

EFFECT OF N-TETRADECANE AND UNDECANE ON SOMATIC CELLS OF ALLIUM SATIVUM L.

Ch. Srinivasulu

Department of Zoology, SR & BGNR Govt. Arts & Science College Khammam
Telangana State , India. Email:chsri39@gmail.com

ABSTRACT

Most of the pentatomidae bugs secrete a volatile defensive secretions from their metathoracic scent glands in adults and dorso-abdominal scent glands in Nymphs. The mutagenic efficiency of certain secretions like n- tetradecane and undecane of pentatomidae bugs are assayed on the root tips cells of *Allium sativum* L. n- tetradecane and undecane (hydrocarbons) when applied on the root tips cells of *A. sativum* caused considerable disturbance during the course of mitotic division. The decline in mitotic index was greater at higher concentrations. The spectrum of mitotic abnormalities included disturbed chromosomes at prophase, un-orientation of chromosomes at metaphase, due to non-formation of spindles. Unequal separation of chromosomes, formation of anaphase bridges, tripolar grouping and unoriented chromosomes, fragmented chromosomes at prophase, disturbed chromosomes at metaphase, chromosomal stickiness, and disturbed anaphase due to un-orientation spindle. The chromosomal aberrations induced by these test compounds were gaps, chromatids breaks, pulverizations, clumping and cells with sticky chromosomes dicentric chromosomes and centromeric breaks, more frequently and regularly appeared in all the time intervals.

KEYWORDS: Pentatomidae bugs, scent components, n-tetradecane, Undecane, *Allium sativum* L.

INTRODUCTION

Most of the heteropteran bugs secrete a pungent volatile compounds from the abdominal and metathoracic scent glands. In order to test the mutagenicity of these scent compounds from insects. *Allium* is being used as an experimental materials. Certain aldehydes like octenal, 2-octenal, and 4-hydroxy-2-octenal acted as antirespiratories, cytological aberrations they induce are very few (Canuto et al., 1985). n-butyl butyrate, n-dodecane and n-pentadecane are the scent components of *C. purpureus* causing cytological aberrations in the root tips cells of *Allium sativum* (Sunder et al., 1987). They showed inhibition effect may be due to the blockage of DNA synthesis (Digamber Rao et al., 1987). 1-Butanol, 1-Hexanol were the scent components reported from the scent glands of *Libyaspis angoleunsis* (Cmelic, 1969) and *Gelastocoris oculatus* (Staddon, 1973). Most of the chemicals were previously treated for inducing chromosomal aberrations (Sago et al., 1993) Sadasivaiah et al (1973), studied the effect of lysergic acid, diethyl amide on onion, rye and barley. Action of antihistamine, dimecras and leaf extracts of *Azadirachta indica* were observed as somatic cells of *Allium sativum* L (mogili and vidyavathi, 1985b; chandrakala and vidyavathi 1992). In tumor cells, Aliphatic aldehydes react with cellular thiol compounds (Guidotti et al 1965; sessa et al 1977) and cause ultra-structural alterations of plasma membranes of Golgi vesicles, mitochondria and nuclei (Bassi, 1967). Brickis et al (1969) have reported the effect of 4-hydroxy-2,3, trans-pent-2n-1-al (HPE), on respiration and glycolysis. Perin et al (1978) observed the antitumor activity of 2,3 dihydroxy butyraldehyde on Enrich Carcinoma sarcana 180 and Yoshida AH-130 hepatoma (Zundel et al., 1978). Kochi et al (1980) and watanuki and sakaguchi (1981) demonstrated the antitumor activity of benzaldehyde, the mutagenic effect as dividing cells of meristematic root tips of *Allium cepa* L. by buthionine sulfoxamine (Kamal et al., 1994), extract of *Epuedia foliota* (sagoo et al., 1993) were reported. Effect of mitomycin-c was studied on root tips cells of *Cuminum cyminum* (mogili et al., 1984) and BIT-4 on root tips of *A. sativum* (sadanandam et al., 1984). Teratogenicity and abnormal chromosomal recombination by selenium compounds have been reported (Ray et al., 1978; Mirimoto et al., 1982). Effect of polluted water, heavy metals, fertilizer, pesticides as chromosomal of *Allium* sps. Roots were also studied.

MATERIALS AND METHODS

Actively growing root tips of *A. sativum* L were harvested and treated with various concentrations (0.01, 0.5, 1.0 and 2.0%) of scent components from heteropteran bugs for a duration of 2, 4 and 6 hours. The required concentrations were prepared in acetone as these components do not dissolve in distilled water. After each duration of treatment of the root tips from all the samples were fixed in carnoys fluid (1 part of acetic acid and 3 parts of absolute alcohol) and kept in the refrigerator . The root tips were allowed to remain in the fixative for 24 hours and then transferred into 70% alcohol . About 2000 randomly selected dividing cells from ten different root tips were washed thoroughly with distilled water

and then temporary squash preparations were prepared for cytological observations employing the acetocarmine as the nuclear stain. Various mitotic aberrations of metaphase, anaphase and telophase stages have been observed under meopta compound microscope (CZECHOSLOVAKIA) with the aid of X10 as eye piece and X 60 as objective. The photomicrographs of aberrated stages were taken from LEITZ LABORLOYS TRINOCULOAS MICROSCOPE (model: LEITZ) with green filter. All the photographs were taken by the author himself and are of equal magnification Cax 2000. All the Chromosomal aberrations were photographed and printed on hard glassy paper .Mitotic induces were calculated along with chromosomal aberrations .

$$\text{Mitotic index (MI)} = \frac{\text{Number of cells in division}}{\text{Total number of cells scored}} \times 100$$

Estimation of mitotic index is an important protocol as it reflects the action of a given compound on cell division and DNA synthesis. 1500 to 2000 cells were scored from each time interval i.e., 24,48 and 72 hours.

RESULTS

n-tetradecane an irritant hydrocarbon was identified from the scent glands of certain heteropteran bugs. n-tetradecane is an irritant hydrocarbon reported from the metathoracic scent glands of Biprorulus bibax (Macleod et al., 1975). The root tips of Allium sativum were treated with various concentrations of n-tetradecane and an attempt is being made to study its mitotic toxicity on the chromosomes of cells of roots tips of A.sativum. It has been observed that this component caused considerable abnormalities in the various phases of mitotic cell division. The data presented in table.1 clearly shows that mitotic indices were consistently low with increasing percentage of concentration. The maximum decline was observed with higher concentrations (1.0% and 2.0%) treated with for 6 hours when compared to the 12.4% of control. Total abnormalities were also recorded hours durations. The stickiness of chromosomes, fragments and bridges were observed in all the treatments. The spectrum of mitotic abnormalities included fragmented chromosomes at prophase (Fig.1), unoriented chromosomes at metaphase (Fig. 2) and disturbed metaphase (Fig.3). Other abnormalities were also observed like diagonal spindle, fragments, bridges and clumping of chromosomes. The chromosomal abnormalities in A. sativum has earlier been reported (Surender et al., 1987,Srinivasulu,2013).

Concentration Percentage	Duration of treatment (hr)	Mitotic Index (MI)	Types of Abnormalities						Diagonal Spindles	Total aberrations
			Metaphase		Anaphase		Telophase			
			Stickiness	Fragments	Stickiness	Bridges	Stickiness	Bridges		
Control		12.4	0.05	--	--	--	--	--	0.04	0.05
	2	12.2	0.2	--	--	--	--	--	0.3	0.5
	4	12.0	0.3	--	--	--	0.1	--	0.4	0.8
0.01	6	11.5	0.4	--	0.2	--	0.2	--	0.5	1.3
	2	12.0	0.1	0.2	0.2	0.1	0.2	--	0.1	0.9
	4	12.0	0.3	0.4	0.4	0.3	0.4	0.2	0.4	2.4
0.5	6	11.6	0.5	0.5	0.6	0.5	0.5	0.3	0.8	3.7
	2	12.1	0.2	0.1	0.3	--	0.3	0.2	0.3	1.4
	4	11.4	0.4	0.5	0.5	0.4	0.5	0.3	0.6	3.2
1.0	6	10.8	0.7	0.8	0.6	0.5	0.5	0.3	0.8	4.2
	2	12.3	0.6	0.5	0.4	0.2	0.5	0.5	0.2	2.9
	4	11.8	1.2	1.6	0.7	0.6	0.6	0.5	0.8	6.0
2.0	6	11.0	1.8	2.0	1.2	1.0	1.8	0.6	1.0	9.4

TABLE-1 Mitotic Index (MI) and percentage aberrations in Allium Sativum L.with n-tetradecane a scent component of Heteropteran bugs

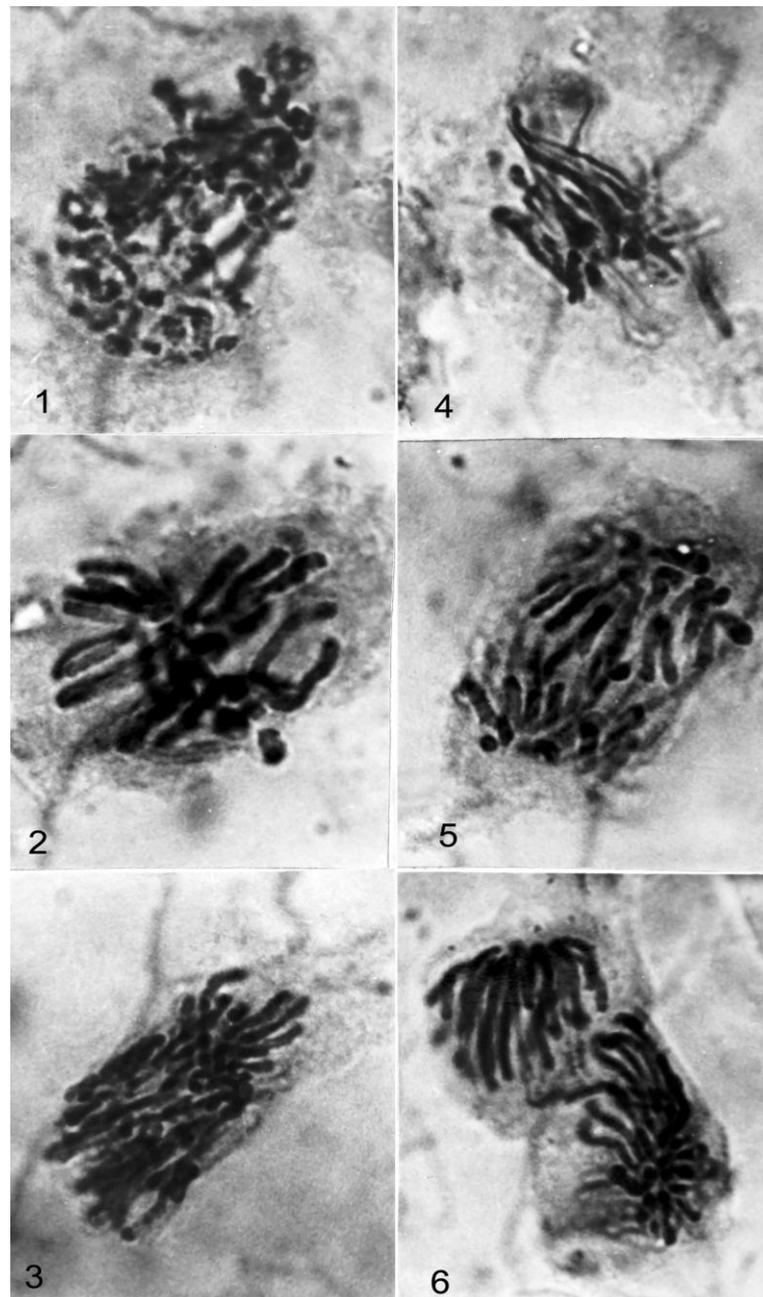


Fig: 1-6: n-tetradecane and undecane induced cytological aberrations in the root tips of *Allium Sativum* (x2000)

Fig 1 : Fragmented Chromosomes at prophase at 2% n-tetradecane treated for 4 hours.

Fig 2: Un orientation of chromosomes at metaphase at 1% n-tetradecane treated for 6 hours.

Fig. 3: Disturbed metaphase at 1% n-tetradecane treated for 6 hours

Fig. 4: Chromosomal stickiness at 2% Undecane treated for 4 hours

Fig.5: Formation of Anaphase bridges at 2% Undecane treated for 4 hours.

Fig. 6: Disturbed Anaphase due to unorientation of spindle at 1% Undecane treated for 6 hours.

Cytotoxic effects of fytolon on somatic cells of *Allium sativum* were observed (Padmaja and Vidyavathi, 1994). Undecane another hydrocarbon in various concentrations treated with root tips cells of *Allium sativum*. The data that mitotic indices were consistently low with increasing percentages of concentrations (Table. 2). The spectrum of mitotic aberrations included chromosomal stickiness (Fig.4) formation of Anaphase bridges (Fig.5) and disturbed anaphase due to unoriented of spindles(Fig. 6). The maximum aberrations observed were in 1.0 and 2.0% of concentration for both the compounds treated for 4 and 6 hours durations. During the present investigations with n-tetradecane and Undecane on root tips of *Allium sativum* showed more effect and the chromosomal aberrations observed in all the cases were similar and comparable (Table.1 & 2). The frequency of chromosomal aberrations was increased with the increasing concentrations and duration for all the compounds.

The formation of anaphase bridge may be due to the development of dicentric chromosomes with two centromeres of each dicentric chromosomes being oriented towards opposite poles(Aly Mohammed et al., 1966). The dicentric chromosomes, presumably, arose by the reunion of two sister chromatids of the broken chromosomes. (Koller, 1952, Grant and Harnery, 1960). Fragmentation of Chromosomes in *A. sativum* has earlier been reported (Digamber Rao et al., 1987). Mitodepressive and Clastogenic activity of Crude drug Combination on the Somatic cells of *Farniculum vulgare* is reported (Krishna Reddy et al., 1984), and also earlier reported the Influence of Floral pigments of Periwinkle plant on Cell division(Mogili et al., 1985). Diagonal spindles were frequently observed in all concentrations for both these chemicals under study. Antimutagenic potential of curcumin on chromosomal aberrations in *Allium cepa*(Ragunathan et al 2007),cytotoxic and genotoxic impact of silver nanoparticle using root tips cells of *Allium cepa* as an indicator(Kumari M et al 2009)

Concentration Percentage	Duration of treatment (hr)	Mitotic Index (MI)	Types of Abnormalities						Diagonal Spindles	Total aberrations
			Metaphase		Anaphase		Telophase			
			Stickiness	Fragments	Stickiness	Bridges	Stickiness	Bridges		
Control		12.4	0.05	--	--	--	--	--	0.04	0.05
0.01	2	12.3	--	--	--	--	--	--	0.2	0.2
	4	12.1	--	--	--	--	--	--	0.2	0.2
	6	12.2	0.4	0.2	--	--	--	--	0.3	0.9
0.5	2	12.1	0.4	0.3	0.2	0.2	0.3	0.1	0.4	1.9
	4	12.0	0.4	0.4	0.6	0.3	0.5	0.3	0.4	2.9
	6	11.8	0.6	0.6	0.5	0.6	0.4	0.5	0.6	3.8
1.0	2	11.8	0.4	0.3	0.3	0.3	0.4	0.3	0.2	2.2
	4	11.6	0.6	0.6	0.6	0.8	0.5	0.6	0.5	4.2
	6	11.0	0.7	0.7	0.8	0.6	0.6	0.6	0.5	4.5
2.0	2	10.8	0.8	0.8	0.2	0.8	0.5	0.6	0.3	4.0
	4	10.6	1.3	1.4	0.7	0.9	0.8	0.8	0.8	6.7
	6	10.0	1.5	1.5	1.2	1.5	1.0	1.2	1.0	8.9

TABLE-2 Mitotic Index (MI) and percentage aberrations in *Allium Sativum* L. with Undecane a scent component of Heteropteran bugs

DISCUSSION

If certain heteropteran bugs on being disturbed they discharge a pungent, volatile liquid from both,abdominal and metathoracic scent glands.Some hydrocarbons and unsaturated aliphatic aldehydes have been reported from the scent secretion of dorsal abdominal scent glands of certain heteropteran bugs (Baggini et al., 1966;)Watanuki and sakaguchi(1981) demonstrated the antitumor activity of benzaldehyde. Canuto et al.(1985)reported some of the aldehydes like octanal, 2-octenal,4-hydroxy-2-octenal(HOE),nonal,2-nonenal,4-hydroxy-2-nonenal(HNE) acted as antirespiratory.Some of the scent components are carcinostatic (schaurenstein et al.,1987),anti fungal (Surender et al 1987) and antibacterial;(Surender et al 1988)sex-attractant(park and sutherland,1962)and neurotoxins(Escousbas et al.,1994).Surender et al (1987)reported the mitotic effect due to n-dodecane and n-pentadecane,the hydrocarbons which act as contact poison reported from the abdominal scent glands of larvae *Chrysocoris purpureus* (Janaiah et al.,1988). Watanuki and sakaguchi (1981) demonstrated the antitumor activity of benzaldehyde.Canuto et al (1985) reported some of the aldehydes like octanal, 2-octenal,4-hydroxy-2-octenal(HOE), 2-nonenal acted as antirespiratory.In recent years

some of the antibiotic have proved to be effective, cytotoxic and mutagenic in both animals and plants many antibiotics. Which interfere with DNA synthesis, also effect cell division process and induce chromosomal aberrations (Rendi and Ocha, 1962). Digambar Rao et al. (1987) studies the mutagenic efficiency of n-butyl-butyrate. The ester component, found in the scent secretion of *Amorbus rhombifer* on the root tips of *Allium sativum* with different concentrations. It was found that higher concentration of n-butyl-butyrate showed greater effect on the mitosis of *A. sativum*, inhibition of cell division, spindle apparatus and cell wall development. The inhibitory effect may be due to the blockage of DNA synthesis. (Heiner, 1971).

The treatment of two hydrocarbons (n-tetradecane and Undecane) caused fluctuations at different stages of mitotic cells division clearly show that mitotic induces were consistently low with increasing percentage of concentration (Table 1 & 2). The spectrum of mitotic abnormalities included fragmented chromosomes at prophase, unoriented chromosome at metaphase, disturbed metaphase, chromosomal stickiness, formation of anaphase bridges, disturbed anaphase due to unorientation of spindle. The abnormalities were also observed in the form of bi and tri-nucleate cells, diagonal spindles and clumping of chromosomes. It was found that higher concentrations of n-tetradecane and Undecane caused greater effect. The spectrum of cellular responses included scattering of chromosomes and the daughter chromatid held together only at the centromeric region and these were referred to as diplochromosomes. The spectrum of abnormalities also included irregular grouping of chromosomes, bridges, non-orientation, micronuclei and unequal groupings, contraction, different stages of condensation stickiness and clumping of chromosomes were commonly observed in both the chemical treatment of n-dodecane and n-pentadecane (Surender et al., 1987). Diagonal spindle and the formation of tri and multinucleate cells were frequently observed at high concentration. Interestingly, little fragmentation was noticed in the 2% concentration of n-pentadecane, treated for 6 hours only. n-dodecane and n-pentadecane showed greater effect at higher concentration. Vidyasagar (1995) studied mitotic effect due to (1-hexanol and 1-butanol) the alcoholic compounds on the root tips of *Allium cepa* with different concentration. It was found that higher concentration of these compounds showed greater effect. The action of these two alcoholic compounds at the mitosis of *A. cepa* showed irregular anaphase, extreme fragmentation with laggards. Diagonal spindles anaphase bridges, binucleate cell, clumping of chromatin material. The unoriented chromosomes may be due to the disfunctioning of centromeric parts of chromosomes or failure of asral rays to drag the chromosomes to their respective poles. Multipolar grouping presumbaly be due to the splitting of spindle apparatus. The dicentric chromosomes possibly arose by the reunion of two sister chromatids of the fragmented chromosomes (Grant and Horney, 1960). Thus it may be observed that the scent components induced cytological aberrations and possibly acted as mitotic inhibitor; The inhibitory effect may be due to the blockage of DNA synthesis in general. The spindle inhibiting action may mostly be caused by other C-mitotic agents (Deysson Gay, 1975).

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