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Colchicine induced variability in *Zanthoxylum armatum* Rox. (Rutaceae)

RAMDAS, G.K. DHINGRA AND M.A. RATHER

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SUMMARY

The genus *Zanthoxylum* is a member of family Rutaceae. The family Rutaceae embraces 1,800 species in 150 genera. It is found as forest undergrowth. During the present investigation, *Z. armatum* was explored for the colchicine induced variable characteristics. For the colchiploid plant study, seed samples were soaked in distilled water in a beaker for 10-15 minutes then were treated with various concentrations (1.0 M, 0.50 M and 0.05 M) of colchicine solution for half to two hours in small vials and then were kept in a refrigerator. After the treatment, seeds were washed thoroughly with distilled water for 4-5 times and then were planted in polybags having mixture of soil, sand and cocopit (2: 2: 1 V/V/V). The analysis was carried out for control and treated plants using parameters *viz*., total length of the plants, length of unbranched stem, length of primary, secondary and tertiary branches, diameters of unbranched stem, stem girth, number of leaflets, length and breadth of leaflets, diameter of seed and seeds per panicle etc. Studies on developmental and morphological characters of control and treated plants showed that the colchiploids exhibited delayed flower and leaf emergence. The leaves were thicker, shorter and darker green in colour with reduction in number of leaves per plant. There was reduction in the number of flower per plant. Floral variants observed included smaller sized flowers, flowers with deep pigmentation and orange red pigmented anthers. Total mean length of control and treated plants ranged from 160.45 – 181.00 and 118.50 -1.48.50, respectively. Mean number of leaflets in control ranged from 3-13 while in colchicine treated plants mean number of leaflets ranged from 2-9, respectively. Length and breadth of leaflets in control ranged from 2.97 x 1.46 to 4.02 x 1.59 cm., while in treated plants it ranged from 1.47 x 0.83 to 2.49 x 1.30 cm. Diameter of seeds in treated plants were observed higher than to control plants.

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Key words : Cocopit, Colchiploidy, Distilled water, Leaf emergence, Polybags, Vial tubes

The genus *Zanthoxylum* is distributed worldwide from tropical to temperate zones. There are over 200 species from small shrubs to large trees. It has some other synonyms as *Z. planispinum, Z. alatum subtrifoliolatum* (French.), etc. It is known as winged prickly ash, tejbal, tejphal, timroo timber or Nepali dhaniya. It is widely distributed throughout the warmer region of the world, extending into temperate region of Europe, Asia and Australia. About 50 species among 20 genera are reported from India. Out of which 9 species are classed as commercial timbers (Pearson and Brown, 1932). About 50 species of *Zanthoxylum* among 20 genera are reported from India. The Uttarakhand Himalaya harbours 4 species of *Zanthoxylum,* namely *Z. armatum* DC. *Z. acanthopodium* DC. *Z. oxyphyllum* Edgew and Z. *budrunga*. The genus is represented by *Z. limonella* in the plains but the other species are restricted to montane and sub-montane regions. All the 8 species, namely *Z. ovalifolium*, n=18, 34; 2n=ca. 136; *Z. acanthopodium,* n=32; *Z. armatum*, n=33; *Z. nitidum*, n=34; *Z. scandens*, n=34; *Z. limonella*, n=34; *Z. oxyphyllum*, n=36; and Z. *tomentella* n=36 are cytologically investigated.

All the plant parts like seeds, bark, fruits, branches, thorns are used in different ailments (Gupta, 1945; Uphof, 1959; Gamble, 1972; Usher, 1974; Chopra *et al.,* 1986). During winter, a soup made from the dried fruit (locally known as hag) is consumed by the local people to keep them warm. A chutney (like a sauce), locally known as dunkcha, is also a popular food item. It is also used as a spice, and as pepper substitute (Gupta, 1945; Tanaka, 1976). The seed is ground into a powder and used as a condiment (Facciola, 1990). The fruit is rather small but is produced in clusters which make harvesting easy. Each fruit contains a single seed and young leaves are used as

Correspondence to:
RAMDAS, Department of Botany, R.C.U. Gov.t P.G. College, UTTARKASHI (UTTARAKHAND) INDIA
Email : ram84.uki@gmail.com

Authors' affiliations:
G.K. DHINGRA, Department of Botany, R.C.U. Gov.t P.G. College, UTTARKASHI (UTTARAKHAND) INDIA

M.A. RATHER, Department of Chemistry, R.C.U. Gov.t P.G. College, UTTARKASHI (UTTARAKHAND) INDIA

condiments (Gupta, 1945; Tanaka, 1976; Facciola, 1990). The different plant parts have a number of alkaloids like dictamine, γ-fagarine, etc. The oil obtained from plant is known as Zanthoxylum oil or Nepali pepper oil. The essential oil is obtained by stem distillation of the dried fruits. The oil being rich in linalool, and also containing limonene, methyl cinnamate and cineole. It is used as antibacterial, anti- infectious, anthelmintic, antifungal, antistomachic, sedative, and for curing diseases like arthritis, cholera and toothache.

MATERIALS AND METHODS

For detailed morphological analysis of plant, matured fruits which were noticed red to dark brown in colour were collected from the different research sites (Z-1 to Z- 3). These seeds were dried in the sun light for 5-10 days. Seeds were subjected to various treatments of colchicine (1.0 M, 0.50 M and 0.05 M concentrations) except control with soap and water as described by Rodriguez (1995) and then were sown in research field. The sowing of the seeds in lines was carried out between first and second week of October 2007. The distance between two lines was kept about 30 cm. and plant to plant distance was about 10 cm. and the length of a line was kept about 1.5 meters. The mature seeds of the different sites (Z -1 to Z - 3) were sown in the last week of the October 2007 at the research field of Botany Department of R.C.U. Govt. P.G. College, Uttarkashi. The vegetative characteristics of control and colchicine treated (0.50 M and 0.05 M) plants were evaluated in mature plants about 15 days before harvesting. The floral characteristics of control and treated plants of different sites were studied when the plants exhibited optimum bloom. Yield related parameters were also worked out at the harvesting stages of plants. Various concentrations of colchicine and different treatment durations were tested.

The morphological studies were carried out using parameters related to vegetative reproduction and yield characteristics as - total length of the plant, total length of unbranched stem, number of primary, secondary and tertiary branches, diameter of unbranched stem. Following parameters were taken into consideration for reproductive studies of the plant *viz*., colour of the florests, number of heads per plant, diameter of a head, number of fertile and sterile florets, seeds set per plant. Total height of plant, length of the unbranched stem, number of primary, secondary and tertiary branches were measured by using measuring tape having calibration in inches. The phenotypic variability between the control and colchicine treated accessions of *Z. armatum* was critically evaluated using the vegetative reproductive and yield related parameters.

RESULTS AND DISCUSSION

A significant morphological characteristics in root system of control and treated plantlets were recorded. Control plantlets generally initiated more roots from the base of the shoots, and these roots were thinner and had fewer branches in comparison to those of the treated plantlets. After transfer to pots, the treated plants produced relatively broader leaves but shorter petioles than control plants. The mature plants of different sites were differed in their total height. In colchicine treated plants, a reduction in plant height, length and breadth of leaf was reported as compared to control. Treated plants also exhibited delayed flowering and flowered about twenty days later than the control. Treated plants had larger flowers and larger seeds than control. Only few seedlings were survived in 1.0 M concentration of colchicine. After six months, 1.0 M treated seedlings were died, so no any morphological parameters at the time of maturity were calculated in this concentration. Seedlings of *Z. armatum* treated with 1.0 M colchicine for 24 h demonstrated high rates of abnormalities (Plate 1).

The mean height of mature plants of control plots were recorded and ranged from 160.45 to 181.00 cm while in 0.50 M and 0.05 M treated plants, mean plant height ranged from 118.50 to 142.00 cm and 136.20 to 148.50 cm, respectively. A significant reduction in height at the time of flowering was noticed in all the treated plants from all sites and a reduction of ca. 40 % was reported in 0.50 M and 0.05 M colchicine treated plants. The range of the mean number of primary branches per plant (control and treated) differed significantly. In control plants, range of the mean number of primary branches was from 7.10 to 8.60 cm, while in 0.5 M treated plants, it ranged from 5.30 to 6.30 cm and in 0.05 M it was reported 5.10 cm, respectively (Table 2 and Fig. 2).

The leaf abnormalities induced changes in shape, size, margin, apex and colours of leaflets etc. No abnormalities were noticed in control condition. Colchiploids (1.0 M) exhibited delayed leaf emergence. Plants treated with 0.50 M and 0.05 M concentrations of colchicine showed thicker, shorter and darker green coloured leaves along with reduction in number of leaves and leaflets per plant. The range of the mean number of secondary branches per plant (control and treated) were differed significantly. In control plants, range of the mean number of secondary branches ranged from 14.10 to 14.60 cm while in 0.5 M treated plants, it ranged from 10.10 to 10.60 cm and in 0.05 M treated plants it was observed

Table 1 : Length and breadth of leaflet in control and colchicine treated seedlings (Z-1 to Z-6) of *Z. armatum*

Sr. No.	Parameters	Mean	SD	± SE	Range
Z–1 Con	LL	4.02	1.90	± 0.60	1.7 – 6.9
	BL	1.59	0.36	± 0.11	1.1 – 2.3
1.0 M	LL	1.47	0.62	± 0.20	0.8 – 2.5
	BL	0.83	0.23	± 0.07	0.5 – 1.2
0.50 M	LL	1.91	0.94	± 0.30	09 – 3.2
	BL	1.21	0.44	± 0.14	07 - 1.7
0.05 M	LL	2.01	0.91	± 0.29	1.1 – 3.4
	BL	1.27	0.36	± 0.11	0.8 – 1.7
Z-2 Con.	LL	3.28	0.99	±0.31	2.0 – 5.2
	BL	1.46	0.29	±0.09	1.0 – 1.9
1.0 M	LL	2.10	0.52	± 0.16	1.5 – 3.1
	BL	1.20	0.29	±0.09	0.8 – 1.7
0.50 M	LL	2.49	0.46	± 0.15	1.9 – 3.3
	BL	1.30	0.38	± 0.12	0.8 – 1.9
0.05 M	LL	2.13	0.53	± 0.17	1.5 – 3.1
	BL	1.13	0.26	± 0.08	0..9 – 1.6
Z-3 Con.	LL	2.97	1.11	± 0.35	1.7 – 5.4
	BL	1.54	0.30	± 0.09	1.1 – 2.0
1.0 M	LL	2.40	0.60	± 0.19	1.6 – 3.1
	BL	0.76	0.22	± 0.07	0. 5 – 1.2
0.50 M	LL	2.41	0.61	± 0.19	1.6 – 3.5
	BL	1.13	0.18	± 0.06	0.9 – 1.4
0.05 M	LL	2.41	0.61	± 0.19	1.6 – 3.5
	BL	0.80	0.24	± 0.07	0.9 – 1.4

LL = Length of leaflet (cm) BL = Breadth of leaflet (cm)

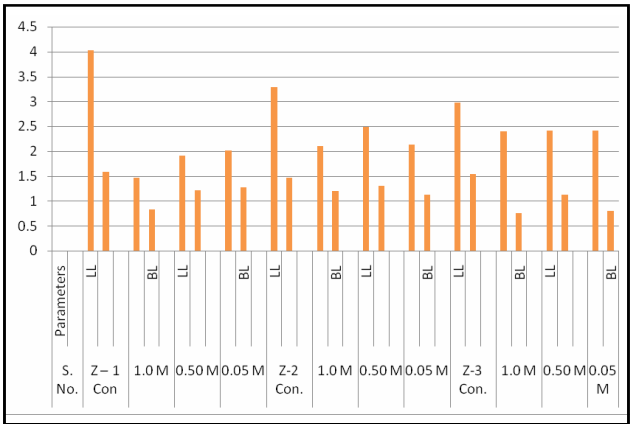


Fig. 1 : Graphical representation of length and breadth of control and colchicine treated (1.0 M, 0.50 and 0.05 M) seedlings of *Zanthoxylum armatum*

Table 2 : Vegetative morphology of mature plant of *Z. armatum*. (control and treated)

Sr. No.	Parameters	Mean	SD	±SE	Range
Z-1 Con	PH	164.00	40.06	±12.67	105-220
	LUS	78.50	16.68	±5.27	65-100
	PBP	8.18	2.68	±0.85	04-13
	SBP	14.50	2.76	±8.87	11.0-18.0
	TBP	21.70	4.19	±1.33	15.0-28.0
	SD	5.86	1.45	±0.46	3.4-8.3
0.50 M	PH	118.50	34.96	±11.06	50-170
	LUS	59.20	17.01	±5.38	40-78
	PBP	5.30	2.06	±0.65	02-07
	SBP	10.10	2.51	±0.70	4.0-11.0
	TBP	16.70	3.92	±1.24	11. 0-23.0
	SD	6.98	2.55	±0.81	3.5-12.5
Z-2 Con.	PH	181.00	39.07	±12.36	110-245
	LUS	66.00	15.24	±4.82	40-90
	PBP	7.10	3.28	±1.04	3.0-13
	SBP	14.60	3.50	±1.11	10-20
	TBP	21.50	3.27	±1.04	15.0-25.0
	SD	7.80	1.81	±0.57	5.5-10.2
0.05 M	PH	148.50	47.96	±15.17	55-205
	LUS	49.90	18.99	±6.00	25-88
	PBP	5.10	2.02	±0.64	3. 0-8. 0
	SBP	10.20	2.70	±0.85	3.0-9.0
	TBP	16.90	2.64	±0.84	12. 0-15.0
	SD	9.17	2.72	±0.86	4.5-13.3
Z-3 Con.	PH	160.45	19.16	±6.06	140-195
	LUS	57.00	8.23	±2.60	45-70
	PBP	8.60	2.80	±0.88	5.0-13.0
	SBP	14.10	3.38	±1.07	11.0-21.0
	TBP	26.20	4.85	±1.53	18.0-30
	SD	7.22	1.59	±0.50	5.2-10.0
0.50 M	PH	142.00	20.71	±6.55	45-100
	LUS	46.30	9.63	±3.04	33-65
	PBP	6.30	2.67	±0.84	3. 0-11.0
	SBP	10.60	4.12	±1.30	3.0-11.0
	TBP	21.70	5.10	±1.61	15.0-27. 0
	SD	8.44	2.12	±0.67	4. 5-10. 2

*No these morphological parameters were recorded during 1.0 M Conc. As seedlings were died within 6 months in this concentration of colchicine. In Z-1, Z-3, Z-5 and Z-6, 0.50 M treated plants were survived at the maturity period, while in Z-2 and Z-4, 0.05 M treated plants were survived.
PH= plant height (cm)
LUS = Length of unbranched stem (cm)
PBP = Number of primary branches per plant (cm)
SBP = Number of secondary branches per plant (cm)
TBP =Number of tertiary branches per plant (cm)
SD = Stem diameter (cm)

10.20 cm, respectively. Colchicine treated plants showed low frequency of secondary branches as compared to control groups. Length and breadth of leaflets per plant was counted at harvesting stage. Seeds and seedlings treated with various concentrations (1.0 M, 0.50 M and 0.05 M) showed decreased length and breadth of leaflets as compared to control. Mean length and breadth of

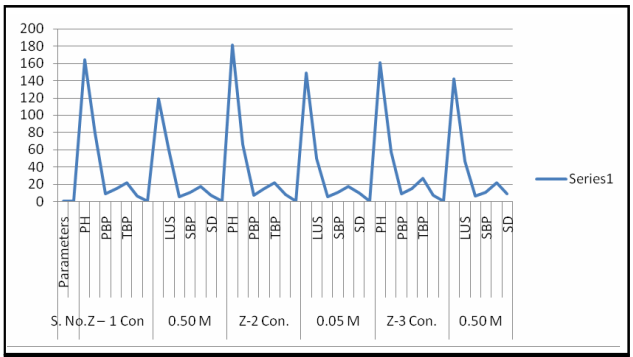


Fig. 2 : Graphical presentation of vegetative parameters *viz.*, plant height, number of primary branches per plant (cm), number of secondary branches per plant (cm), number of tertiary branches per plant (cm) and stem diameters of control and colchicine treated plants of *Zanthoxylum armatum*

leaflets of control plants (all sites) were found to be 2.97 to 4.02 cm and 1.46 to 1.59 cm while in treated plants, mean length and breadth of leaflets were ranged from 1.47 to 2.40 cm, 0.76 to 1.20 cm in 1.0 M; 1.91 to 2.49, 1.13 to 1.30 cm in 0.50 M and 2.13 to 2.41 cm, 0.80 to 1.27 cm in 0.05 M, respectively. In 1.0 M concentration, most of the seedlings were died and some seedlings showed brown coloured leaflets and three cotyledons (Table 1 and Fig. 1).

The minimum number of heads was found in 0.50 M and 0.05 M as compared to control plants of all sites. Mean number of heads ranged from 6.80 to 14.80 in control while in different concentrations of colchicine, heads number ranged from 5.40 to 5.90 in 0.50 M and 4.60 in 0.05 M concentration, respectively. Mean of the head diameter in control ranged from 3.98 to 5.52 while in 0.50 M treated mature plants of *Z. armatum,* mean head diameter was ranged between 6.03 to 7.24 and in 0.05 M it was recorded 5.08, respectively (Table 3).

The number of fertile and sterile florets in control and treated plants of *Z. armatum* was calculated in all the selected sites. The mean number of fertile florests per head in control plants ranged between 5.20 to 7.30 while in colchicine treated mature plants, the mean number of fertile florest per head was ranged from 5.30 to 7.00 in 0.50 M and 4.70 in 0.05 M concentrations of colchicine, respectively. A significant variability between the control and treated flowers of different sites were also observed for the mean number of sterile florests per head. Mean number of sterile florests per head in control ranged from 1.20 to 3.20. In 0.50 M treated plants, mean number of sterile florests per head was ranged from 3.20 to 3.50 and in 0.05 M concentration it was reported 1.80, respectively. Number of heads per branch (control and treated) and fertile florets per head directly affected the number of seeds set per plant. During the present investigation, the mean seeds per branches (control) ranged from 665.00 to 731.00 and in 0.50 M treated plant, seeds per branch ranged from 525.00 to 709.00 and in 0.05 M 461.00 was noticed. A characteristic variability

Table 3 : Distribution pattern of heads florests and seed size (control and treated)

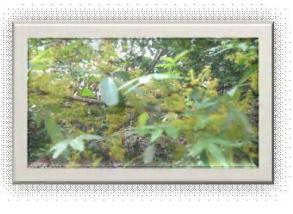
Sr. No.	Parameters	Mean	SD	± SE	Range
Z-1 Con	NH	7.60	2.80	± 0.88	4.0-12.0
	FFH	6.20	1.93	± 0.61	3.0-9.0
	SFH	3.20	0.92	± 0.29	2.0 – 5.0
	TFH	7.60	2.84	± 0.90	3.0-12.0
	HD	4.35	1.54	± 0.49	2.3-7.1
	SPB	690.00	371.78	±117.57	200-1350
0.50 M	NH	5.40	1.78	± 0.56	3.0-9.0
	FFH	5.30	2.21	± 0.70	3.0-9.0
	SFH	3.20	1.55	± 0.49	1.0-5.0
	TFH	5.50	1.16	± 0.37	4..0-8.0
	HD	6.03	1.25	± 0.40	2.9-7.5
	SPB	525.00	277.14	± 87.64	200-1000
Z-2 Con.	NH	6.80	2.39	± 0.76	3.0-10.0
	FFH	7.30	1.89	± 0.60	5.0-11.0
	SFH	1.20	1.23	± 0.39	0.00-3.0
	TFH	8.90	1.97	± 0.62	5.0-14.0
	HD	3.98	1.42	± 0.45	2.1-6.0
	SPB	665.00	296.32	± 93.71	350-1200
0.05 M	NH	4.60	2.07	± 0.65	3.0 – 9.0
	FFH	4.70	1.57	± 0.50	3.0 -8.0
	SFH	1.80	1.32	± 0.42	3.0 -6.0
	TFH	7.80	2.70	± 0.85	5.0- 7.0
	HD	5.08	0.92	± 0.29	3.5 – 6.5
	SPB	461.00	263.71	± 83.39	150 - 900
Z-3 Con.	NH	14.80	2.57	± 0.81	11.0 – 20.0
	FFH	5.20	1.93	± 0.61	3.0 – 9.0
	SFH	2.40	1.51	± 0.48	1.0 -5.0
	TFH	9.40	2.64	± 0.83	5.0 – 14.0
	HD	5.52	1.67	± 0.53	2.5 – 7.5
	SP	731.00	352.69	± 111.53	400- 1100
0.50 M	NH	5.90	2.73	± 0.86	2.0 – 10.0
	FFH	7.00	1.56	± 0.49	5.0 -9.0
	SFH	3.50	0.71	± 0.22	3.0 – 5.0
	TFH	7.70	2.11	± 0. 67	5.0 -9.0
	HD	7.24	1.88	± 0.60	3.5 – 10.0
	SPB	709.00	395.01	±124.16	250 – 1300

NH = Number of heads
FFH = number of fertile florests per head
SFH = Number of sterile florests per head
TFH = Total number of florests per head
HD = head diameter (cm)
SPB = seed/ branch

Z- 1 control male plant

Control male plant (Z- 2)

0.50 M colchicine treated male plant (Z- 2)

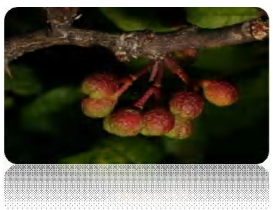


Control male plant (Z- 2)

Control male mature (Z-3)

0.50 M treated male plant (Z-3)

Control female plant of Z. armatum (Z-3)



0.50 M treated female plant (Z-3)

Plate 1 : Colchicine induced variability, *Zanthoxylum armatum* Rox.

was observed in the mean total number of florests per head in control and treated plants of all the sites. Mean total number of florests per head in control ranged from 7.60 to 9.40. In 0.50 M treated plants, mean total number of florests per head was ranged from 7.70 to 8.90 and in 0.05 M it was recorded 7.50, respectively.

The treatment of colchicine to *Zanthoxylum* spp. was found to affect physiology and development of plants in the present study. These findings agree with the report on work done by Sharp and Davidson (1968). Frequency and pattern of colchicine induced mutations depended on concentration, dipping time and variety used (Seneviratne *et al.*, 2002). Induction of mutation by colchicine could provide variability in *Z. armatum*. Cytomorphological studies on *Z. armatum* plants raised from seeds treated with aqueous solution of colchicine showed that lower doses *i.e.* 0.05 M and 0.50 M for 6 to 12 hrs colchicine has stimulatory effect on most of the morphological characters like number of branches, leaf area, fruit diameters, flower colour, flower diameter and seed weight. These findings agree with the report on work done by Dirkes *et al.* (1956), Seneviratne *et al.* (2002), Mensah *et al.* (2007) and Shao *et al.* (2003). The application of chemical mutagen (Colchicine) inhibits the plant height as compared to control. Similar results were also reported by Dirkes *et al.* (1956), Choi *et al.* (2000) and Mensah *et al.* (2007).

Concentration of 1.0 M was found deleterious to branching while in lower concentrations, increased number of branches was reported. Likewise, the findings of Obute *et al.* (2007) proved that stunted growth of plants and decreased number of leaflets were induced by the chemical mutagen (Colchicine) in *Z. armatum* as compared to control. The decrease in plant height has led treated plants to bear less number of leaflets and increase in size of flowers and seeds. Inhibition of root and shoot growth, short structure of root and shoot with thick and wide leaves was reported in treated plants of all the sites. These findings are in agreement with the findings of Kordan (1982) and Mujib (2005) on different plants. Growth rate of treated plants was noticed very slowly in all the treated sites as compared to control. Shortening of internodes was reported in colchicine treated plants as compared to control. Low concentrations of colchicine produced early maturation and yield as compared to control. Similar results were reported by Mensah *et al.* (2007).

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REFERENCES

Choi, H.W., Lemaux, P.G. and Choi, M.J. (2000). Increased Chromosomal variation in transgenic versus nontransgenic barley (*Hordeum vulgare* L.) plant. *Crop Sci.,* **40**: 524- 533.

Chopra, R.N., Nayar, S.L. and Chopra, I.C. (1986). *Glossary of Indian medicinal plants including the supplement*. CSIR, Pub., New Delhi.

Dirks, V.A., Ross, J.G. and Harpstead, D.P. (1956). Colchicine induced true Breeding Chemical sectors in Flax. *J. Heredity,* **47**: 229-233.

Facciola, S. (1990). *Cornucopia-A source book of edible plants*. Kampong Publications.

Gamble, J.S. (1972). *A mannual of Indian timbers*. Bishen Singh Mahendra Pal Singh.

Gupta, B.L. (1945). *Forest flora of Chakrata, Dehradun and Saharanpur*. Forest Research Institute Press.

Kordan, H.A. (1982). Colchicine –light effects on growth and mitotic behavior in germinating *Lettuce achenes*. *Histochemical J.,* **15** (5): 419-426.

Mensah, J.K., Obadoni, B.O., Akomeah, P.A., Ikhajiagbe, B. and Janet, Ajibolu (2007). The effect of sodium azide and colchicine treatments on morphological and yield traits of sesame seed (*Sesame indicum* L.). *African J. Biotechnol*., **6** (5): 534-538.

Mujib, A. (2005). Colchicine induced morphological variants in pineapple. *Plant Tissue Culture & Biotechnol.,* **15** (2): 127-133.

Obute, G.C., Ndukwu, B.C. and Chukwu, O.F. (2007). Targeted mutagenesis in *Vigna unguiclata* (L.) walp. and *cucumeropsis mannii* (NAUD) in Nigeria. *African J. Biotechnol*., **6**: 2467-2472.

Pearson, R.S. and Brown, H.P. (1932). *Commercial timbers of India*, **2**: 1150.

Seneviratne, K.A., Krishnarajah, C.N., D.S.A., Wijesundara, S.A. and Palipane P.W.U.B. (2002). Colchicine induced floral variations in African Voilets *Saintpauli ionantha* H. Wend. *Ann. Sri Lanka Department Agric.*, **4**: 227-232.

Shao, J., Chen C. and Deng X., (2003). *In vitro* induction of Tetraploidy in Pomegranate (*Punica granatum*). Plant Cell, tissue culture, **75** (3): 241-246.

Sharp, W.R. and Davidson (1968). The physiology of tumours of colchicine treated and decapitated *Brassica oleracea*. *Plant Physiol.,* **44**: 468-470.

Tanaka, T. (1976). *Tanaka's encyclopaedia of edible plants of the world*. Keigaku Publishing.

Uphof, J.C. Th. (1959). *Dictionary of economic plants*. Weinheim.

Usher, G.A. (1974). *Dictionary of plants used by man*. Constable. ISBN 0094579202.

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