

Salicylic acid alleviates *Lantana camara* aqueous extract cyto-genotoxicity in *Nigella sativa* (black cumin)

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Abstract: *Lantana camara* L. (2n = 44) is important species of family Verbenaceae and is commonly used in folk medicine in many countries. Root tip cells of *Nigella sativa* (black cumin) were separately treated with different concentrations (20-100 %) of aqueous plant extract for 3, 6, 12 and 24 hours and the results were recorded. The results showed that all concentrations of aqueous extracts significantly reduced the mitotic index and caused a disturbance in the frequencies of mitotic phases. The treatment with 100% of the extract for 24 hours was the most effective in reducing the mitotic activity and inducing the highest percentage of mitotic abnormalities. The different frequent types of abnormalities were irregular prophase, bridges, stickiness at different phases, disturbed chromosomes, forward and lagging chromosomes. In addition to other infrequent types such as micronucleus, binucleate, fragments divided polar and ghost cells. Also, in this study salicylic was used to minimize or recover the cyto-genotoxic effect of *Lantana camara* extract. For this, three concentrations of SA (0.01, 0.1 and 0.2 mM) for 6 and 12 hours were applied for the root tip cells of the tested plant treated with the plant extract concentration of 100% for 3 and 24 hours. This Post treatment with SA resulted in increasing MI and significant reduction of chromosomal abnormalities than that in treated root tips with plant extract. These results illustrate the ameliorating effect of SA under stress conditions and reveal that SA is effective in alleviating the toxic effects of plant extract at all applied concentrations.

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1. Introduction

Lantana camara L. (Verbenaceae) with (2n = 44), a neotropical aromatic shrub, is considered one of the 10 worst weeds of the world because it infests 14 crops in many tropical and subtropical countries [1]. However, it grows as an ornamental plant in different countries. *Lantana camara* is one of the known allelopathic weed plants in many parts of the world [2]. Allelopathic effects of *L. camara* on germination and seedling vigor of many agricultural crops, such as *Triticum aestivum* [3] and [4], *Cucurbita pepo* [5], *Phaseolus mungo* [6], *Triticum aestivum* and *Zea mays* [7] and *Cicer aeritinum* [8] have been reported. Allelopathy is a biological phenomenon in which plants can produce certain secondary metabolites that inhibit the growth, reproduction and survival of other plants to avoid competition in their surroundings [7]. Some of the plant secondary metabolites have phytotoxic effects [9]. Many of these compounds are phytotoxic and have potential as herbicides or as templates for new herbicides classes [10]. Thus, the phytotoxicity of *L. camara* can be considered as a source of potent green herbicide to control *P. hysterophorus* fruiting stage [11]. Allelopathic inhibition is complex and can involve the interaction of different classes of chemicals, such as phenolic compounds, flavonoids, terpenoids, alkaloids,

steroids, carbohydrates, and amino acids. As many as 14 phenolic compounds are present in *Lantana* that can reduce the seed germination and growth of young plants [12] and [13]. A series of aromatic alkaloids and phenolics can be extracted from the various plant parts of *Lantana* [14] and [15]. The leaves, roots and fruits of *L. camara* contain allelochemicals, mainly aromatic alkaloids and phenolic compounds [14] and [16]. Allelochemicals promote or inhibit the crop growth based on their concentration [14] and the concentration increases from root, stem to leaf [17], making the leaf toxic to grazing animals [14]. Allelochemicals affect plant cytological processes such as cell wall expansion, protein synthesis, cell division and nucleic acids level [18]. Decreased rate of protein synthesis is a result of integration of certain amino acids into protein inhibited by phenolic acids [19].

This raise concern about the potential mutagenic or genotoxic hazards resulting from the long-term use of such plants. Therefore, it is extremely important the employment of genotoxicity tests to identify their possible mutagenic potential [20], [21] and [22]. Genotoxic effects of extracts, infusions, essential oils and fractions of extracts of many plants have been widely evaluated using cytogenetic approaches [23], [24], [25] and [26]. In this concern, different studies

were recorded on the effect of *Lantana camara* extract on the mitotic division behavior in different plants by many investigators such as [27], [28], [29], [30], [31], [16], [32], [33], [34] and [35], [36] and [37].

Salicylic acid is a compound that is chemically like aspirin. It functions as a phytohormone, an important factor in environmental stress tolerance in plants [38]. SA produces an ameliorative protective effect in plants in response to abiotic stress, such as metal toxicity, heat, chilling, osmotic and salt stress [39], [40], [41], [42], [43], [44], [45], [46], [47] and drought [48]. SA also elevates negative action of the stress-inducing factors [49], [50]. The considerable interests have been focused on SA due to its ability to induce a protective measure on plant under stress factors [51].

Exogenous SA application may be responsible for activation of defense genes [52] and it is supposed that exogenous SA as antioxidants may have wider application as means for the simultaneously increasing resistance not to one but to several stress-inducing factors. SA may be applied in agriculture for increasing the plant production quality. The majority of using the SA as protective agent before the treatment plant with abiotic factor such as [53], [54] and [55].

The *Nigella sativa* (black cumin) is an efficient test material for chemical screening and *in situ* monitoring for genotoxicity of environmental contaminants and has been widely used to study genotoxicity of many pollutants revealing that these compounds can induce chromosomal aberrations in root meristems of *Nigella sativa* (black cumin) where it has relatively large monocentric chromosomes in reduced numbers and are accepted as suitable test organism for the study of environmental mutagenesis. *Nigella sativa* (black cumin) is recommended as a stranded for environmental monitoring [56]. Since no study was not recorded on the cytotoxic effect of *L. camara* extracts on *Nigella sativa* (black cumin) and for this reason, this study aims to investigate, first: the cytotoxic and genotoxic effects of aqueous extracts from leaves of *Lantana camara* L. on *Nigella sativa* (black cumin) root tip meristem cells using a cytogenetic approach. Secondary: test the ability of the salicylic acid to recover the toxic effects of the used plant extract.

2. Material and Methods

Prepare the extract of *Lantana camara*:

Leaves of *Lantana* is obtained from the local garden; the leaves are crushed manually in a mortar with a pestle. A volume of 100 ml of distilled water was added to 10 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 was filtered through Whatman filter No.1 using Buchner funnel and stored at 4 °C in sterile tubes until use.

To evaluate the cyto-genotoxicity, ten germinated seeds of *Nigella sativa* with radicle 2-3 cm length, were treated with different concentrations of aqueous plant extract (20-100 %) for 3, 6, 12 and 24 hours. Control germinated seeds were placed in distilled water. **To evaluate the ability of the salicylic acid** to recover the toxic effects, Root tip cells were treated with 100% concentration of plant extract for 3 and 24 hours soaked in different concentrations of salicylic acid (SA) for 6 and 12 hours.

Cytological Examination: In this investigation *Nigella sativa* (2n=14) plant used as test plant, (1) Ten germinated seeds of this plant with radicle 2-3 cm long, were treated with different concentrations of aqueous plant extract for different times. 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), 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. (2) Ten germinated seeds of *Nigella sativa*, with radicle 2-3 cm long, were treated with (100 %) concentration of aqueous plant extract for 3 and 24 hours were soaked in solution of SA (0.01, 0.1 and 0.2 mM) for 6 and 12 hours and cytological examination were done as mentioned in no (1). The control of untreated root tips was made for each treatment.

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 $p < 0.05$ [57].

3. Results

The effects of different concentrations of leaves aqueous extracts (20, 40, 80, and 100 %) for different treatment times (3, 6, 12 and 24 hours) on mitotic index of *Nigella sativa* were given in were studied in Petri plates and the results given in Table (1). From the results, in general, the mitotic index values reduced in the treated roots was a dose and time dependent increased with increasing concentrations from 20 to 100 %. It was also observed that, with the same concentration, mitotic index decreased with prolonging treatment period. Thus, after the treatment with 100% concentration at 24 hours the mitotic index reached to a minimum value of 1.66 ± 0.04 compared the control value of $13.45 \pm 0.03\%$ Also, the results in

Tables (1) showed that the percentage of each mitotic phase in treated root tips of *N. sativa* was differed than that in control following the treatments with the extract and did not depend on concentration and time of treatment. the results in Tables (2) showed the total percentages of abnormal mitotic cells induced in

treated root tips in all stages which increased with increasing treatment times and concentrations. The prophase stage was the most affected by *Lantana* extract for all treatments and the total percentage of its abnormalities was higher than those at the other mitotic stages.

Table 1. Mitotic index and mitotic phases of *N. sativa* meristematic cells exposed to different concentrations of *Lantana. camara* aqueous extracts after 3, 6, 12 and 24 hours

Treatments		Counted cells	Divided cells	Mitotic index (MI ± S.E)	Mitotic phases %			
Time(h)	Conc. (%)				Prophase	Metaphase	Anaphase	Telophase
3	Control	1677	218	13.00 ± 0.11	50.91	25.22	18.35	5.52
	20	1670	200	11.97 ± 0.05	52.50	20.00	15.00	12.50
	40	1645	190	11.55 ± 0.02	53.68	17.37	13.16	15.79
	80	1620	170	10.49 ± 0.03	55.88	15.88	11.76	16.47
	100	1610	155	9.62 ± 0.01	58.06	13.55	10.97	17.42
6	Control	1688	225	13.32 ± 0.07	51.11	24.00	18.66	6.23
	20	1660	188	11.32 ± 0.04	53.20	20.20	14.90	11.70
	40	1635	160	9.78 ± 0.05	56.25	18.75	11.87	13.13
	80	1605	120	7.47 ± 0.04	58.34	16.66	8.34	16.66
	100	1575	90	5.71 ± 0.02	61.11	11.11	7.78	20.00
12	Control	1680	220	13.09 ± 0.04	52.72	23.63	16.81	6.36
	20	1650	180	10.90 ± 0.03	55.55	18.33	13.33	12.77
	40	1615	132	8.17 ± 0.02	60.60	15.15	7.57	16.66
	80	1580	100	6.33 ± 0.05	64.00	11.00	7.00	18.00
	100	1555	60	3.86 ± 0.01	66.66	6.66	5.00	21.66
24	Control	1695	228	13.45 ± 0.03	53.50	25.43	15.35	5.70
	20	1630	150	9.20 ± 0.02	56.66	20.00	10.00	13.13
	40	1560	100	6.41 ± 0.04	65.00	15.00	6.00	14.00
	80	1530	55	3.59 ± 0.02	69.09	7.27	5.45	18.18
	100	1500	30	1.66 ± 0.04	73.33	3.33	3.34	20.00

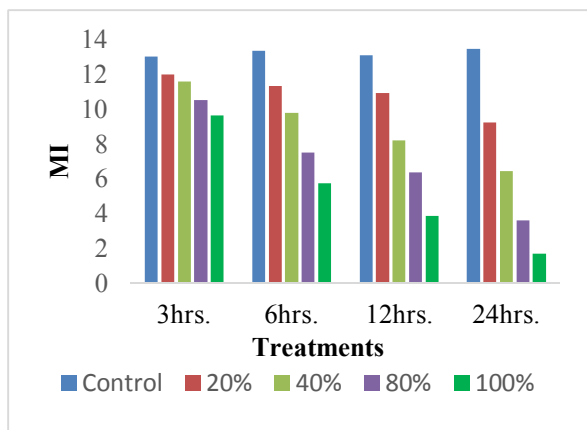
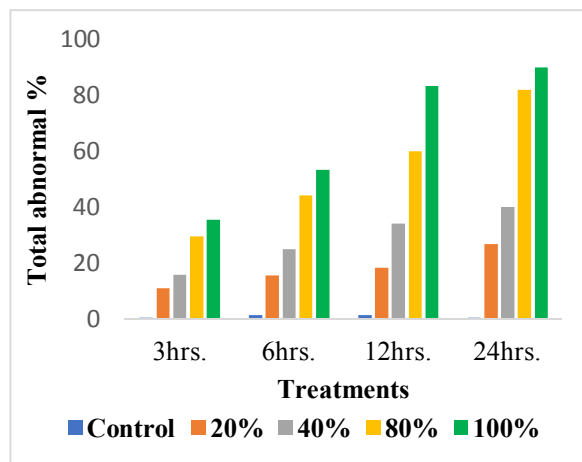


Fig 1. Effect of different concentrations (20 to 100 %) of *Lantana camara* aqueous extract on mitotic index of *Nigella sativa* seeds after four treatment periods (3, 6, 12 and 24 hours).



The total percentage of abnormalities induced at telophase was higher than that in metaphase and

anaphase for all treatments. On the other hand, the total percentage of abnormalities induced at anaphase stage was lower than that present in metaphase for all treatments. Tables (3) showed the different types of chromosomal aberrations induced in tested plant such as irregular prophase and sticky chromosomes in all stages, bridges and disturbed metaphases. In addition to the above- mentioned types, lagging and forward chromosome (s) were recorded in low frequencies in treated root tip cells of tested plant. Moreover, the induction of micronucleus, bi-nucleated cells formation was generally observed in few cells in *Nigella sativa* root tips.

In this study the results showed that, all concentrations of using SA caused an increased in MI in treated root tips compared with untreated root tip cells (control) for each period. This increase more

clearly at lowest concentration (0.01mM), when MI recorded was 15.05 and 14.86 for 6 and 12 hours respectively. Also, all applied concentrations of SA caused elevate in total abnormal cells percentages and the concentration (0.1mM) was the lowest one and caused minimal increase in total abnormalities percentage in treated root tip cells (Table 4). Result presented in Table 5 clearly state that, using SA post-treatment caused a significant increase of MI at high concentrations (0.1- 0.2 mM) in all treated root tip cells. The improvement was more prevalent at higher concentrations of SA in root tips treated with 100 % plant extract for 24 hours. On contrast the lower concentration of SA (0.01mM) did not enhanced the MI in treated cells with 100 % plant extract for 3 hours, but for 24 hours it caused a non-significant increase of MI.

Table 2. Percentages of abnormal cells in different mitotic stages and total abnormalities after treating *Nigella sativa* root tip cells with different concentrations of *Lantana camara* aqueous extract for different treatment times.

Treatments		Divided cells	Total abnormal cells (X ± S.E)	Abnormal mitotic phases %			
Time(h)	Conc. (%)			Prophase	Metaphase	Anaphase	Telophase
3	Control	218	0.46 ± 0.02	0.46	0.00	0.00	0.00
	20	200	11.00 ± 0.05 □	4.50	2.50	2.00	2.00
	40	190	15.79 ± 0.03 □	6.31	3.69	2.64	3.15
	80	170	29.41 ± 0.01 □	11.76	5.30	5.30	7.05
	100	155	35.48 ± 0.02 □□	16.13	5.16	4.52	9.67
6	Control	225	1.33 ± 0.11	1.33	0.00	0.00	0.00
	20	188	15.42 ± 0.02 □	5.32	4.25	3.72	2.13
	40	160	25.00 ± 0.02 □	11.26	5.00	4.37	4.37
	80	120	44.16 ± 0.03 □□	19.16	6.66	5.84	12.50
	100	90	53.33 ± 0.01 □□	24.45	7.77	6.66	14.45
12	Control	220	1.36 ± 0.07	1.36	0.00	0.00	0.00
	20	180	18.33 ± 0.04 □	8.33	5.00	2.77	2.22
	40	132	34.09 ± 0.02 □□	13.63	6.82	6.07	7.57
	80	100	60.00 ± 0.01 □□	27.00	8.00	8.00	17.00
	100	60	83.33 ± 0.04 □□	41.67	8.33	8.33	25.00
24	Control	228	0.45 ± 0.04	0.45	0.00	0.00	0.00
	20	150	26.66 ± 0.05 □□	10.00	6.66	6.66	3.34
	40	100	40.00 ± 0.03 □□	15.00	8.00	7.00	10.00
	80	55	81.81 ± 0.01 □□	45.45	5.45	3.64	27.27
	100	30	90.00 ± 0.02 □□	50.00	3.33	3.33	33.34

Application of SA after 100 % plant extract exposure resulted in the significant reduction of chromosomal abnormalities as shown in the table (4).

In treated cells with 100 % plant extract for 3 hours, the recovery with SA caused more than 10% decrease in CAs at 0.01 and 0.2 mM concentrations of both

duration of recovery but, it showed greatest adaptive response to SA 0.1 mM concentration at which, SA caused 25% decrease in CAs in the root tip cells. in the same manner treated cells with 100 % plant

extract for 24 hours, the recovery with SA caused maximum decreased in CAs (more than 15 %) at 0.1 Mm concentration of both duration of recovery.

Table 3. Types and percentage of chromosomal aberrations induced by *Lantana camara* aqueous extract in root tip cells of *N. sativa*

Treatments		Prophase%		Metaphase%		Anaphase%				Telophase%		
Time (hr)	Conc. (%)	Irreg.	St.	St.	Dist.	Bridg	St.	Fw.	Lagg.	St.	St\Fw	Bridge.
3	Control	0.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	20	4.50	0.00	1.50	1.00	0.50	0.50	0.50	0.50	1.00	0.50	0.50
	40	5.26	1.05	2.11	1.58	1.05	0.53	1.05	0.00	1.05	1.05	1.05
	80	8.82	2.94	2.36	2.94	1.00	1.00	0.50	1.00	1.17	1.17	1.76
	100	12.25	3.87	2.58	2.58	1.28	0.64	1.93	0.64	3.22	3.22	3.23
6	Control	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	20	3.72	1.60	2.66	1.60	1.06	1.06	1.60	0.00	1.07	1.06	0.00
	40	7.50	3.75	2.50	2.50	1.25	1.25	1.87	0.00	1.87	1.25	1.25
	80	12.50	6.66	3.33	3.33	1.66	1.66	1.66	0.83	4.17	4.16	4.17
	100	16.66	7.77	4.44	3.33	2.22	1.11	2.22	1.11	5.55	3.33	5.55
12	Control	1.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	20	5.00	3.33	2.77	2.23	1.11	0.56	1.11	0.00	0.00	1.11	1.11
	40	7.57	6.06	3.78	3.04	1.52	1.51	1.52	1.52	2.27	2.27	3.03
	80	15.00	12.00	4.00	4.00	1.00	4.00	2.00	1.00	7.00	4.00	6.00
	100	25.00	16.67	5.00	3.33	0.00	5.00	3.33	0.00	8.33	6.67	10.00
24	Control	0.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	20	6.66	3.34	3.33	3.33	2.66	2.66	1.33	0.00	2.00	1.33	0.00
	40	10.00	5.00	5.00	3.00	2.00	3.00	2.00	0.00	5.00	3.00	2.00
	80	27.27	18.18	5.45	0.00	1.82	1.82	0.00	0.00	18.81	5.45	2.63
	100	30.00	20.00	3.33	0.00	0.00	3.33	0.00	0.00	20.00	3.34	6.66

4. Discussions

Positive results monitored in higher plant systems like the *N. sativa* indicate the presence of cytotoxic and/or genotoxic attributes of some compounds. This can also be used to monitor ameliorative and protective effects of some other compounds. The toxic potential of *L. camara* was described but not using cytogenetic parameters [58] and [59]. 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 [60] and [61]. Using cytogenetic approach, the mitotic index (indicator of cytotoxicity), the micronuclei formation and chromosomes aberrations (indicators of genotoxicity) are the most common parameters evaluate. The micronuclei can be interpreted as a

consequence of clastogenic (chromosome breakage) or aneugenic (chromosome lagging and interference on the spindle behavior) effects, and chromosomes aberrations can be originated by chromosome breakage and/or chromosome ex-change [62], [24] and [21].

In this study, the results revealed the different concentrations of *lantana camara* applied for different periods caused a reduction in the MI values. This effect was a time and a dose dependent. Similar effects of *Lantana camara* were reported in different plants such as in *Allium cepa* [28] and [16] in *Vicia faba* [27] in *Helianthus annuus*, [33] in *Lathyrus sativa* [32] and in *Oryza sativa*, *Vigna unguiculata* and *Vigna mungo* [35], [36] and [37]. Such inhibition is complex and can involve the interaction of different classes of chemicals, such as phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates, and amino acids present in *Lantana*

plant [30]. Also, [10] reported that various phenolic compounds in this plant inhibited cell division. In this concern, [60] reported that the decrease in mitotic index was the result of cytotoxic effects which could be due to interaction of the extract with protein or inhibition of protein synthesis [63].

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 chemical constituents of *L. camara* extract mainly aromatic alkaloids and phenolic compounds [64] and [65]. The inhibition in mitotic division can be also attributed to increase in mitotic abnormalities and DNA damage [66]. In contrast, [31] reported that the extract of *Lantana camara* did not effect on the mitotic division on *allium cepa* except one concentration (40 ml/L) which caused an increase in MI than the control value. Decrease in mitotic activities indicated mitodepressive effect of *Lantana* leaf extract, while chromosomal abnormalities revealed its chromotoxic potential on

Nigella sativa chromosomes in agreement with reported clastogenic potentials of different plant extract with different plant [32]. The results showed that cell cycle analysis for the number of cells in each phase of mitosis changed in extract-exposed root tips, providing the indication of induction of abnormal progress through mitosis by accumulation cells at prophasic and telophasic stages, reflecting a reduction in MI. The obtained data demonstrate that *L. cammara* extract induces cell cycle checkpoints defects in the tested plant root tip cells as suggested by [67]. The increasing amount of cells in prophase resulted from total inhibition of dividing cells to enter into the subsequent stages, indicating a complete stoppage of cell cycle due to mito-toxic effect of leaf extract.

The results showed that the *L. cammara* extract has a significant effect on the total percentages of the abnormalities induced in root tip cells in *N. sativa* plant and it was found to be positively correlated with the concentration and treatment duration and was observed different from that of the control.

Table 4. Effect of different concentration of Salicylic acid on *N. sativa* root tip cells for different durations.

Treatments	Salicylic acid concentrations. (mM)							
	6 hours				12 hours			
	0	0.01	0.1	0.2	0	0.01	0.1	0.2
Mitotic index	13.02	15.05 [□]	14.11 [□]	13.68	13.4	14.86 [□]	14.63 [□]	13.81 [□]
Total abn. %	0.44	4.18 [□]	2.67	3.18	0.5	6.25 [□]	3.83	5.02 [□]

The highest value of total abnormalities was 90% which recorded at concentration 100% for 24 hours compared with control value of 0.45%. The aberrant mitotic stages might have been the outcome of spindle poisoning that cause chromosome disturbances during mitotic cell division [68]. The total percentage of the abnormalities increased gradually with increase the plant extract concentration and as the time of treatment was prolonged. In general, the highest percentages of abnormalities

induced by the extract in all treatments were recorded in divided cells at prophase and telophase stages in tested plant.

The experimental findings of the present study showed that *Lantana camara* plant extract can induce significant chromosomal abnormalities in the root meristem cells of *Nigella sativa* as compared to control, which indicate the genotoxic potential of *Lantana camara* plant extract.

Table 5. Effect of different concentration of Salicylic acid on treated cells of *N. sativa* with *Lantana camara* aqueous extract (100%) for different durations.

Treatments	Concentrations	100 % Lantana for 3 hours		100 % Lantana for 24 hours	
		Mitotic index	Total abn. %	Mitotic index	Total abn. %
Time (h)	Control	13	0.46	13.45	0.45
	100 % Lantana	9.62	35.48	1.66	90
6	SA (0.01mM)	9.48	31.20 [□]	2.20 [□]	87.09 [□]
	SA (0.1mM)	9.96 [□]	26.87 ^{□□}	5.04 ^{□□}	77.02 ^{□□}
	SA (0.2mM)	9.83 [□]	30.37 ^{□□}	3.05 ^{□□}	80.95 ^{□□}
	SA (0.01mM)	9.58	31.40 [□]	2.04 [□]	86.20 [□]
12	SA (0.1mM)	10.08 ^{□□}	25.77 ^{□□}	6.43 ^{□□}	76.84 ^{□□}
	SA (0.2mM)	9.90 [□]	29.67 ^{□□}	3.43 [□]	80.00 ^{□□}

The results are in line with previous experiment in other plant [27], [29], [30], [32] [35], [36] and [37]

which demonstrated the potential cytotoxic and genotoxic of *Lantana. camara* in *Allium cepa*, *Vicia*

faba, *Lactuca sativa*, *Oryza sativa*, *Vigna unguiculata*, *Vigna Mungo* and *Lathyrus* meristematic cells exposed to different concentrations of plant extract.

Different types of mitotic abnormalities were observed in *Nigella sativa* after treatments with *Lantana camara* plant extract during the mitosis of root tip cells. The variation in the percentages of each of different types of chromosomal aberration observed in this study was dose independent [27], [28], [29], [33], [32] [35], [36] and [37]. In *Nigella sativa* L. these types were: irregular prophase, stickiness at different phases, disturbed metaphase, bridges, forward chromosome and lagging in addition to other types recorded in one or two cells in some treatments such as tripolar, binucleated cells, ghost cell and micronucleus. Of these types of aberrations, stickiness, as a most frequent type, was recorded in the different mitotic phases in root tip cells of tested plant treated with plant extract. This type of abnormality was recorded by many investigators following the treatment of different plants by *Lantana camara* plant extracts [30], [32], [69], [70], [71], [35], [36] and [37].

In this study, Irregular prophase or abnormal prophase (where the DNA in chromatin threads may be despiraled and/or depolarized or the chromatin threads were not typically arranged) was a common type of abnormalities induced in treated root tip cells *N. sativa* by *Lantana camara* plant extract and was recorded in a relatively high percentage in all treatments. The percentage of irregular prophase was higher than those in other types of abnormalities. Formation of irregular prophase resulted from its effect on the process of individualization of chromatin threads to normal chromosomes [72].

The induction of Sticky chromosomes indicates 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 [73]. Also, the induction of sticky chromosomes; laggards and vagrant chromosomes are indicators of spindle poisoning [74]. Bridges at anaphase and telophase can result from the terminal deletion or loss of telomeres. In according to suggestion of [67], chromosomal bridge could be a result of the failure of free anaphase separation, unequal translocation or inversion of chromosome segments. This type of abnormality was recorded by [30], [31], [32], [35], [36] and [37].

Binucleated cells have been observed in few cells. The occurrence of binucleated cells is the result of prevention of cytokinesis or cell plate formation. Also, Microtubules have been implicated in cell plate formation and the leaf extract of the plant contains

phenolic compound and alkaloid which can be one of the involved factors, resulting in inhibition of cytokinesis. Similar inhibition of cytokinesis cells was also reported by [27]. In view of this induced aberration, it can be concluded here that *L. camara* leaf extract showed its effect on spindle, process of cytokines and DNA [27]. The ghost cell induced effect of *Lantana camara* was not reported by other authors. Ghost cell is a dead cell in which the outline remains visible, but whose nucleus and cytoplasmic structures are not stainable. It is a possibility that substances in the high concentrations of *Lantana camara* leaf extract leading to nucleus damage and prevention of cytoplasmic structures resulted in ghost cells. Also, the laggards and chromosome fragments induced in study became surrounded by nuclear membrane forming micronucleus in accordance the explanation of [34] following the treatments of different plants with *Lantana camara* extract. In general, as we observed, of the extract *Lantana* species studied, suggesting of the extracts for the species studied, suggesting that the effect can be considered dose dependent. Thus, our results suggest caution in the use of *L. camara* in folk medicine. In this investigation the results showed that, all concentrations of using SA caused an increase in MI to the highest value of 15.05 which recorded with (0.01mM for 6 hours) compared with control value of 13.03%. Also increased the total abnormalities percentage in treated root tips to the value of 6.25 compared with the value of untreated root tip cells (control). In similar, [75] reported that there is significant increase in mitotic index in root tip cells of *Vicia faba* at majority of the treatments with salicylic acid for exposure time 24 and 48 h. In this respect, our observation was in contrast to the results obtained by [76] which demonstrated a dose dependent decreased in MI of *Allium cepa* treated with different concentration of SA for different duration's treatments.

SA post-treatment resulted in significant decrease in the frequency of cells with chromosomal abnormalities (CAs) from 35.48 to 25.76 for *N. sativa* treated root tips with *Lantana camara* plant extract for 3 hours and the period recovery 6 hours and from 90.00 to 76.84 for *N. sativa* treated root tips with *Lantana camara* plant extract for 24 hours and the period recovery 12 hours. So, our results revealed that exogenous SA ameliorate the toxic effect of *Lantana camara* plant extract. Also, this result implies that SA conditioning conferred adaptive response to genotoxic stress of *Lantana camara* plant extract. This result is in accordance with the earlier reports demonstrating SA-induced protective response to DNA damaging agents [77], [78] and [79]. Responses to genotoxic stress include activation of distinct stress signaling

pathways, delay of cell cycle progression, and induction of DNA repair [80], but it is not known which signal transduction pathways sense genotoxic stress in plants [81]. The application of SA may either

be harmful as reported by [76] at higher concentration or provide protection during abiotic stress, depending on the plant species, the concentration used and the mode of application [49].

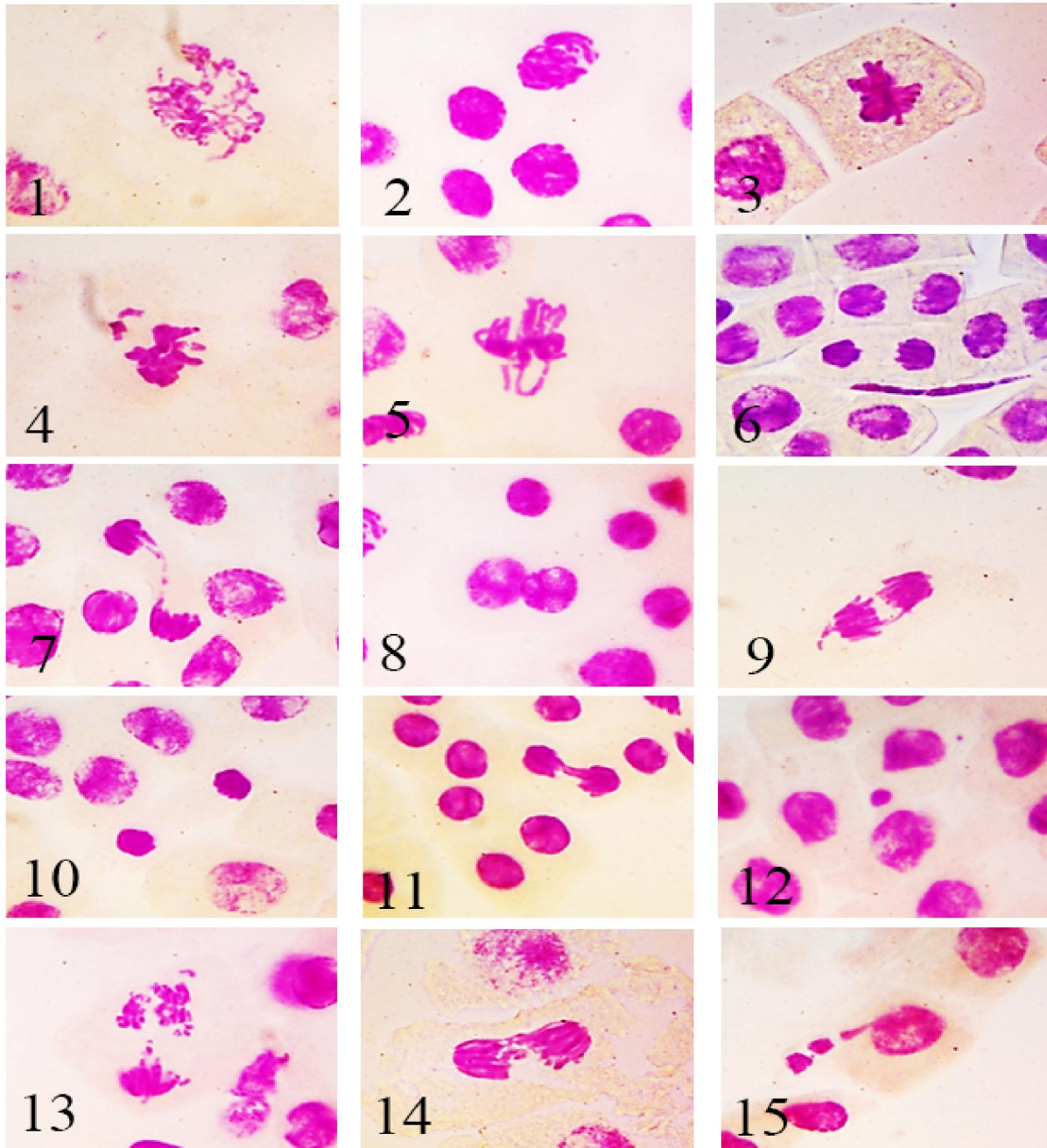


Plate (1) Chromosome and cellular aberrations observed in *N. sativa* root meristem exposed to *Lantana camara* aqueous extracts.

(1) Irregular prophase (2) Sticky prophase (3) Sticky metaphase (4) Fragments (5) Disturbed metaphase (6) Sticky anaphase (7) Anaphase with chromatinal bridge (8) Bi nucleated cell (9) Anaphase with single bridge and forward chromosome (10) Sticky telophase (11) Telophase with multiple bridge (12) Micronucleus (13) Divided polar of anaphase (14) Anaphase with lagging chromosome (15) Ghost cell.

In conclusion: the experimental findings from the present study could explain that SA is effective in inducing the adaptive response to genotoxic stress since it significantly reduces the chromosomal aberrations induced by the *L. camara* leaves extract. Efforts to understand the different mechanisms by which SA influence biological activities could help to better distinguish the advantages and disadvantages of its use and to clarify its possible protective role against environmental toxicants.

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