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# EVALUATION OF MITO-INHIBITORY AND CHROMOTOXIC ACTION OF PESTICIDES ON THE MERISTEMATIC ROOT TIP CELLS OF *Vicia faba* L.

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## ABSTRACT

**The present paper reports the cytotoxic potential of pesticides viz. Aldrin ($C_{12}$ $H_8$ $Cl_6$), Bavistin ($C_9$ $H_9$ $N_3$ $O_2$), Monocrotophos ($C_7$ $H_{14}N$ $O_5$ P) and Chlorpyrifos ($C_9$ $H_{11}Cl_3$ N $O_3$P S) in *Vicia faba* L (Fabaceae). Seeds of *Vicia faba* L were treated with different concentrations (0.12%,0.25%.0.50%,0.75%,1.00%) of pesticides for four hours at room temperature and for control some seeds were kept in distilled water for the same duration under identical conditions. Root tips were squashed in 2% aceto-carmine and mitotic index was calculated from each treatment. All four pesticides depressed the mitotic index with level to 3.16±0.46 (Aldrin), 3.46±0.12 (Chlorpyrofos), 3.90 ±0.96 (Monocrotophos) and 4.26± 0.18 (Bavistin) in comparison to control ( 22.80±0.16). The mitotic index of *Vicia faba* L was found inversely correlated with almost all the doses of pesticides. The percent chromosomal aberrations such as fragments, bridges, stickiness, micronuclei and disturbed metaphase/anaphase were noted in each treatment which ranged from 10.80±0.95 to 40.86±0.94 in comparison to control (0.38±0.23). The degree of mitoticinhibition and induction of chromosomal aberrations due to pesticides was noted as Aldrin>Chlorpyrifos>Monocrotophos>Bavistin. Evidently the organochlorine pesticides were more effective than the organophosphate pesticides. The ANOVA test confirmed that a significant variation of mitotic depression (P<0.001) and induction of chromosomal aberrations (P<0.01) due to the action of pesticides. So it is advisable to secure the genotoxicity test of the pesticides before their use to agricultural crops.**

**KEYWORDS:** Pesticides, Mitotic Index, *Vicia faba* L.

The use of pesticides in agriculture is increasing rapidly for improved agricultural yields through inhibition of diseases in the field as well as their storage (Taylor *et al.*, 1997). Extensive use of pesticides caused genotoxicity and muta-genotoxicity for non-target organisms and their influence on ecosystems are of worldwide concern (Pimental *et al.*, 1998). Pesticides have also been used to control weeds, insects and fungi in a wide range of application. The mutagenic and or carcinogenic potentials to the non-target organism have been demonstrated by (Ahmad and Yasmin, 1992; Asita and Makhalemele, 2008; Kumar and Kumar, 2000; Sinha and Kumar, 2014).The mito-depressive activities of pesticides in crop plants have earlier been reported (Badar, 1983; Wuu and Grant, 1967). In order to enhance the agricultural yields, several million tons of organic and inorganic chemicals with anti-microbial and insecticidal properties are added annually to soil and environment which may upset the ecosystem.

Grant, 1978 pointed out that plant chromosomes are sensitive indicators to environmental pollution and suggested that the higher plant system appears to be an excellent indicators of the cytotoxic /genotoxic/mutagenic effects of environmental mutagens, therefore, the plant system must be accepted as a first tier assay for detection of the possible genetic damage due to use of genotoxic chemicals.

The present investigation has been carried out to evaluate the mito-inhibitory action and possible genetic damage induced by pesticides (Aldrin, Chlorpyrifos, Monocrotophos and Bavistin) in *Vicia faba* L.

## MATERIALS AND METHODS

### Test Material

In the present investigation healthy and dry seeds of *Vicia faba* L. (Fabaceae) were procured from National Seeds Corporation, New Delhi and used as a test material. *Vicia faba* L. is an important pulse crop commonly known as broad bean and is used as vegetable, silage, forage, and stock feed. For assessing genotoxicity of chemical pesticides root tips have widely been used as a bioassay test system which has shown extremely good correlation with the mammalian system also (De Kergommeaux *et al.*, 1983) and *Vicia faba* L. has the advantage of having relatively large chromosomes that are excellent for assessing chromosomal aberration.

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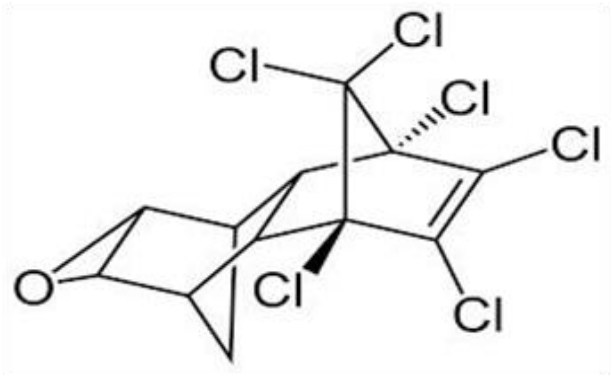
**Pesticides**

Aldrin: It is a colorless solid highly lipophilic organochlorine insecticide used to treat seeds and soil. Its molecular formula is $C_{12}$ $H_8$ $Cl_6$, molar mass is 364.90 g/mol, melting point 104°C and density is 1.60 g/mm.

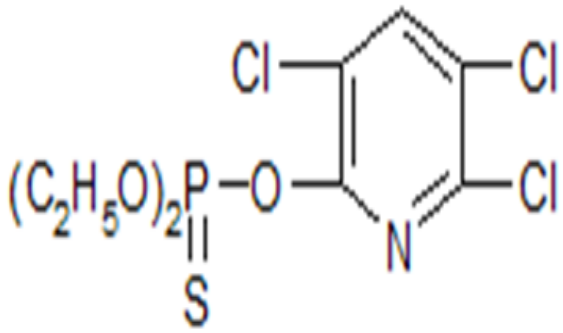
Chlorpyrifos: It is a white or colorless crystalline organophosphate insecticide having slightly skunky odor, like rotten eggs or garlic. Its molar mass is 350.59 g/mol, molecular formula $C_9H_{11}Cl_3NO_3PS$, melting point 42 °C and density 1.40 g/cm³.

Monocrotophos: It is an organophosphate insecticide and it is acutely toxic to birds and humans. Its molecular formula is $C_7H_{14}NO_5P$; molar mass is 223.2 g/mol, melting point 55 °C, boiling point 120 ° and density 1.33 g/cm³.

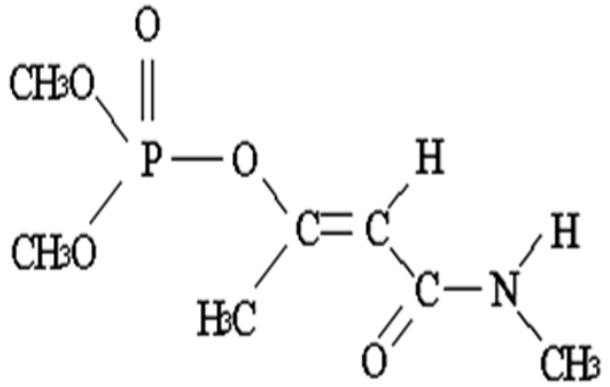
Bavistin: It is methyl N-(1H-benzimidazol-2-yl)carbamate with molecular formula C9 $H_9$ $N_3$ $O_2$ and molar mass 191.186660 g/mol.



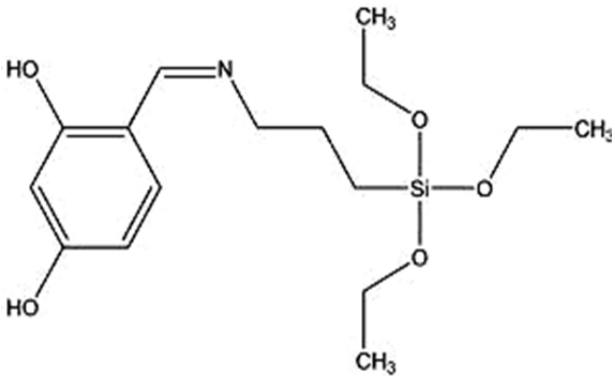

Aldrin




Chlorpyrifos




Monocrotophos




Bavistin

**Preparation of Pesticides Solutions**

The solutions of varying concentrations (0.12%, 0.25%, 0.50%, 0.75%, 1.00%) were prepared by dissolving pesticides in desired amount of distilled water.

**Treatment**

Seeds of *Vicia faba* L. were first pre-soaked in distilled water for 18 hours at room temperature and then treated with different concentrations of pesticides (0.12%, 0.25%, 0.50%, 0.75%, 1.00%) for 4 hrs. Some of the seeds were kept in distilled water for the same duration under identical conditions for the control. The treated seeds were thoroughly washed in running tap water 2-3 times and put in petridishes for germination.

**Squash Preparation**

The treated root tips along with the control were fixed in freshly prepared 1:3 acetobutanol for 24 hrs and squashed in 2% aceto-carmine to study the mitotic index (MI) in each pesticide treatment. The mitotic index (MI) was calculated by number of dividing cells / total number of cells scored x 100 while percentage of chromosomal aberrations by number of abnormal cells / total number of dividing cells x 100. The data were statistically analyzed by applying ANOVA test.

## RESULTS

In *Vicia faba* L the somatic chromosome number was noted to be 2n=12. Pesticides (Aldrin, Chlorpyrifos, Monocrotophos and Bavistin) treated cells had reduced mitotic index (MI) as compared with control. The depression of mitotic index was gradually increased with the increasing concentrations of the pesticides and reached the level of 3.16±0.46due to Aldrin,3.46±0.12 due to Chlorpyrifos, 3.90 ±0.96due to Monocrotophos and 4.26± 0.18 due to Bavistin while chromosomal abnormalities such as stickiness, fragments, bridges, laggards, disturbed metaphase/anaphase were gradually increased with increasing doses of the pesticides ranging from 10.80±0.95 to 30.80±0.69 in comparison to control (Table 1). The degree of mitotic inhibition potential was noted as Aldrin>Chlorpyrofos>Monocrotophos>Bavistin. The ANOVA test confirmed that the inhibitory potential of the pesticides and their different doses varied significantly (P<0.001) in *Vicia faba* L (Table 2, 3).

**Table 1: Effect of pesticides on mitotic index and chromosomal aberrations in *Vicia faba* L.**

| Pesticides | Parameters | Control | Concentration of Pesticides (%) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0.12 | 0.25 | 0.50 | 0.75 | 1.00 |
| Aldrin | M I | 22.80±0.16 | 15.40±0.62 | 11.70±0.23 | 7.80±0.24 | 5.62±0.71 | 3.16±0.46 |
| | C A | 0.38±0.23 | 12.16±0.87 | 22.61±0.67 | 30.26±1.09 | 35.80±0.69 | 40.86±0.94 |
| Chlorpyrifos | M I | 22.80±0.16 | 15.80±0.59 | 12.60±0.66 | 08.10±0.21 | 6.16±0.15 | 3.46±0.12 |
| | C A | 0.38±0.23 | 11.96±0.49 | 20.44±0.93 | 27.09±0.84 | 32.12±1.09 | 39.10±0.65 |
| Monocrotophos | M I | 22.80±0.16 | 16.65±0.23 | 13.46±0.12 | 9.42±0.18 | 6.83±0.15 | 3.90±0.96 |
| | C A | 0.38±0.23 | 11.36±0.98 | 18.86±0.89 | 26.06±0.87 | 30.84±0.88 | 38.78±0.87 |
| Bavistin | M I | 22.80±0.16 | 16.42±0.12 | 13.12±0.28 | 9.82±0.49 | 7.10±0.62 | 4.26±0.18 |
| | C A | 0.38±0.23 | 10.80±0.95 | 16.80±0.69 | 24.34±0.87 | 29.38±1.09 | 36.76±0.29 |

**Table 2: ANOVA for mitotic index in *Vicia faba* L.**

| Source of Variation | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Between treatment | 974.77 | 5 | 194.95 | 2052.11*** | > 0.001 |
| Between pesticides | 5.72 | 3 | 1.9 | 20.11*** | > 0.001 |
| Residual | 1.43 | 15 | 0.095 | - | - |
| Total | 981.92 | 23 | - | - | - |

**Table 3 ANOVA for chromosomal aberrations in *Vicia faba* L.**

| Source of Variation | S S | df | MS | F | P |
|---|---|---|---|---|---|
| Between doses | 1908.14 | 5 | 381.62 | 183.03*** | < 0.001 |
| Between pesticides | 35.84 | 3 | 11.94 | 5.72** | < 0.01 |
| Residual | 31.28 | 15 | 2.085 | - | - |
| Total | 1975.26 | 23 | - | - | - |

## DISCUSSION

In recent years many cytological studies have been carried out to detect the mito-inhibitory / genotoxic/cytotoxic /chromo toxic effects of various pesticides on different plants (Amer and Farah, 1974; Inceer and Beyazoglu, 2000; Kumar *et al.*, 2011; Lerda, 1992; Nandi, 1985). Most of these chemicals have been reported to have detrimental effect on the natural ecosystem and their residues in plants may also affect human health through nutritional cycles (Fujii and Inoue, 1983; Sinha and Kumar, 2014). Mitotic inhibition by pesticides has been attributed to blocking of mitotic cycle during interphase which may result from a prolonged $G_2$ period or arrest cell division at $G_1$ phase.

Mohandas and Grant, 1972 reported similar results by the application of herbicides. Reduction of MI might have been achieved by the inhibition of DNA synthesis at S-phase (Sudhakar *et al.*, 2001). Decrease in the mitotic index as a result of treatment with a particular substance shows its capacity to arrest cell divisions together with its ability to kill the actively dividing cells (Tijo and Thoppil, 1998). Several investigators have used mitotic index as endpoint for the evaluation of genotoxicity or antigenotoxicity of different chemical

treatments (Sharma *et al.*, 2012; Panda and Sahu, 1985). Kara *et al*., 1994 reported that if ATP level in the cell decreases by 50%, mitosis can completely be prevented.

The mitotic poison may cause metabolic imbalances which may interfere with the synthesis, state and structure of nucleic acid including physiological effects and structural changes in chromosomes during cell division which may lead to mitotic delay and mitotic inhibition (Soni *et al.*, 1982). Many other investigations were attributed to depression of mitotic activity due to inhibition of protein synthesis (Kim and Bendixen, 1987).The induction of genetic damage may cause an increased incidence of genetic disease in future generations and may contribute to somatic cell diseases including cancer in the present generation (Connell, 1997).

Increased concentration of the pesticides interferes with the normal sequence of cell cycle that reduces the number of cell to enter prophase and succeeding divisional stages. Such types of mito-depressive action of chemicals have been reported earlier (De Campos and Viccini, 2003). Cumminis *et al.*, 1966 reported that the proteins which determine the duration of transition from metaphase to onwards are concerned with the transformation of chemical energy into the mechanical work of mitosis.

Pesticides (Aldrin, Bavistin, Monocrotophos, Chlorpyrifos) caused cytological changes and induced a wide range of mitotic abnormalities (stickiness, bridges, laggards, micronuclei, fragments etc) in the root tip cells of *Vicia faba* L. Our results matched with earlier findings made by (Ajay and Shorbhay, 1987; Kumar *et al.*, 2011; Sinha, 2009). Chromosomal abnormalities are considered as reliable indicators of mutational changes and are used as reliable evidence for screening the mutational activity (Kihlman, 1966). Stickiness comprises the most dominant type abnormalities observed in all concentrations ofthe pesticides. Patil and Bhat, 1992 suggested that stickiness is a type of physiological adhesion involving mainly the proteinous matrix of chromatin material. The fragments have been observed in all concentrations of the pesticides, it may be formed due to DNA breakage by endonuclease (Grant, 1978). Alkylating agents are known to cause chromosomal fragments by binding to DNA region rich in GC pairs causing instability (Patra and Sharma, 2002). Kaeppler and Rhee, 2008 has reported that the fragments may be the result of change in the level of DNA methylation. Multipolar anaphase abnormalities are caused due to inhibition of spindle formation (Amar and Ali, 1983). Bridges and laggards were reported in all concentrations of the pesticides. Bridges are caused due to breakage of chromosomes (Tomkins and Grant, 1972) while laggards due to stickiness of chromosomal end (Kaur and Grover, 1985). Micronuclei might be due to the aggregation of chromatin materials into massages of various number and size. It has been reported that the appearance of micronuclei is due to fragmentation of chromosomes by the action of monosodium glutamate (Omanakumari *et al.*, 2006) and chlorpyrifos (Ali *et al.*, 2008).

Conclusively our result shows an inverse correlation between the pesticides doses and mitotic index (MI) which is compatible with the hypothesis that inhibition of mitosis may be due to inhibition of DNA synthesis / protein synthesis /prolonged $G_2$ period.

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