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GENOTOXIC EFFECT OF FOUR MEDICINAL PLANT EXTRACTS ON *PISUM SATIVUM* CV. ARIKIL

SAZADA SIDDIQUI*

College of Science, Department of Biology, King Khalid University, Al-Lassan Campus, Abha 61413, Kingdom of Suadi Arabia

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Abstract

The leaf extracts from four medicinal plants *viz.*, *Azadirachta indica* A. Juss., *Tectona grandis* L.f., *Dalbergia sissoo* Roxb. and *Eucalyptus tereticornis* J.E. Smith were evaluated using *Pisum sativum* (Linn.) reduced mitotic index in a dose-dependent manner. The percentage of increasing abnormal mitotic plates was also concentration and time dependent. Commonly observed abnormalities were c-mitosis, laggard, bridges, stickiness, precocious separation, vagrant and fragments. The results indicate that commonly used aqueous leaf extracts of above plants has significant mutagenic action on plant model *P. sativum* var. *Arikil*.

Herbal medicines prepared using various plant parts of the plant are being used all over the world to cure human diseases. It is well known that the medicinal property of plants or plant extracts is due to the phytochemical substances present in it (Prabhu *et al.* 2011). The World Health Organization estimates that up to 80% of the world's populations rely on the traditional medicinal system for some aspects of primary health care (Farnsworth *et al*. 1985). *Azadirachta indica* A. Juss., *Tectona grandis* L.f., *Dalbergia sissoo* Roxb. and *Eucalyptus tereticornis* J.E. Smith are well known because of their medicinal use (Majumdar *et al.* 2007, Asif and Kumar 2011, Sengottayan 2007).

However, these plants may exert toxic effect on other plants as the genetic constitution and metabolic pattern are different. In the present investigation the genotoxic effects of four medicinal plant *(A. indica*, *T. grandis*, *D. sissoo* and *E. tereticornis*) extracts in root tip cells of *P. sativum* var. *Arikil* were carried out.

Fresh leaves of *A. indica*, *T. grandis*, *D. sissoo* and *E. tereticornis* were collected from different parts of the campus of Bundelkhand University, Jhansi, India. The leaves were collected from the middle region of the tree canopy, washed and shade-dried in the laboratory. The leaves were powdered with the help of a blender and different concentration of the extract (12.5, 25 and 50 g/l) was prepared by using double distilled water. The solutions were kept in water bath maintained at 45 - 55ºC for 24 hrs. The resultant extracts were filtered through sterile cotton followed by Whatman No.1 filter paper, sealed and stored in a refrigerator until further use.

Healthy uniform grains of *P. sativum* cv. Arikil (2n = 14) were obtained from the Agriculture Seed Store, Govt. of Uttar Pradesh, Jhansi, India. Before germination, the grains were surface sterilized with 1% sodium hypochlorite for 20 min followed by rinsing with distilled water for several times to remove excess of chemical. The seeds were pre-soaked in distilled water for 3 hrs and then soaked in aqueous extracts of leaves of different medicinal plants for 6 hrs. The seeds from different treatment groups (30 each) were placed on moistened pre-sterilized cotton in Petri dishes (15 cm dia) and incubated in a plant growth cabinet for 120 hrs in dark at 20 ± 2°C. Seeds in the control groups (Ck) were treated with the double distilled water. The entire experiment was repeated thrice under similar experimental conditions.

*Author for correspondence. <kalasaz@yahoo.co.in>.

Chromosome preparation was made from the root tips following the methods described by Qian (1998) with minor modification (Siddiqui *et al.* (2007). Chromosome spreads were made by using the squash technique (Savaskan and Toker 1991). A total of 500 cells was scored from each preparation to study the mitotic index and expressed in percentage. Various types of chromosomal aberrations (CA) such as fragments (FR), precocious separations (PS), sticky chromosomes (STC), laggards (Lag), bridges (BR) were studied in a minimum of 100 metaphase-anaphase (M-A) plates. Data were compared by ANOVA using the STATVIEW 4.5 (abacus concept; Berkeley, USA) software package and difference considered statistically significant at $p < 0.05$.

Leaf extract of all the medicinal plants had significant inhibitory effect on the mitotic activity, even at the lowest concentration. *Dalbersia sissoo* and *T. teretocornis* extracts had significantly higher inhibitory effect on mitosis compared to that of *A. indica* and *T. grandis* extract (Table 1). The lowest mitotic index was observed in seeds treated with (50 g/l) *T. tereticornis* extract which was ~5 times lower than that of control group. At the highest dose tested (50 g/l) mitotic index of 5.2 ± 1.3, 5.4 ± 1.51, 3.2 ± 1.91 and 2.6 ± 1.14 was observed in root tip cells treated with extracts of *A. indica, T. grandis, D. sissoo and T. teretocornis* groups, respectively (Table 1). Even though the mitotic index was found to be marginally higher in *A. indica* (12.5 v/s 25 g/l) and *T. grandis* (25 v/s 50 g/l) with increase in dose, it was statistically non-significant. The result indicates that the order of inhibitory action on mitosis is as follows: *E. tereticornis* > *D. sissoo* > *T. grandis* >*A. indica.*

Table 1. Mitotic index in root tip cells of *P. sativum* cv. Arikil after treatment of seeds with different plant extracts*.

Treatment groups	Concentration of extract (g/l)		
	12.5	25	50
A. indica	10.6 ± 5.59b	11.8 ± 3.03c	5.20 ± 1.3b
T. grandis	10.4 ± 2.88b	4.60 ± 1.94b	5.40 ± 1.51b
D. sissoo	7.20 ± 1.30b	3.80 ± 1.91b	3.20 ± 1.91b
T. tereticornis	5.60 ± 3.20b	2.80 ± 0.83b	2.60 ± 1.14b

*Ck = 16.25 ± 2.72. b = $p < 0.01$ very significant; c = $p < 0.05$ significant compared to control.

The mitotic preparation from root tips of *P. sativum* seeds treated with leaf extracts of four medicinal trees exhibited concentration dependent chromosomal abnormalities such as STC, Lag, C-m, PS, FR, BR and vag (Table 2, Fig. 1). Among these abnormalities C-m was the most common whereas vag. was the rarest. The leaf extracts exhibited a concentration dependent activity on inducing chromosomal aberration. The occurrence of different chromosomal abnormalities induced by *A. indica* at the highest concentration tested was in the following order, C-m (1.8 %), STC and PS (~1.2%) > Lag (~`0.8%)> FR and BR (~0.4%). Vagrant was not observed.

In the case of *T. grandis*, the most frequently reported CA were STC (~1.6%) and C-m (~1.6%) followed by Vag (~1.2%), FR (~1.0%), PS (~1.0%), BR (~0.8%) and Lag (~0.2%). However, BR and vag were not observed in lower dose (12.5 g/l). (Table 2).

In *D. sissoo* treated seeds the most frequent reported chromosomal aberration was C-m (~1.2%). The next abnormalities are STC (~1.0%) and FR (~0.2%). The abnormalities Lag, PS and BR were not observed in lower and higher doses (Table 2).

Table 2. Chromosomal aberration in *P. sativum* cv. Arikil seeds treated with different plant extracts.

Treatment groups	Chromosomal aberrations (mean ± SD)						
	STC	Lag	C-m	PS	FR	BR	Vag
A. indica (g/l)							
Ck	0.00 ± 0.00	0.01 ± 0.001	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
12.5	0.40 ± 0.24	0.20 ± 0.09	0.60 ± 0.40 b	0.20 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
25	1.00 ± 0.67b	0.20 ± 0.01	1.2 ± 0.58b	0.60 ± 0.24b	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50	1.20 ± 0.34b	0.80 ± 0.37b	1.8 ± 0.4b	1.20 ± 0.37b	0.40 ± 0.24b	0.40 ± 0.24	0.00 ± 0.00
T. grandis (g/l)							
Ck	0.01 ± 0.001	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
12.5	0.40 ± 0.24	0.00 ± 0.00	0.20 ± 0.01	0.20 ± 0.07	0.20 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
25	0.80 ± 0.37b	0.00 ± 0.00	1.00 ± 0.3b	0.40 ± 0.20b	0.20 ± 0.09	0.20 ± 0.14	0.40 ± 0.24b
50	1.60 ± 0.60b	0.20 ± 0.37	1.60 ± 0.24b	1.00 ± 0.44b	1.00 ± 0.54b	0.80 ± 0.37b	1.20 ± 0.37b
D. sissoo (g/l)							
Ck	0.00 ± 0.001	0.00 ± 0.00	0.00 ± 0.00	00.0 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
12.5	0.40 ± 0.24c	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
25	0.80 ± 0.37b	0.00 ± 0.00	0.40 ± 0.24b	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50	1.00 ± 0.44b	0.00 ± 0.00	1.20 ± 0.37b	0.00 ± 0.00	0.20 ± 0.08b	0.00 ± 0.00	0.00 ± 0.00
E. tereticornis (g/l)							
Control	0.01 ± 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
12.5	0.80 ± 0.48b	0.20 ± 0.01b	0.60 ± 0.03b	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
25	1.20 ± 0.48b	0.20 ± 0.09b	1.00 ± 0.44b	0.40 ± 0.001b	0.40 ± 0.24b	0.40 ± 0.24b	0.20 ± 0.08
50	1.60 ± 0.50b	1.40 ± 0.24b	1.80 ± 0.37b	1.80 ± 0.37b	1.80 ± 0.37b	1.00 ± 0.44b	0.60 ± 0.40b

a = $p < 0.001$ highly significant; b = $p < 0.01$ very significant; c = $p < 0.05$ significant compared to control. Ck = Control.
STC = Sticky chromosome; Lag = Laggard; C-m = C-mitosis; PS = Precocious Separation; FR = Fragment; Br = Bridge; Vag = Vagrant.

In case of seeds treated with *E. tereticornis* extract, the most frequent abnormalities were reported C-m (1.8%) followed by PS and FR (~1.8%), STC (~1.6 %), BR (~1.0%) and Vag (~0.6%). The overall order of genotoxicity of four medicinal plants that caused chromosomal aberration is as follows*: E. tereticornis* > *T. grandis* > *A. indica* > *D. sissoo*.

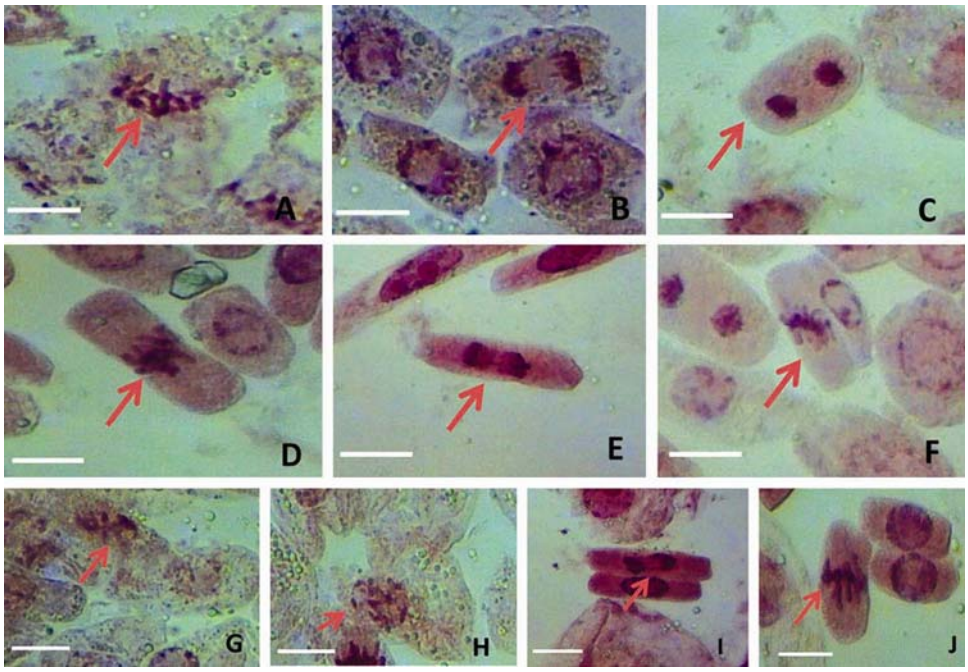

Fig. 1. Representative images showing the effects of aqueous leaf extracts of four medicinal trees on chromosome aberrations in root tip cells of *P. sativum* cv. Arikil. (A-C)- Control metaphase, anaphase and telophase. (D-J)- Sticky chromosome, laggard, C-mitosis, precocious separation, fragment, bridge at anaphase and vagrant (Scale bar = 10 µm).

In the present study, the potential genotoxic effects of leaf extract of four medicinal plants on *P. sativum* root cells were tested. Leaf extract of medicinal plants have a complex mixture of certain phytochemicals that may contain both mutagenic and antimutagenic properties. The suppression of mitotic index in *P. sativum* by leaf extract of medicinal plants is observed in the present study. Inhibition of mitotic index of *P. sativum* might be due to the presence of mutagenic compounds in the extract which might be related to its action on spindle assembly or cell cycle regulators (Al- Moaruf *et al.* 2004, Haider *et al.* 2004). Similar results were reported earlier in *Allium cepa*, cucumber, Lettue, millet and *Cicer arietinum* (Siddiqui *et al.* 2010, Ali *et al.* 2012).

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