

**Research Article**



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**Evaluation of the cytotoxic and genotoxic effects of Cinnamon aqueous extract in *Allium cepa* and *Vicia faba***

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**Abstract**

In the present study, the effect of various concentrations of cinnamon bark extracts (10-150%) for 6, 12 and 18 hrs duration was investigated on seed germination and radicle growth of *Allium cepa* and *Vicia faba*. Moreover, we achieved a genotoxicity study at cytological level in root meristems of both plants by means of traditional cytogenetic approach, to evidence possible alterations in mitotic activity, chromosomal aberrations, and micronuclei release. From these analyses it comes out that the cinnamon extract, after short-term exposure and under our experimental conditions, in both plants the germination percentage was significantly reduced with all treatments and radicle elongation was affected and significantly inhibited and the *Allium cepa* was more affected than *Vicia faba*. Both investigated criteria were concentration and treatment time dependent. The reduction was correlated with a decrease in the mitotic index where the lowest mitotic index value was found to be at 150% of extract treatment for 18 hrs in both plants. A dose-dependent increase in total mitotic aberrations was also observed according to concentration and time of treatment of the extract. Significantly higher frequencies of cells with mitotic aberrations indicated the primary action of cinnamon to involve chromatin organization and mitotic spindles, leading to the induction of several abnormalities. The various abnormalities scored were irregular prophase, stickiness, C-mitosis, bridges, diagonal and lagging or vagrant chromosomes. Micronucleated cells were also observed at interphase and other phases. The induction of chromosomal aberrations that evidenced a cinnamon induced cytotoxic and genotoxic effect for both tested plants.

**Keywords:** Cinnamon bark, *Allium cepa*, *Vicia faba*, seed germination, radicle length, root tip cells, mitotic activity, chromosomal aberrations

**Introduction**

Many of the plants species used in the medicinal have been found to contain therapeutic substances which can be extracted and used in preparation of drugs, but the plant itself can also be used either directly or as an extract for medication, a practice that is particularly popular in developing countries (Hoyos *et al.*, 1992). In this concern, there is increasing evidence that many current chemically synthesized medicines simply suppress symptoms of the diseases and ignore the underlying causes. Therefore, crude plant extracts in the form of decoction, infusion or tincture are traditionally more used by the population for the

treatment of several diseases, as well as an anti-inflammatory and healing agent (Holetz *et al.*, 2005). Despite preliminary findings about therapeutic advantages of medicinal plants, some of their constituents may be potentially toxic, mutagenic, carcinogenic, or teratogenic (Gadano *et al.*, 2006). Therefore, therapeutical plants must be tested with regard to quality, safety and efficiency, like conventional drugs (Simaan, 2009). As a general rule crude therapeutic products are less toxic than their synthetic counterparts because they are found in their natural source and hence offer less risk of side effects

(Koul *et al.*, 2005). Plant extracts have great potential as antimicrobial compounds against several pathogenic microorganism which cause infectious disease and resistant towards synthetic drugs. Plants have been considered as raw materials for alternative medicine and have antimutagenic effects against chemicals and environmental factors. On the other hand, these extracts may also have mutagenic and cytotoxic effects on microorganisms, plants and human cells (Lee, *et al.*, 2004).

In screening for genotoxic and cytotoxic effects, extracts of different plant parts have been used, ranging from leafy vegetables, fruits, and underground storage organs to whole plants. The extracts were prepared mainly in water or organic solvents. The short term tests for genotoxicity and cytotoxicity are typically used to identify potential mutagens and carcinogens, but the same methods can also be used to identify anti-genotoxic agents. The effects of toxicants can be observed at the level of chromosomes (clastogenesis) through alterations in chromosome structure (chromosomal aberrations) and number (aneuploidy, polyploidy). A wide range of short-term and long term screening procedures is available (Askin Celik, 2012). This raises concern about the potential toxic effects resulting from the short-term and long-term use of such plants. Therefore, evaluating the toxicological effects of any herbal extract intended to be used in humans is of utmost importance. Thus, an assessment of their cytotoxic and mutagenic potential is necessary to ensure a relatively safe use of medicinal plants.

Cinnamon is used to treat nausea and diarrhea and in wound healing (Kamath *et al.*, 2003) and it has anti-bacterial and anti-fungal properties (Nir *et al.*, 2000 and Thakur *et al.*, 2013). It also showed anti-inflammatory (Tung *et al.*, 2008 and Thakur *et al.*, 2013), antioxidant (Singh *et al.*, 2007, Su *et al.*, 2007 Thakur *et al.*, 2013, Sathya *et al.*, 2014 and Lamfon, 2014) and hypotensive effect (Preuss *et al.*, 2006). The hepatoprotective of cinnamon was reported by some investigators (Eidi *et al.*, 2012, Sakr and Al-Barakati, 2014). It is not an easy task to study chemical pollution that contains complex organic–inorganic mixtures and assess its genotoxicity (Minissi *et al.*, 1998). Sensitive plant bioassays, as well as various other systems, may be suitable for measuring the genotoxicity of aqueous leachates. Several plant species have been used as bioindicators, and a variety of tests, including cytogenetic tests, have been employed (Juchimiuk and Maluszynska, 2005). These tests are commonly used to biomonitor the extent of

pollution and to evaluate the toxicity and mutagenicity of environmental contaminants on vegetal organisms (Liu *et al.*, 2005b; Matsumoto *et al.*, 2000). In addition, roots are the primary point of contact with contaminants (Liu *et al.*, 2005b).

The genus *Cinnammum* is belonging to family Lauraceae and represented by about 250 species worldwide many of them are aromatic and flavouring (Lee and Balck, 2005). It is native to South–East Asia, some Pacific islands and Australia growing mainly in tropical rain forests at varying altitudes. Lauraceae is an economically important family consisting mostly of trees or tree-like shrubs. There are no or very few reports, to date, of plant bioassays that have been used to detect the toxic effects of the cinnamon (*Cinnammum zeylanicum*) aqueous extracts on mitotic division in plant. However, there is great interest in identifying toxicity to safety use it and byproducts, to avoid environmental toxicity and to increase the value of these byproducts. *Allium cepa* and/or *Vicia faba* test plant is one of reliable model for screening drugs, chemical, pollutants and contaminations because root growth inhibition and adverse effects on chromosomes provide likely indication of toxicity.

The purpose of the present investigation was to evaluate the influence of cinnamon extract concentrations on somatic cells of *Allium cepa* and *Vicia faba*, since the most pronounced effect of this extract on human being rather than on plant development is growth inhibition, which is inseparably connected with cell division. Thus, the aim of this study was to investigate the effects of cinnamon aqueous extracts on seed germination, radicle growth and the cell cycle of onion and broad bean to establishing a screening test to evaluate the toxicity of this plant extract.

## Materials and Methods

**Prepare the extract of cinnamon:** Bark of cinnamon is obtained from the local supplier; the bark is crushed manually in a mortar with a pestle. A volume of 100 ml of distilled water was added to 20 g of dry powder. It was vortexed continuously until there was no further change in color of the solution. This solution was centrifuged at 5000 rpm for 15 min. The supernatant (brownish-orange in color) was filtered through Whatman filter No.1 using Buchner funnel and stored at 4 °C in sterile tubes until use.

**Seed Germination Test:** To evaluate the rate of seed germination of onion and broad bean under the effect

of plant extract, seeds of the plant presoaked in distilled water for 6hrs were placed directly in different concentrations of cinnamon, ranging from 10 to 150% for different treatment times (6, 12 and 18hrs). For each treatment, a triplicate of 25 seeds was used. Seeds were surface sterilized with 10% sodium hypochlorite for ten minutes then washed with sterile distilled water and placed on sterile filter paper in the Petri dishes. Fresh test solutions were added to the Petri dishes and the plates were placed in the dark for different treatment times at  $25 \pm 1^\circ\text{C}$  to facilitate linear growth. The treated seeds (25 for each treatment) were washed carefully with distilled water then transferred to Petri-dishes containing filter paper moistened with distilled water and allowed to germinate at room temperature  $25 \pm 1^\circ\text{C}$  for 5-7 days. Control seeds were treated with distilled water. Radicle length 5 mm was considered as germinated. The germinated seeds were counted and the percentages were calculated.

**Radicle Growth Investigation:** To study the effect of different concentration of plant extract on radicle growth: The seeds were germinated in distilled water till appearance the radicle. Twenty five of the germinated seeds were immersed in suitable amount of each tested concentrations of plant extract of cinnamon (10, 25, 50, 75,100 and 150 %)for the different times of 6 , 12 and 18 hours. Similarly 25 germinated seeds were soaked in distilled water for the same period was run with each treatment as the control. Following the treatments, the treated seedlings together with each control samples were kept in the dark at  $23\text{-}25^\circ\text{C}$ , in order to minimize the fluctuation in the rate of cell division (Evans *et al.*, 1957). At the end of each treatment time, the length of the radicle was measured. The relative change of radicle length was calculated as a percentage of the variance from the control or expressed as percent of controls (T/C ratio).

**Cytological Examination:** Ten germinated seeds, with radicle 2-3 cm long, were treated with different concentrations for different times. Control germinated seeds were placed in distilled water. After each treatment, the roots were cut off and immediately fixed in glacial acetic acid: absolute ethyl alcohol (1:3 v/v) for overnight. The root tips were stained by using the Feulgen squash technique. At least three slides for each treatment were examined to determine the mitotic index (MI), limited of mitotic inhibition and the frequency of mitotic phases. Dividing cells in the same slides were analyzed for determination of the

percentage of different types of abnormalities and their total percentages of abnormalities were also calculated.

**Statistical Analysis:** Each treatment was made in three replicates. For statistical analysis, one-way ANOVA (Sigma Plot13.0 software) SPSS was used to determine significance at  $p < 0.05$  (Duncan, 1955).

## Results

The effects of different concentrations of cinnamon bark aqueous extracts (10, 25, 50, 75,100 and 150%) for different treatment times (6, 12 and 18 hours) on seed germination and radicle growth of *Allium cepa* and *Vicia faba* were studied in Petri plates and the results given in Table (1). From the results, it was observed that different concentrations of cinnamon aqueous extract at different times caused a markedly decrease the germination percentage and radicle length of both plants from the control value with increase of the extract concentration.

In *Allium cepa*, all treatments affected and significantly inhibited germination process (Table 1). The germination percentage was less than the control value and recorded a value of about 50%at concentrations more than 25%, following treatment for 12 and 18 hrs then the percentage was decreased at higher concentrations till reached a value of 12 % with 150% concentration at 18 hrs. In the same manner, the radicle growth was inhibited and its length as express by T/C gradually decreased from the control value of 100% until reached to the value of 18.08% with the higher concentration of 150 % for 18 hrs. Also at 6 and 12 hrs treatment periods, the radicle length decreased gradually from the control value of 100 % to a minimum value of 37.66 and 24 % with 150 % concentration, respectively.

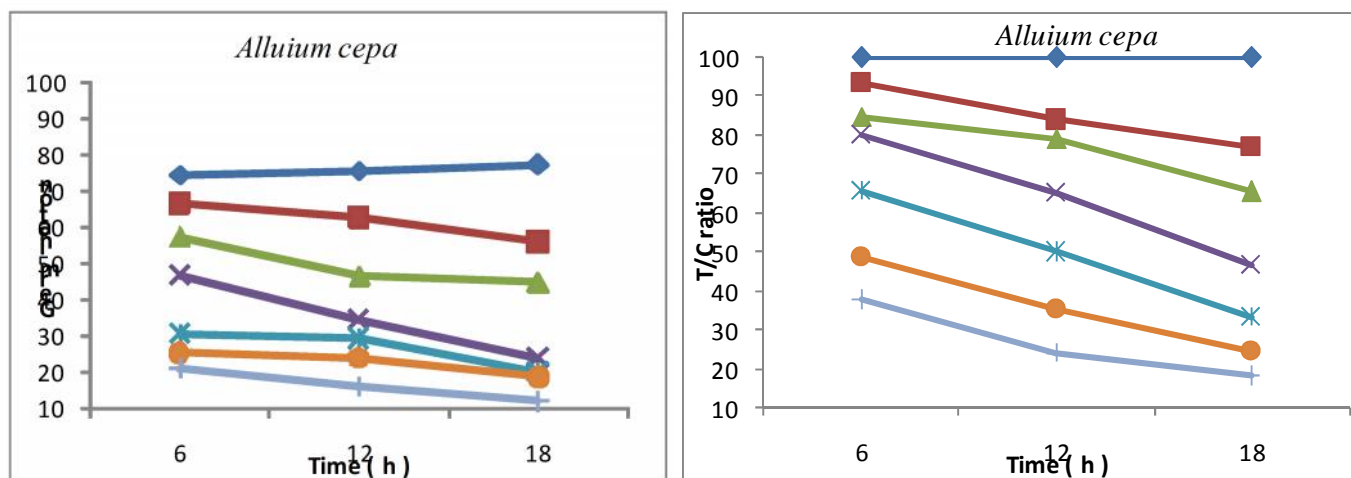
In *Vicia faba*, germination percentage of seeds was significantly decreased with all treatments (Table 1). The germination percentage was concentration and time dependent. The germination percentage significantly was reduced to the value below 50% at 75 % concentration with 6 hrs, and at 75% and 25% with 12 and 18 hrs, respectively. At 6 hrs, germination percentage of seeds was reduced to the value of 26.67 % with 150 % concentration compared with control value of 77.33%. At the higher concentration with the 12 and 18 hrs treatment times, germination percentage of *Vicia faba* seeds reduced to the value of 24 % and 17.33 % compared with control value of 82.67% and 89.33%, respectively. Similarly, the radicle length of

germinated seeds of *Vicia faba* was gradually decreased from the control value of 100 % until reached to a minimum value of 40.56,32.53 and 21.07

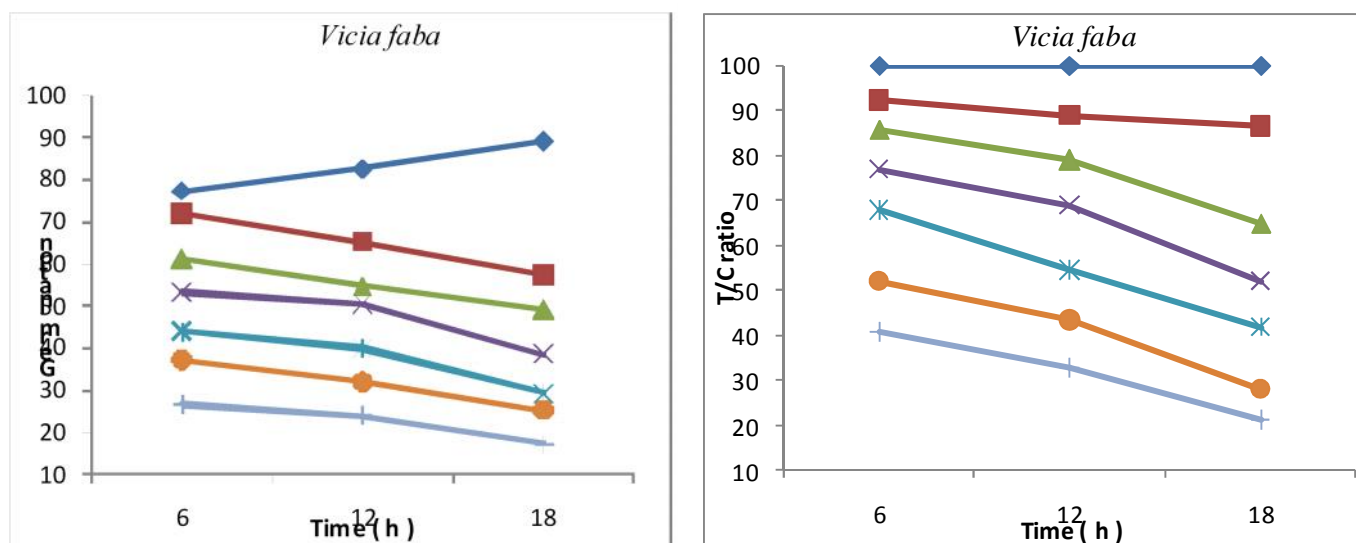
% with 150 % concentration at 6, 12 and 18 hrs respectively.

Table 1: Effect of different concentrations of cinnamon aqueous extract for different times on the seed germination and radicle of *Allium cepa* and *Vicia faba*

Time (hr.)	Conc. (%)	Germination (X ± S.E.)		Radicle Length			
		<i>Allium cepa</i>	<i>Vicia faba</i>	<i>Allium cepa</i>		<i>Vicia faba</i>	
				Radicle Length (cm)	T/C Ratio	Radicle Length (cm)	T/C Ratio
6	control	74.66 ± 1.33	77.33 ± 1.33	2.39 ± 0.04	100.00	3.55 ± 0.02	100
	10	66.66 ± 1.33**	72.00 ± 0.0**	2.24 ± 0.05**	93.72	3.27 ± 0.04*	92.11
	25	57.33 ± 1.33**	61.33 ± 1.33**	2.03 ± 0.03**	84.94	3.04 ± 0.02**	85.63
	50	46.66 ± 3.53**	53.33 ± 1.33**	1.92 ± 0.03**	80.33	2.73 ± 0.04*	76.90
	75	30.66 ± 1.33**	44.00 ± 0.0**	1.57 ± 0.04**	65.69	2.40 ± 0.02**	67.61
	100	25.33 ± 1.33**	37.33 ± 1.33**	1.16 ± 0.04**	48.54	1.84 ± 0.04*	51.83
	150	21.33 ± 1.33**	26.67 ± 1.33**	0.90 ± 0.02**	37.66	1.44 ± 0.06*	40.56
12	control	76.00 ± 0.00	82.67 ± 1.33	2.50 ± 0.06	100.00	3.72 ± 0.02	100
	10	62.66 ± 2.67**	65.33 ± 1.33**	2.10 ± 0.03*	84.00	3.30 ± 0.04**	88.71
	25	46.66 ± 1.33**	54.67 ± 1.33**	1.98 ± 0.04*	79.20	2.93 ± 0.02**	78.76
	50	34.66 ± 1.33**	50.67 ± 1.33**	1.63 ± 0.04*	65.20	2.56 ± 0.02**	68.82
	75	29.33 ± 1.33**	40.00 ± 0.0**	1.25 ± 0.03 *	50.00	2.02 ± 0.02**	54.30
	100	24.00 ± 2.31**	32.00 ± 0.0**	0.88 ± 0.10*	35.20	1.61 ± 0.03**	43.28
	150	16.00 ± 0.00**	24.00 ± 0.0**	0.60 ± 0.02*	24.00	1.21 ± 0.03**	32.53
18	control	77.33 ± 2.67	89.33 ± 1.33	2.60 ± 0.14	100.00	3.94 ± 0.04	100
	10	56.00 ± 0.00**	57.33 ± 1.33**	2.00 ± 0.04**	76.92	3.40 ± 0.06*	86.29
	25	45.33 ± 2.66**	49.33 ± 1.33**	1.70 ± 0.05**	65.38	2.55 ± 0.09*	64.72
	50	24.00 ± 0.30**	38.67 ± 1.33**	1.21 ± 0.04**	46.54	2.05 ± 0.02*	52.03
	75	20.00 ± 0.00**	29.33 ± 1.33**	0.86 ± 0.02**	33.08	1.64 ± 0.02*	41.62
	100	18.66 ± 1.33**	25.33 ± 1.33**	0.64 ± 0.02**	24.62	1.10 ± 0.04**	27.92
	150	12.00 ± 2.30**	17.33 ± 1.33**	0.47 ± 0.03**	18.08	0.83 ± 0.05**	21.07



**Fig 1.** Effect of different concentrations (10 to 150 %) of *Cinnamomum zeylanicum* aqueous extract on (A) Germination (B) Radicle length (T/C ratio) on *Allium cepa* seeds after three treatment periods (6, 12 and 18 hrs).



**Fig 2.** Effect of different concentrations (10 to 150 %) of *Cinnamomum zeylanicum* aqueous extract on (A) Germination (B) Radicle length (T/C ratio) on *Vicia faba* seeds after three treatment periods (6, 12 and 18 hrs).

The effect of *cinnamon* aqueous extract on mitotic index of *Allium cepa* and *Vicia faba* were given in (Tables 2 and 3). In general, the mitotic index values reduced in the treated roots of both *Allium cepa* and *Vicia faba* was a dose and time dependent increased with increasing concentrations from 10 to 150 %. It was also observed that, in both plants, with the same concentration, mitotic index decreased with prolonging treatment period. Thus, after the treatment with 150% concentration at 18 hrs the mitotic index reached to a minimum value of  $3.44 \pm 0.30$  compared the control value of  $5.90 \pm 0.32\%$  in *Allium cepa* and to the value of  $3.79 \pm 0.42\%$  compared the control value of  $7.30 \pm 0.55\%$  in *Vicia faba*. Also, the results in Tables (2 and 3) showed that the percentage of each

mitotic phase in treated root tips of both plants was differed than that in control following the treatments with the extract and did not depend on concentration and time of treatment.

The limit of mitotic inhibition was increased with increasing exposure time and concentration. The minimum value of mitotic inhibition frequency in *Allium cepa* root tips was 1.19% and scored at 10 % concentration for 6 hrs treatment whereas the maximum value of mitotic inhibition frequency was 41.50 % scored at 150% concentration for 18 hrs treatment (Table 2). Also, the minimum value of mitotic inhibition frequency in *Vicia faba* root tips was 3.28% and scored at 10 % concentration for

**Table 2:** Frequency of mitotic phases and the percentage of total abnormal mitotic phases after treatment of *Allium cepa* root tips with different concentration of *cinnamon* aqueous extract for 6, 12, 18 hours.

Treatment		Mitotic phase %				MI ± S.E	% limit of Mitotic Inhibition	Abnormal mitotic phase %				Total abnormal. Cells % (X ± S.E.)
Time (hr.)	Conc. (%)	Prophase	Metaphase	Anaphase	Telophase			Prophase	Metaphase	Anaphase	Telophase	
6	control	56.45	25.81	6.45	11.29	5.90 ±0.32	0.00	1.61	3.23	3.23	0.00	8.06 ±2.28
	10	48.28	28.74	6.90	16.09	5.83 ±0.21	1.19	14.94	19.54	5.75	10.34	50.57±1.29*
	25	42.86	19.64	16.07	21.43	5.63 ±0.65	4.58	16.07	14.29	14.29	8.93	53.57±2.40
	50	53.23	33.87	6.45	6.45	5.35 ± 0.24*	9.32	19.35	32.26	4.84	4.84	61.29±2.89
	75	48.00	30.00	16.00	6.00	5.07 ±0.30**	14.07	22.00	26.00	14.00	4.00	66.00±1.63
	100	37.18	38.46	14.10	10.26	4.77 ±0.20	19.15	11.54	37.18	12.82	8.97	70.51±2.21*
	150	37.93	41.38	15.52	5.17	4.45 ±0.08	24.58	15.52	39.66	15.52	3.45	74.14±4.82
12	control	56.96	29.11	2.53	11.39	5.87 ± 0.20	0.00	2.53	3.80	0.00	2.53	8.86± 1.55
	10	55.56	27.78	1.85	14.81	5.66 ± 0.47	3.57	20.37	14.81	1.85	12.96	50.00±4.75
	25	56.00	22.67	5.33	16.00	5.49 ± 0.06*	6.47	17.33	20.00	4.00	14.67	56.00±2.66
	50	39.42	44.23	4.81	11.54	5.13 ± 0.15*	12.06	10.58	43.27	3.85	10.58	68.27±1.07
	75	32.65	44.90	8.16	14.29	4.66 ± 0.89	20.61	8.16	44.90	8.16	10.20	71.43±2.58
	100	28.74	42.53	13.79	14.94	4.30 ± 0.14*	26.92	4.60	42.53	13.79	13.79	74.71±1.22
	150	35.00	35.00	12.50	17.50	4.18 ± 0.17*	28.79	17.50	35.00	12.50	16.25	81.25±2.18
18	control	61.43	27.14	5.71	5.71	5.88 ±0.11	0.00	4.29	1.43	1.43	1.43	8.57±1.25
	10	50.00	32.93	10.98	6.10	5.30 ±0.41	9.86	13.41	32.93	9.76	4.88	60.98±2.73
	25	45.21	28.77	15.07	10.96	5.10 ±0.19	13.26	9.59	28.77	15.07	9.59	63.01±2.10
	50	29.31	50.00	12.07	8.62	4.76 ±1.00	19.04	3.45	50.00	12.07	6.90	72.41±1.66
	75	26.42	35.85	28.30	9.43	4.52 ±0.45	23.12	5.66	35.85	28.30	7.55	77.36±4.20
	100	22.41	44.83	25.86	6.90	3.96 ±0.12**	32.65	5.17	44.83	24.14	5.17	79.31±2.37
	150	18.52	50.00	24.07	7.41	3.44 ±0.30	41.50	5.56	50.00	24.07	5.56	85.19±4.78

S.E., Standard error

\* Significant at level 5% (p 0.05) \*\* Significant at level 1% (p 0.01)

**Table 3:** Frequency of mitotic phases and the percentage of total abnormal mitotic phases after treatment of *Vicia faba* root tips with different concentration of *Cinnamon* aqueous extract for 6, 12, 18 hours.

Treatment		Mitotic phase %				MI ± S.E	%limit of Mitotic Inhibition	Abnormal mitotic phase %				Total abnormal. Cells % (X ± S.E.)
Time (hr.)	Conc. (%)	Prophase	Metaphase	Anaphase	Telophase			Prophase	Metaphase	Anaphase	Telophase	
6	control	55.38	12.31	20.00	12.31	7.30 ± 0.55	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00
	10	50.25	14.93	29.85	4.48	7.06 ± 0.24	3.28	5.97	4.48	28.36	1.49	40.30 ± 0.10
	25	34.29	42.86	9.52	13.33	6.18 ± 0.44	15.34	2.86	33.33	4.76	1.90	42.86 ± 0.22
	50	40.00	44.29	11.43	3.33	5.93 ± 0.59	18.76	14.35	43.06	11.48	2.87	71.77 ± 0.62
	75	49.18	39.34	9.84	1.64	5.73 ± 0.51*	21.50	22.95	39.34	9.84	1.64	73.77 ± 0.11
	100	46.78	40.35	7.02	5.26	5.35 ± 0.20	26.71	15.70	40.12	6.98	5.23	68.02 ± 0.23
	150	52.24	23.88	19.40	4.48	4.75 ± 0.40	34.93	38.81	23.88	19.40	4.48	86.57 ± 0.23
12	control	37.72	31.58	17.11	13.16	7.20 ± 0.62	0.00	0.00	0.00	1.31	0.00	1.31 ± 0.42
	10	38.94	54.87	4.42	1.77	6.69 ± 0.32	7.08	7.08	54.87	4.42	1.77	68.14 ± 2.04
	25	47.30	45.95	2.70	4.05	5.99 ± 0.15	16.80	12.16	45.95	2.70	4.05	64.86 ± 2.02
	50	49.71	42.11	8.77	0.00	5.50 ± 0.68	23.61	15.79	40.35	8.77	0.00	64.91 ± 1.63
	75	42.86	44.29	12.86	0.00	5.31 ± 0.36	26.25	21.43	42.86	12.86	0.00	77.14 ± 2.12
	100	49.38	35.80	14.81	0.00	4.34 ± 0.27	36.72	32.10	35.80	14.81	0.00	82.72 ± 1.36
	150	48.10	36.71	15.19	0.00	4.13 ± 0.55	42.63	35.44	36.71	15.19	0.00	87.34 ± 1.69
18	control	28.68	44.19	19.77	6.98	7.02 ± 0.33	0.00	0.00	0.00	3.47	0.00	3.47 ± 0.92
	10	34.88	51.16	10.47	3.49	6.17 ± 0.18	12.10	7.00	51.36	10.51	3.50	72.37 ± 1.94
	25	34.15	50.00	12.20	3.66	5.88 ± 0.15	16.23	13.41	50.00	12.20	3.66	79.27 ± 0.55
	50	36.67	37.78	12.22	13.70	5.26 ± 0.24	25.07	22.22	37.78	12.22	13.33	85.56 ± 1.76
	75	41.59	44.04	1.83	12.84	4.91 ± 0.23	30.05	34.86	44.04	1.83	12.84	93.58 ± 0.25
	100	41.55	49.32	2.74	6.85	4.29 ± 0.42	38.88	35.62	49.32	2.74	6.85	94.52 ± 0.22
	150	52.46	44.26	3.28	0.00	3.79 ± 0.42	46.01	52.46	44.26	3.28	0.00	100.00 ± 0.00

S.E., Standard error \* Significant at level 5% (p 0.05) \*\* Significant at level 1% (p 0.01)

6 hrs treatment whereas the maximum value of mitotic inhibition frequency was 46.01% scored at 150% concentration for 18 hrs treatment (Tables 3).

In both plants, the results in Tables (2&3) showed the total percentages of abnormal mitotic cells induced in treated root tips in all stages which increased with increasing treatment times and concentrations. In *Allium cepa*, the metaphase stage was the most affected by cinnamon extract for all treatments and the total percentage of its abnormalities was higher than those at the other mitotic stages. The total percentage of abnormalities induced at prophase was higher than that in anaphase and telophase for 6 and 12 hours treatment. On contrast, the total percentage of abnormalities induced at anaphase stage was higher than that present in prophase and telophase for 18 hours treatment (Table 2).

The results in (Table3) revealed that in *Vicia faba* treated root tip cells, the metaphase stage was also the most affected stage by extract treatment and the total percentage of its abnormalities was higher than those at other mitotic stages. In this respect, the total percentage of abnormalities in the other mitotic stages was in the following sequence Prophase>anaphase>telophase for all treatments.

Tables (4 & 5) showed the different types of chromosomal aberrations induced in both plants such as irregular prophase, vacuolated nuclei, and sticky chromosomes in all stages, C-metaphase, bridges and disturbed metaphases. In addition to the above-mentioned types, fragments, diagonal and forward chromosome(s) were recorded in low frequencies in treated root tip cells of both plants. Moreover, the induction of micronucleus formation was generally observed in all tested concentrations in *Allium cepa* and *Vicia faba*. Micronuclei with different size and number were, also, observed in all treatments. The percentages of micro-nucleated cells in *Allium cepa* were more than those in *Vicia faba*.

## Discussion

Phytotoxic effects of cinnamon extracts were indicated by decreases in number of germinated seeds and roots growth in a dose response relationship. Literature review on cinnamon revealed that it mainly contains essential oils and important compounds like Cinnamaldehyde, eugenol, cinnamic acid and cinnamate (Meena et al., 2012). Extracts of cinnamon bark has revealed the presence of flavonoids, glycosides, coumarins, alkaloids, anthraquinone, steroids, tannins and terpenoids (Shihabudeen et al.,

2011). The different components of cinnamon oil bark extract were in total 19 compounds ((Joy *et al.*, 2005, Singh *et al.*, 2007 and Wang *et al.*, 2008) and a 9 major constituents in water extract of bark (Araar, 2009). The water extract exerts its toxic effects on germination percentage and radicle length where the root seems to be the object of extract toxicity, particularly the apex, producing a rapid inhibition of cell division and elongation of the root. The phytotoxic effects, it can be due to the phenolic compounds catechins and proanthocyanidins of cinnamon water extract (Araar, 2009). In similar to our results, the toxic effect of cinnamon bark extract decreased the seed germination and root growth of different plants (*Lactuca sativa*, *Triticum aestivum*, *Vicia faba*, *Lolium perenne*, *Lycopersicum esculentum*, *Lepidium sativum* and *Arachis hypogaea*) and this action increased with increasing concentration and treatment duration and can be related to the composition of this extract (Alves *et al.*, 2004, Liu *et al.*, 2005a, Araar, 2009 and Soares *et al.*, 2015) and also Cavalieri (2009) reported that cinnamon bark extract exerted its inhibition or retardation effects on seed germination, radicle root and shoot development of seven common weed species from the Mediterranean environment: *Amaranthus retroflexus* L., *Solanum nigrum* L., *Portulaca oleracea* L., *Chenopodium album* L., *Sinapis arvensis* L., *Lolium perenne* L., and *Vicia sativa* L. and the inhibition was a dose-dependent and the lethal dose able to completely inhibit weed seed germination.

Thus, germination rate and root growth are variables that can be measured (Vadillo *et al.*, 2004) and can be associated with cytogenetic assays. Valerio *et al.* (2007) found that the reduction in germination percentage is the most sensitive parameter that can be observed to infer the different levels of phytotoxicity of components of the extract. One suggested mechanism for the inhibition of seed germination is the disruption of mitochondrial respiration (Weir *et al.*, 2004). The interference of allelochemicals on seed germination depends on the concentration used (Ciarka *et al.*, 2002). Furthermore, the influence of the type of extract also depends on the sensitivity of the tested plant to allelopathic compounds present; with certain substances it can inhibit germination or growth, and with others it can be the same or stimulating innocuous (Almeida *et al.*, 1998). Also, root growth inhibition upon exposure to cinnamon extract has been used extensively as one of the most distinct and earliest symptoms of cinnamon plant extract toxicity (Inderjit *et al.*, 2008). In this study, the inhibition effect of cinnamon extract on root

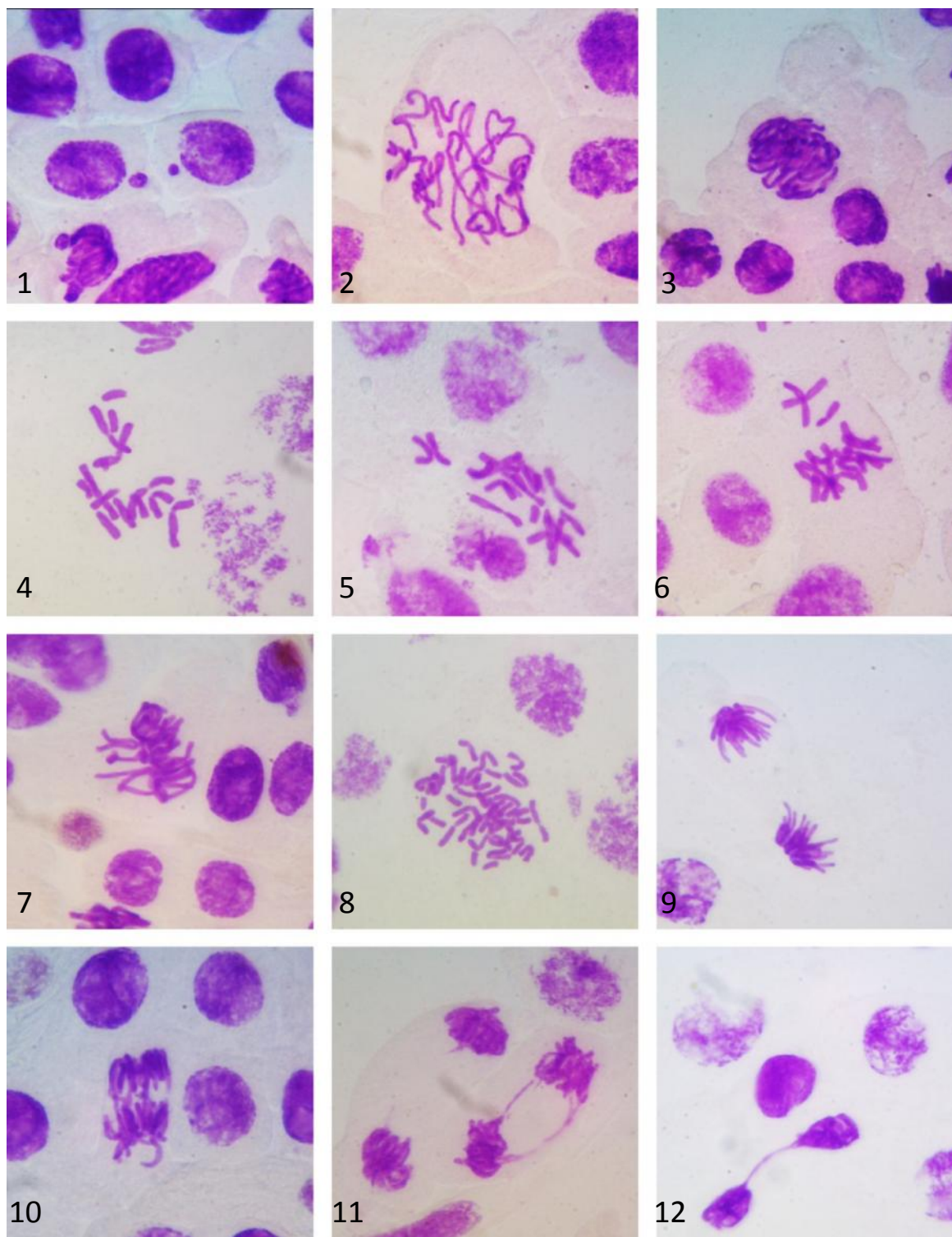


Table 4 Percentage of abnormalities and types of mitotic abnormalities in *Allium cepa* root-tip meristems after root treatments for different time and concentrations with *cinnamon* aqueous extract.

Treatment		prophase		metaphase			anaphase						Telophase			% of Micro-nucleus
Time (hr.)	Conc. (%)	irregular	sticky	disturbed	c-metaphase	sticky	sticky	s/bridges	Sticky / forward	c-anaphase	Diagonal	multipolar	sticky	sticky / bridges	sticky / forward	
6	control	1.61	0.00	0.00	0.00	3.23	1.61	1.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30
	10	12.64	2.30	12.64	0.00	6.90	2.30	2.30	1.15	0.00	0.00	0.00	9.20	1.15	0.00	0.71
	25	14.29	1.79	8.93	0.00	5.36	5.36	5.36	3.57	0.00	0.00	0.00	5.36	1.79	1.79	0.64
	50	16.13	3.23	11.29	0.00	20.97	1.61	1.61	1.61	0.00	0.00	0.00	4.84	0.00	0.00	1.19
	75	16.00	6.00	2.00	0.00	24.00	6.00	6.00	2.00	0.00	0.00	0.00	4.00	0.00	0.00	0.53
	100	8.97	2.56	1.28	0.00	35.90	5.13	5.13	2.56	0.00	0.00	0.00	6.41	1.28	1.28	0.39
	150	10.34	5.17	1.72	0.00	37.93	6.90	5.17	1.72	0.00	1.72	0.00	3.45	0.00	0.00	0.64
12	control	2.53	0.00	0.00	0.00	3.80	0.00	0.00	0.00	0.00	0.00	0.00	2.53	0.00	0.00	0.24
	10	14.81	5.56	9.26	0.00	5.56	1.85	0.00	0.00	0.00	0.00	0.00	11.11	1.85	0.00	0.11
	25	12.00	5.33	9.33	0.00	10.67	2.67	0.00	1.33	0.00	0.00	0.00	12.00	2.67	0.00	0.47
	50	7.69	2.88	8.65	3.85	30.77	0.96	0.96	0.00	0.96	0.00	0.96	4.81	2.88	2.88	0.62
	75	4.08	4.08	6.12	6.12	32.65	4.08	2.04	2.04	0.00	0.00	0.00	6.12	0.00	4.08	2.50
	100	3.45	1.15	3.45	2.30	36.78	5.75	4.60	2.30	0.00	1.15	0.00	10.34	2.30	1.15	0.88
	150	11.25	6.25	2.50	1.25	31.25	6.25	5.00	0.00	0.00	1.25	0.00	12.50	2.50	1.25	0.76
18	control	4.29	0.00	0.00	0.00	1.43	0.00	1.43	0.00	0.00	0.00	0.00	1.43	0.00	0.00	0.45
	10	8.54	4.88	19.51	0.00	13.41	6.10	1.22	2.44	0.00	0.00	0.00	2.44	1.22	1.22	0.41
	25	5.48	4.11	12.33	0.00	16.44	8.22	4.11	1.37	1.37	0.00	0.00	5.48	2.74	1.37	0.52
	50	1.72	1.72	3.45	3.45	43.10	5.17	3.45	0.00	0.00	0.00	3.45	3.45	1.72	1.72	0.43
	75	3.77	1.89	3.77	1.89	30.19	11.32	9.43	1.89	5.66	0.00	0.00	3.77	1.89	1.89	0.36
	100	3.45	1.72	10.34	1.72	32.76	10.34	8.62	1.72	1.72	1.72	0.00	3.45	1.72	0.00	0.28
	150	3.70	1.85	7.41	1.85	40.74	12.96	5.56	3.70	0.00	1.85	0.00	3.70	1.85	0.00	0.54

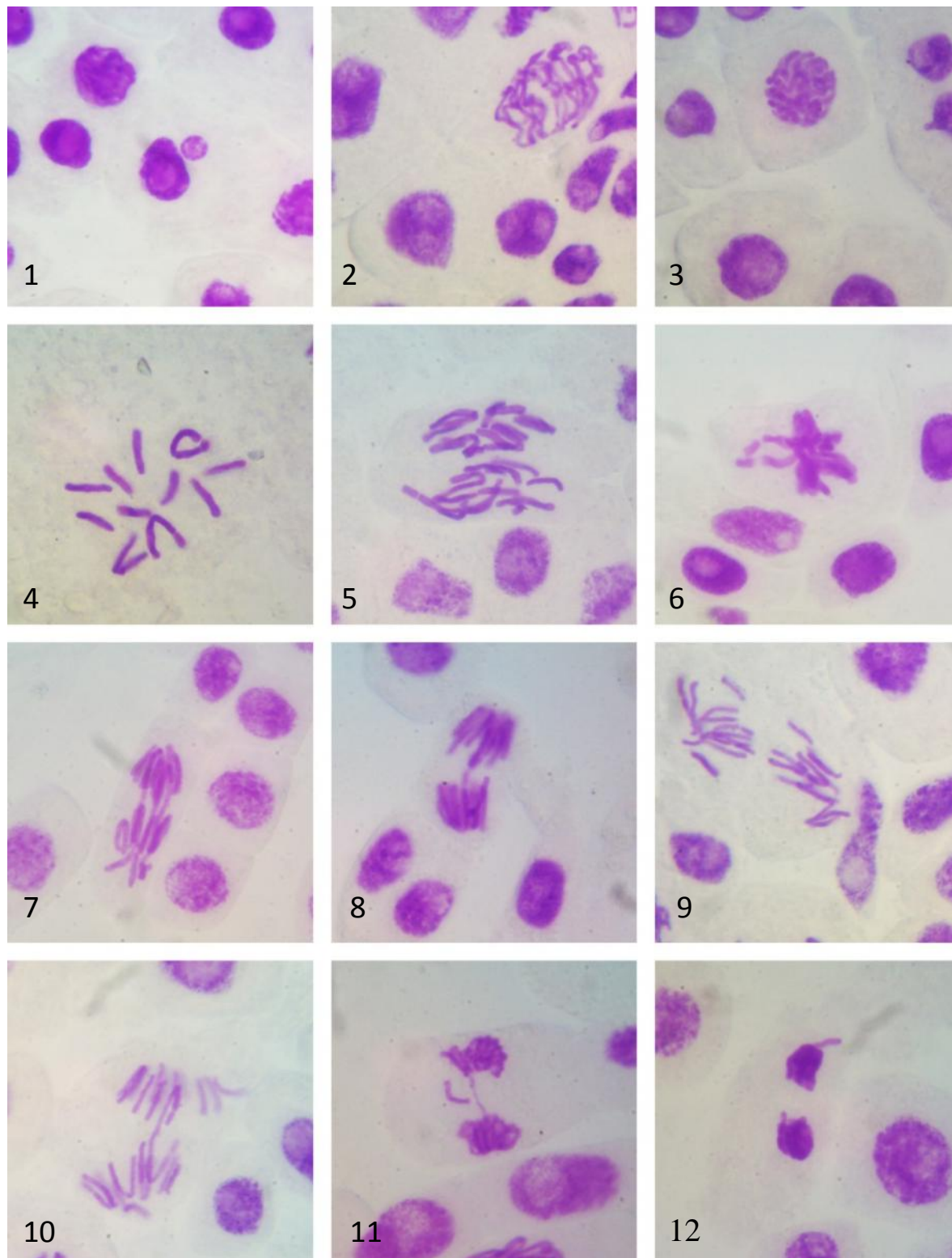
**Table 5** Percentage of abnormalities and types of mitotic abnormalities in *Vicia faba* root-tip meristems after root treatments for different time and concentrations with *Cinnamon* aqueous extract.

Treatment		prophase			Metaphase			Anaphase					Telophase	% of Micro-nucleus
Time (h.)	Conc. (%)	Irregular.	Vacuolated	Sticky	Disturbed	sticky	C-meta	sticky	Stk. / Fr.	sticky / bridges	C-Ana.	multipolar	sticky	
6	control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10	5.97	0.00	0.00	0.00	4.48	0.00	17.91	0.00	10.45	0.00	0.00	1.49	0.11
	25	1.90	0.00	0.95	24.76	7.62	0.00	4.76	0.00	0.95	0.00	0.00	1.90	0.38
	50	14.35	0.00	0.00	0.00	38.76	0.00	8.61	0.00	4.31	0.00	1.44	4.31	0.00
	75	18.03	0.00	0.00	1.64	39.34	0.00	9.84	0.00	1.64	0.00	0.00	3.28	0.00
	100	15.70	0.00	0.00	0.00	36.63	0.00	6.98	0.00	3.49	0.00	0.00	5.23	0.10
	150	35.82	0.00	0.00	0.00	25.37	0.00	11.94	1.49	2.99	0.00	4.48	4.48	0.22
12	control	0.00	0.00	0.00	0.00	0.00	0.00	1.31	0.00	0.00	0.00	0.00	0.00	0.20
	10	3.54	0.88	2.65	36.28	3.54	12.39	1.77	0.88	0.88	1.77	1.77	1.77	0.25
	25	5.41	0.00	0.00	40.54	9.46	0.00	0.00	0.00	2.70	0.00	1.35	5.41	0.26
	50	14.04	0.00	0.00	26.32	14.04	0.00	7.02	0.00	1.75	0.00	1.75	0.00	0.20
	75	18.57	0.00	0.00	7.14	37.14	0.00	7.14	1.43	5.71	0.00	0.00	0.00	0.24
	100	32.10	0.00	0.00	0.00	35.80	0.00	9.88	0.00	4.94	0.00	0.00	0.00	0.22
	150	35.44	0.00	0.00	2.53	35.44	0.00	7.59	1.27	2.53	0.00	2.53	0.00	0.16
18	control	0.00	0.00	0.00	0.00	0.00	1.16	1.16	0.00	1.16	0.00	0.00	0.00	0.09
	10	5.84	0.00	1.17	32.68	9.34	9.34	3.50	0.00	1.17	5.84	0.00	3.50	0.08
	25	10.98	0.00	0.00	30.49	15.85	4.88	6.10	0.00	2.44	3.66	1.22	3.66	0.23
	50	12.22	3.33	4.44	1.11	36.67	0.00	7.78	1.11	2.22	0.00	2.22	14.44	0.12
	75	28.44	3.67	2.75	0.00	43.12	0.00	1.83	0.00	0.92	0.00	0.00	12.84	0.14
	100	36.99	0.00	0.00	0.00	49.32	0.00	2.74	0.00	1.37	0.00	0.00	4.11	0.18
	150	49.18	0.00	1.64	0.00	44.26	0.00	1.64	0.00	3.28	0.00	0.00	0.00	0.19



**Fig. 3.** Some types of abnormalities induced in *Allium cepa* root tip cells produced by different treatments of *cinnamonaqueous* extract.

(1) Micronuclei at interphase. (1) Irregular prophase (3) sticky prophase (4) C-metaphase. (5) C-metaphase (6) Disturbed metaphase with lagging chromosome. Diagonal anaphase. (10) Sticky anaphase with multibridge and forward chromosomes. (11) A- Sticky telophase B- Sticky telophase with double bridge. (12) Sticky telophase with single bridge.



**Fig. 4.** Some types of abnormalities induced in *Vicia faba* root tip cells produced by different treatments of *cinnamon* aqueous extract.

- (1) Micronuclei at interphase. (1) Irregular prophase (3) sticky prophase (4) C-metaphase. (5) Disturbed metaphase (6) Sticky metaphase (7) Anaphase with multibridge and forward chromosomes. (8) Sticky anaphase with broken bridge. (9) Anaphase with lagging chromosomes. (10) Single bridge at anaphase with vagrant chromosome. (11) Telophase with single bridge and lagging chromosome. (11) Sticky telophase with forward chromosome.

elongation could be due to interference its components with cell division, including inducement of chromosomal aberrations and abnormal mitosis.

The cyto-genotoxicity level of a plant extract can be determined based on the increase or decrease in the mitotic index (MI) which can be used as a reliable parameter for determining the presence of cytotoxic and genotoxicity compounds in the studies extract or environment and is a suitable test for bio-monitoring (Smaka-Kincl *et al.*, 1996 and Fernandes *et al.*, 2007). A dose dependent decrease in MI as observed in the present study. Interaction of the extract with protein or inhibition of protein synthesis (Unyayar *et al.*, 2006) may be the cause of inhibition of MI. Smaka-Kincl *et al.* (1996) reported that the decrease in mitotic index was the result of cytotoxic effects. The cytotoxic threshold was estimated as the concentration causing 40-50% mitotic depression compared to the control (Sharma, 1983). We observed that the mitotic index was lower than the cytotoxic limiting value at the highest cinnamon extract concentration (150 %) in 18 hrs periods. However, the cytotoxic effect of cinnamon extract treatments was evident as observed by a significant decrease in the mitotic index. In the current study, a lethal effect was not observed, because, among all the applied cinnamon extract concentrations and periods, the lowest mitotic index value was only 3.44% and 3.97% at the highest gel extract concentration for 18 hrs duration in *Allium cepa* and *Vicia faba* respectively.

Cell cycle analysis indicate that the number of cells in each phase of mitosis changed in extract-exposed root tips, providing the indication of extract-induced abnormal progress through mitosis by reducing the percentage of prophase and metaphase cells, reflecting a reduction in MI. These data demonstrate that cinnamon extract induces cell cycle checkpoints defects in both plants root cells. The reduction of MI in treated roots is probably due to disturbances in the cell cycle as well as chromatin dysfunction, which is induced by interactions between DNA and the components of cinnamon extract (Chandra *et al.*, 2005; Glinska *et al.*, 2007). In this respect, the induction of micronuclei, in this study, indicates that some mitotic cells can enter into mitotic (M) phase with DNA damage. Schulze and Kirscher (1996) stated that the decrease in the mitotic index was the result of the suppressive effect of some components of cinnamon extract on DNA and nucleoprotein synthesis. It can be concluded that, together with the increase in cinnamon extract concentrations, the mitotic index of *A. cepa* and *Vicia faba* root tip cells

decreased due to the blocking effect of some components of extract in the G1 phase or DNA synthesis inhibition in the S phase as stated by El-Ghamery *et al.* (2000) or stopped the cell cycle in G1 phase (Assadollahi *et al.*, 2013).

Cytogenetic analysis of the effect of cinnamon extract has revealed the presence of abnormally dividing cells. Different concentrations of cinnamon extract induced different types of chromosomal aberrations at various stages of cell division and the total percentages of abnormalities increased with increasing extract concentrations and treatment times. The most frequent aberration that we observed upon cinnamon extract treatment was stickiness, followed by C- metaphase, bridges and lagging chromosomes.

Sticky chromosomes indicate the toxic effect of some components of the extract on the organization of chromatin. This property is related to a disturbed balance of histones or other proteins that are responsible for controlling the proper structure of nuclear chromatin; generally, this imbalance leads irreversibly to cell death (El-Ghamery *et al.*, 2003). The interesting feature noticed after treatment with cinnamon bark extract was strong C-mitotic effect in the investigated plants. The normal mitotic stages were modified into C-metaphase due to inactivation of spindle followed by random scattering of chromosomes. The C-metaphase that we observed in treated meristems suggests that some constituents of the extract act on the mitotic spindle apparatus, probably interfering with the polymerization and depolymerization of microtubules (Seth *et al.*, 2008). Bridges and laggard chromosomes were also observed, though at low frequency. Bridges can be attributed to chromosome breaks, stickiness, and the breakage and reunion of broken ends (El-Ghamery *et al.*, 2003). Bridges at anaphase and telophase can result from the terminal deletion or loss of telomeres. In according to suggestion of Andrade *et al.* (2010), chromosome bridge could be a result of the failure of free anaphase separation, unequal translocation or inversion of chromosome segments. This bridge cause structural chromosome mutations (El-Ghamarey *et al.*, 2001). Lagging chromosome and vagrant chromosome were observed after treatment with cinnamon extract which may be due to the disruption of the spindle organization. Lagging chromosomes or vagrant chromosomes are probably induced by interference with normal chromatid separation, which leads to an unequal number of chromosomes in the daughter nuclei and subsequently to the formation of daughter cells with unequally sized or irregularly shaped nuclei

at interphase (El-Ghamery *et al.*, 2003) The spindle organization and movement of chromosomes during cell division is ATPdependent process, may fail to migrate towards either of the daughter nuclei during telophase of the mitotic cell (Iarmarcovai *et al.*, 2006). Laggards may produce micronuclei if they fail to reach the poles in time to be included in the main nucleus.

The induction of micronucleated cells in treated root tips with all treatments is another interesting feature in this study. Micronucleus is the small nucleus that forms whenever a chromosome or a fragment of a chromosome is not incorporated into one of the daughter nuclei during cell division. The lagging chromosome or the fragments are surrounded by a nuclear membrane to form micronuclei. According to

Sudhakar *et al.* (2001), micronuclei can originate from acentric fragments, chromosome laggards during anaphase, or even a malfunctioning spindle. Thus, cinnamon bark extract may induce the formation of micronuclei by disturbing the spindle and inducing chromosome breaks, which are an anomalous disjunction of chromosomes at anaphase. Additionally, the increase in the frequency of condensed nuclei suggests that some component of the extract cause DNA damage. The condensation of the nucleus is one of the first steps in the cell death process (Vermes *et al.*, 2000). In *Allium cepa* vagrant was also noticed due to the treatment and the abnormality vagrant was results of impaired spindle function (Patel and Patel, 2013).

The cytotoxic activity of bark extract at low concentration exerts cytotoxicity and showed a dose dependent inhibitory effect on the growth of MCF 7 cells and breast cancer cell lines (Priyarani *et al.*, 2010). Also, the studies established that this plant extract as a genotoxic and cytotoxic reagent in human cells (Lee *et al.*, 2004). From the previous studies and our results we can be concluded that cinnamon bark extract is a cytotoxic factor in both human and plant cells. Since the plant system can detect the cytotoxicity more efficiently and quickly than animal bioassays, we could use these simple and economical plant bioassays for clastogen screening and environmental monitoring in general.

Finally, this work provides the evidence for cinnamon cyto-genotoxicity in plant cells and also confirms the efficiency of the plant cytogenetic assays on monitoring the cyto-genotoxicity of the plant extract. So, we conclude that when applied in high doses

cinnamon bark extract shows cytotoxic and genotoxic activity. We used in this study crude extract of cinnamon bark which is appropriate because traditional medicinal herbs are generally used as crude extracts. However, working with crude extracts also means working with complex mixtures of biologically active compounds. This might be due partly to the heat involved in the extraction process since this would have probably caused a good extraction to release more compounds into extract. Some of these compounds can be cytotoxic and/or genotoxic; others can be cytoprotective and/or antigenotoxic. The results of this study suggest that, although *A. lappa* has beneficial effects as a medicinal herb, it can cause serious problems and damage on cells when used improperly.

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