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Research Article

Assessment of mutagenic sensitivity in jasmine (*Jasminum spp*.) to chemical mutagen

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Abstract
The present experiment was carried out to determine mutagenic sensitivity on Jasmine (*Jasminum spp*.). In this experiment, terminal cuttings of *Jasminum grandiflorum* cv. White Pitchi and semi hardwood cuttings of *J. nitidum* (clone Acc.Jn-1 selected at TNAU) and *J. multiflorum* cv. Arka Arpan were treated with ten different concentrations of EMS ranging from 5 mM to 50 mM. . The results revealed gradual and significant reduction in survival of cuttings, shoot length, number of leaves, leaf length and leaf width with increase in dosage of EMS. The probit curve analysis based on survival percentage and growth rate of treated cuttings revealed LD_{50} dosage of EMS to be 37.15 mM, 40.7 mM and 42.66 mM for White Pitchi, Arka Arpan and ACC Jn-1 respectively.

Key words
Mutation breeding, *Jasminum*, probit analysis, EMS.

Introduction

India has a long tradition of floriculture, with flowers forming an integral part of almost all religious, social and cultural activities of the society. Jasmine belonging to family *Oleaceae* is one of the most important traditional flowers in India. Jasmine is grown commercially in India, Thailand, China, Sri Lanka and the Phillippines for its fresh flowers. India is the largest exporter of jasmine oil in the world accounting for over 40 per cent of total world export. The genus *Jasminum* comprises of around 200 species (Rendle, 1925; Bailey, 1958) which are native to tropical and warm temperate regions of Europe, Asia and Africa. India is one of the centers of origin of jasmine.

Among the species of *Jasminum*, only three species *viz., J. sambac, J. grandiflorum, J. auriculatum)* have attained commercial significance (Rimando, 2003; Green and Miller, 2009). *J. multiflorum* (Syn: *J. pubescens*) is commercially cultivated to some extent in Karnataka. Since all these species are commonly propagated by asexual means, limited variability exists in each of these species. Therefore, mutation breeding is a viable option to enhance genetic variability and to improve economic traits such as floral traits, flowering pattern, plant architecture, *etc*. Induced mutagenesis is a potential plant breeding tool which can induce the generation of allelic variants of genes that modulate the expression of traits. Mutation breeding which leads to altered phenotypes after permanent heritable change in the structure of genetic material (Rego and Faria, 2001), is now established as a time-saving and inexpensive approach for flower improvement (Datta and da Silva, 2006).

Chemical mutagens and ionizing radiations have been used as mutagens in plant breeding research and genetic studies for a long time (Guenet, 2004). Chemicals induce mainly point mutations while ionizing radiations normally induce chromosomal rearrangements and deletions (Bhat *et al.,* 2007). Among the alkylating chemical mutagens, ethylmethanesulfonate (EMS) is the most commonly used mutagen in plants. EMS alkylates guanine bases and leads to mis-pairing of alkylated G pairs with T instead of C, resulting in primarily G/C- to -A/T transitions (Bhat *et al.,* 2007).

To avoid excessive loss of actual experimental materials, sensitivity tests must be conducted to determine LD_{50} (the safe dose at which half population of the planting material survive) doses before massive exposure of similar materials. LD_{50} dose is considered as the dose at which highest frequency of mutation occurs. With this background, the present investigation was undertaken aiming to determine the optimum lethal dose (LD_{50}) for EMS in jasmine (*Jasminum* spp.).

Materials and Methods

Uniform sized healthy terminal cuttings of *J. grandiflorum* cv. White Pitchi and semi hard wood cuttings of *J. multiflorum* cv. Arka Arpan, *Jasminum nitidum* clone Acc.Jn-1 were collected. The terminal and semi hardwood cuttings were soaked in distilled water for one hour and 6 hours respectively. Water was decanted and after shade drying, the cuttings were soaked in EMS solution (freshly prepared in phosphate buffer at pH 7.0) of different concentrations *viz.*, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 mM. The soaking duration was one hour for terminal cuttings and 6 hours for semi hardwood cuttings. After incubation at room temperature, the cuttings were thoroughly rinsed with running tap water for 1 hour to wash out the chemical residues. Thirty treated cuttings per treatment per replication were planted in nursery polythene bags filled with red soil: FYM: sand at (1:1:1 ratio) along with untreated cuttings as control. Planted cuttings were placed inside polytunnels (wherein the temperature was 28–30 °C and relative humidity was 80-85%) for 25-30 days until rooting of cuttings and then the rooted plants were shifted to 50% shade house. The percentage of survival, shoot length, number of leaves, leaf length and leaf width were measured at eight weeks after planting. The experiment was laid out in Completely Randomized Design with three replicates. The LD_{50} value was calculated based on probit analysis using the survival of treated cuttings compared to that of control.

The LD_{50} values were determined based on Probit analysis (Finney, 1978). The probit function is the inverse cumulative distribution function (CDF) or quantile function associated with the standard normal distribution. Data obtained for growth related traits were subjected to the standard analysis of variance procedure using AGRES statistical package to identify the lethal dose (LD_{50}). The LD_{50} for each variety was estimated through the simple linear regression model by fitting the straight line equation $y= a + bx$; where y is the response variable (percent survival), x is the independent variable (irradiation dose), while a and b represent the slope and constant, respectively.

Results and Discussion

In this present study, a gradual reduction in survival rate of cuttings with increase in dose of EMS was observed (Table 1). LD_{50} for EMS was fixed based on survival percentage and growth rate of cuttings in three varieties. Probit analysis was carried out based on survival rate of the stem cuttings after treatment with different doses of EMS compared with that in untreated control (Table 2). In the present study, LD_{50} value for EMS as assessed from the probit curve analysis and the results are depicted in Fig. 1, Fig. 2 and Fig. 3. From the results it was found that the LD_{50} valuefor the three genotypes viz., of *J. grandiflorum* cv. White Pitchi, *J. multiflorum* cv. Arka Arpan, *J. nitidum* clone Acc Jn 1 was 37.15, 40.7 and 42.66 mM respectively.

A declining trend with increase in EMS concentration similar to that observed for survival rate was observed for growth related traits namely, shoot length, number of leaves, leaf length and width. The least shoot length, leaf length, number of leaves, leaf width were recorded with 50 mM EMS concentration while maximum was observed in control (Table 3-5). The results showed that the differences among mutation treatments considerably influence the survival, shoot length, leaf length leaf width and number of leaves. Moreover, with increase in the intensity of EMS concentration, the shoot length, leaf length and width shoot length proportionately decreased in a linear trend (Fig. 4, 5 and 6).

Among the chemical mutagens and alkylating agents, EMS has especially been demonstrated to be the most potent mutagen. Similar results of decline in the survival percentage as well as shoot length with increase in EMS concentration have been reported in Bougainvillea cultivars Roseville's Delight (Banerji *et al.* 1987) and Mahara (Datta and Banerji 1994) and in cassava (Kanagarasu *et al.*, 2014). To identify the biological influences of different chemical mutagens, seedling height is mostly utilized as an index (Bhat *et al*., 2007). It has been shown that a linear dependency exists between plant height and the dosage of chemical mutagens. Observations of the present study are in agreement with the above reports.

Determination of LD_{50} value for any mutagen is necessary to produce maximum viable mutants with minimum damage to the plants. The present study indicated that based on survival percentage and growth rate, LD_{50} values for EMS were 37.15, 40.7 and 42.66 mM for White Pitchi, Arka Arpan and Acc.Jn-1 respectively. These optimum mutagen doses determined for the jasmine genotypes could be useful for mutation breeding studies in jasmine improvement.

Acknowledgment
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Table1. Effect of mutagen on survival of cuttings in *J. grandiflorum* cv. White Pitchi, *J. multiflorum* cv. Arka Arpan and *J. nitidum* cv. Acc.Jn-1

Concentrations (mM)	*J. grandiflorum* cv. White Pitchi			*J. multiflorum cv.* Arka Arpan			*J. nitidum* cv. Acc.Jn-1		
	Survival percentage (%)	Per cent survival over Control	Per cent reduction over control (%)	Survival percentage (%)	Per cent survival over control	Per cent reduction over control (%)	Survival percentage (%)	Per cent survival over control	Per cent reduction over control (%)
0	90.00	100	-	90.00	100	-	93.33	100	-
5	86.67	96.30	3.7	86.67	96.3	3.7	90.00	96.43	3.57
10	83.33	92.58	7.42	83.33	92.58	7.42	86.67	92.86	7.14
15	76.67	85.18	14.82	76.67	85.18	14.82	83.33	89.28	10.82
20	73.33	81.47	18.53	73.33	81.47	18.53	80.00	85.71	14.29
25	66.67	74.07	25.93	66.67	74.07	25.93	73.33	78.57	21.43
30	63.33	70.36	29.64	63.33	70.36	29.64	60.00	64.28	35.72
35	50.00	55.55	44.45	56.67	62.96	37.04	56.67	60.72	39.28
40	43.33	48.14	51.86	46.67	51.85	48.15	53.33	57.14	42.86
45	33.33	37.03	62.97	36.67	40.74	59.26	43.33	46.42	53.58
50	20.00	22.22	77.78	23.33	25.92	74.08	23.33	24.99	75.01
SE(d)	0.11			0.22			0.16		
CD(5%)	0.25			0.48			0.24		

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Table 2. Probit analysis for calculating LD_{50} in *J. grandiflorum* (White Pitchi), *J. multiflorum* (Arka Arpan) and *J. nitidum* cv. Acc.Jn-1

Concen-tration (mM)	*J. grandiflorum* (White Pitchi)					*J. multiflorum* (Arka Arpan)				*J. nitidum* (Acc.Jn-1)			
	Log_{10} of doses	Observed mortality percentage	Corrected mortality percentage	Emprical probit unit	LD_{50} value	Observed mortality percentage	Corrected mortality percentage	Emprical probit unit	LD_{50} value	Observed mortality percentage	Corrected mortality percentage	Emprical probit unit	LD_{50} value
0	-	10	-	-	**37.15**	10	-	-		7	-	-	**42.66**
5	0.69	13	3	3.12	**mM**	13	3	3.12	**40.7**	10	3	3.12	**mM**
10	1.00	17	8	4.08		17	8	4.08	**mM**	13	6	3.44	
15	1.17	23	14	3.92		23	14	3.92		17	11	3.77	
20	1.30	27	19	4.12		27	19	4.12		20	14	3.92	
25	1.39	33	26	4.36		33	26	4.36		27	22	4.23	
30	1.47	37	30	4.48		37	30	4.48		40	35	4.62	
35	1.54	50	44	4.85		43	37	4.67		43	39	4.72	
40	1.60	57	52	5.05		53	48	4.95		47	43	4.82	
45	1.65	67	63	5.33		63	59	5.23		57	54	5.1	
50	1.69	80	78	5.77		77	74	5.64		77	75	5.67	

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Table 3. Effect of chemical mutagen on vegetative parameters of *J. grandiflorun* cv. White Pitchi

Concentrations (mM)	Shoot length (cm)			No. of leaves			Leaf length (cm)			Leaf width (cm)		
	Actual	Per cent over control (%)	Per cent reduction over control (%)	Actual	Per cent over control (%)	Per cent reduction over control (%)	Actual	Per cent over control (%)	Per cent reduction over control (%)	Actual	Per cent over control (%)	Per cent reduction over control (%)
0	26.63	100	-	9.87	100	-	7.88	100	-	4.45	100	-
5	22.35	83.92	16.08	8.11	82.16	17.84	6.84	86.8	13.2	4.36	97.97	2.03
10	21.98	82.53	17.47	7.25	73.45	26.55	6.25	79.31	20.87	3.87	86.96	13.04
15	21.25	79.79	20.21	6.85	69.4	30.6	5.79	73.47	26.53	3.53	79.32	20.68
20	20.14	75.62	24.38	6.15	62.31	37.69	5.45	69.16	30.84	3.15	70.78	29.22
25	19.25	72.28	27.72	5.24	53.09	46.91	5.23	66.37	33.63	2.85	64.04	35.96
30	17.24	64.73	35.27	5.11	51.77	48.23	4.35	55.2	44.8	2.45	55.5	44.5
35	14.56	54.67	45.33	4.98	50.45	49.55	3.62	45.93	54.07	2.11	47.41	52.59
40	13.11	49.23	50.77	4.67	47.31	52.69	3.42	43.4	56.6	1.67	37.52	62.48
45	10.54	39.57	60.43	3.87	39.2	60.8	2.87	36.42	63.58	1.52	34.15	65.85
50	9.57	35.93	64.07	3.11	31.5	68.5	2.32	29.44	70.56	1.24	27.86	72.14
SE(d)	0.14			0.88					0.63			
CD(5%)	0.59			1.56					0.89			

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Table 4. Effect of chemical mutagen on vegetative parameters of *J. multiflorum cv.* Arka Arpan

Concentrations (mM)	Shoot length (cm)			No. of leaves			Leaf length (cm)			Leaf width (cm)		
	Actual	Per cent over control (%)	Per cent reduction over control (%)	Actual	Per cent over control (%)	Per cent reduction over control (%)	Actual	Per cent over control (%)	Per cent reduction over control (%)	Actual	Per cent over control (%)	Per cent reduction over control (%)
0	17.11	100	-	14.11	100	-	5.24	100	-	2.45	100	-
5	15.45	90.29	9.71	12.48	88.44	11.56	5.21	99.42	0.48	2.23	91.02	8.98
10	14.59	85.27	14.73	12.23	86.67	13.33	4.88	93.12	6.88	2.11	86.12	13.88
15	13.25	77.44	22.56	11.69	82.84	17.16	4.53	86.45	13.55	1.96	80	20
20	12.24	71.53	28.47	11.53	81.71	18.29	4.12	78.62	21.38	1.92	78.36	21.64
25	11.12	64.99	35.01	10.56	74.84	25.16	3.86	73.64	26.36	1.87	76.32	23.68
30	10.29	60.14	39.86	9.45	66.97	35.03	3.67	70	30	1.83	74.69	25.31
35	9.68	56.67	43.33	9.13	64.7	35.3	2.74	52.29	47.71	1.63	66.53	33.47
40	8.56	50	50	7.11	50.38	49.62	2.57	49.04	50.96	1.33	54.28	45.72
45	6.53	38.16	61.48	6.53	46.27	53.73	1.74	33.2	66.8	1.21	49.38	50.62
50	5.58	32.61	67.39	5.36	37.98	62.02	1.39	26.52	73.48	1	40.81	59.19
SE(d)	0.88			0.38						0.33		
CD(5%)	1.14			0.59						0.69		

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Table 5. Effect of chemical mutagen on vegetative parameters of *J. nitidum* cv. Acc.Jn-1

Concentrations (mM)	Shoot length (cm)			No. of leaves			Leaf length (cm)			Leaf width (cm)		
	Actual	Per cent over control (%)	Per cent reduction over control (%)	Actual	Per cent over control (%)	Per cent reduction over control (%)	Actual	Per cent over control (%)	Per cent reduction over control (%)	Actual	Per cent over control (%)	Per cent reduction over control (%)
0	17.46	100	-	12.54	100	-	6.3	100	-	2.75	100	-
5	16.25	93.06	6.94	11.96	95.37	4.63	5.23	83.01	16.99	2.24	81.45	18.55
10	15.33	87.8	12.2	11.23	89.55	10.45	5.12	81.26	18.74	2.14	77.81	22.19
15	14.63	83.79	16.21	10.85	86.52	13.48	4.89	77.61	22.39	1.96	71.27	28.73
20	13.25	75.88	24.12	10.25	81.73	18.27	4.57	72.53	27.47	1.92	69.81	30.19
25	12.28	70.33	29.67	9.74	77.67	22.33	4.12	65.39	34.71	1.75	63.63	36.67
30	11.59	66.38	33.62	8.56	68.26	31.74	3.89	61.74	38.26	1.46	53.09	46.91
35	11.12	63.68	36.32	7.29	58.13	41.87	3.56	56.5	43.5	1.42	51.63	48.37
40	9.63	55.15	44.85	6.35	50.63	49.37	3.11	49.36	50.64	1.38	50.18	49.82
45	8.35	47.82	52.18	6.12	48.8	51.2	2.96	46.98	53.02	1.32	48	52
50	6.25	35.79	64.21	4.32	34.44	65.56	1.74	27.61	72.39	0.96	34.9	65.1
SE(d)	0.33			0.81			0.75			0.38		
CD(5%)	0.69			1.71			1.76			0.52		

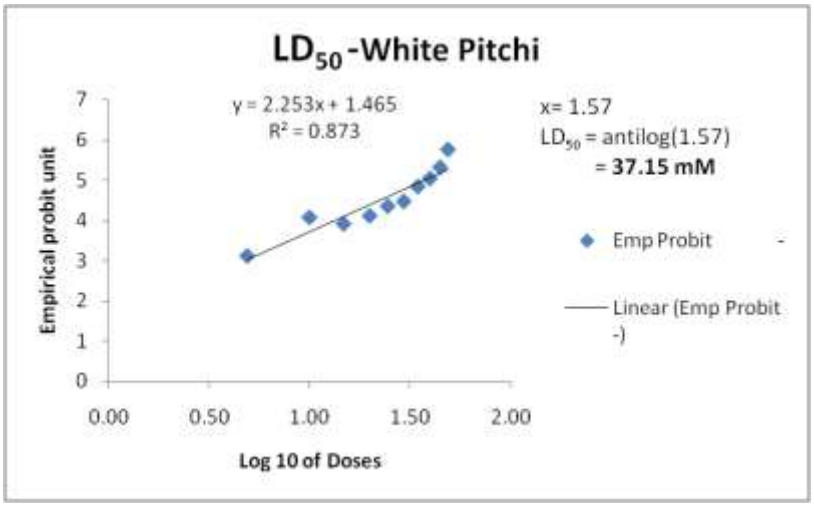

Fig. 1. Probit analysis based on corrected mortality rates of *J. grandiflorum* cv. White Pitchi

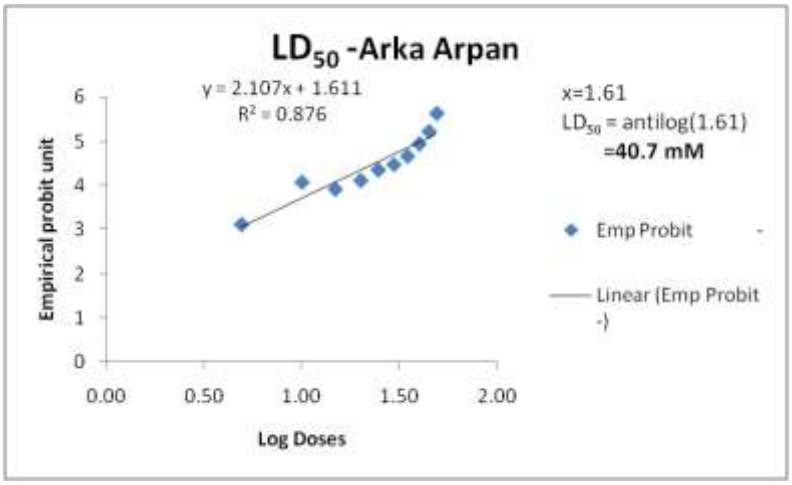

Fig. 2. Probit analysis based on corrected mortality rates for *J. multiflo*rum cv. Arka Arpan

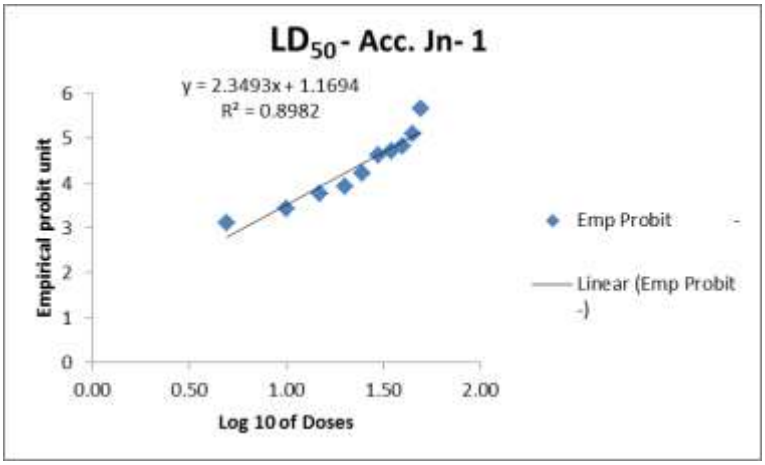

Fig. 3. Probit analysis based on corrected mortality rates of *J. nitidum* cv. Acc Jn-1

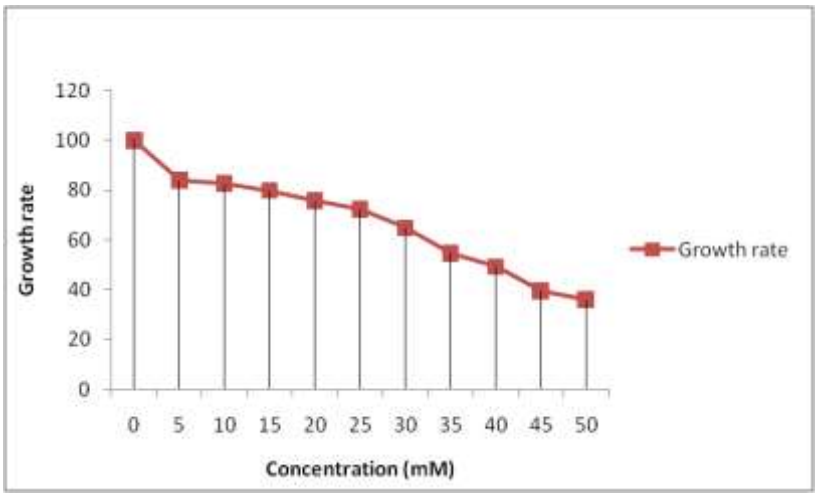

Fig. 4. Mutagenic sensitivity and comparative growth rate in *Jasminum grandiflorum* cv. White Pitchi

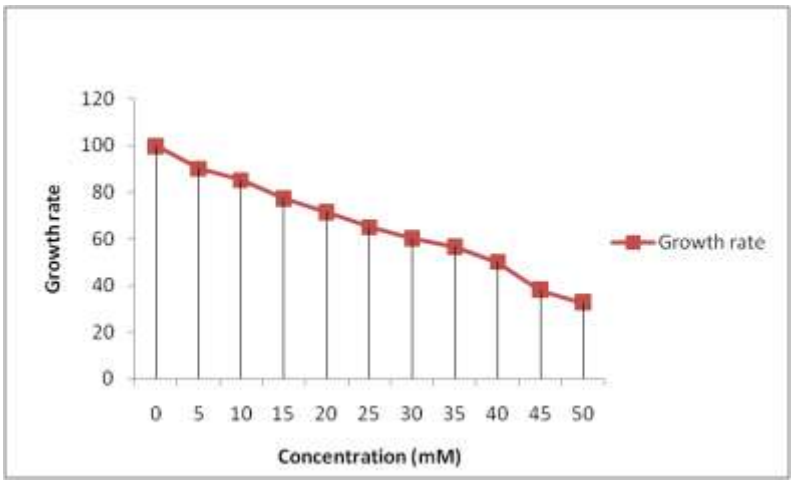

Fig. 5. Mutagenic sensitivity v and comparative growth rate in *Jasminum multiflorum* cv. Arka Arpan

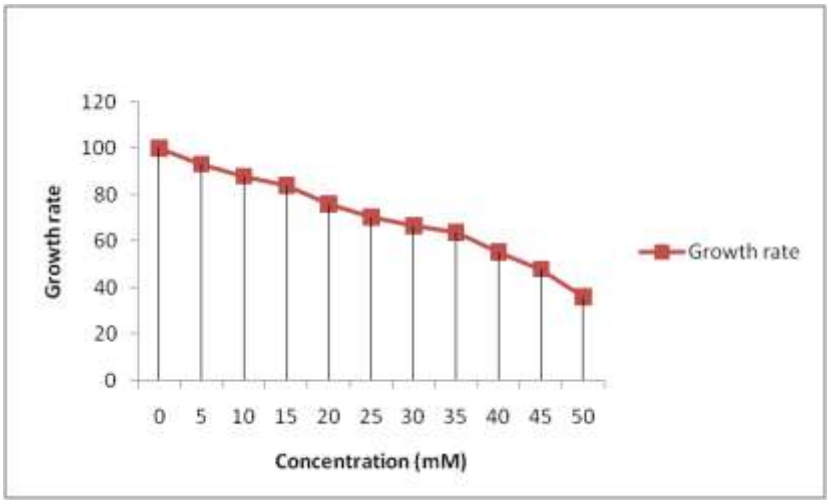

Fig. 6. Mutagenic sensitivity v/s Comparative growth rate in *Jasminum nitidum cv.* Acc Jn-1