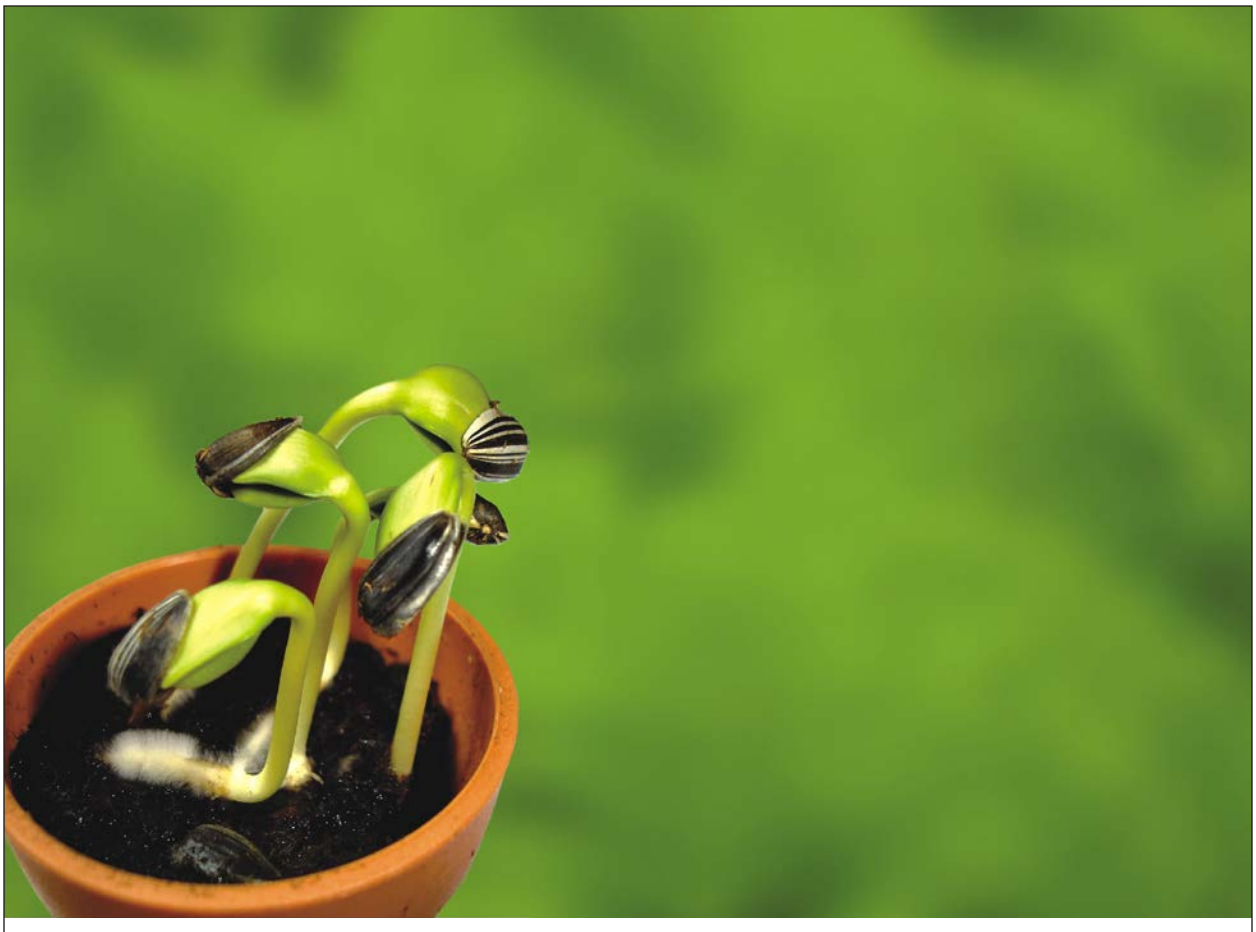

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Effect of some Chemical Mutagens on the Growth, Phytochemical Composition and Induction of Mutations in *Khaya senegalensis*

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ABSTRACT

Induced mutation using chemical mutagen is a method to create genetic variation resulting in new varieties with better characteristics. However, their effects in forest trees have received relatively little attention, particularly in *Khaya senegalensis*. Here, I study the effect of sodium azide and dimethyl sulphate on the growth and phytochemical composition of *Khaya senegalensis*, in addition to produce genetic variation on the vegetative growth. Seeds of *Khaya senegalensis* were soaked in dimethyl sulphate solutions (0, 1000, 2000, 3000, 4000 and 5000 ppm) and sodium azide solutions (200, 400, 600, 800 and 1000 ppm) for 15 h. Number of branches and leaves increased significantly on plants treated with 2000 ppm dimethyl sulphate in both seasons. The concentration of 3000 ppm dimethyl sulphate (DMS) increased significantly plant height in the first season but did not differ significantly in the second season. Plants treated with all concentrations of sodium azide increased alkaloid contents in the leaves and bark in both seasons. In addition, they enhanced the accumulation of saponins. The treatments of 4000 ppm dimethyl sulphate in the second season produced dwarfed plant with reddish stem. While, the treatment of 3000 ppm dimethyl sulphate produced plant having reddish pedicel leaflets. In addition, plant with biggest and fast growth was found using 300 ppm dimethyl sulphate in the second season, this last mutant was more genetically distinct to control as found by peroxidase isozyme patterns.

Key words: Dimethyl sulphate, genetic variation, mutation, peroxidase isozyme, sodium azide

INTRODUCTION

Khaya is a genus of trees in the mahogany family (Meliaceae), native to tropical Africa. The wood is used for a variety of purposes. Bark, leaves and seeds are used for a variety of medical purposes. They are taken against fever caused by malaria, stomach complaints and headaches. They are applied externally to cure skin rashes, wounds, or any abnormality. Bark and leaves of *Khaya* contain tannins, saponins, sterol, flavonoids, alkaloids and phenols (Adeiza *et al*., 2009; Sexton *et al*., 2010).

Induced mutation using physical and chemical mutagens is a method to create genetic variation resulting in new varieties with better characteristics (Arulbalachandran *et al*., 2009).

Sodium azide (NaN_3) and dimethyl sulphate $(Ch_3O)_2SO_2$ are chemical mutagens that act as alkylating agents and consider as the most powerful mutagens in plants. Their applications on plant are easy, inexpensive and create mutation to improve their traits. The efficiency of mutant production depends on many conditions such as pH, soaking into water, temperature, concentration and treatment duration. They create point mutation, damage the chromosomes and thus produce tolerance in plant for numerous conditions (Al-Qurainy and Khan, 2009).

Sodium azide was used in many studies to induce mutation as found by El-Nashar (2006) on *Amaranthus caudatus*, Al-Gawwad and Makka (2009) on *Mirabilis jalapa* and Mostafa (2011) on *Helianthus annuus*.

Dimethyl sulphate (DMS) was used to induce mutation as reported by Jain (2006) on *Vigna mungo*, Mostafa (2009) on *Balanites aegyptiaca*.

The aim of this research was to study the effect of sodium azide and dimethyl sulphate on the growth and phytochemical composition of *Khaya senegalensis* and to produce genetic variation on the vegetative growth.

MATERIALS AND METHODS

The study was carried out at the Nursery of Ornamental Plants, Faculty of Agriculture, South Valley University, Qena, Egypt during the period of 2010 to 2013.

Seeds were soaked in dimethyl sulphate solutions (0, 1000, 2000, 3000, 4000 and 5000 ppm) and sodium azide solutions (200, 400, 600, 800 and 1000 ppm) for 15 h. Then seeds were washed with tap water and sown in 20 cm plastic pots containing a soil mixture of clay and sand (1:1 v/v). One hundred and fifty seeds were sown for each treatment on October 17th 2010 of first seasons and 2011 of second season. The seeds were sown in three replications; each replication contained five pots (ten seeds in each pot). After 3 months from sowing, seedlings were transplanted into a 25 cm plastic pots containing the soil mixture of clay and sand (3:1 v/v).

Estimated data: All data were recorded after one and half year from sowing. Plant height (cm), number of branches and leaves, number of leaflets per leaf, leaf area (cm^2), length of leaf (cm), stem diameter, fresh and dry weights of vegetative growth and roots were recorded. Total chlorophyll content (SPAD unit) was determined with SPAD meter apparatus as described by Yadawa (1986). The phytochemical constituents such as phenols, saponins, alkaloids and flavonoids content were determined in the leaves and bark. Phenols were determined according to Cheng and Hanning (1955), while saponins and alkaloids were carried out as the methods of Osuagwu *et al*. (2007). Flavonoids were determined as reported by Huang *et al*. (2004).

All changes in the vegetative growth were recorded. Peroxidase isozyme activities were studied on the leaf of the mutants and the control of *Khaya senegalensis*. Agar-starch-polyvinyl pyrrolidine (PVP), gel electrophoresis was carried out according to the procedures described by El-Metainy *et al*. (1977) and Rida (2003).

Similarity values were calculated to determine the genetic relationships between the mutant plants. Bands on agarose gels were scored as present or absent and a pairwise similarity matrix was constructed using Dice coefficient after Sneath and Sokal (1973), followed by the UPGMA (unweighted pair grouping method of average method) to construct the dendrogram (Mostafa *et al*., 2014).

Experimental layout and statistical analysis: The experimental layout was a randomized complete block design containing three replications (Steel and Torrie, 1982). Each replication contained 11 treatments and every treatment consisted of 15 plants.

RESULTS AND DISCUSSION

As shown in Table 1, the concentration of 3000 ppm dimethyl sulphate (DMS) increased significantly plant height in the first season but did not differ significantly in the second season

Table 1: Effect of dimethyl sulphate and sodium azide concentrations on plant height, number of branches, number of leaves, number of leaflets per leaf , leaf length and leaf area (cm^2) of *Khaya senegalensis* plants

	Plant height (cm)		No. of branches		No. of leaves		No. of leaflets per leaf		Length of leaf (cm)		Leaf area (cm^2)	
Treatment (ppm)	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control												
0.0	40.0^{b}	45.6^{ab}	2.3^{b}	2.6^{b}	54.6^{b}	56.6^{c}	4.6^{a}	5.0^{a}	10.0^{a}	11.1^{a}	128.3^{a}	132.6^{a}
Dimethyl sulphate												
1000	22.0^{ef}	27.5^{ef}	1.0^{c}	1.5^{c}	33.6^{e}	35.6^{e}	2.0^{b}	3.0^{bc}	3.50^{d}	4.1^{de}	32.7^{de}	41.1^{a}
2000	36.6^{bc}	39.6^{bc}	3.0^{a}	3.6^{a}	66.3^{a}	71.0^{a}	4.3^{a}	4.6^{a}	7.30^{b}	8.0^{bc}	60.6^{c}	78.7^{a}
3000	46.3^{a}	48.3^{a}	2.3^{b}	2.6^{b}	55.0^{b}	58.0^{bc}	4.0^{a}	5.0^{a}	8.10^{b}	8.9^{ab}	77.6^{b}	93.1^{a}
4000	26.0^{de}	27.0^{ef}	1.0^{c}	2.0^{bc}	51.5^{b}	67.5^{ab}	2.0^{b}	2.5^{c}	2.80^{d}	4.1^{de}	30.8^{e}	35.4^{a}
5000	23.5^{def}	25.0^{f}	1.5^{c}	2.0^{bc}	53.5^{b}	56.5^{c}	1.0^{c}	3.0^{bc}	1.40^{e}	3.2^{e}	22.7^{e}	34.1^{a}
Sodium azide												
200	26.6^{d}	31.3^{de}	1.3^{c}	1.3^{c}	52.6^{b}	55.0^{c}	4.0^{a}	4.0^{ab}	5.10^{c}	5.9^{cd}	48.2^{cd}	51.3^{a}
400	34.5^{c}	36.5^{cd}	1.3^{c}	1.3^{c}	45.3^{c}	54.0^{cd}	4.3^{a}	4.3^{a}	7.40^{b}	10.4^{ab}	56.9^{c}	66.7^{a}
600	25.3^{de}	32.6^{de}	1.5^{c}	2.0^{bc}	54.6^{b}	56.3^{c}	1.5^{bc}	2.0^{cd}	2.70^{d}	3.7^{de}	22.9^{e}	24.8^{a}
800	20.5^{f}	31.5^{de}	1.0^{c}	1.5^{c}	39.6^{d}	44.6^{de}	1.5^{bc}	2.0^{cd}	2.70^{d}	3.5^{de}	26.0^{e}	29.2^{a}
1000	20.0^{f}	27.0^{ef}	1.0^{c}	1.5^{c}	43.0^{cd}	54.0^{cd}	1.0^{c}	1.0^{d}	1.40^{e}	2.0^{e}	25.1^{e}	30.4^{a}
$LSD_{0.05}$	4.5**	6.2**	0.6**	0.9**	5.5**	9.5**	0.7**	1.2**	1.10**	2.5**	15.9**	NS

Values in the same column not followed by the same letter are significantly different at the 5% level of probability, NS: Not significant, **Significant at p = 0.01, respectively

(46.3 and 48.3 cm for first and second season, respectively) compared to control (40 and 45.6 cm for first and second season, respectively). Other all treatments decreased significantly plant height in both seasons.

Number of branches and leaves increased significantly on plants treated with 2000 ppm dimethyl sulphate in both seasons. All the concentrations of both mutagens decreased number of branches in both seasons except for the concentration of 3000 ppm dimethyl sulphate did not differ significantly from the control.

The high concentrations of both mutagens decreased significantly number of leaflets per leaf in both seasons, while the concentrations of 2000 and 3000 ppm dimethyl sulphate and 200 and 400 ppm sodium azide did not differ significant compared to control.

All treatments decreased length of leaf in both seasons and leaf area in the first season. This decrease was not significant in case of plants treated with 3000 ppm dimethyl sulphate and 400 ppm sodium azide for length of leaf in the second seasons. No significant differences were obtained for leaf area among the treatments in the second seasons.

Reduced growth may be attributed to the increase in destruction on growth inhibitors, drop in the auxin level or inhibition of auxin synthesis as reported by Roychowdhury and Tah (2011) and Mostafa *et al*. (2014).

All treatments decreased stem diameter, fresh and dry weight of vegetative growth for both seasons compared to control as shown in Table 2. This reduction on the growth might be attributed to the physiological damage produced by chemical mutagens and its hydrolysis products as reported by El-Torky (1992) and Gvozdenovic *et al*. (2009).

Plants treated with 3000 ppm dimethyl sulphate gave the largest root length in the first and second seasons (45.3 and 57.8 cm, respectively) compared to control (36.3 and 41.6 cm), followed by plants treated with 600 ppm sodium azide (39.3 and 47 cm).

Table 2: Effect of dimethyl sulphate and sodium azide concentrations on stem diameter, fresh and dry weights of vegetative growth and roots and root length of *Khaya senegalensis* plants

	Stem diameter (cm)		Fresh weight of vegetative growth (g)		Dry weight of vegetative growth (g)		Fresh weight of roots (g)		Dry weight of roots (g)		Root length (cm)	
Treatments (ppm)	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control												
0	1.50^{a}	1.56^{a}	77.0^{a}	84.3^{a}	21.3^{a}	23.7^{a}	77.0^{a}	84.3^{a}	16.5^{b}	17.9^{ab}	36.0^{b}	39.9^{b}
Dimethyl sulphate												
1000	0.90^{e}	0.95^{cd}	24.9^{de}	25.7^{ef}	3.7^{c}	8.1^{fg}	24.9^{de}	25.7^{ef}	3.1^{f}	5.1^{d}	13.4^{d}	29.6^{cde}
2000	1.30^{b}	1.36^{ab}	49.5^{b}	59.4^{abc}	12.3^{b}	14.4^{cd}	49.5^{b}	59.4^{abc}	14.8^{bc}	15.8^{bc}	33.4^{b}	34.9^{bc}
3000	1.30^{b}	1.43^{ab}	57.8^{b}	69.4^{ab}	18.4^{a}	21.4^{ab}	57.8^{b}	69.4^{ab}	21.9^{a}	24.9^{a}	46.0^{a}	49.0^{a}
4000	1.10^{cd}	0.95^{cd}	22.9^{def}	40.8^{cdef}	4.8^{c}	8.9^{efg}	22.9^{def}	40.8^{cdef}	6.4^{de}	9.0^{bcd}	14.0^{d}	20.7^{f}
5000	0.70^{f}	0.80^{d}	17.9^{ef}	27.8^{def}	2.7^{c}	4.6^{g}	17.9^{ef}	27.8^{def}	6.3^{de}	9.1^{bcd}	15.2^{cd}	20.9^{f}
Sodium azide												
200	1.00^{de}	1.23^{bc}	29.8^{cd}	49.1^{bcde}	10.4^{b}	12.2^{def}	29.8^{cd}	49.1^{bcde}	12.0^{c}	15.3^{bc}	31.2^{b}	31.7^{cd}
400	1.20^{bc}	1.33^{ab}	38.9^{c}	51.9^{bcd}	11.4^{b}	14.1^{cde}	38.9^{c}	51.9^{bcd}	14.2^{bc}	15.9^{bc}	30.0^{b}	32.3^{cd}
600	0.65^{fg}	0.86^{d}	29.8^{cd}	38.2^{cdef}	4.6^{c}	18.1^{bc}	29.8^{cd}	38.2^{cdef}	6.1^{def}	14.0^{bcd}	21.6^{c}	30.9^{cd}
800	0.60^{fg}	0.85^{d}	15.9^{ef}	20.7^{f}	3.9^{c}	10.4^{def}	15.9^{ef}	20.7^{f}	3.8^{ef}	11.8^{bcd}	17.4^{cd}	23.1^{ef}
1000	0.50^{g}	0.75^{d}	14.3^{f}	16.4^{f}	1.7^{c}	4.1^{g}	14.3^{f}	16.4^{f}	7.8^{d}	7.7^{cd}	17.2^{cd}	25.8^{def}
$LSD_{0.05}$	0.18**	0.30**	9.4**	25.1**	4.0**	5.3**	9.4**	25.1**	3.2**	9.0**	6.8**	7.1**

Values in the same column not followed by the same letter are significantly different at the 5% level of probability, **Significant at p = 0.01, respectively

The concentration of 3000 ppm dimethyl sulphate produced the largest fresh weight of roots in the first and second seasons (46 and 49 g, respectively). These increments may be due to the physiological stimulation of the chemical mutagen as reported by El-Torky (1992). In addition, it may be attributed to cell division rates as well as an activation of growth hormones e.g., auxin (Joshi *et al*., 2011; Mostafa *et al*., 2014).

Plants treated with 3000 ppm dimethyl sulphate gave the heaviest dry weight of the roots in the first and second seasons (21.9 and 24.9 g, respectively) compared to the control (16.5 and 17.9 g, respectively). Other treatments decreased dry weight compared to control.

The highest chlorophyll content was found in plants treated with 0 and 3000 ppm dimethyl sulphate with insignificant differences between the two treatments in both seasons as shown in Table 3.

The highest values of leaf saponin content were found using the concentrations of 4000 ppm dimethyl sulphate and 400 and 600 ppm sodium azide in the first season (1.9, 2.0 and 1.9) compared to control (1.57). In the second season the highest values were found in plants treated by 2000 ppm dimethyl sulphate and 400 ppm sodium azide (2.2 and 2.0) compared to control (1.98).

Saponin content in the bark was increased significantly by using the high concentrations of dimethyl sulphate in both seasons, all concentrations of sodium azide in the first season and the concentrations of 400 and 600 ppm sodium azide in the second seasons compared to control.

With regard to alkaloids content, plants treated with all concentrations of sodium azide increased significantly alkaloid contents in the leaves and bark in both seasons except for that of 200 ppm in the leaves for the first season.

Table 3: Effect of dimethyl sulphate and sodium azide concentrations on chlorophyll, saponins and alkaloids content in the leaves and bark of *Khaya senegalensis* plants

	Chlorophyll content (SPAD unit)		Saponins content (mg/g dry weight)				Alkaloids content (mg/g dry weight)			
			In the leaves		In the bark		In the leaves		In the bark	
Treatments (ppm)	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control										
0.0	39.1^{a}	40.8^{a}	1.57^{d}	1.98^{b}	1.67^{fg}	2.02^{c}	2.00^{c}	1.98^{c}	1.69^{d}	1.26^{c}
Dimethyl sulphate										
1000	29.0^{cd}	32.8^{cde}	1.61^{cd}	1.67^{d}	1.78^{ef}	1.48^{e}	2.05^{c}	2.21^{b}	0.86^{g}	0.87^{e}
2000	34.4^{b}	35.1^{bcd}	1.58^{d}	2.20^{a}	1.56^{g}	1.83^{d}	2.06^{c}	2.02^{c}	0.82^{g}	1.03^{d}
3000	37.0^{a}	39.2^{ab}	1.59^{cd}	1.62^{def}	2.42^{a}	2.22^{ab}	2.04^{c}	1.23^{c}	1.18^{f}	0.82^{ef}
4000	25.8^{d}	28.6^{e}	1.90^{a}	1.56^{f}	1.79^{e}	2.22^{ab}	2.17^{b}	1.93^{c}	1.31^{e}	0.75^{f}
5000	29.7^{cd}	31.0^{de}	1.74^{b}	1.66^{de}	2.20^{cd}	2.25^{ab}	1.90^{d}	2.29^{ab}	1.88^{c}	2.04^{b}
Sodium azide										
200	33.9^{b}	37.6^{abc}	1.03^{e}	1.92^{b}	2.09^{d}	1.40^{e}	2.1^{bc}	2.30^{ab}	2.05^{b}	2.04^{b}
400	32.2^{bc}	36.3^{abc}	2.00^{a}	2.00^{a}	2.11^{d}	2.20^{b}	2.16^{b}	2.33^{a}	1.92^{c}	2.14^{a}
600	32.0^{bc}	32.9^{cde}	1.91^{a}	1.57^{ef}	2.37^{ab}	2.32^{a}	2.34^{a}	2.37^{a}	1.94^{c}	2.21^{a}
800	34.6^{b}	35.5^{bcd}	1.71^{bc}	1.95^{b}	2.28^{bc}	2.03^{c}	2.18^{b}	2.20^{b}	2.17^{a}	2.19^{a}
1000	31.5^{bc}	39.6^{ab}	1.62^{bcd}	1.79^{c}	2.23^{c}	2.22^{ab}	2.38^{a}	2.40^{a}	2.10^{ab}	2.03^{b}
$LSD_{0.05}$	3.4**	5.0**	0.13**	0.1**	0.12**	0.11**	0.12**	0.12**	0.09**	0.08**

Values in the same column not followed by the same letter are significantly different at the 5% level of probability, **Significant at p = 0.01, respectively

Concerning to dimethyl sulphate the concentrations of 4000 ppm in the first season, 1000 and 5000 ppm in the second season increased significantly alkaloids content in the leaves. Alkaloids content in the bark were increased significantly using the concentrations of 5000 ppm in both seasons compared to control. The concentrations of 2000 and 3000 ppm in the first season and 3000 ppm in the second season gave the highest content of phenols in the leaves compared to control.

Sodium azide at the concentrations of 1000 ppm in the first season, 6000 and 1000 ppm in the second season increased significantly phenols content in the leaves as shown in Table 4.

The concentration of 200 ppm sodium azide increased significantly phenols content in the bark in the first season, while the treatments of 1000, 2000 and 3000 ppm gave the highest values in the second season compared to control.

No significant differences were found with respect to flavonoids content in the leaves in both seasons and for flavonoids content in the bark in the first season as shown in Table 4. In the second season, the concentration of 200 ppm sodium azide gave the highest value compared to control.

The secondary metabolites of the medicinal plants have antimutagenic activities, which capable of lowering the frequency of mutation by diverse mechanisms of action. They have the capacity to scavenge mutagens or free radicals. Thus, increasing the photochemical composition after mutagens treatments may be due to reduce the deleterious effects of oxidative stress as a natural mechanism in plants after abiotic stresses (Mezzoug *et al*., 2006).

Induction of variations: In the first season, the treatments of 2000, 5000 ppm dimethyl sulphate and 400 ppm sodium azide produced changes in the leaf form as shown in Fig. 1. In addition, the concentrations of 2000, 3000, 4000 and 5000 ppm dimethyl sulphate and 200 ppm sodium azide produced leaves abnormalities in the second season as shown in Fig. 1. These changes of leaf form

Table 4: Effect of dimethyl sulphate and sodium azide concentrations on phenols and flavonoids content in the leaves and bark of *Khaya senegalensis* plants

	Phenols content (mg/g dry weight)				Flavonoids content (mg/g dry weight)			
	In the leaves		In the bark		In the leaves		In the bark	
Treatments (ppm)	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control								
0.0	9.80^{cd}	9.76^{e}	9.68^{b}	8.51^{f}	0.61^{a}	0.55^{a}	0.54^{a}	0.56^{bcd}
Dimethyl sulphate								
1000	9.06^{f}	9.00^{h}	9.11^{e}	9.43^{bc}	0.54^{a}	0.58^{a}	0.47^{a}	0.50^{e}
2000	9.99^{ab}	9.41^{f}	8.45^{f}	8.82^{e}	0.57^{a}	0.56^{a}	0.43^{a}	0.43^{f}
3000	9.86^{bc}	11.03^{a}	9.53^{c}	9.55^{b}	0.56^{a}	0.56^{a}	0.46^{a}	0.51^{de}
4000	9.55^{e}	7.57^{i}	9.13^{de}	8.54^{f}	0.57^{a}	0.59^{a}	0.52^{a}	0.54^{bcde}
5000	9.92^{abc}	9.87^{d}	9.48^{c}	9.38^{c}	0.57^{a}	0.58^{a}	0.62^{a}	0.52^{cde}
Sodium azide								
200	10.00^{a}	9.67^{e}	10.13^{a}	9.13^{d}	0.53^{a}	0.55^{a}	0.56^{a}	0.62^{a}
400	9.71^{d}	7.56^{i}	7.26^{h}	9.36^{c}	0.53^{a}	0.54^{a}	0.61^{a}	0.56^{bcd}
600	9.07^{f}	10.62^{b}	9.08^{e}	10.08^{a}	0.63^{a}	0.55^{a}	0.57^{a}	0.57$^{ab\ c}$
800	9.55^{e}	9.52^{g}	9.22^{d}	9.42^{bc}	0.60^{a}	0.58^{a}	0.52^{a}	0.54^{bcde}
1000	9.52^{e}	10.25^{c}	7.97^{g}	8.50^{f}	0.57^{a}	0.58^{a}	0.59^{a}	0.59^{ab}
$LSD_{0.05}$	0.14**	0.10**	0.11**	0.15**	NS	NS	NS	0.06*

Values in the same column not followed by the same letter are significantly different at the 5% level of probability, NS: Not significant, *,**Significant at p = 0.05 and 0.01, respectively

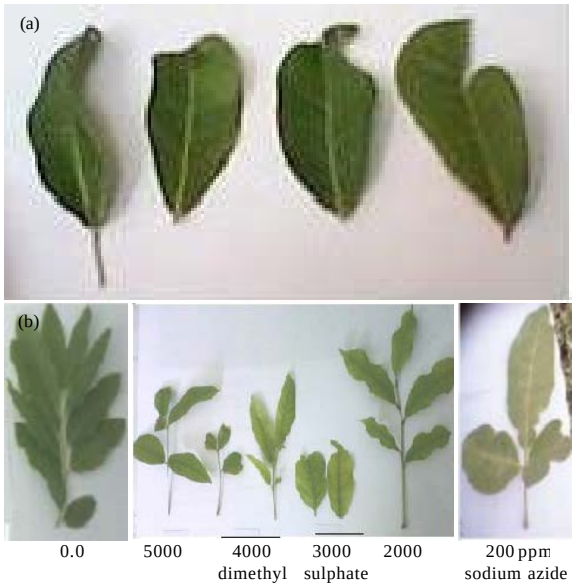

Fig. 1(a-b): Changes in the leaf form in the (a) First season (Frome left to right; Control, 2000, 5000 ppm DMS and 400 ppm sodium azide) and in the (b) Second season (From left to right; Control, 2000, 3000, 4000 and 5000 ppm DMS and 200 ppm sodium azide, respectively)

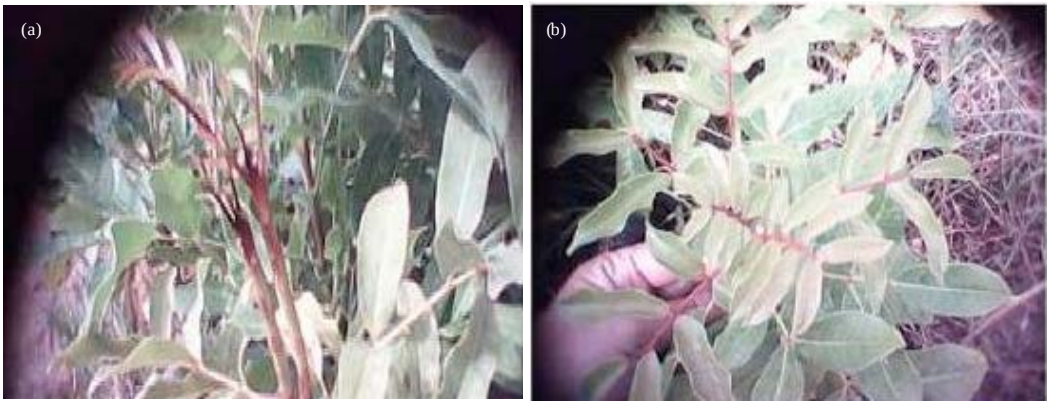

Fig. 2(a-b): Plants with (a) Reddish stem and (b) Reddish pedicel leaflets as a result of the treatments of 4000 and 3000 ppm dimethyl sulphate in the second season, respectively

Table 5: Mutants of *Khaya senegalensis* selected for peroxidase isozyme analysis

Mutant No.	Variation characteristic
Control (M0)	Normal plant
M1	Reddish stem
M2	Dwarfed plants
M3	Changes the form of leaves
M4	Reddish pedicel leaflets
M5	Greater and faster growth with biggest leaves

or shape may be due to chromosomal disturbances. These changed could be referred also to the layer rearrangement as a result of the chemical mutagens effect (El-Nashar, 2006).

The treatments of 4000 ppm dimethyl sulphate in the second season produced dwarfed plant with reddish stem as shown in Fig. 2. At the same time, the treatment of 3000 ppm dimethyl sulphate produced plant having reddish pedicel leaflets as shown in Fig. 2. This dwarfed growth may be due to physiological damage resulted in the alteration from normal to dwarf growth (El-Maksoud and El-Mahrouk, 1993). Joshi *et al*. (2011) explained the dwarfed growth to auxin destruction, changes in ascorbic acid content and physiological and biochemical disturbances. Plant having greater and faster growth was found using 3000 ppm dimethyl sulphate in the second season, their biggest leaves were shown in Fig. 3. The stimulatory effect of the mutagen may be attributed to the increase in the rate of cell division or cell elongation as reported by Joshi *et al*. (2011).

Isozyme analysis using electrophoresis offers a very well defined effective tool for breeders to detection the genetic differences among individuals (Arulsekar and Parfitt, 1986). Five mutants and the control plant were selected for isozyme analysis as shown in Table 5. The electrophoretic banding patterns presented in Fig. 4 indicate different profiles among mutants. It can be concluded that a total number of five loci control the production of peroxidase in *Khaya senegalensis*. One band migrated toward the anode (+) and the others migrated toward the cathode (-) in the electrophoresis field and were designed as prx-1 and prx-5. The band of the locus prx-2 presented with different intensity in all the evaluated genotypes. Locus prx-1 was found only in the mutant 5. This might be related to the improvement of these mutants' traits comparable to control

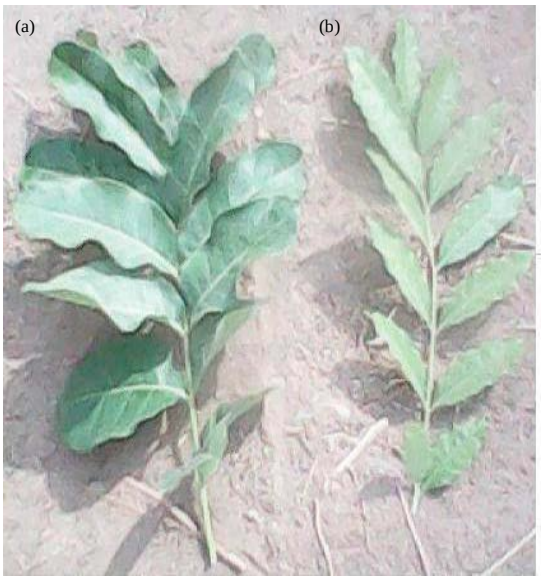

Fig. 3(a-b): (a) Biggest leaves as a result of the treatment 3000 ppm dimethyl sulphate and (b) Control plant in the second season

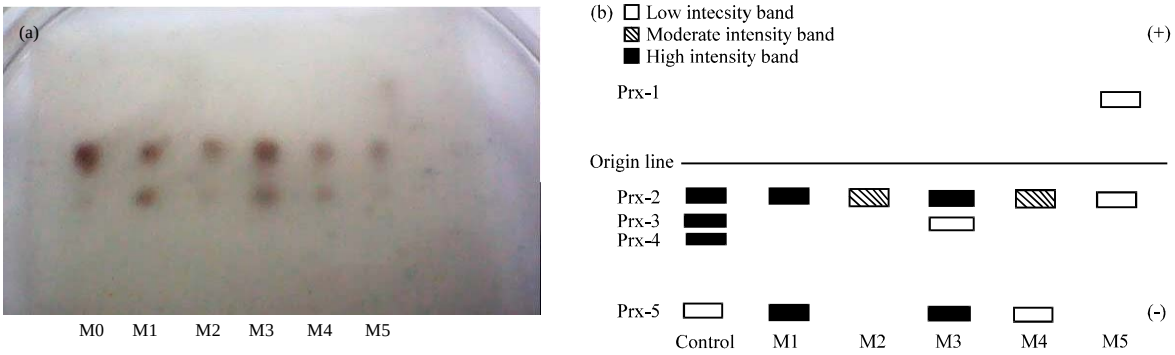

Fig. 4(a-b): (a) Electrophoretic separation pattern of peroxidase isozyme of *Khaya senegalensis* mutants and (b) Zymogram of electrophoretic separation pattern of peroxidase isozyme of *Khaya senegalensis* mutants

(Talukdar, 2010; El-Mokadem and Mostafa, 2014). The mutagenesis treatments seemed to activate expression of some genes which resulted in the appearance of some new bands. Mutations have been identified as one of the sources of isozyme variation in higher plants. These results are almost in agreement with those of Bartosova *et al*. (2005) and Malaviya *et al*. (2006). The loci prx-4 was found only in the control this may be due to the mutagenic effects (Aly and Elsayed, 2006). Regarding the similarity values, Table 6 and Fig. 4 show that, the mutant 3 was more genetically related to control with similarity value of 85.7% and the dendrogram of mutants shown in Fig. 5. On the other hand, mutant 5 was more genetically distinct to control (33.3%).

Table 6: Similarity value among mutants of *Khaya senegalensis* produced by dimethyl sulphate and sodium azide

Mutants	M0	M1	M2	M3	M4	M5
M0	100.0					
M1	66.6	100				
M2	66.6	100	100			
M3	85.7	80	80	100		
M4	66.6	100	100	80	100	
M5	33.3	50	50	60	50	100

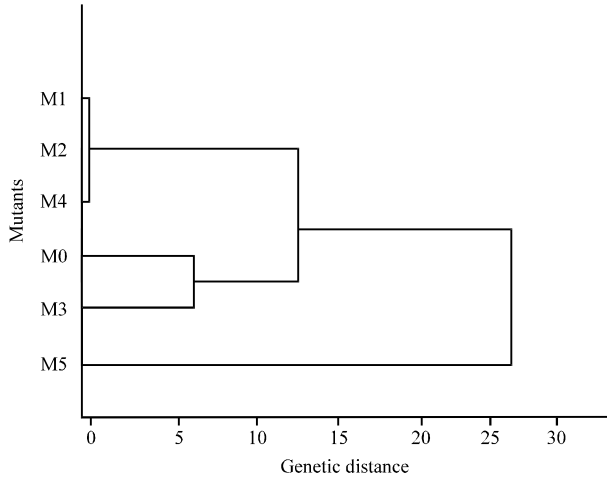

Fig. 5: Dendrogram constructed on the basis of peroxidase isozyme profile for control plant as M0 and five mutants (M1, M2, M3, M4 and M5) of *Khaya senegalensis*

CONCLUSION

Finally, it can be concluded that sodium azide and dimethyl sulphate are powerful mutagens for the induction mutations in *Khaya senegalensis* plant and increasing its phytochemical compositions. Peroxidase isozyme could act as a useful biochemical marker for mutant identification.

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