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GENOTOXIC EFFECT OF APPLE GREEN COLOUR ADDITIVE ON GERM CELLS OF SHORT HORNED GRASSHOPPER *POECILOCERUS PICTUS* F. (INSECTA: ORTHOPTERA: ACRIDIIDAE)

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ABSTRACT
Colour additives, preservatives and food retention agents in our daily food is a matter of serious concern. Such food additives might be mutagenic to human genome. The long term exposure of humans to different synthetic food additives leads to several health hazards,of which genotoxicity is of prime importance.The investigation on the effect of Apple green which is a combination of chemical compounds such as Tartrazine (1914 0), Brilliant Blue (42090) and Sodium chloride of Alpana's Natraj brand on grasshoppers shows that this compound induces chromosomal aberrations significantly with increase in concentration and exposure duration of the organism to such chemical. Hence, nonuse of the stated colour additive is suggested for the better health status of human beings.

INTRODUCTION

Now-a-days a drastic change in the life style of human beings are seen. The significant change has taken place in food items. Fast foods or junk foods have dragged the attention of both young and old individuals. Different foods available in the market are attractive and palatable because of colour and flavour. These colours, flavour enhancers and preservatives are synthetic chemicals which are reported to have adverse effect on health (Conners *et al.,* 1976; Schab and Trinh, 2004; Wajih *et al*., 2007; Weiss, 2012). The emphasis is clearly on taste, look and flavour of such food items. The widespread use of food additives including different colours in the preparation of these items is a matter of concern and opens a rich field for research concerning their long-term impact on human health. A lot of works have been reported on the effect of various chemicals such as herbicides, pesticides, insecticides and drugs on insects, amphibians and mammals as experimental material (Auerbach and Robson, 1944; Abrahamson and Friedman, 1964; Klassen *et al*., 1969; Abdel Hameed *et al.,* 1970; Manna and Parida, 1972; Bhunya and Das, 1976; Fulton and Chambers, 1985; Behera and Bhunya,1986; Bhunya and Behura, 1986; Cakmak *et al*., 2004; Koseoglu *et al.,* 2004; Robichova *et al.,* 2004; Ithoh *et al.,* 2005; Mohanty *et al.,* 2007). To know the impact of food additive, an *in vivo* investigation was undertaken to study the direct effect of Apple green on a type of short horned grasshopper test system.

MATERIALS AND METHODS

For the present investigation, the experimental animal is short-horned grasshopper (*Poecilocerus pictus* F.) which was collected from different regions of Jajpur and Cuttack of Odisha, India during July, 2008 to December, 2008. The testes lobules were used as the test materials for the study. The animals were injected with Apple green colour additive for 6, 12 and 24h interval after which specimens were sacrificed and testes lobules were collected. Parallel controlled sets were also prepared in the same intervals by injecting distilled water to the specimens. 10 per cent, 15 per cent and 20 per cent concentration of Apple green colour were prepared and injected to the specimens separately. For each concentration of dose, the treated animals were kept for 6h, 12h and 24h of duration to study their effects on germ cells. Slides were prepared for both controlled and treated sets and stained by haemotoxylene. Cells and chromosomes were observed, analysed and photomicrographed. Z- value was calculated for each dose and duration of treatment .

RESULTS

From the normal squash preparation, a good number of metaphase plates were observed. The chromosomes are rod shaped and acrocentric in nature. In the control set, the percentage of abnormal cells having chromosomal abnormalities such as corrosive effect, woolly appearance, fragment,

Table 1: Percentage and proportion of abnormal cells in control series

S no	Solvent	Duration of treatment (in h)	Total no cells	Types of chromosomal abnormalities found in no of cells							No of abnormal cells	Percentage of abnormal cell	Proportion of abnormal cells
				Corrosive counted	Woolly effect	Fragment appearance	Clumping	Breaks	Centromeric fission	Bridge			
1	Distilled water	6	350	01	02	01	01	03	-	-	08	2.2	0.022
2	Distilled water	12	350	01	01	02	01	04	01	01	11	3.14	0.031
3	Distilled water	24	350	02	03	03	03	02	02	01	16	4.57	0.045

Table 2: Percentage and proportion of abnormal cells found in different doses of treatment of colour additive, Apple green

S no	Dose of treatment in per cent	Duration of treatment (in h)	Total no cells counted	Types of chromosomal abnormalities found in no of cells							No of abnormal cells	Percentage of abnormal cell	Proportion cells of abnormal	Z- Value
				Corrosive effect	Woolly apearance	Stickiness	Clumping	Breaks	Bridge	Centromeric stretching				
1	10	6	350		15	10	-	12	02	09	48	13.71	0.137	5.623*
		12	350	02	22	06	03	13	02	04	52	14.85	0.148	5.421*
		24	350	03	08	15	06	16	02	04	54	15.42	0.154	4.817*
2	15	6	350	01	11	18	03	08	02	06	49	14	0.14	5.721*
		12	350	02	10	13	03	09	07	08	52	14.85	0.148	5.421*
		24	350	04	05	24	02	09	03	09	56	16	0.16	5.015*
3	20	6	350	03	06	17	06	07	04	07	50	14.28	0.142	5.785*
		12	350	02	03	15	20	08	02	03	53	15.14	0.151	5.519*
		24	350	04	07	15	22	09	03	03	63	18	0.18	5.652*

*Significance at 1% level; The tabulated value at 1% is 2.58.

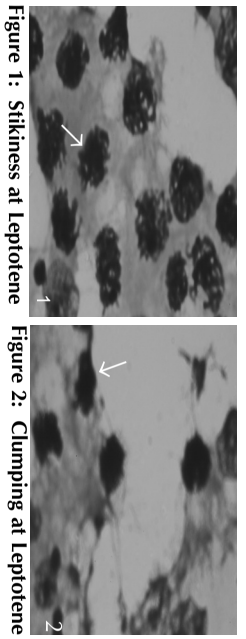

Figure 1: Stikiness at Leptotene

Figure 2: Clumping at Leptotene

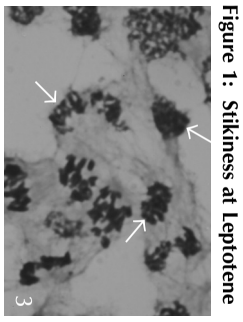

Figure 3: Fragment, Break and Clumping at Leptotene

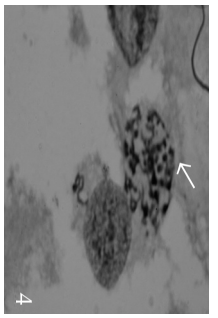

Figure 4: Corrosive effect at Leptotene

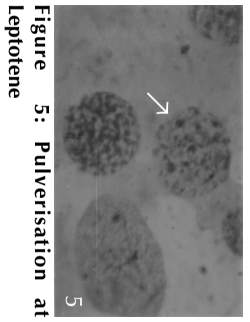

Figure 5: Pulverisation at Leptotene

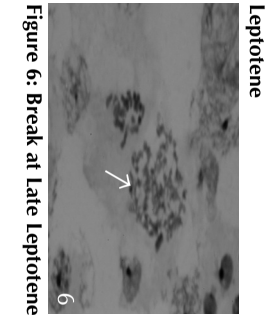

Figure 6: Break at Late Leptotene

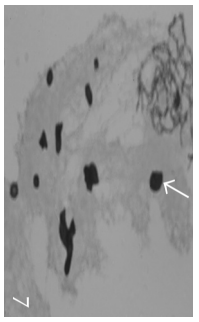

Figure 7: Clumping in Diakinesis

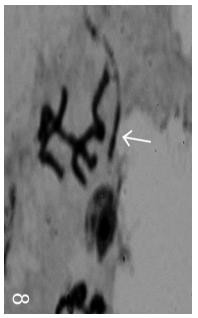

Figure 8: Stretching and Break at Diakinesis

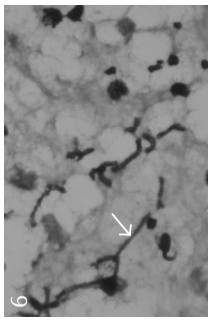

Figure 9: Stretching at Diakinesis

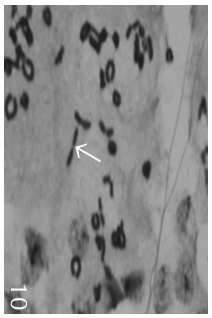

Figure 10: Breaks at Diakinesis

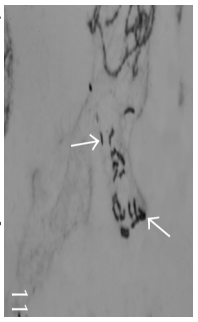

Figure 11: Fragment and Improper orientation at Metaphase

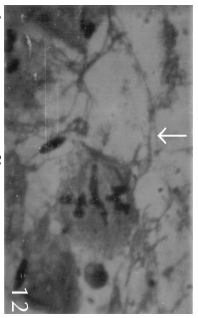

Figure 12: Woolly appearance at Metaphase-I

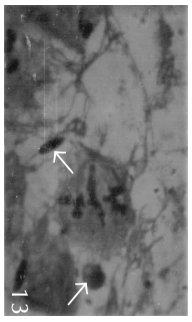

Figure 13: Clumping of chromosomes at Metaphase-I

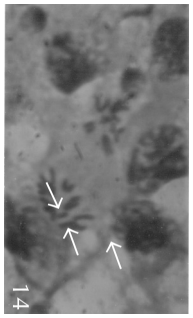

Figure 14: Centromeric fission in Anaphase-I

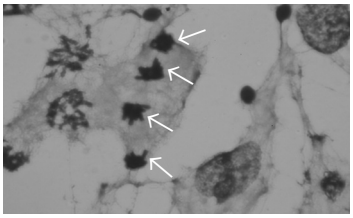

Figure 15: Clumping at Anaphase-II

clumping, breaks, centromeric fission and bridge are found to be 2. 2, 3. 14 and 4.57; proportions 0.022, 0.031 and 0.045 for 6, 12 and 24 h respectively (Table 1). In treated sets (Table 2), the chromosomal abnormalities encountered during our observation were corrosive effect (Fig. 4), woolly appearance (Fig. 12), pulverisation (Fig. 5) fragments (Figs. 3 and 11), clumping (Figs. 2, 3, 7, 13 and 15), breaks (Figs. 3, 6, 8 and 10), centromeric fission (Fig. 14) bridge of chromosomes, stickiness (Fig. 1), Stretching (Figs. 8 and 9) and improper orientation (Fig. 11). Treatment of 10 per cent dose of Apple green for 6, 12 and 24h of duration showed the percentage of abnormal cells (having chromosomal abnormality) to be 13.71,14.85 and 15.42; proportions 0.137, 0.148 and 0.154 and calculated Z values 5. 623, 5.421 and 4.817 respectively. Similarly for 15 per cent, percentage of abnormal cells was observed to be 14, 14. 85 and 16; proportions 0.14, 0. 148 and 0.16 and Z values 5.721, 5.421 and 5.015 respectively . Percentage of abnormal cells in 20 per cent was calculated to be 14.28, 15. 14 and 18; proportions 0.142, 0.151 and 0.18 whereas calculated Z values 5.785, 5.519 and 5.652 respectively. From this observation, it is evident that the percentage of abnormal cells was more with the increase of dose and duration of exposure of the used Apple green compound.

DISCUSSION

In the last four decades, the progress on mutagenic effect of the chemicals on different animal test system has increased vastly. Auerbach and Robson (1944) first demonstrated the chromosomal aberration by chemical mutagen. Effects of organic pesticides on the chromosomes of mammalian cells have been reported by Abrahamson and Friedman (1964); Abdel Hameed *et al*. (1970); Bhunya and Das (1976); Behera and Bhunya (1986); Bhunya and Behura 1986; Cakmak *et al*. (2004); Koseoglu *et al*. (2004); Robichova *et al*. (2004); Ithoh *et al*. (2005); Mohanty *et al*. (2007) have reported the genotoxic potential of different mutagens and chromosomal aberrations such as woolly appearance, pulverization, corrosive effect, stickiness, laggard, stretching, breakage, clumping, bridge, fragmentation, gaps, centromeric stretching and centromeric fission. The effect of organophosphorous compounds have been found to be mutagenic in the embryo of amphibians (Fulton and Chambers, 1985). The chemically induced chromosomal aberration in insects has been reported by a good number of researchers (Klassen *et al.,* 1969; Manna and Parida, 1972; Bhunya and Das, 1976; Bhunya and Behura, 1986; Mohanty *et al.,* 2007).

From the above analyses, it is observed that chromosomal anomalies are caused due to chemicals. In this study Apple green is found to be clastogenic and mitotoxic to genetic material of grasshopper. The percentage of abnormal cells has been noticed to be increased with increased concentration of dose and duration of treatment. From the statistical analysis, it is inferred that Apple green has significant mutagenic effect on genetic material of grasshopper test system. Hence, nonuse of this food additive is suggested for the better health status of the organisms including human beings.

In this study, results indicate that such colour additive like Apple green is observed to be cytotoxic and genotoxic to grasshoppers. The percentage of abnormal cells having chromosomal abnormalies has been recorded to increase with increased concentration of dose and duration of the treatment. From the statistical analysis, it is evident that Apple green has significant mutagenic effect on genetic material of grasshopper. Therefore, nonuse of this food colour additive is suggested to maintain the genetic stability and sustained genetic make up of animals including human population.

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