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Short Communication

Effects of distillery effluent on chrysanthemum (*Dendrathema grandiflora* Tzvelve)

S. K. Datta, N. Singh[1], R. Shukla and Z. Hossain

Botanic Garden and Floriculture, National Botanical Research Institute, Rana Pratap Marg, Lucknow 226 001

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Two chrysanthemum (*Dendranthema grandiflora* Tzvelve) *viz.*, Ajay (small flowered, dwarf, mauve flower, October blooming) and Flirt (small flowered, decorative type, red flower, early December blooming) were selected for the study of effluent effect on morphological characters. The cv. Maghi Yellow (small flowered, pompon type, yellow flower, January-February blooming) was selected for the study of cytotoxic effect of effluent.

Rooted suckers of Ajay and Flirt were planted in 30 cm earthen pots containing soil : leaf mould (1:2) as growing medium. All the plants were irrigated periodically (twice in a week) with 100%, 50% and 25% distillery effluents. Equal number of suckers were planted separately and irrigated with tap water which served as control.

Rooted suckers of Maghi yellow were dipped in 100% effluent for 1, 3, 4, 5 and 6h. After each treatment the roots were thoroughly washed in running tap water. Roots (1.5 cm long) were fixed in propionic acid : alcohol (1:2 v/v) for three hours. The root tips were hydrolysed in (N) HCl at 60°C for 13 min. After thorough washing, the root tips were stained in Feulgen solution. Temporary squash preparations were made using 45% propionic acid.

After collecting the roots from 6h treated suckers, they were replanted in the sand:soil (1:1) for the study of mitotic behaviour again after 7 and 12 days.

Physico-chemical properties of waste water (distillery effluent) collected from the outlet of effluent treatment plant (E.T.P.) of Mohan Meakin Ltd., a distillery unit, which produces alcohol from molasses, were analysed. Colour was dark brown, odour was burning sugar and the temperature and pH was 30°C and 5.5 respectively. The BOD, COD and Total Solid were 37,000 mg/l, 98000 mg/l and 9042 mg/l respectively. The nitrate, phosphate, potassium, sodium, iron, calcium, sulphate and chloride were 12.5, 736, 4045, 900, 55, 1250, 3600 and 4200 ppm respectively.

Plant height of control and effluent treated populations was recorded from planting to flowering at the interval of 15 days. Height was significantly more in cv. Ajay after treatment with 50% and 100% from 6th fortnight whereas in Flirt height was more only after treatment with 100% effluent from 3rd fortnight. There was no significant change in branch number in treated and control populations of both the varieties (Table 1). Leaf number significantly increased in all treated populations of Ajay but no change was observed in Flirt. No significant change was observed in leaf size and floral characters (number of buds and number and size of flower and floret) after effluent treatment in both the cultivars except in Ajay where number of flowerheads per plant was more in all the treated population. Survival percentage was found to be more in all the treated populations of Ajay over the control. However, in case of Flirt, both treated and control plants depicted similar survival percentage.

Normally formation of suckers (propagules) in chrysanthemum takes place when the blooming period is over. It was very interesting to observe that formation of suckers took place in all the treated populations of Ajay during blooming period while there were no suckers in control population. The number of suckers was 4, 42 and 97 in 25%, 50% and 100% effluent treatment, respectively. No sucker formation was observed during blooming period of cv. Flirt.

Root tip mitosis was normal in control plants of cv. Maghi yellow except 0.076% cells showed chromosome bridges. Effluent showed severe effects on cell division. Cell division was arrested and the

[1]Present address: Environmental Sciences, Dept. of Botany, University of Lucknow, Lucknow 226 020

Table 1. Effect of distillery effluents on chrysanthemum cultivars.

Characters	Flirt				Ajay			
	Control	25%	50%	100%	Control	25%	50%	100%
Number of branches	11.67±2.49	6.67±1.25	11.00±3.85	11.00±2.38	14.89±3.16	13.33±1.54	17.00±1.48	13.50±2.02
Size of leaf								
Length (cm)	6.97±0.72	6.36±0.72	6.26±0.81	7.01±0.49	3.95±1.37	3.91±1.14	4.31±1.24	4.03±1.36
Width (cm)	4.40±0.74	3.85±0.31	3.64±0.53	4.27±0.41	2.83±0.96	2.95±0.86	3.40±0.91	3.04±0.95
Floral characteristics								
No. of buds (flowers)	71	69	76	83	135	279	399	429
No. of florets/head	159	159	160	161	151	146	160	134
Flower head size (diameter in cm)	7.02±0.32	6.96±0.72	6.96±0.89	7.01±1.10	3.53±0.21	3.37±0.24	3.47±0.19	3.40±0.08
Floret size								
Length (cm)	3.37±0.16	3.02±0.15	3.29±0.16	3.25±0.21	1.28±0.10	1.16±0.96	1.29±0.07	1.44±0.19
Width (cm)	0.73±0.18	0.69±0.06	0.69±0.07	0.71±0.07	0.34±0.07	0.38±0.06	0.38±0.06	0.31±0.06

percentage of dividing cells reduced with increase in treatment period. Maximum mitotic effect was observed in 100% effluent treatment for 6h where only 1.78% dividing cells were recorded. Mitotic index is represented in the form of relative division rate (RDR) [1]. The RDR at each treatment period was calculated and in all cases the values were negative indicating also inhibition of mitotic division. Increase in the negative value of RDR was directly proportional to the severity of the mitotic inhibition. It was interesting to note that the frequency of dividing cells was found to increase again when the same treated plants were studied after 7 and 12 days. RDR values were also found to decrease after 7 and 12 days.

A wide spectrum of chromosomal abnormalities were recorded in effluent treated populations at all treatment periods. Chromosomal abnormalities detected at each duration of treatment were bridges, clumping, disturbed metaphase, early separation, micronuclei, stickiness, nuclear budding, changes in nuclear morphology, increased nucleolar and cell volume, exclusion, grouping of chromosomes, granulated cells etc. There was no time specific abnormality. However, some type of abnormalities increased with increase in treatment period and also the frequency of cells with chromosomal abnormalities increased with increase in treatment period [Table 2, Fig. 1 (a-l)].

In the present study, two Chrysanthemum cultivars were found to be differentially sensitive to the distillery effluent. Effluent showed positive increase in some vegetative and floral characters in cv. Ajay. Effluent was found to have immediate effect on different morphological characters as normally observed after treatment of plants with different mutagens. Survival of

Table 2. Cytological abnormalities exerted by effluent on chrysanthemum var. Maghi Yellow

		100% effluent treatment					After*	
Abnormalities (%)	Control	1h	3h	4h	5h	6h	7 days	12 days
Bridge	0.076	0.000	0.091	0.000	0.000	0.000	0.000	0.000
Chromosome clumping	0.000	0.000	0.000	0.079	0.000	0.174	0.000	0.000
Early separation	0.000	0.389	0.091	0.237	0.000	0.348	0.000	0.193
Micronuclei	0.000	0.324	0.364	0.237	0.255	0.305	0.000	0.000
Stickiness	0.000	0.064	0.091	0.079	0.063	0.087	0.000	0.000
Nuclear budding	0.000	0.000	0.000	0.001	0.005	0.000	0.000	0.000
Changed nuclear morphology	0.000	0.909	1.002	1.029	1.339	1.307	0.000	0.000
Increased nucleolar volume	0.000	0.001	3.555	4.988	6.122	3.311	0.020	0.000
Giant cell	0.000	0.000	7.748	7.125	5.867	8.132	0.035	0.030
Exclusion	0.000	0.029	0.045	0.046	0.090	0.098	0.003	0.001
Chromosomes in groups	0.000	0.064	0.546	0.237	0.127	0.043	0.000	0.096
Granulated cell	0.000	0.000	0.000	0.000	0.001	1.525	0.023	0.000
Total aberrations	0.076	9.528	12.91	12.80	16.13	17.23	00.08	00.29
Total dividing cells	159	122	58	53	32	40	81	84
Total cell counted	1310	1540	1097	1263	1568	2250	1119	1032
Mitotic index	12.13	7.922	5.287	4.190	2.041	1.777	7.238	8.139

*Suckers treated with 100% effluent for 6 h were replanted in the sand : soil (1:1) & re-examined for cytological abnormalities (long-term effect)

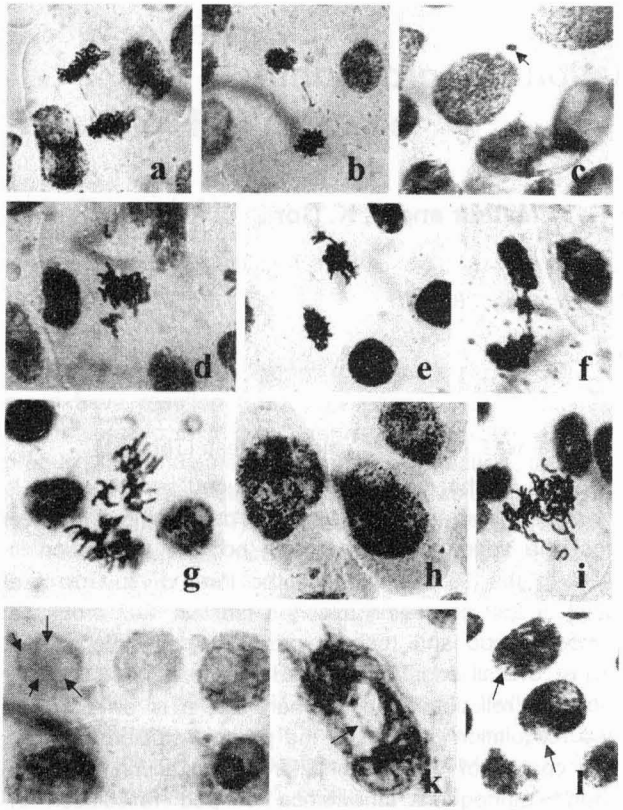

Fig. 1(a to l). Showing chromosomal aberration induced by distillery efflient in chrysanthemum (*Dendrathema grandiflora* Tzvelve) cv Maghi Yellow; (a) bridge; (b) laggard; (c) micronuclei (arrow marked); (d) early separation; (e) exclusion; (f) abnormal nuclear bridge; (g) chromosomal groups; (h) giant cell with granulated nucleus; (i) abnormal metaphase; (j) increased nucleolar (four in number) volume [arrow marked]; (k) increased nucleolar volume [enlarged view (arrow marked)]; (l) bursting of nucleolus (arrow marked)

plants after treatment with effluent was more than the control in cv. Ajay. Significant increase was found in plant height, number of leaves and flower heads per plant as recorded in cv. Ajay after effluent treatment. In addition to these, formation of suckers even during blooming period in cv. Ajay after effluent treatment is highly desirable. There are many Chrysanthemum cultivars where sucker formation is very poor. Present effluent can be tested on various such cultivars for commercial use. Flirt cultivar, on the other hand, did not show any significant negative changes in all these characters after treatment with effluent.

Studies on the effect of dairy, sugarmill and fertilizer factory effluents on seed germination and seedling growth of some crop plants had been carried out by several authors [2-4]. Effluent showed cytotoxic effect in the form of lethal effect on cell division and induction of various chromosomal abnormalities during root tip mitosis of cv. Maghi Yellow. Similar type of cytotoxic effects of different chemicals have been carried out on different plant materials by several workers [5-8]. Effluent treated plants when allowed to grow, frequency of chromosomal abnormalities reduced indicating that the harmful effect of effluent reduced and the plants returned to normalcy. Damaged cells were being eliminated after 7-12 days through *diplontic selection.*

In the present experiment it has been clearly observed that although the distillery effluent had temporary immediate cytotoxic effect, at the initial stage, it showed some positive effect on different morphological characters. It is clear that on the basis of only cytotoxic effect pollutants should not be declared dangerous for crops. Present study gives positive indication that such type of environmental pollutants can be exploited commercially for better growth of cash crops such as ornamentals.

References

1. **Hoda Q., Bose S. and Sinha S. P.** 1991. Vitamin C mediated minimization of Malathion and Rogor induced mitoinhibition and clastogeny. Cytologia, **56**: 389-397.
2. **Gautam D. D., Kumar K. and Bishnoi S.** 1992. Effect of dairy effluent on seed germination of some *rabi* and *kharif* crop plants. J. Environ. Biol., **13**: 7-12.
3. **Singh D. K., Kumar D. and Singh V. P.** 1985. Studies on pollutional effects of sugar mill and distillery effluent of seed germination and seedling growth of three varieties of rice J. Environ. Biol., **6**: 31-35.
4. **Neelam and Sahai R.** 1998. Effect of fertilizer factory effluent on seed germination seedling growth, pigment content and biomass. J. Environ. Biol., **9**: 45-50.
5. **Adam Z. M. Ebab F. A. and Ei-Shaikh I. A.** 1990. Alteration in nucleic acid, protein content and mitotic division of *Vicia faba* root tip cells as effected by Malathion and Tamron insecticides. Cytologia., **55**: 349-355.
6. **Bellani L. M., Binallo C. and Bennici A.** 1991. Cytomorphological alterations in *Allium* roots induced by surfactants. J. Environ. Exp. Biol., **31**: 179-185.
7. **Datta S. K., Pandey R. K. and Shukla R.** 1998. *Allium* test for the assessment of oxytocin toxicity. Sci. Lett., **21**: 305-308.
8. **Shukla R. and Datta S. K.** 1999. Cytotoxic effect of artificial blue dye. J. Cytol. Genet., **34**: 95-97.