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Induction of mitotic-chromosome anomalies and micronucleus test in *Anabas testudineus* by single superphosphate

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Abstract
Phosphate, a common fertilizer used to supplement soil with phosphorous induced 1.10%, 1.26% and 1.43% of micronuclei and 18.3%, 21.0% and 24.6% of chromosomal abnormalities upon three different doses. The frequency of abnormalities increased with increase of doses. Polyploidy and aneuploidy were common among gross, while acentric fragments, minute fragments, chromatid breaks were more common among individual type of abnormalities. The individual type of damages were more prominent than gross type. This might be due to the formation of electrophilic radicals/ions during the metabolization of mutagens that attack the nucleophlic site of DNA leading to structural changes in chromosomes. These chemicals probably cause an increase formation of a base analogue to act as a mutagen that induce chromosomal and cellular abnormalities.

Keywords: Phosphate, genotoxicity, micronuclei, chromosomal abnormalities, *Anabas testudineus*

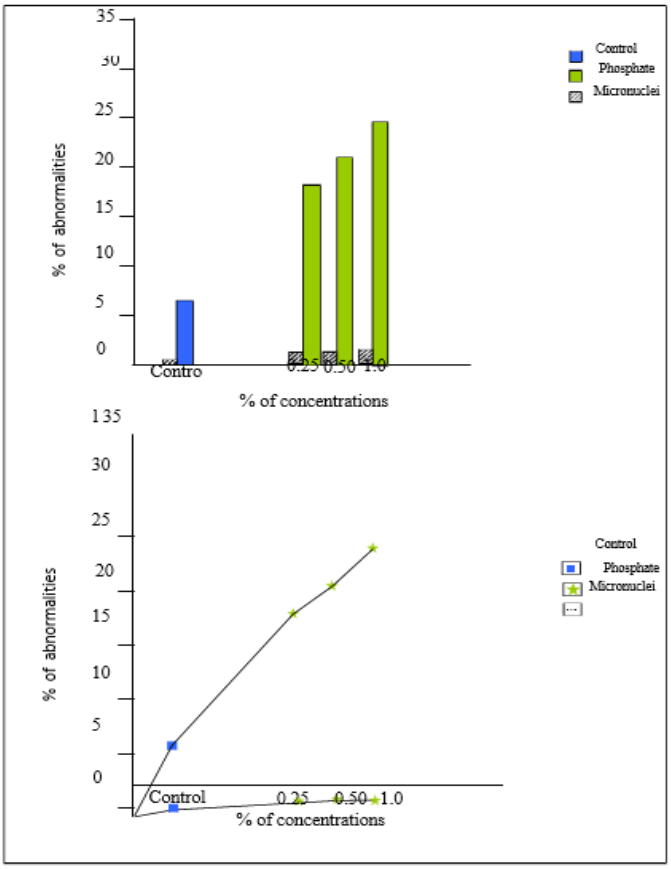
Introduction
Wide spread use of synthetic fertilizers in agriculture has led to the large scale contamination of our living environment (Petrovic *et al.*, 1981) [34]. The Agrochemicals like pesticides and fertilizers contribute in great to reduce the irrigated and well water quality (http://www.fao.org /docrep /w2598E/w2598e 0.4htm 6/22/2005). As agricultural run-off they pose a serious effect that induce various histopathological (Srivastava and Sravastava 1979; Nanda *et al.*, 2004; Ravindar, 2000) [32, 35, 38] and cytogenetical changes (Jha, 1998; Kohlpoth, 1999; Dashwood, 1998; Baski, 1990) [20, 28, 30] in the plants, aquatic animals, cattles and humans (Dravyam and Rajamanickam 2003, Neff, 1985; Bhaskaran, 1988; Singh *et al.*, 1998; Gupta 2000, http:/www3.Interscience wiley.com/cgibin/abstract/ABSTRACT?CRETRY=1&SRETRY=6/15/06) [6, 23, 33, 36-37].
Phosphate is an important and conventional fertilizer that enriches the soil with phosphorous. This agrochemical is taken up by the growing plants as orthophosphate. This fertilizer has been found to be present either in residual or some metabolized/derived from among the plants grown over them, and thus get accessed to the body of the animal that feed upon these plants (Baker and Chesnin 1975; Chaurasia and Sinha 1989; Current Science, Nov 2000) [1, 2, 4, 7-19, 21, 31]. The genotoxic effects of agrochemicals has been reported in various test system (Chaurasia and Sinha 1987, 1988, 1990; Chaurasia 1991) [1, 2, 7-19, 21, 31], but the genotoxicity of this relevant fertilizer is poorly known and limited to plant system only (Chaurasia and Rathore 1980, Chaurasia and Sinha 1986) [1, 2, 7-19, 21, 31], mice (Chaurasia and Sinha 1988, Chaurasia 1991, Chanda kumari & Chaurasia O.P 2008) [1, 2, 7-19, 21, 31], but very few reports are available in the air-breathing fishes. So, the present investigation was therefore taken up to study the hitherto almost unknown genotoxic effect of phosphate on chromosomal abnormalities and incidence of micronuclei in *Channa punctatus*.

Material and Methods
Two test system *viz.* micronucleus test (from peripheral blood cells) and mitotic chromosomes (form head kidney) were used. 10-15 days acclimatized fishes were treated with freshly prepared doses of phosphate with three different concentration i.e. Sub-lethal (SL-1.0%), half of the sub-lethal (HSL- 0.5%) and quarter of the Sub-lethal (QSL-0.25%) for

7 consecutive days. The animals were sacrificed after seven days of the termination of treatment. The micronucleus test was conducted in peripheral blood cells. A film of blood smear was prepared after mixing with few drop of anticoagulant (0.1% trisodium citrate solution) on a greese free clean slide. Preserved in methanol for 10 minutes, stained with 0.15% Leishman's stain for 20-25 minutes and cleared in xylene for 5 minutes. 3000 RBC cell were screend. A concurrent control were carried out where animals were kept in fresh water. For studying the chromosomal abnormalities, tissue from head kidney were taken and the slides were made by the conventional Colchicine – hypotonic – acetoalcohol- flame drying- iemsa staining technique. 300 well spread and randomly selected metaphase plates were screened and data were analyzed by statistical procedure. A separate common control was also carried out.

Results and Discussion

Amidst 3000 RBCs, only 0.5% micronuclei were found in the control group while 1.10%, 1.26% and 1.43% micronuclei upon three doses of phosphate (SL, HSL & QSL) were observed (Table – 1). A close observation of data revealed that the effect was dose – dependent (Graph – 1). Most of the cells were found to have only one micronucleus of very small size (due to acentric fragment) or bigger size (due to lagging of whole chromosome) but very few cells were found to have more than one micronucleus. Amidst 300 metaphase plates, 18.3%, 21.0% and 24.6% chromosomal abnormalities were found upon treatment with three doses of phosphate in contrast to 6.6% in the control (Table – 1). The abnormalities that were found can be put in two categories – gross and individual ones. The insignificant gross changes were the stickiness, polyploidy, hypoploidy etc. The significant individual changes were mostly breaks in the chromosomes (Chromatid break, chromatid gap). Acentric fragment and minute fragment were also observed that might be due to breaks and deletion of certain part of chromosomes (telomeric or interstitial part). A quantitative estimation revealed that the abnormalities increased with the increase of the doses. Thus the effect was dose dependent (Graph-1). The individual type of damages were more prominent than gross type. While Chaurasia and Sinha 1987 [1, 2, 7-19, 21, 31], 1988, 1990, Chaurasia *et al.*, (2005) [1, 2, 8-10, 14-19] were studying on genotoxicity induced by fertilizer and silk dyeing wastes; Kumar and Sinha (1989) [1, 2, 7, 10-14, 18, 21, 31] on doses – dependent genotoxic effects of synthetic pesticides, they observed that the individual type of damages were more frequent than the gross type. Bose and Sinha (1994) [1, 2, 7, 10-14, 21, 31], Dharmashila and Sinha (1994) and Awasthy *et al.* (2000) [1, 2, 7, 10-14, 21, 31], could find that the biomutagens induced more gross type of abnormalities than individual types. This differential sensitivity might be occurred at two different levels. First, the damages at protein level either on spindle protein or on protein packing. Second, by the production of electrophilic ions and reactive radicals during the metabolization of mutagens (Klopman *et al.*, 1985) [29]. Such electrophilic reactive radicals/ ions might attack to nucleophilic site of DNA leading to structural changes in chromosomes (Awasthy *et al.*, 1999) [1, 2]. Phosphate is present only in nucleic acid, and it is only the DNA that could have got changed. This fact is in conformity with mutagenic nature of various nitrogen bases of DNA. The excess may induce mutation as chromosome break (Kihlman 1952, Freese, 1963, Chaurasia, 1992, 1994, 2005, 2007) [1, 2, 3, 8-10, 14-19, 22] in the form of micronuclei in peripheral blood cells (Hayashi *et al.*, 1984, 1990, 1994, Hayashi, 1994) [24-27]. These chemicals probably cause an increase formation of a base analog to act as a mutagen that induce chromosomal and cellular abnormalities. The final effect would be delayed DNA synthesis or some inhibition in it (Chaurasia, 1987) [1, 2, 8-10, 14-19]. The result thus shows that the fertilizer phosphate was mutagenic and harmful to the fishes with a regular deterioration of their population and thus affecting the economy of our country.



Graph 1: Comparative graph of micronucleus and chromosomal abnormalities induced by SSP in *Anabas testudineus*

Table 1: Frequency of Micronuclei and Cromosomal abnormalities (% ± S. E., N = 300) induced by SSP in *Anabas testudineus*

Treatment	Micronuclei		Chromosomal Abnormalities							
			Abnormal Metaphase		Gross		Individual		Grand Total	
	No.	% ± S.E	No.	% ± S.E	No.	% ± S.E	No.	% ± S.E	No.	% ± S.E
Control SSP	15	0.5 ± 0.12	20	6.6 ± 1.43	3	1.00 ± 0.57	17	5.6 ± 1.32	20	6.6 ± 1.43
0.25%	33	1.10 ± 0.19*	55	18.3 ± 2.23*	3	1.00 ± 0.57	52	17.3 ± 2.18*	55	18.3 ± 2.23*
0.50%	38	1.26 ± 0.20*	63	21.0 ± 2.35*	4	1.33 ± 0.66	59	19.6 ± 2.29*	63	21.0 ± 2.35*
1.00%	43	1.43 ± 0.21*	74	24.6 ± 2.48*	7	2.33 ± 0.87	67	22.3 ± 2.40*	74	24.6 ± 2.48*

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