a]	
	48	0.34 ± 0.03[a]	
	72	0.40 ± 0.36[a]	
	96	0.49 ± 0.57[a]	
C^{-ve}	24	1.33 ± 0.04[d]	
	48	2.17 ± 0.15[c]	
	72	2.26 ± 0.36[e]	
	96	5.92 ± 0.69[d]	
0.125 %	24	1.03 ± 0.03[c]	0.97 ± 0.03[c]
	48	2.1 ± 0.12[c]	1.89 ± 0.22[c]
	72	3.1 ± 0.10[d]	1.9 ± 0.19[c]
	96	3.33 ± 0.19[c]	2.8 ± 0.15[b,c]
0.25 %	24	0.96 ± 0.03[c]	0.93 ± 0.67[c]
	48	1.64 ± 0.07[b]	1.60 ± 0.17[b]
	72	2.27 ± 0.15[b,c]	1.8 ± 0.19[c]
	96	2.90 ± 0.12[c]	2.8 ± 0.15[b,c]
0.5 %	24	0.7 ± 0.12[b]	0.74 ± 0.13[b]
	48	1.50 ± 0.09[b]	1.2 ± 0.15[b]
	72	1.93 ± 0.03[b,c]	1.58 ± 0.03[b,c]
	96	2.77 ± 0.67[b,c]	2.63 ± 0.18[b,c]
1 %	24	0.92 ± 0.0[c]	0.64 ± 0.05[b]
	48	1.36 ± 0.16[b]	1.33 ± 0.14[c]
	72	2.41 ± 0.14[c]	1.48 ± 0.03[b]
	96	2.60 ± 0.16[b,c]	2.17 ± 0.17[b]
2 %	24	0.43 ± 0.55[a]	0.40 ± 0.07[a]
	48	1.45 ± 0.05[b]	1.21 ± 0.06[b]
	72	1.75 ± 0.07[b]	1.24 ± 0.05[b]
	96	1.96 ± 0.07[b]	1.83 ± 0.17[b]

C^{+ve}, positive control (0.01 % methyl parathion); C^{-ve}, negative control (distilled water). SE, standard error. Means within a column followed by the same letters are not significantly different ($P < 0.05$) as determined by DMRT (Duncan's multiple range test)

Table 2 Effect of varying concentrations of *H. suaveolens* and *L. indica* leaf extracts on the mitotic indices and percentage of chromosomal aberrations of *Allium cepa* root meristems

C	T	Number of Abnormal cells/1000 cells in different mitotic phases										Mitotic index % (±SE)		Abnormality % (±SE)	
		I		P		M		A		T					
		H	L	H	L	H	L	H	L	H	L	H	L	H	L
C^{+ve}	½	623		97		48		27		16		18.8 ± 5.45[a]		81.1 ± 2.5[a]	
	1	736		107		51		19		9		43.46 ± 19.98[a]		92.2 ± 1.2[a]	
	2	804		86		31		13		7		13.7 ± 6.79[a]		94.2 ± 2.28[a,b]	
	3	832		92		21		9		7		12.9 ± 7.3[a]		94.8 ± 0.9[a,b]	
C^{-ve}	½	3		–		–		–		–		76.9 ± 1.1[c]		0.3 ± 0.4[a]	
	1	2		6		1		2		–		77.3 ± 1.1[c]		1.2 ± 0.6[a]	
	2	7		1		–		–		–		78.1 ± 1.0[c]		0.9 ± 0.4[a]	
	3	8		–		1		–		–		76.6 ± 1.1[c]		0.9 ± 0.6[a]	
0.125 %	½	34	31	187	117	46	59	40	4	9	0	28.2 ± 0.9[b]	18.0 ± 0.47[b]	31.6 ± 0.89[b]	21.1 ± 0.39[b]
	1	101	14	129	139	99	37	21	20	3	4	25.2 ± 0.19[b]	20.0 ± 0.73[b]	35.3 ± 0.19[b]	21.4 ± 0.50[b]
	2	132	195	93	127	9	30	3	39	1	0	23.8 ± 0.73[b]	19.6 ± 0.59[b]	37.0 ± 1.52[b]	31.1 ± 0.58[b]
	3	144	211	146	109	74	52	11	16	7	2	23.8 ± 0.62[b]	17.9 ± 0.32[b]	38.2 ± 1.78[b]	39.0 ± 0.80[b]
0.25 %	½	104	9	197	137	36	54	6	9	2	0	24.1 ± 0.35[c]	20.0 ± 1.24[b,c]	34.5 ± 2.73[b]	20.9 ± 0.49[b]
	1	146	51	130	120	49	59	33	9	4	2	21.6 ± 0.68[c]	19.0 ± 0.34[b,c]	36.3 ± 3.41[b]	24.1 ± 1.01[c]
	2	157	152	112	99	17	70	24	19	4	0	21.4 ± 1.42[b]	18.8 ± 0.48[b]	37.1 ± 1.15[b]	34.0 ± 1.36[b]
	3	213	245	121	104	69	63	7	4	0	4	19.7 ± 0.58[c]	17.5 ± 0.52[b,c]	41.0 ± 4.41[b]	42.0 ± 1.59[b,c]
0.5 %	½	151	99	156	187	29	8	8	7	0	1	19.3 ± 0.37[d]	20.3 ± 1.46[b]	34.4 ± 1.17[b]	30.2 ± 0.89[c]
	1	81	171	117	102	53	53	12	40	4	4	18.6 ± 0.46[d]	19.9 ± 0.72[b]	36.7 ± 1.39[b]	37.0 ± 1.51[d]
	2	203	251	96	124	49	42	30	20	2	1	17.7 ± 0.72[c]	18.7 ± 0.62[b]	38.0 ± 2.78[b]	43.8 ± 1.56[c]
	3	275	283	99	99	37	29	3	30	0	9	13.9 ± 1.24[e]	16.7 ± 0.86[b,c]	41.4 ± 1.63[b]	45.0 ± 2.43[c]
1 %	½	215	116	127	106	53	63	10	24	3	1	19.3 ± 0.37[d]	19.4 ± 0.91[b,c]	40.8 ± 0.59[c]	31.0 ± 1.35[c]
	1	298	224	113	97	69	41	4	48	0	0	18.6 ± 0.46[d]	18.6 ± 0.35[b,c]	48.4 ± 2.05[c]	41.0 ± 0.38[e]
	2	317	277	109	84	47	60	20	15	1	2	17.7 ± 0.72[c]	16.1 ± 0.72[c]	49.4 ± 1.34[c]	43.8 ± 1.77[c]
	3	439	303	89	113	39	27	7	14	4	1	13.9 ± 1.24[e]	15.5 ± 1.12[c,d]	57.8 ± 1.18[c]	45.8 ± 1.92[c]
2 %	½	305	233	112	109	33	55	10	7	1	0	15.6 ± 0.37[e]	17.1 ± 1.14[c]	46.1 ± 0.69[d]	40.4 ± 0.40[d]
	1	338	330	77	110	70	63	3	5	0	2	15.0 ± 0.23[e]	18.0 ± 0.21[c]	48.8 ± 1.65[c]	51.0 ± 0.79[f]
	2	386	401	89	102	37	21	4	17	0	0	13.0 ± 0.89[d]	14.0 ± 0.78[c]	51.6 ± 1.16[c]	54.1 ± 1.25[d]
	3	459	420	80	87	17	24	7	16	2	3	10.6 ± 0.48[f]	13.0 ± 0.49[d]	56.5 ± 1.81[c]	55.0 ± 1.44[d]

C, treatment; T, time duration (h); C^{+ve}, positive control (0.01 % methyl parathion); C^{-ve}, negative control (distilled water); I, interphase, P, prophase; M, metaphase, A, anaphase, T, telophase; H, *Hyptis suaveolens*; L, *Leucas indica*; SE, standard error. Means within a column followed by the same letters are not significantly different ($P < 0.05$) as determined by DMRT (Duncan's multiple range test)

Microscopic studies

Mitotic index (MI)

The effect of both aqueous extracts of *Hyptis* and *Leucas* on the MI (%) of *Allium cepa* root meristem cells was also determined (Table 2). There were significant differences between both tested extract concentrations and the control ($P < 0.05$). MI significantly decreased in both cases compared to control at each exposure time. The percentage of MI was significantly lower after the treatment with both the extracts of *Hyptis* and *Leucas* at a concentration of 2 % for 3 h and it was found to be 10.6 ± 0.48 and

13.0 ± 0.49, respectively, when compared to other concentrations at 1/2, 1, 2 and 3 h.

The inhibition of cell division by different concentrations of *Hyptis* and *Leucas* extracts on treated cells was confirmed by MI values, which were lower than control (78.1 ± 1.0). Significant reduction in the MI was observed in both treatments, in which *Hyptis* treatment revealed maximum reduction in MI (10.6 ± 0.48) at a concentration of 2 % at 3 h. In *Leucas* treatments, MI reached 13.0 ± 0.49 at the highest concentration (2 %) after treatment for 3 h.

Effects on chromosome aberrations

The results showed that the aqueous extract of both selected plants had excellent cytotoxic activity. Clastogenic and non-clastogenic abnormalities have been observed (Fig. 1). Tables 2, 3 and 4 shows the data on the mitotic indices, percentage and number of types of chromosome aberrations observed in *A. cepa* root tip cells treated with the aqueous leaf extracts of the test plant material. Major CA noticed are nuclear lesion, binucleate cell, chromosome bridge, abnormal condensation, hyperchromasia, chromosome pulverisation. Minor aberrations include-aberrant cell wall formation, ball metaphase, chromosome fragments and shift in microtubule-organizing center (MTOC), chromosome laggards and vagrants, coagulation, misorientation, dissolution of chromosome, formation of macro and micro cells, nuclear budding, nuclear fragmentation and diagonal metaphase and anaphase. The total chromosome aberrations (%) were significantly higher at the highest concentration. The results revealed the fact that the treatments with the plant extracts showed extremely significant chromosome abnormality ($P < 0.05$) when compared to the negative control and were lower when compared to positive control. Thus, it can be stated that the clastogenic and non-clastogenic abnormalities observed were due to the effect of toxic chemicals present in both tested extracts.

The mitotic indices of all the extract treated roots were significantly lower than that of the control. Also, the MI values were observed to be decreasing with increasing concentrations of the extracts. Whereas the number of aberrant cells were observed to be increasing with the concentration of the extract. The MI of *L. indica* at higher concentration (2 %) was 17.1 ± 1.14, 18.0 ± 0.21, 14.0 ± 0.78, 13.0 ± 0.49 and at lower concentration (0.125 %) was 18.0 ± 0.47, 20.0 ± 0.73, 19.6 ± 0.59, 17.9 ± 0.32 and that of *H. suaveolens* at higher concentration (2 %) was 15.6 ± 0.37, 15.0 ± 0.23, 13.0 ± 0.89, 10.6 ± 0.48 and at lower concentration (0.125 %) was 28.2 ± 0.9, 25.2 ± 0.19, 23.8 ± 0.73, 23.8 ± 0.62 for 30 min, 1, 2, 3 h, respectively. These results show that both extracts are cytotoxic but *L. indica* shows lowest MI at lower concentration, as a consequence it can be concluded that *L. indica* is more cytotoxic than *H. suaveolens*. Cell division was normal in the root tips kept as control.

A one way ANOVA showed that there was a significant effect of the treatment on mitotic activity. Post-hoc analysis using Duncan's multiple range test showed that the activity of all the different extracts were significant when compared with that of control.

Discussion

The CA (Fig. 1) induced in the treated onion root cells were definitely caused by the chemical ingredients in the aqueous leaf extracts of the tested plant species, since such aberrations were not observed in the control. The reduction of MI might be explained as being due to the obstruction of the onset of prophase, the arrest of one or more mitotic phases, or the slowing down of the rate of cell progression through mitosis (Yuet-Ping et al. 2012) and interfere in the normal cell cycle resulting in a decrease in the number of dividing cells (Sharma and Vig 2012b).

The MI is a reliable parameter which allows estimating the frequency of cellular division (Fernandes et al. 2007). MIs higher than negative control may be derived from the result of the induction of cell division while MIs lower than negative control may be derived from the growth and development of exposed organisms having been affected by test compounds (Hoshina 2002). The cytotoxic level of a test chemical/compound can be determined based on the increase or decrease in the mitotic index (MI), which can be used as a parameter of cytotoxicity in studies of environmental biomonitoring (Amin 2002). Significant reduction in MI, noted in the present study may be due to the inhibition of DNA synthesis or the blocking in the G2 phase of the cell cycle (Sudhakar et al. 2001). Several other chemicals have been reported to inhibit mitosis (Turkoglu 2007a, b). The cytotoxic level can

be determined by the decreased rate of MI. A MI decrease below 22 % of the negative control causes lethal effects on test organism while a decrease below 50 % has sublethal effects (Sharma and Vig 2012a) and is called cytotoxic limit value. Several investigators have used MI as an endpoint for the evaluation of genotoxicity or antigenotoxicity of different chemical treatments (Sharma et al. 2012a, b).

In this study both *Leucas* and *Hyptis* extracts showed changes in the MI that depended on the duration of treatment and concentration of the extract. Root length is an important parameter, reflecting the toxicity of the additive in the elongation zone of onions, which may serve as a sensitive external signal of ongoing internal cellular events. Root growth in *A. cepa* like other plants is due to expansion of cells in the elongation zone of the root tip where cellular differentiation occurs (Cordoba-Pedrosa et al. 2004). The biological processes involved in cellular expansion include water uptake, nitrogen mobilization, increased sugar synthesis, plasma and tonoplast membrane flexibility etc. (Budentsev et al. 2010; Gonzalez-Reyes et al. 1999). Plant roots interact with each of the physical factors in their environment and this interaction can lead to modification of the roots. Metabolites such as ascorbate and enzymes such as asparagine synthase and membrane ATPases have been described as promoters and mediators of these biological processes (Budentsev et al. 2010). Alterations in these biological processes, including disrupted lipid biosynthesis by plant extracts and toxins have been linked to reduced cell wall expansibility, loss of vacuolar homeostatic regulation, cellular cytotoxicity, cell necrosis and root growth inhibition (Gonzalez-Reyes et al. 1999).

Plant extracts and toxins have been described to impact negatively on these processes to cause root growth inhibition/arrest (Sehgal et al. 2006). In the *A. cepa* assay, inhibition of root growth has been shown to indicate retardation of growth and cytotoxicty (Grant 1999; Odeigah et al. 1997). Also, growth inhibition can be caused by a reduction in mitotic activities and occurrence of various CA. Therefore, the observed inhibition of root growth by *Hyptis* and *Leucas* aqueous plant extracts in this study suggests that the extract contains substances that impair one or more biological processes which mediate cell expansion and differentiation at the elongation region of *A. cepa* root tip.

Fig. 1 Chromosomal aberrations induced by aqueous leaf extracts of *H. suaveolens* and *L. indica.* **a** Aberrant cell wall formation at cytokinesis, **b** abnormal condensation at metaphase, **c** ball metaphase, **d** binucleate cell, **e** chromosome bridge at anaphase, **f** chromosome bridge at early telophase, **g** chromosome fragments at early telophase and shift in MTOC (microtubule-organizing center), **h** chromosome laggards and vagrants at anaphase, **i** chromosome pulverisation, **j** coagulated telophase, **k** diagonal chromosome bridge at telophase, **l** diagonal metaphase, **m** dissolution of chromosomes and misorientation at telophase, **n** disturbed metaphase, **o** double nuclear lesion, **p** formation of macro and micro cells at cytokinesis, **q** hyperchromasia at interphase, **r** nuclear budding at interphase, **s** nuclear fragmentation at interphase, **t** trinucleate cell at interphase

Major types of chromosome aberrations were observed in this study and are discussed. Earlier reports suggest that the presence of nuclear lesions offer cytological evidences for the inhibitory action on DNA biosynthesis (Akaneme and Iyioke 2008). Ball metaphase results from the complete destruction of spindle fibres and a subsequent clumping of chromosomes into a tight ball. Chromosome bridges may be caused by stickiness of chromosome which makes their separation and free movements incomplete and thus they remain connected by bridges (Hoseiny Rad et al. 2011). Jabee et al. (2008) also reported that chromosome bridges may arise due to stickiness or due to formation of dicentric chromosome by breakage and reunion. Inhibition of cytokinesis following telophase is responsible for binucleated cell formation (Majewska et al. 2003).

Akaneme and Amaefule (2012) reported the occurrence of nuclear lesions which may be due to the inhibitory action of phytochemicals on DNA biosynthesis. Chromosome or chromatin fragmentation is the result of breakage at the fragile sites (Lukusa and Fryns 2008). The changes brought about in the viscosity of cytoplasm are responsible for CA like fragmentation during anaphase, micronuclei formation, multinucleate cells and unequal grouping of chromosomes.

In plants, the absence of organelles such as the centrosome has led to the belief that MTOCs originate on the nuclear envelope and are transported to specific intracellular locations by microtubule proteins (Mathur and Chua 2000). Alternatively, spontaneous and de novo assembly of such MTOCs may occur in the cell by enhanced microtubule stability (Rieder et al. 2001). Studies in both plant and animal system

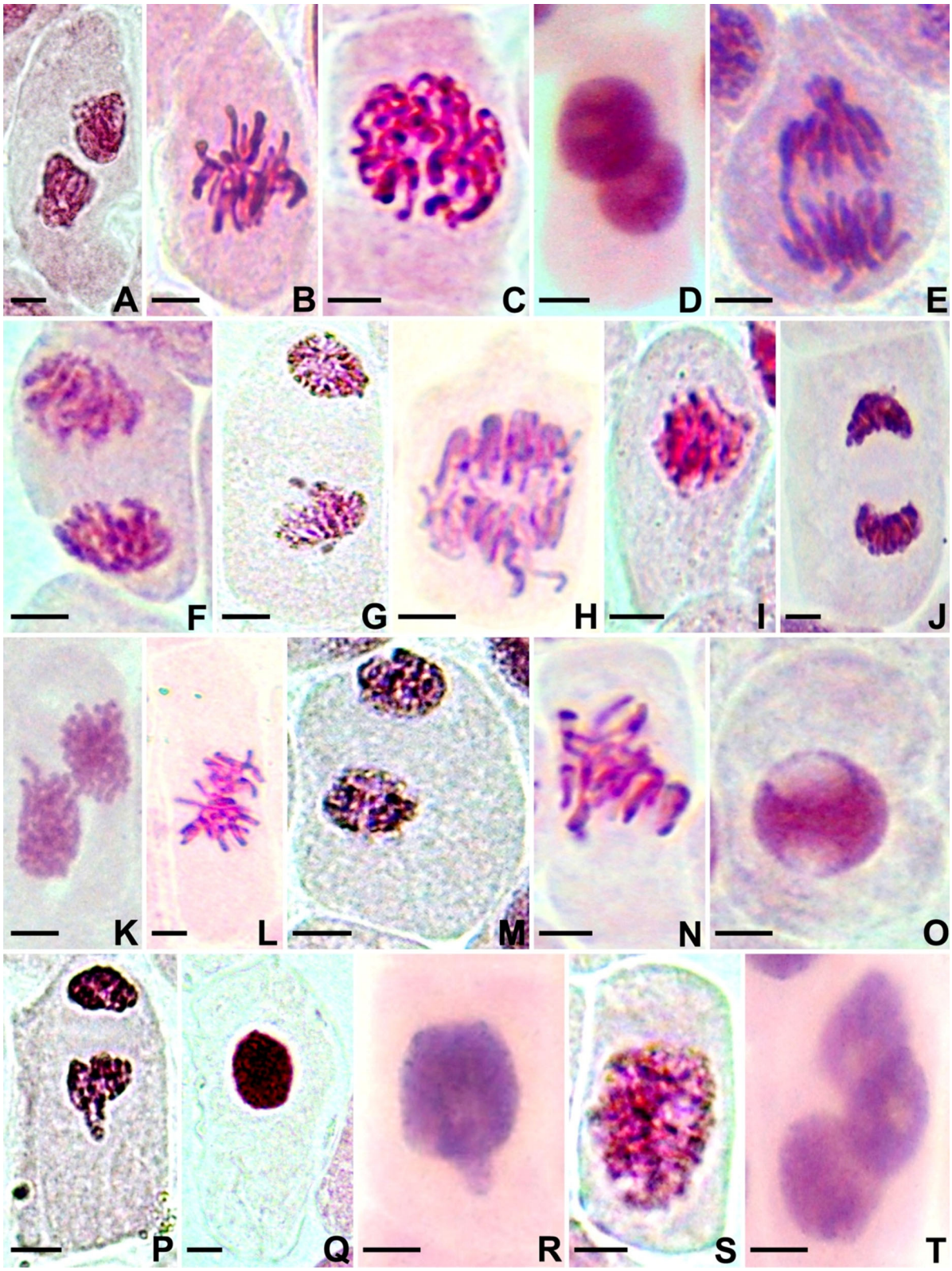
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Table 3 Effect of varying concentrations of *H. suaveolens* leaf extracts on number and types of chromosomal aberration in *A. cepa* root meristems

Conc	T (h)	Total number of cells	Abnormal cells							Abnormality % (±SE)
			Major aberrations						Minor aberrations	
			NL	BC	CB	AC	HC	CP		
C^{+ve}	½	1000	352	235	94	–	89	17	24	81.1 ± 2.5^{a}
	1	1000	541	176	82	12	32	–	79	92.2 ± 1.2^{a}
	2	1000	493	217	–	118	47	–	67	$94.2 \pm 2.28^{a,b}$
	3	1000	448	218	39	–	75	34	134	$94.8 \pm 0.9^{a,b}$
C^{-ve}	½	1000	–	–	–	–	–	3	–	0.3 ± 0.4^{a}
	1	1000	4	–	–	2	–	–	6	1.2 ± 0.6^{a}
	2	1000	2	–	–	–	–	–	7	0.9 ± 0.4^{a}
	3	1000	3	–		1	–	–	5	0.9 ± 0.6^{a}
0.125 %	½	1000	221	46	46	–	–	–	3	31.6 ± 0.89^{b}
	1	1000	230	–	–	99	–	–	24	35.3 ± 0.19^{b}
	2	1000	132	93	13	–	23	32	77	37.0 ± 1.52^{b}
	3	1000	144	146	10	–	–	–	82	38.2 ± 1.78^{b}
0.25 %	½	1000	104	197	2	36	–	–	6	34.5 ± 2.73^{b}
	1	1000	276	–	26	–	–	–	61	36.3 ± 3.41^{b}
	2	1000	269	37	20	–	12	8	25	37.1 ± 1.15^{b}
	3	1000	121	–	7	69	213	–	–	41.0 ± 4.41^{b}
0.5 %	½	1000	151	156	–	–	–	29	8	34.4 ± 1.17^{b}
	1	1000	181	117	16	–	–	–	53	36.7 ± 1.39^{b}
	2	1000	96	–	–	–	203	49	32	38.0 ± 2.78^{b}
	3	1000	374	–	–	–	–	–	40	41.4 ± 1.63^{b}
1 %	½	1000	342	–	13	–	–	–	53	40.8 ± 0.59^{c}
	1	1000	113	–	4	–	–	–	367	48.4 ± 2.05^{c}
	2	1000	109	–	–	–	–	47	338	49.4 ± 1.34^{c}
	3	1000	439	128	11	–	–	–	–	57.8 ± 1.18^{c}
2 %	½	1000	417	–	8	33	–	–	3	46.1 ± 0.69^{d}
	1	1000	100	77	3	–	–	–	308	48.8 ± 1.65^{c}
	2	1000	85	89	–	–	301	–	41	51.6 ± 1.16^{c}
	3	1000	539	17	–	–	–	–	9	56.5 ± 1.81^{c}

C, concentration; T, time duration (h); C^{+ve}, positive control (0.01 % methyl parathion); C^{-ve}, negative control (distilled water); NL, nuclear lesion, BC, binucleate cell, CB, chromosome bridge, AC, abnormal condensation, HC, hyperchromasia, CP, chromosome pulverisation. Minor aberrations include—aberrant cell wall formation, ball metaphase, chromosome fragments and shift in MTOC (microtubule-organizing center), chromosome laggards and vagrants, coagulation, misorientation, dissolution of chromosome, formation of macro and micro cells, nuclear budding, nuclear fragmentation and diagonal metaphase and anaphase. SE, standard error. Means within a column followed by the same letters are not significantly different ($P < 0.05$) as determined by DMRT (Duncan's multiple range test)

have suggested that stable microtubules form an integral component of microtubule organizing centres or MTOCs.

According to Lera and Burkard (2012) lagging of chromosomes is due to abnormal spindle activity. The failure of normal organization and function of spindle apparatus may lead to formation of laggards (Neelamkavil and Thoppil 2014). Vagrant chromosomes are indicators of spindle poisoning (Rank 2003a, b). Rybaczek and Kowalewicz-Kulbat (2011) suggested that pulverisation of chromosomes is due to the premature condensation of chromosomes as a result

Table 4 Effect of varying concentrations of *L. indica* leaf extracts on number and types of chromosomal aberration in *A. cepa* root meristems

C	T (h)	Total number of cells	Abnormal cells							Abnormality %
			Major aberrations						Minor aberrations	
			NL	BC	CB	AC	HC	CP		
C^{+ve}	½	1000	352	235	94	–	89	17	24	81.1 ± 2.5[a]
	1	1000	541	176	82	12	32	–	79	92.2 ± 1.2[a]
	2	1000	493	217	–	118	47	–	67	94.2 ± 2.28[a,b]
	3	1000	448	218	39	–	75	34	134	94.8 ± 0.9[a,b]
C^{-ve}	½	1000	–	–	–	–	–	3	–	0.3 ± 0.4[a]
	1	1000	4	–	–	2	–	–	6	1.2 ± 0.6[a]
	2	1000	2	–	–	–	–	–	7	0.9 ± 0.4[a]
	3	1000	3	–		1	–	–	5	0.9 ± 0.6[a]
0.125 %	½	1000	–	117	3	–	–	–	91	21.1 ± 0.39[b]
	1	1000	100	39	16	–	14	37	8	21.4 ± 0.50[b]
	2	1000	127	30	21	–	–	–	133	31.1 ± 0.58[b]
	3	1000	320	–	17	52	–	–	1	39.0 ± 0.80[b]
0.25 %	½	1000	146	–	–	–	–	–	63	20.9 ± 0.49[b]
	1	1000	171	–	7	–	–	–	63	24.1 ± 1.01[c]
	2	1000	141	99	–	–	–	–	100	34.0 ± 1.36[b]
	3	1000	227	104	7	–	–	–	82	42.0 ± 1.59[b,c]
0.5 %	½	1000	187	–	7	–	99	–	9	30.2 ± 0.89[c]
	1	1000	273	–	37	53	–	–	7	37.0 ± 1.51[d]
	2	1000	375	–	–	–	–	–	63	43.8 ± 1.56[c]
	3	1000	382	–	–	–	–	29	39	45.0 ± 2.43[c]
1 %	½	1000	–	106	–	–	116	63	25	31.0 ± 1.35[c]
	1	1000	321	–	40	41	–	–	8	41.0 ± 0.38[e]
	2	1000	84	102	15	20	34	–	183	43.8 ± 1.77[c]
	3	1000	303	113	–	–	–	27	15	45.8 ± 1.92[c]
2 %	½	1000	233	109	4	55	–	–	3	40.4 ± 0.40[d]
	1	1000	330	110	5	–	–	–	65	51.0 ± 0.79[f]
	2	1000	503	–	–	21	–	–	17	54.1 ± 1.25[d]
	3	1000	507	–	16	–	–	24	3	55.0 ± 1.44[d]

C, concentration; T, time duration (h); C^{+ve}, positive control (0.01 % methyl parathion); C^{-ve}, negative control (distilled water); NL, nuclear lesion, BC, binucleate cell, CB, chromosome bridge, AC, abnormal condensation, HC, hyperchromasia, CP, chromosome pulverisation. Minor aberrations include—aberrant cell wall formation, ball metaphase, chromosome fragments and shift in MTOC (microtubule-organizing center), chromosome laggards and vagrants, coagulation, misorientation, dissolution of chromosome, formation of macro and micro cells, nuclear budding, nuclear fragmentation and diagonal metaphase and anaphase. *SE* standard error. Means within a column followed by the same letters are not significantly different ($P < 0.05$) as determined by DMRT (Duncan's multiple range test)

of the action of chemical substances found in the extract.

Coagulation of chromosomes is an after effect of chromosome stickiness, where the chromosomes seem to be adhering together to form an intact mass of aberrant chromosome group. Coagulation of chromosomes suggests that changes have occurred in the viscosity of their constituent materials. It has frequently been assumed that such changes in viscosity are due to depolymerisation of deoxyribonucleic acid. Moreover, Ramesh et al. (2012) stated that some plant extracts can cause DNA depolymerization and

partial dissolution of nucleoproteins, breakage and exchanges of the basic folded units of chromatids and the stripling of the protein covering of DNA in chromosomes. In other cases, most chromosome breaking agents also have the ability to affect the synthesis, state or structure of DNA.

Chromosome bridges arise as a result of stickiness, which hinders separation of chromosomes. Telophase bridges were observed by Cabaravdic (2010) when exposing *Allium cepa* cells to Benzo(a)pyrene. According to Teerarak et al. (2010) the improper functioning of spindle apparatus causes the diagonal orientation of chromosomes. This phenomenon has also been observed on treatment with extracts of other plants as well (Leeja and Thoppil 2004; Asha and Thoppil 2011).

Misorientation of chromosomes can be due to the disturbed functioning of the spindle apparatus, a tilt in the equatorial organization of metaphase chromosomes or a change in the direction of movement of daughter chromosomes during anaphase. Disturbed metaphase may be the result of disturbances in the spindle apparatus or due to inhibition of spindle formation (Hsieh et al. 2012; Cuyacot et al. 2014; Tripathy and Rao 2015). Adam and El-Ashry (2010) attributed macro and micro cells to the failure of normal organization and function of spindle apparatus.

Hyperchromasia is the most distinguishable state of aberration, where the nucleus takes up intense stain than normal, probably due to heterochromatinisation. It is an extremely condensed and thereby deeply staining state of the nucleus observed during stress induced by the influence of toxic environmental chemicals or during incompatible conditions. Progressive heterochromatinisation seems to be responsible for this aberration (Gernand et al. 2005).

Naturally occurring nuclear budding, apart from the induced bud or appendage formation occurs as a result of the selective entrapment of extra chromosomal amplified DNA by the nucleus and which can probably end in micro nucleation during S-phase. Probably these nuclear buds arise as a result of excessive production of nucleic acids and proteins, induced by the cytotoxicants (Fenech et al. 2011).

Mitotic index is an acceptable measure of cytotoxicity in all living organisms. The cytotoxicity levels can be determined by the decreased rate of MI. MI was found to be decreasing with increasing extract concentration and duration of treatment. MI observed in the treated root tip meristems were significantly different from the control group. Significant difference in the percentage of CA was also observed in the test samples, when compared to the distilled water control.

The decreased MI values in the treated onion root may be an indication of the presence of cytotoxic substances in the aqueous leaf extracts, which causes inhibition of mitotic activities, while the aberrant cells in the treated onion root tip meristems indicates genotoxic effects of the leaf extract (El-Shahaby et al. 2003). Chromosome aberrations were observed in all stages of mitosis. The abnormalities of chromosomes could be due to the blockage of DNA synthesis or inhibition of spindle formation. The results of the present investigation suggest that aqueous leaf extract containing polar and non-polar compounds showed maximum cytotoxic potential than those containing polar compounds alone. The results also revealed that extracts of *L. indica* is more cytotoxic than *H. suaveolens*. The mitodepressive effects induced by the plant extracts suggest that it has some effect on the cell division of *A. cepa* which may be due to the conditions induced by the chemical components of the extracts.

Volatile terpenic and phenolic components of essential oils can function as prooxidants by affecting the cellular redox status. This may lead to late apoptosis and/or necrosis including damage to proteins and DNA and overall cytotoxic effects (Bakkali et al. 2006). Generally, the major components are found to reflect quite well the biophysical and biological features of the essential oils from which they were isolated (Ipek et al. 2005), the amplitude of their effects being just dependent on their concentration when they were tested alone or comprised in essential oils. Thus, synergistic functions of the various molecules contained in an essential oil, in comparison to the action of one or two main components of the oil, seems questionable. However, it is possible that the activity of the main components is modulated by other minor molecules (Hoet et al. 2006). Moreover, it is likely that several components of the essential oils play a role in defining the fragrance, the density, the texture, the colour and above all, cell penetration (Cal 2006), lipophilic or hydrophilic attraction and fixation on cell walls and membranes, and cellular distribution. This last feature is very important because the distribution of the oil in the cell determines the different types of radical

reactions produced, depending on their compartmentation in the cell. In that sense, for biological purposes, it is more informative to study entire oil rather than some of its components because the concept of synergism appears to be more meaningful.

Until now, because of their mode of action affecting several targets at the same time, generally, no particular resistance or adaptation against essential oils has been described.

Conclusion

Cytotoxicity assessment is a warning against the non-judicious use of plants in folk medicine (Neelamkavil and Thoppil 2014). Further studies are required to isolate the compounds, responsible for the cytotoxic activity, from these plants and establish the activity of the isolated compounds in vivo and in vitro with different animal systems. Present study results can be successfully utilized in developing safe plant based bio-pesticides in public health sector and more works are also essential to prove the specificity of these plants to be used as ecofriendly pesticidal agents.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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