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RESEARCH ARTICLE

Pesticide Toxicity: A study of effects of pesticide Monocrotophos on root tip mitosis of *Pennisetum glaucum* (L)RBr

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Abstract

In the present world where food security is given utmost importance and the focus is mostly on increasing the production and decreasing the loss due to pests etc, pesticides have become a virtual necessity. In spite of their undebatable benefits, pesticides have definite harmful effects on the plants and through them to the human beings as well. These ill effects operate through small chromosomal mutations which may lead to unknown genetic effects in the long run. In countries like India where agriculture is mostly done by the less educated people, this situation becomes worse as they apply these pesticides indiscriminately. Thus, bioassaying of each pesticide on all the major crops must be done in order to select lesser damaging ones and recommend them to the farmers for application. The present study is an effort towards this goal where a common and comparatively safe pesticide Monocrotophos was tested for its effects on the mitosis of Pearl Millet to enact similar conditions which roots face when excess pesticides leach into the soil. Various abnormalities were encountered in the mitosis along with chromosomal aberrations. The paper discusses the mode of action and reasons for the abnormalities.

INTRODUCTION

Plants are the main recipients of pesticides, regardless of whether they themselves represent the target organism (e.g., weed) or whether the targets are pests, pathogenic fungi, etc. They are exposed to pesticides from direct application, through the uptake from soil and water, and from atmospheric drift. It has been shown (Ware et al., 1970) that about half of pesticides applied by aircraft land outside the target cropland or forest and fall out either on adjoining ecosystems or drift into distant ecosystems.

Pesticides tend to be very reactive, mostly electrophilic, compounds that can react with various nucleophilic centers of cellular biomolecules, including DNA (Crosby, 1982), or form even more reactive electrophilic products that either modify cellular components or are metabolized to more or less stable products. Ideally, pesticides should affect only the target organism; however, this ideal is rarely attained because of similarities in the basic life processes of both target and non-target plants. Toxicity, including mutagenicity, is the consequence; it is mediated by various changes in the metabolism of plants, including formation of metabolic pathways of pesticide degradation.

Differential sensitivity of plant species to toxic and genotoxic effects of pesticides has been shown to cause overall changes in species ratios, both among weeds in a crop field and in natural plant communities, due to the reduced abundance of susceptible species with concurrent increases in naturally tolerant species. Another important consideration is the possible formation of stable metabolites and their accumulation in plants to such an extent that they can become harmful to both human and animal populations via their respective food chains. Attention must be

given to this problem despite the observation that bound residues incorporated into lignin, hemicellulose, and other carbohydrate components of the cell wall of plants are generally considered to be less toxic to the biosphere than corresponding adducts produced in animal cells (Lamoureux and Rusness, 1986).

Monocrotophos belongs to the Organophosphate group of pesticides. It has the Chemical formula: $C_7H_{14}NO_5P$ and molecular weight 223.21. It has the Chemical name dimethyl (E)-1-methyl-2-(methyl carbamoyl) vinyl phosphate and sold under the Trade names of Monocil, Nuvacron, Azodrin, Monocil besides Monocrotophos in India.

It is used against a wide variety of pests infesting crop plants, specially those of Graminae. Although, Pearl Millet or *Pennisetum* is one of the hardiest and most resistant member of the grass family yet it shows various genotoxic effects of pesticide application at micro levels. The study was conducted as pilot project to show that if such hardy grasses are affected by high doses of pesticides, then all crops are vulnerable.

Material and Methods

For the pesticide treatment, dry and healthy seeds of pearl millet were soaked overnight in distilled water. The presoaked seeds were then allowed to germinate on wet filter papers. When the emergent roots reached 4-5 mm, some seeds were transferred to freshly-prepared aqueous solutions of test pesticide having different concentrations viz 0.1%, 0.25%, 0.50%, 0.75% and 1.00% (v/v) and allowed to remain immersed for 5h. For control, some germinated seeds were soaked for the same period in distilled water. All the seeds were subsequently washed in running tap water for at least 30 min and then their roots fixed in the usual manner. The pesticide of commonly used brand was obtained from local market.

For the analysis, the fixed roots were first hydrolyzed in 1N HCl at 60^{O}C for 15 min. The roots were subsequently washed in distilled water and stained with 2% aceto-carmine for 45 min. Slides were prepared using the standard squash technique. A total of 10 root tips were analyzed for each set, thus studying on an average 1000 cells / set.

Result and Discussion

Table 1 presents the cytological behaviour of control and treated root tip cells of pearl millet. The mitosis in controls was normal with 12.40% AMI. 0.1% Monocrotophos as perhaps the only pesticide concentration that was able to cause mitoactivation of 1.61% in the root tip cells. Higher doses of this pesticide were however highly mitoinhibitory. Maximum mitoinhibition was observed in case of 0.75% monocrotophos (16.71%).

Most common metaphase anomalies included unorientation of chromosomes, scattering of chromosomes, stickiness and clumping of chromosomes. Highest frequency of unorientation was observed in case of 1.0% monocrotophos (2.46%) and scattering of chromosomes was also highest (1.23%) at the same treatment. Stickiness and clumping of chromosomes showed linear dose based increases. Comparatively fewer incidences of precocious movement and fragmentation were encountered in the treated sets. Fragmentation of chromosomes was virtually absent at the lower doses and in low frequencies at higher doses.

Cells with anaphase abnormalities were fewer. Major aberrations included lagging chromosomes and clumping of chromosomes. Laggards were evident at almost all the doses. Clumping was lesser evident and Chromatin bridges were seen in very few cells. Disturbed polarity at anaphase was evident in some cells. Total abnormality percentage at the highest dose reached 13.11%. Higher doses of monocrotophos were found to be highly mitotoxic ie growth retarding.

Analysis of cytological behaviour of somatic cells under the influence of the pesticide provides us with a valuable tool for assessing the genotoxic potentialities. This study on one hand gives us information about the effect of these toxins on numerical increase in the cells (cell division) and on the other; it provided an insight into the mechanism of action of each.

As far as the effects of pesticide doses on normal division are concerned, it was observed that most of these were able to depress the AMI even when used in recommended doses. A drop in AMI indicated that these interfere in the normal sequence of events in the mitosis, thus preventing a number of cells in interphase from entering into prophase. Such interference might be a result of inhibition of DNA synthesis (Schniedermen et al 1971). Movement of chromosomes is a prerequisite for cell division and this movement needs energy. The pesticides might also interact with the respiratory energy producing pathways, resulting in low accumulation of energy-containing compounds like ATP. This assumption is further strengthened by the fact that cell division is inhibited by agents that suppress the energy processes viz glycolysis, respiration and oxidative phosphorylation (Jain and Sarbhoy 1988). Epel (1963) reported that the rate of mitosis was closely related to resultant levels of ATP and mitosis could be blocked at any stage at appropriate time by adding optimum concentration of respiratory inhibitors.

A clear-cut predominance of Metaphase over other phases could be observed in almost all the treatment sets. It can thus be said that there was a tendency towards the arrest of division at Metaphase or towards lengthening of metaphase period of division. It has been reported that the proteins, which determine the duration of transition from metaphase to anaphase, are concerned with transformation of chemical energy into mechanical energy. A lower accumulation of chemical energy in form of ATP due to pesticide or heavy metal mediated inhibition of respiratory pathways, leads to inactivation of these proteins. This in turn causes metaphase arrest and a consequent increase in metaphase abnormalities.

A reduction in mitotic activity with increase in cytological anomalies seems to be common effect of most pesticides and other toxins on different plants (Wuu and Grant 1966, Singh and Sharma 1980, Chand and Roy 1981, Kaur and Grover 1985, Amer and Ali 1986, El Khodary et al 1990, Singh 1991, Kumar and Sinha 1991, Anis et al 1998, Gomurgen 2000, El Ghamery et al 2000, Sharma 2001, Kumar and Sharma 2002, Kumar and Kumar 2004, Turkoglu 2007, Yugbasioglu et al 2008, Fisun and Rasgele 2009, Asita and Mokhobo 2013).

With the onset of M-phase, preparations for precise distribution of already duplicated genetic material begin with the help of spindle apparatus. The distribution of daughter chromosomes between daughter nuclei requires (i) congression of chromosomes on the equatorial plate of the spindle apparatus (ii) separation of sister chromatids (iii) migration of chromosomes towards opposite poles of the spindle. Since mitosis is a temporally expressed multi-step process under precise genetic and biochemical controls, any physical or chemical agent, which affects it adversely, is a potent clastogen. Thus clastogeny of the test pesticides and heavy metals was evidenced by induction of significant amounts of mitotic anomalies.

In the present investigation, mitotic anomalies related to chromatin agglutination, spindle dysfunction, chromosome fragmentation and unnatural association were observed. Chromosome agglutination characterized by chromosome stickiness and clumping was significantly higher than most other anomalies. The biochemical mechanism for chromosome agglutination is not precisely understood. In case of metals, it seems probable that the positively charged metals may interact with non-histone and the DNA itself, resulting in structural changes and stickiness. McGill et al (1974) and Klasterska et al (1976) have attributed stickiness and clumping to improper folding of chromatin fibres leading to creation of sub-chromatid bridges between the chromosomes. Jayabalan and Rao (1987) have attributed stickiness to changes in cytochemically-balanced reactions, which in turn lead to changes in cytoplasmic viscosity.

Inhibition of spindle organization by various chemicals, can lead to conditions of scattering of chromosomes, also known as C-metaphase. It can be said that the signal given after prophase for organization of spindle had been blocked (Sybenga 1992). Metals like mercury and cadmium, have high affinity for the –SH group of the spindle monomers. The attachment of metal ions with these groups, leads to inability of the monomers to polymerize and form fibres (Ramel 1969, Verschaeve 1978). Some protein kinases, which play an important function in organization of the spindle might also get inactivated by unavailability of ATP for phosphorylation or direct interaction with the toxins (Murray 1991a).

Spindle dysfunction also produced anomalies like lagging chromosomes at anaphase. Gomez-Arroyo and Villiobos-Pietrini (1983) used the term ‘chromosomes with inactivated centromeres’ for lagging chromosomes, as they linked this anomaly to absence of centomeres, localized stickiness at the centromeric portions of the chromosomes or to inability of centromere to condense microtubules. Similarly, precocious movement, another spindle anomaly can be attributed to spindle dysfunction (Lawley and Brooks 1963).

Chromosome fragmentation was observed in almost all sets at higher doses. This may be assigned to the failure of broken chromosomes to recombine or due to misrepair of DNA (Evans 1976). Sharma and Sharma (1960) suggested that the upset of nucleic acid metabolism ultimately results in disturbed protein re-duplication causing chromosomes to break at several loci. However no definite mode of action for causing fragmentation can be given to pesticide or heavy metal.

Chromatin bridges may arise due to stickiness in localized portions of chromosomes leading to retardation in chromatin disjunction (Abraham and Koshy 1979). Bridges might also be viewed as indicators of exchange between the chromosomes involving breakage and proximal reunion (Tomkins and Grant 1976).

Among other abnormalities were chromosome erosion, multipolarity, polyploid cells etc. Pool et al (1989) established that the chemicals may lead to less localized inhibition of DNA synthesis. This may in turn cause less or unstained regions on the chromosomes and giving an eroded appearance. Kumar and Sinha (1978) reported that pole formation in dividing cells depends upon the number of points of RNA and polysaccharide assemblage. Interaction of some chemicals leads to disturbed assemblages and multipolar condition. Formation of polyploid cells may result due to failure of scattered chromosomes to separate into two nuclei (Jain and Sarbhoy 1988). Micronuclei at Telophase represent the remnants of laggards and fragments of earlier phases, which fail to reach the poles.

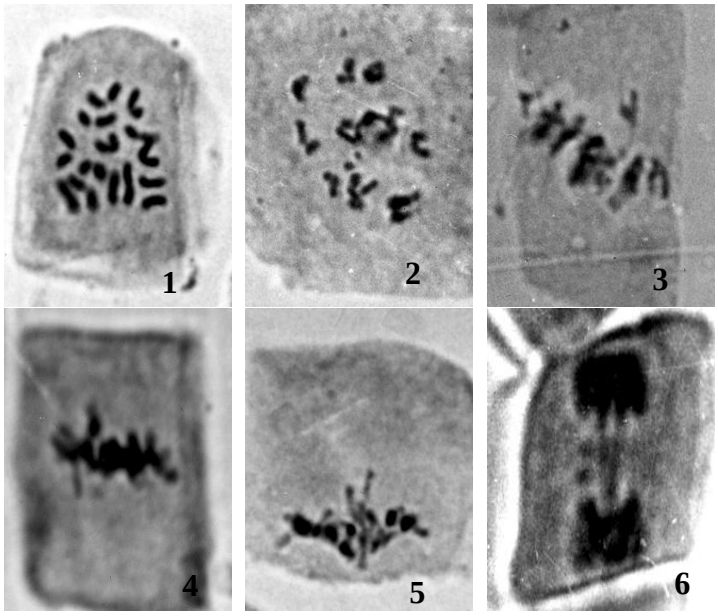

Figure 1: Various chromosomal abnormalities caused by pesticide treatments. 1. Unorientation at Metaphase 2. Scattering and secondary associations at Metaphase 3. Precocious movement of chromosomes from Metaphase plate 4. Stickiness and clumping of chromosomes at Metaphase 5. Disturbed polarity of Metaphase plate 6. Chromosomal bridges at Anaphase.

Table 1: Major chromosomal anomalies induced by pesticide Monocrotophos in mitosis of pearl millet

Treatment(%)	TAd(%)	AMI(%)	MIn(%)	TAb(%)	Metaphase abnormalities (%)						Anaphase Abnormalities (%)				Others(%)	TAb(%)
					Un	Sc	Pm	Fr	St	Cl	Lg	Dp	Br	Cl		
CONTROL	620	12.40	-	6	0.32				0.48			0.16				0.97
MONOCROTOPHOS																
0.10	630	12.60	-1.61	24	0.79	0.63			0.95	0.48	0.32	0.16		0.63		3.81
0.25	414	8.28	6.03	25	0.97	0.72	0.48		1.69	0.72	0.48	0.24		0.72		6.03
0.50	395	7.90	11.39	45	1.77	1.26		0.51	2.53	1.01	1.26	0.25	0.51	1.52	0.76	11.39
0.75	383	7.66	16.71	64	2.09	1.57	0.52	1.31	3.65	1.57	2.09	0.26	1.04	1.57	1.04	16.71
1.00	244	4.88	13.11	32	2.46	1.23		0.82	4.10	1.64	2.05			0.82		13.11

TAd=Total number of Actively dividing cells; AMI=Active mitotic Index; MIn=Mitoinhibition; TAb=Total number of abnormal cells; Un=Unorientation of chromosomes; Sc=Scattering of chromosomes; Fr=Fragmentation of chromosomes; Pm=Precocious movement of chromosomes from the Metaphase plate; St=Stickiness of chromosomes; Cl=Clumping of chromosomes; Lg=Lagging chromosomes; Dp=Disturbed polarity of chromosomes; Br=Chromatin bridge between the poles; TAb (%)=Total percentage of abnormal cells.

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