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Genotoxicity Testing of Neem Plant (*Azadirachta indica* A. Juss.) Using the *Allium cepa* Chromosome Aberration Assay

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Abstract: The genotoxic effects of aqueous extracts of neem *Azadirachta indica* A. Juss., Meliaceae leaves, kernels and seed coats was evaluated using *Allium cepa* chromosome aberration assay according to the standard protocol of the plant assays established by The International Program on Chemical Safety and The World Health Organization. Neem extracts suppressed the mitotic activity of *Allium* root meristems after 24 and 48 h treatment with all concentrations. Neem seed coat extract was the least effective in its ability to inhibit cell division, whereas kernel extract was the most effective. Analysis of the data of phase index indicate that all treatments reduced prophase percentage, mean while the percentage of metaphases and ana-telophases rose over those of control. Also, the extracts caused different kinds of chromosome aberrations in dividing and non dividing cells of *Allium cepa* such as micronucleus and multinucleated cells observed in the interphase stage; bridges, stickiness, non-congression metaphase, laggards, polyploidy and disturbed ana-telophase observed in dividing cells. Bridges was the most frequent kind of aberration in dividing cells. Neem seed kernel extract proved to be induce more chromosomal aberrations than neem leaf extract and neem seed coat extract. Accordingly, neem extracts should not be used for internal medicinal purpose until more thoroughly tested, calls for a closer look at the genetic toxicological effects in different test systems for human welfare.

Key words: Genotoxicity, neem, *Allium cepa*

Introduction

The neem tree (*Azadirachta indica* A. Juss., Meliaceae) is one of the most promising botanical insecticide at present (Lowery and Isman, 1995). Its products are known to have strong pesticidal properties (Schmutterer *et al*., 1981; Schmutterer and Ascher, 1984, 1987; Jacobson, 1986).

Evaluation of seed kernel extracts and pure compounds against numerous species of insect pests have demonstrated neem's diverse biological effects: repellence (Jacobson *et al*., 1978; Quadri, 1973), feeding deterrence (Pradhan *et al*., 1962; Singh, 1987), reduced growth and abnormal development (Koul, 1985; Schmutterer and Rembold, 1980), oviposition deterrence (Saxena *et al*., 1981; Singh and Srivastava, 1983), reduced egg laying due to sterilizing effect (Rembold and Sieber, 1981; Gujar and Mehrotra, 1984) and also direct toxicity (Goyal, 1971; Singh *et al*., 1988).

Studying the effects of neem seed kernel extracts on egg and larval survival of the Sorghum shoot fly *Atherigona soccata* Rondani, Zongo (1993) found that unhatched eggs were completely decomposed.

Radcliffe *et al*. (1991) found in field experiments that a water extract of neem kernels significantly prevented feeding on Sorghum seedlings by the Sahelian grasshopper, *Kraussaria angulifera* krauss. Dreyer (1984) found that simple aqueous extract of neem seed and neem oil reduced significantly damage done by *Bemisia tabaci* Genn. on Courgettes, *Cucurbita pepo* L.

Dreyer (1987) studying the effects of weekly applications of aqueous extracts of neem kernel and neem oil on several insect pests of vegetables and field crops, obtained positive results in controlling *Plutella xylostella* L. and *Hellula undalis* F. on cabbage, *Jacobiella facialis* Jac. on egg plant and *Scrobipalpa ergasima* Mayr. on gboma (*Solanum aethiopicum* L.).

Neem leaves are eaten as vegetable, and twigs are used as toothbrushes. Neem is used in the treatment of various skin diseases and it has antibiotic properties (Sharma, 1993). The people of India have taken neem tea as a tonic, oral birth control pill for men which have reduced fertility, and placed neem leaves in their beds, books, grain bins, cupboards and closets to keep away troublesome bugs. In certain parts of India (Andhra Pradesh), farmers specially feed neem leaves to cattle and goats immediately after parturition for increasing milk secretion (CSIR, 1985).

Toxicological studies have shown that neem alkaloids are safe for humans and neem oil is edible after processing (Rukmini, 1987).

The active ingredients in *Azadirachta indica* A. Juss. (Fig. 1) belong to a general class of natural products called "triterpenes"; more specifically, "Limonoids". Four new Limonoids are discovered in neem, azadirachtin, salannin, meliantriol, and nimbin are the best known (Vietmeyer, 1992). Azadirachtin is structurally similar to insect hormones called "ecdysones" which control the process of metamorphosis. On average, neem kernels contain 2 to 4 mg of azadirachtin per gram of kernel. The highest figure so far reported 9 mg per gram. Meliantriol feeding inhibitor while is able in extremely low concentration. Salannin has also powerful feeding inhibit, Nimbin and Nimbidin have been found to have antiviral activity. Other minor ingredients as antihormones including deacetyl-azadirachtinol. Although bioactive compounds are found throughout the tree, those in the seed kernels are the most concentrated (Jhansi and Singh, 1993).

Neem extract is used to inhibit insect growth. This may be mainly due to its ability to inhibit cell division which is a prerequisite to the growth process. Chromosomal changes resulting from the application of agricultural chemicals e.g. pesticides, therefore, have been regarded as reliable evidence of the genotoxicity of these compounds (Grant, 1978; Ma, 1982). Genotoxicity studies have led to the development of more than 200 short term assays to evaluate the genotoxicity of unknown environmental agents (Grant, 1994). *Allium cepa* chromosome aberration assay is highly effective and sensitive for the detection of mutagens (Rank and Nielsen, 1994).

This experiment was done to evaluate the genotoxic effects of aqueous extracts of neem leaves, kernels and seed coats using *Allium cepa* chromosome aberration assay according to the standard protocol of the plant assays established by the International Program on Chemical Safety and the World Health Organization.

Materials and Methods

Neem tree is native of Pakistan, then it introduced to Egypt. For this experiment the neem plant samples were collected

from the cultivated trees grown in the research farm of Mansoura University, Egypt.

Water extraction: The simplest technique is to crush or grind the leaves, kernels and seed coats and extract them with water. About 100 gm powder steeped overnight in a cloth bag suspended in 1 L distilled. water. The resulting crude suspension used for preparing different concentrations of neem leaf, neem seed kernel and neem seed coat. The concentrations are 1, 2, 4, 6 and 8% (containing 10,000, 20,000, 40,000, 60,000 and 80,000 ppm respectively) from the stock solution.

Chromosome aberration assay: The Allium test was carried out as described by Fiskesjo (1985) and later modified by Rank and Nielsen (1993). Commercial onion bulbs of *Allium cepa*, were obtained from the National Institute of Agriculture, Giza, Cairo. The onions were not treated with any growth inhibitors. Before use, the loose outer scales were carefully removed, and the dry bottom plates were scraped away without destroying the root primordia.

For each water sample six onions were set up and allowed to produce roots in tap water for 2 days. The tap water was changed every day. On second day the bulb with the poorest growth in each set was discarded and the other five transferred to the test solutions. Roots (2-3 cm) were treated with the solutions of 1, 2, 4, 6 and 8% for 24 and 48 hours. After treatment, the root tips were fixed immediately in aceto-alcohol (1:3). After fixation, the slides were prepared for examination using 5-6 root tips from each bulb. The root tips were hydrolyzed in 1N HCl at 60°C for 3-5 min. followed by staining in Carbol Fuchsin stain (Kao, 1975a&b). Root tips were then squashed in a 2% aceto-orcein stain in 45% acetic acid. Slides were made permanent, mounted in Canada balsam, examined and photographed.

The mitotic index, phase index and chromosome aberrations were determined by examination of at least 1000 cells. Chromosome aberrations were characterized and classified in the following categories: micronucleus and multinucleated cells in the interphase stage and bridges, stickiness, non-congression metaphase, laggards, polyploidy and disturbed ana-telophase in dividing cells. Statistical analysis was performed using the X^2-test for chromosome aberrations. Snedecor and Cochran, 1968 and Dougherty, 1990.

Results and Discussion

The use of biological active substances from neem for the control of agricultural pests, has been undertaken in different parts of the world. The studies done in Egypt indicate the possibility of using the neem seed kernel extract with insecticidal properties, for the control of different pests (Dimetry, 1993). Also, the data obtained by Moustafa (1993) showed that spraying cotton leaf worm (*Spodoptera littoralis* Boisd.) with neem water extract caused developmental retardation and complete sterility of the pest and concluded that the plant materials (polar and non polar components) seem to be responsible for antifeedant properties.

In this study, neem extracts suppressed the mitotic activity of *Allium* root meristems after 24 and 48 h treatment with all concentrations (Tables 1,2,3).

Njagi and Gopalan (1980) reported that the effect of food preservatives, sodium sulphite and sodium benzoate might be related to inhibitory effect on DNA synthesis. Similarly the effect of neem components might interact with DNA nucleotides thus inhibiting DNA synthesis (DNA/nucleoprotein equilibrium) and subsequent mitotic inhibition.

In general, neem seed coat extract (Table 3) was less effective in its ability to inhibit cell division compared with leaf extract (Table 1) and kernel extract (Table 2). The neem kernel extract was the most effective in this respect.

Many other investigations showed that the reduction in cell division activity could be due to change in the duration of the mitotic cycle. Van't Hoff (1968) suggested that, the inhibition of mitotic activity by chemical compounds is due to an increase in the G2 period. This view was supported by the results of Bruneri (1971) who obtained a complete arrest of mitotic cycle at the G2. Other authors (Webster and Davidson, 1969; Macleod, 1969) attributed the inhibition of mitosis to the increase in S-phase duration.

Studying the data of phase index (Tables 1-3) it is obvious, that all treatments reduced prophase percentage, mean while the percentage of metaphases and ana-telophases rose over those of control. So it is now clear that the components of the extract may interfere with or lead to blockage of DNA synthesis rather than retardation of the spindle formations, where mitotic index depression was not accompanied by lowering the metaphase and ana-telophase percentage.

Treatments significantly increased the percentages of aberrant dividing and non-dividing cells of *Allium cepa* root meristems than their respective control (Tables 1,2,3).

Seven main types of chromosome aberrations were recorded these are; micronucleus and multinucleated cells observed in the interphase stage; bridges, stickiness, non-congression metaphase, laggards, polyploidy and disturbed ana-telophase observed in dividing cells.

The most frequent kind of aberration is bridges. Single (Fig. 3A&B), double (Fig. 3C) and multiple bridges (Fig. 3D) were recorded in anaphase and telophase configuration. They are the result of chromosome breakage and reunion. The stickiness of chromosomes made the separation of daughter-chromosomes incomplete and thus they remained connected by bridges (Kabarity *et al.*, 1974; Badr *et al.*, 1992).

Chromosome stickiness was also one type of the abnormalities observed in metaphase and anaphase in root tips of *Allium* (Fig. 2F). Darlington and Mc Leish (1951) suggested that stickiness might be due to degradation or depolymerization of chromosomal DNA. Stickiness had been shown to be a result of entanglement of inter-chromosomal chromatin fibers and this led to subchromatid connections between chromosomes (Chauhan *et al.*, 1986).

Moreover, cells containing non-congression metaphase where the whole chromosome set had the tendency to build up the equatorial plate, a part from one or two chromosomes were usually noticed to lie free in the cytoplasmic area as shown (Fig. 3F). This may also form micronuclei in the mitotic cycle. The production of micronuclei in living cells after chemical treatment, was considered as a true mutagenic chemical (Auerbach, 1976). It was also used as a reliable parameter for chromosome aberration in *Vicia faba* test system (Degrassi and Rizzoni, 1981).

In addition to the types of chromosomal anomalies induced in dividing cells, micronuclei in interphase cells (Fig. 2A) have been recorded (Tables 1-3). Such abnormality may originate from a preceding multipolar telophase (Amer and Farah, 1974) or as a result of the disruption of spindle apparatus (Stroev, 1970). Micronuclei may also originate from lagging chromosomes or chromosome fragments in a preceding mitosis. Amer and Mikhael (1972) found that micronuclei represent a dominant type of the anomalies produced by radiations and were recorded in interphase as well as in different mitotic stages. Micronuclei were considered as an indication of a true mutation effect (Auerbach, 1962).

Table 1: Mitotic index, phase index and percentage of abnormalities in each phase and interphase cells in treated *Allium cepa* root with different concentrations under different treatments of neem leaf extracts

Abnormalities frequency (%)												
	Total .	Mistosis	24.0	33.6	49.1	34.9	69.7	27.8	27.3	35.6	45.7	44.0	6.01
	Abn.	±S.E.	±0.2NS	±0.2NS	±0.5NS	±0.5*	±0.5NS	±0.3NS	±0.2NS	±0.3NS	±0.1NS	±0.5*	±0.4
	%	Interphase	2.5	2.2	2.8	2.9	1.1	0.0	0.1	1.5	4.7	3.2	0.0
		±S. E.	±0.5*	0.2NS	±0.5	±0.4*	±0.3NS		±0.1NS	±0.3NS	±0.4*	±0.3NS	
		Unequal nucleus	0.0	19.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Telo.	Bridege	0.0	9.5	23.5	22.7	65.6	0.0	3.1	14.3	71.4	28.6	0.0
		Asynchrony	0.0	4.8	0.0	4.5	0.0	0.0	0.0	7.1	0.0	0.0	0.0
		Micronucleus	0.0	0.0	35.5	4.5	12.5	0.0	3.1	14.3	42.9	28.6	1.1
		Diagnoal	5.3	3.7	0.0	0.0	0.0	3.2	3.3	0.0	0.0	0.9	0.0
	Ana	Late separation	5.3	7.4	0.0	7.7	0.0	9.7	23.3	18.8	11.1	6.7	4.9
		Bridge	36.8	44.4	83.3	69.2	100	22.6	36.7	37.5	11.1	26.7	3.3
		Disturbed	21.0	3.7	0.0	7.7	0.0	32.3	20.0	25.0	11.1	20.0	23.0
		Chromosome ring	0.0	0.0	0.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0
		Polyploidy	0.0	0.0	3.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Meta.	Non-congression	5.6	0.0	17.9	0.0	10.0	2.6	0.0	0.0	7.7	2.6	0.0
		Micronucleus	8.3	31.7	17.9	19.0	30.0	7.9	10.3	22.9	30.8	47.4	0.9
	Pro.	Micronucleus	0.0	0.0	28.6	0.0	0.0	0.0	10.5	0.0	0.0	0.0	0.0
		Multinucleat	0.0	0.0	0.0	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0
	Inter.	Mincronucleus	2.5	2.0	2.6	2.4	0.9	0.0	0.1	1.4	4.4	3.1	0.0
		Telophase %	17.3	19.6	29.8	34.9	48.5	10.0	26.4	19.2	20.0	19.3	26.2
		± S. E.	±4.9*	±3.8NS	±6.4NS	±5.6NS	±6.9NS	±2.9*	±4.0NS	±3.2*	±4.8*	±5.7NS	±2.1
	Phase	Anaphase %	25.3	25.2	10.5	20.6	18.2	34.4	24.8	21.9	25.7	27.5	17.8
	Index	± S. E.	±5.7NS	±4.1NS	±5.3NS	±3.7NS	±5.3NS	±4.8*	±3.8NS	±4.6NS	±4.9NS	±5.6NS	±2.1
		Metaphase %	48.0±	38.3	49.1	33.3	30.0	42.2	32.2	47.9	37.1	34.9	33.5
		± S. E.	6.1NS	±4.0NS	±10.7*	±6.1NS	±6.2NS	±5.1NS	±4.0NS	±6.9*	±5.5*	±7.5NS	±2.2
		Prophase %	14.7	16.8	12.3	11.1	10.6	12.2	15.7	11.0	17.1	18.3	23.6
		± S. E.	4.3NS	±4.0NS	±5.0NS	±3.6*	±3.4*	±2.8*	±5.8NS	±3.8*	±2.6*	±4.2*	±2.2
		Mitotic index %	5.9	8.3	4.1	4.4	4.8	6.6	8.4	5.0	2.4	3.7	11.4
		± S. E.	±0.5*	±0.5*	±0.5*	±0.5*	±0.5*	±0.5*	±0.4*	±0.5*	±0.4*	±0.6*	±0.3
		Total divided %	58.6	83.3	41.2	44.3	48.1	65.5	83.6	49.8	24.4	36.6	114.4
		± S. E.	±5.2*	±5.6*	±5.1*	±5.4*	±4.9*	±5.1*	±4.8*	±5.4*	±3.7*	±5.7*	±3.3
		Concentration	1%	2%	4%	6%	8%	1%	2%	4%	6%	8%	Control
		Time			24h					48h			

*Significant at 0.05 level from control, NS: Not significant at 0.05 level from control, Total examined cells = 1000

Table 2: Mitotic index, phase index and percentage of abnormalities in each phase and interphase cells in treated *Allium cepa* root with different concentrations under different treatments of neem leaf extracts

Abnormalities frequency (%)												
	Total .	Mistosis	47.9	63.5	77.5	50.0	81.1	58.8	51.3	77.3	52.1	105.3	3.2
	Abn.	±S.E.	±0.4*	±0.4*	±0.3NS	±0.3NS	±0.4NS	±1.2NS	±0.4*	±0.4*	±0.4*	±0.2N	±0.4
	%	Interphase	2.7	8.5	2.0	3.1	7.2	0.7	4.2	1.0	0.5	1.8	0.0
		±S. E.	±0.5*	±0.5*	±0.5*	±0.4*	±1.2*	±0.2NS	±0.3*	±0.3NS	±0.3NS	±0.3*	
	Telo.	Bridge	65.9	25.0	90.0	80.0	94.7	95.8	38.7	56.3	63.6	100.0	8.9
		Micronucleus	24.4	31.3	10.0	20.0	±47.4	0.0	29.0	25.0	22.7	16.7	1.1
	Ana	Bridge	69.0	77.8	80.0	0.0	100.0	80.6	72.2	0.0	66.7	0.0	0.0
		Diagnoal	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Polyploidy	0.0	20.0	8.3	0.0	7.1	14.3	21.1	0.0	11.8	0.0	0.0
	Meta.	Non-congression	0.0	0.0	8.3	0.0	0.0	17.9	0.0	24.0	0.0	0.0	0.0
		Stickiness	0.0	0.0	41.7	25.0	0.0	0.0	0.0	60.0	0.0	88.9	0.0
	Pro.	Stickiness	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	125	0.0
		Multinucleat	0.0	6.0	1.4	2.2	6.8	0.0	3.0	0.1	0.0	1.4	0.0
	Inter.	Mincronucleus	2.7	2.5	0.6	0.9	0.5	0.7	1.2	0.9	0.5	0.4	0.0
		Telophase %	33.9	50.8	50.0	35.7	51.4	24.7	38.8	36.4	31.0	31.6	26.2
		± S. E.	±5.9NS	±7.5*	±6.7NS	±6.4NS	±7.0*	±4.9NS	±5.1NS	±6.8NS	±5.7NS	±6.1NS	±2.1
		Anaphase %	24.0	28.6	12.5	0.0	5.4	32.0	22.5	0.0	33.8	0.0	17.8
		± S. E.	±5.2NS	±6.8NS	±3.4*		±3.0*	±4.8*	±4.4NS		±5.6*		±2.1
	Phase	Metaphase %	24.0	15.9	30.0	57.1	37.8	28.9	23.8	56.8	23.9	47.4	33.5
	Index	± S. E.	±3.8	±4.1*	±4.8*	±7.6NS	±5.1*	±5.4NS	±5.0NS	±7.3*	±6.4NS	±6.9NS	±2.2
		Prophase %	19.0	7.9	10.0	0.0	5.4	14.4	17.5	6.8	9.9	21.1	23.6
		± S. E.	±4.2NS	±3.6*	±1.5*		±3.2*	±3.3*	±4.0NS	±2.1*	±3.4*	±5.2*	±2.2
		Mitotic index %	9.1	5.6	3.4	1.8	3.3	7.3	6.5	3.3	5.6	1.5	11.4
		± S. E.	±0.8*	±0.5*	±0.7*	±0.4*	±0.5*	±0.4*	±0.4*	0.2*	±0.5*	±0.2*	±0.3
		Total divided %	91.5	56.1	33.6	17.8	33.2	73.4	64.9	32.8	55.9	15.0	114.4
		± S. E.	±7.9*	±5.2*	±6.7*	±4.1*	±5.1*	±4.0*	±4.2*	±2.5*	±4.6*	±2.5*	±3.3
		Concentration	1%	2%	4%	6%	8%	1%	2%	4%	6%	8%	Control
		Time			24h					48h			

*Significant at 0.05 level from control, NS: Not significant at 0.05 level from control, Total examined cells = 1000

Table 3: Mitotic index, phase index and percentage of abnormalities in each phase and interphase cells in treated *Allium cepa* roots with different concentrations under different treatment of neem seed coat extracts

Total .	Mistosis	21.7	11.7	11.8	46.9	25.0	28.0	36.2	42.9	15.9	26.3	8.2
Abn.	±S.E.	±0.2NS	±0.2NS	±0.2NS	±0.3NS	±0.6NS	±0.2NS	±0.4NS	±0.6NS	±0.3NS	±0.3NS	±0.4
%	Interphase	0.0	0.3	0.2	2.0	0.0	0.2	0.2	1.4	0.8	1.5	
	±S. E.		±0.2NS	±0.2NS	±0.6NS		±0.1NS	±0.1NS	±0.4NS	±0.3NS	±0.4NS	0.0
	laggard	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Telo.	Micronucleus	0.0	0.0	0.0	45.5	0.0	4.5	7.1	0.0	0.0	0.0	1.1
	Bridge	0.0	0.0	0.	9.1	0.0	4.5	7.1	50.0	0.0	0.0	0.0
	Disturbed	5.9	10.0	33.3	0.0	0.0	45.5	14.3	0.0	0.0	0.0	8.9
	Diagnoal	8.0	0.0	0.0	0.0	0.0	14.3	0.0	0.0	0.0	0.0	0.0
Ana	Micronucleus	0.0	0.0	0.0	40.0	0.0	0.0	27.3	0.0	0.0	0.0	4.9
	Bridge	32.0	0.0	18.2	10.0	0.0	42.9	45.5	0.0	40.0	100.0	3.3
	Disturbed	12.0	21.4	27.3	0.0	0.0	7.1	36.4	0.0	40.0	0.0	23.0
	Chromosome ring	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Stickness	16.1	0.0	0.0	0.0	0.0	4.2	28.1	50.0	17.6	20.0	0.0
Meta.	Non-congression	0.0	4.8	0.0	11.5	0.0	0.0	0.0	0.0	0.0	10.0	0.0
	Micronucleus	0.0	0.0	0.0	7.7	0.0	4.2	0.0	0.0	0.0	0.0	0.0
Pro.	Micronucleus	0.0	13.3	0.0	68.4	66.7	0.0	0.0	0.0	0.0	0.0	0.0
Inter.	Multinucleat	0.0	0.3	0.2	2.0	0.0	0.2	0.2	1.4	0.8	1.5	0.0
	Telophase %	18.5	16.7	17.6	17.2	25.0	26.8	20.3	57.1	22.7	5.3	26.2
	± S. E.	±4.1NS	±5.2NS	±5.3NS	±7.5NS	±16.4NS	±6.2NS	±5.1NS	±15.2NS	±7.1NS	±1.7*	±2.1
	Anaphase %	27.2	23.3	12.9	15.6	25.0	17.1	15.9	0.0	11.4	10.5	17.8
	± S. E.	±4.2*	±6.2NS	±3.2NS	±4.4NS	±16.4NS	±5.3NS	±4.9NS		±5.6NS	±5.5NS	±2.1
Phase	Metaphase %	33.7	35.0	43.5	40.6	12.5	29.3	46.4	28.6	38.6	52.6	33.5
Index	± S. E.	±5.1NS	±7.4NS	±6.3NS	±7.1NS	±12.5NS	±5.0NS	±6.1*	±12.2NS	±7.3NS	±9.9NS	±2.2
	Prophase %	20.7	25.0	25.9	29.7	37.5	26.8	17.4	14.3	27.3	31.6	23.6
	± S. E.	±4.0NS	±5.6NS	±4.7NS	±6.5NS	±18.3NS	±4.0NS	±4.0NS	±9.1NS	±6.0NS	±9.2NS	±2.2
	Mitotic index %	7.1	4.6	6.7	9.6	2.6	6.0	5.2	1.4	5.5	2.1	11.4
	± S. E.	±0.5*	±0.4*	±0.5*	±0.9NS	±0.2*	±0.4*	±0.3*	±0.4*	±1.0*	±0.5*	±0.3
	Total divided %	70.9	45.8	67.2	95.5	26.0	59.5	52.1	14.0	55.1	20.6	114.4
	± S. E.	±5.0*	±4.3*	±5.1*	±9.4NS	±1.1*	±3.9*	±2.8*	±3.5*	±10.1*	±4.9*	±3.3
	Concentration	1%	2%	4%	6%	8%	1%	2%	4%	6%	8%	Control
	Time	24h					48h					

*Significant at 0.05 level from control, NS: Not significant at 0.05 level from control, Total examined cells = 1000

Polyploidy was observed in treated *Allium cepa* cells only with neem kernel and leaf extracts (Fig. 2D, Tables 1&2). Sudharson Raj and Reddy (1971) attributed the polyploid cells they observed in both mitosis and meiosis to the inhibition of spindle mechanism by the *Lathyrus sativus* leaf extract.

The bi- and multinucleated cells (Fig. 2B) observed in this investigation might be due to the suppression of phragmoplast formation in the early telophase by the extract treatment. Therefore neither cell plate nor cell wall appeared in treated cells (Sato and Tanaka, 1972; Shehab *et al.*, 1978).

Lagging chromosomes and chromosome fragments were observed in a law percentage (Table 3). Adhesion of the centromers of one or more chromosomes to the outer layer of the plasma and movement of the others towards the equatorial plate led to appearance of such lagging chromosomes (Barthelmess, 1957; Amer, 1965; Amer and Ali, 1968).

Disturbed metaphase and ana-telophase (Fig. 3E) might be due to the disturbance of the spindle apparatus. The chromosomal damage produced by chemicals may be due to their effect on DNA (Grant, 1978).

Thakur *et al.*, 1988 reported that 5% neem seed kernel extract can be used, in place of highly toxic synthetic insecticides because of its safety to beneficial insects and for its lower cost. On the other hand Makanjuola (1989) concluded that neem seed extracts were more effective than leaf extract, while Sallam (2000) noticed that leaf extract of neem proved to be more effective against insects than seed extract. In this study seed kernel extract of neem (Table 2) proved to be induce more chromosomal aberrations than neem leaf extract (Table 1) and neem seed coat extract (Table 3 and Fig. 4). All parts of neem are biologically active. The maximum bioactivity against insect pest is in seed kernel where azadirachtin and salannin are the major compounds present in the seed kernel (Jhansi and Singh, 1993).

In fact that neem extracts are natural products does not mean that they are benign. Indeed, there is evidence that they have negative effects. For instance, affect on certain aquatic life tadpoles and mosquito-eating fish died when neem extracts were applied to water and neem seeds falling into fish ponds killed tilapia fry. The leaves or leaf extracts also should not be consumed by people or fed to animals over a long period. Renal failure in Ghanaians who were drinking leaf teas as a malaria treatment also may lead to liver damage. In other studies, neem extracts were found to be toxic to guinea pigs and rabbits, and leaves fed to goats and guinea pigs reduced their rate of growth. Others found no toxicity problem of neem oil obtained from clean, fungus free seed kernels showed no oral toxicity in rats (Vietmeyer, 1992).

However, the strong correlation between mitotic inhibition caused by neem extracts through the inhibition of DNA synthesis, the induction of chromosome aberration leading to a loss of genetic material and most probably it interfere with protein synthesis, however this needs further investigation.

A strong correlation between the ability of the chemicals to cause chromosome aberrations and its capacity to induce point mutation had been reported (Njagi and Gopalan, 1981). Such effect should involve an action on chromosomal DNA. The induction of chromosome breaks, in particular, may lead to structural rearrangements and is regarded to be a rapid indication of mutagenic activities of their inducers. The results of the present study, therefore, point out a potential mutagenicity of the used neem. Neem extracts should not be used for internal medicinal purpose until more thoroughly tested, calls for a closer look at the genetic toxicological effects in different test systems for human welfare.

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