Cytological Effects of Insecticides on Onion *(Allium cepa)*

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Abstract

Living organism such as insects, pest, rodents and weeds which compete with man for food, cause damage to the plants. The chemicals which are used to control them are called pesticides. Insecticides are chemicals, which are used to kill or repel especially the insects. Here two insecticides i.e. Rogor and Malathion were taken to evaluate the cytological effects of the insecticides on the plants. Onion was taken as a test material, which is an important medicinal plant. It is also used in culinary preparations and as a flavouring agent. For this study onion bullbs were treated separately by Rogor and Malathion with different concentrations i.e. 0.1%, 0.15%, 0.2% and 0.25%. There are different cytological parameters were considered to evaluate the effect of both insecticides. These parameters were studied in root tip cells. These were namely mitotic index, percentage of different mitotic stages and percentage of mitotic abnormalities like anaphase bridges, laggards, disturbed spindle, chromosome condensation, precocious movement of chromosome and micronuclei. As insecticides concentration increases, the reduction in mitotic index and in the percentage of different stages were observed. Mitotic abnormalities were increased with increased doses of Rogor and Malathion both. The result showed that the higher concentrations 0.2% and 0.25% of insecticides were found more toxic for inducing cytological abnormalities. Overall results conclude that insecticides used plant protection may induce cytological damage. It may pass to next generation and tends to accumulate due to excessive use.

Introduction

Farmers use some common insecticides Rogor and Malathion in their crop in order to control the occurrence of diseases in them. These chemicals besides checking plant diseases also cause various types of damages in the plants, thereby invariably affecting viability and yield of crop plants. They invariably get entry into the human system through food chain. Hence to evaluate the toxic effects of these commonly used insecticides is carried out through the treatments on onion bulbs. Onion (*Allium cepa*) is an important crop of the world. Like other condiments it is widely used in culinary preparations and as a flavouring agent. As a pharmaceutical value, onion possesses stimulant, diuretic, expectorant, antibiotic, hyperglycaemiant, anti-inflammatory and antihelminthic and many other properties. It is probably a native of Asia, perhaps from Palestine to India. China and India are the leading onion producing countries in the world.

Rogor and Malathion both insecticides are of organophosphate group. The organophosphate insecticides are all phosphours derived insecticides. These insecticides block the phosphorylation of a serine hydoxyl group on the active site of the enzyme cholinesterases. These enzymes are responsible for normal neural transmission in insects (Fest and Schmidt, 1970, 1973). The primary routes for the metabolism of organophosphates are oxidation and hydrolysis (Hutson et. al. 1971; Bedford and Robinson, 1972).

Materials and Methods

The sun dried onion of particular size was first pre-soaked in distilled water for 6 hours. They were later on treated separately with different concentrations of both chemicals in staining jars for 72 hrs. A control was maintained for each treatment. For each treatment 10 bulbs were used. Three replicates of each treatment were employed. After these treatments, the bulbs were washed in tap water for some time and later on grown in field beds. From these bulbs, some root tips were collected for cytological studies.

For mitotic studies root tips of about 5 mm. size were used. These tips were separately washed in water after each treatment. They were fixed in acetoalcohol (1:3) of 24 hrs. and were stored in 70% alcohol. Later on these root tips were hydrolysed in acetocarmine and (N) HCl (9:1) schedule for 5 minutes at 65^0C. These tips were then squashed and stained with 1% acetocarmine solution.5 slides were prepared for each dose for mitotic studies. 1000 cells were observed for each dose for microscopic examinations.

These cells were studied at ×400 and ×1000 magnifications. Many cytological characters were studied on the basis in both control as well as treated mitotic cells.

Mitotic Index (MI) percentage (%) was computed by the following formula -

$$\text{MI}(\%) = \frac{\text{Total no. dividing cells}}{\text{Total no. of cells observed}} \times 100$$

Quantitative characters were subjected to statistical analysis. Following statistical formulae were used for observation data.

Arithmetic Mean ($\overline{\mathbf{X}}$) : $\overline{\text{X}} = \frac{S}{n}$

Where S = Sum of the observation

n = Number of observations

Standard Deviation (SD) :

$$\text{SD} = \sqrt{\frac{(\text{x}_1 - \bar{\text{x}})^2 + (\text{x}_2 - \bar{\text{x}})^2 + (\text{x}_3 - \bar{\text{x}})^3 + .. + (\text{x}_\text{n} - \bar{\text{x}})^2}{\text{n}}}$$

Where X_1, X_2, X_3 ..... Xn = Individual observations

$\overline{\text{X}}$ = Arithmetic mean

n = Total number of observations

Standard Error (S.E.) : $\text{S. E.} = \frac{\text{S.D.}}{\sqrt{\text{n}}}$

Where S.D. = Standard deviation of Individual observations

n = Total number of observations

Coefficient of Variation (C.V.) : $\text{CV}\ (\%) = \frac{\text{SD}}{\overline{\text{X}}} \times 100$

Were SD = Standard deviation of sample

$\bar{X}$ = Arithmetic mean

Results and Discussion

The effect of to insecticides Rogor and Malathion (0.1%, 0.15%, 0.2% and 0.25%) mitotic index in root tip cells of onion were studied. In control, the arithmetic mean ($\overline{\text{X}}$) for mitotic index was calculated as 65.64%, the value of S.E. was 0.5527% and the value of C.V. was observed 1.4582 (Table

The minimum $\bar{X}$ value was calculated 43% in 0.25% dose of Rogor and 43.65% in this dose of Malathion whereas maximum $\bar{X}$ value was 57.32% in 0.1% dose of Rogor and 56.99% in 0.1% dose of Malathion. Hence the MI values of observed root tip cells of onion after treatment of Rogor as well as Malathion, gradually decreased as the concentration of both insecticides increased. Therefore from the observation data, it appears that both Rogor and Malathion affect similarly the rate of mitotic index in onion.

Effect of Rogor and Malathion on percentage of different stages of Mitosis in root tip cells of onion was also observed (Table 2). In Rogor treatment, in control, (%) of cells showing prophase was observed 18.84. The minimum (%) was found 13.62 in 0.2% dose and maximum was 18.0 in 0.1% dose. Thus in control, the highest % of prophase was observed. It was gradually decreased in increased concentration of doses. In control, the (%) of metaphase showing cells, was found 14.0. The minimum (%) found in 0.25% dose, was 10.0 and maximum found in 0.2% dose, was 12.5. 11.05% cells, observed in anaphase of control. The minimum (%) showing by 0.25% dose was 5.0 and maximum showing by 0.1% was 8.47. In control, the (%) of telophase showing cells, was 21.05. It was observed minimum in 0.2% dose, as 15.37 and maximum in 0.1% dose, as 20.0(%) of cells found in decreasing order with increased doses of Rogor. Malathion also show similar effect on this parameter of cytological study.

As far as effects of Rogor and Malathion on mitotic abnormalities in root tip cells of onion is concerned, I have observed common mitotic abnormalities namely anaphase bridge, laggards, disturbed spindle, chromosome condensation, precocious movement of chromosome and micronulei after both treatments (Table 3). In control, these abnormalities did not appear in the cells observed by me. However their frequencies (%) varied invariably in root tip cells after these treatments. These individual abnormalities were observed in very rare frequencies, e.g. The frequency of anaphase bridge was 1.0% in 0.1% dose of Rogor's treatment and it was highest 3.97% in 0.25% dose of this treatment. Likewise in Malathion treatment, the frequency of this abnormality was 1.8% in 0.1% dose, and 4.72% in 0.25% dose. Hence these abnormalities were also appeared to be dose dependent. They were maximum at the highest concentration and minimum at lowest concentration. However total mitotic abnormalities were statistically significant over control. All mitotic abnormalities were observed in more or less similar frequencies in root tip cells after both treatments. (as shown in graph). The perusal of available literature on effects of insecticides on cytological characters of onion, is scanty. Not much work has been done on cytological effects of insecticides in various crop plants.

Sharma and Vardhani (1981) studied the cytological effects of two fungicides (Cupramar, Phygon-XL) on nuclear cytology of *Spirogyra paradoxa* Rao. Three different concentrations of Cupramar (10, 20 and 30 ppm) and Phygon-XL (0.1, 1.0 and 5 ppm) were applied for 1 and 2 hrs. Duration. In case of Cupramar concentration of 50 ppm for 48 hrs. and 100 ppm for 24h proved to be sublethal and lethal doses respectively. Whereas in case of phygon-XL 10 ppm for 24 hrs. and 50 ppm for 2 hrs. proved to be sublethal and lethal doses respectively. The various nuclear and chromosomal aberrations as a result of treatment with different concentration of the two fungicides were assessed qualitatively and quantitatively.

Choudhary and Sajid (1986) studied the effect of Carbendazim (Bavistin) on chromosome structure in *Pisum sativum* L. cv. T/169. Emerging young roots were treated with 0.1% and 0.2% aqueous solution of Carbendazim. Mitotic karyotypic studies revealed an increase in total chromatin length in *P. sativum* under the influence of 0.2% Carbendazim.

Das (1986) studied the observations on effects of Rogor (insecticide) on germination and root meristem cells of *Vicia faba* L. He concluded that the aqueous concentrations (0.02-1.00%) of Rogor

produced significant reduction in germination and growth of radicle of *Vicia faba* in reference to higher concentration. Rogor also produced significant dose dependent retardation effects on mitotic index.

Bakele and Srinivasu (1986) studied the effects of weedicides 2, 4 - D, Gramoxone and Stam F-34 on mitosis in *Parthenium hysterophorus* Linn. 500 ppm of 2, 4 - D, 25000 ppm of Gramoxone and 200 ppm of Stam F-34 proved to be the lethal doses. Chromosomal aberrations such as fragments, laggards, grouping, precocious movement of chromosomes were found at different concentration of all the three weedicides.

Mosuro et. al. (1999) studied the genetic safety evaluation of pesticides using the *Allium cepa* assay. He used Lindane, Pirimiphos methyl, Glyphosphate and 2:1 Metachlor : Atrazine pesticides of commonly used in agriculture for pest control. Their cytotoxic effects on root meristem cells of *A. cepa* were investigated. It was found that 1 ppm, 5 ppm, 10 ppm, 25 ppm, 50 ppm and 100 ppm, induced a variety of chromatid and chromosome type structural aberrations. All the pesticides were effective in the disturbance of the spindle fibre apparatus at all the concentration used.

Table No. 1
Comparative effects of Rogor and Malathion on mitotic index in root tip cells of Onion

Dose (%)	Mitotic Index (%)					
	Rogor			Malathion		
	$\bar{X}$	S.E.	C.V.	$\bar{X}$	S.E.	C.V.
C	65.64	0.5527	1.4582	65.64	0.5527	1.4582
0.1	57.32	0.5403	1.6325	56.99	0.9404	2.8582
0.15	52.78	0.7954	2.6072	54.33	0.7201	2.2956
0.2	45.99	0.9404	3.5418	49.74	1.8235	2.6835
0.25	43.0	0.9428	3.7974	43.65	0.9561	3.7938

Table No. 2
Comparative effects of Rogor and Malathion on percentage of different stages of mitosis in root-tip cells of Onion

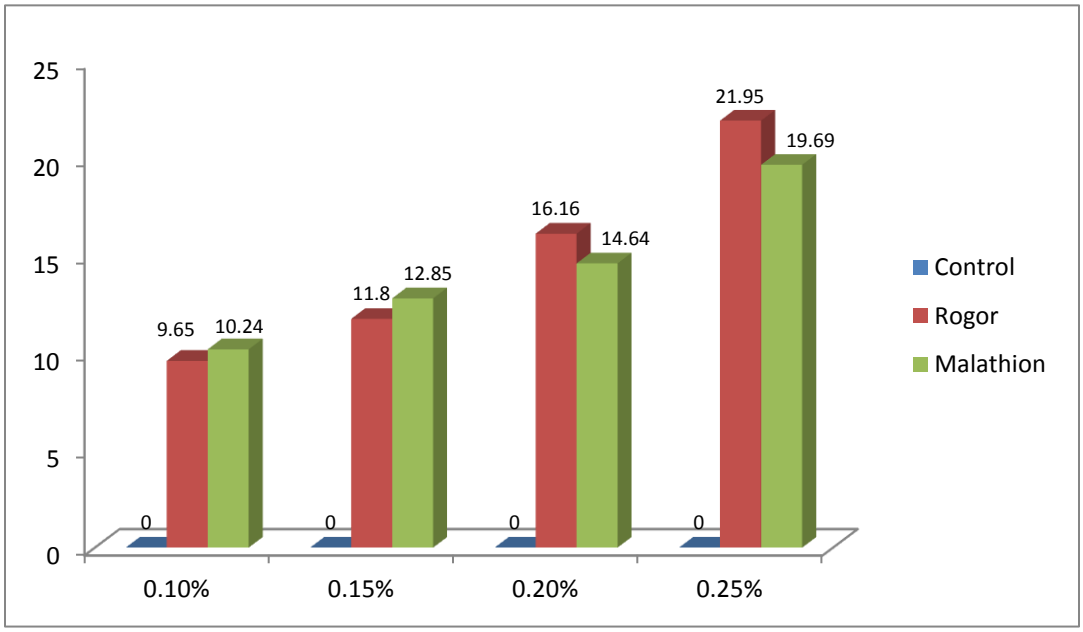
Dose (%)	% of different stages of mitosis							
	Rogor				Malathion			
	Prophase	Metaphase	Anaphase	Telophase	Prophase	Metaphase	Anaphase	Telophase
Control	18.84	14.0	11.05	21.05	18.84	14.0	11.05	21.05
0.1	18.0	11.5	8.47	20.0	17.25	12.58	9.74	19.39
0.15	17.05	11.0	7.3	19.0	16.2	11.8	9.3	18.7
0.2	13.62	12.5	6.5	15.37	14.0	12.81	10.0	15.0
0.25	14.0	10.0	5.0	16.0	10.8	11.67	9.5	14.0

Table No. 3
Comparative effects of Rogor and Malathion on mitotic abnormalities (%) in root tip cells of Onion

	Rogor (%)					Malathion (%)				
	Control	**0.1**	**0.15**	**0. 2**	**0.25**	**Control**	**0.1**	**0.15**	**0.2**	**0.25**
1. Anaphase bridge	-	1.0	1.50	2.70	3.97	-	1.80	4.65	3.10	4.72
2. Laggards	-	1.35	1.15	3.27	4.17	-	1.17	2.44	2.99	4.23
3. Disturbed spindle	-	2.30	2.63	2.91	3.20	-	1.95	1.04	1.19	2.38
4. Chromosome condensation	-	1.29	1.84	2.34	3.67	-	1.02	2.61	3.06	3.08
5. Precocious movement of chromosome	-	1.37	1.46	2.19	3.25	-	2.41	1.18	3.20	2.33
6. Micronuclei	-	2.34	3.22	2.75	3.69	-	1.89	3.93	1.10	2.95
Total abnormalities (%)	**-**	**9.65***	**11.80***	**16.16***	**21.95***		**10.24***	**12.85***	**14.64***	**19.69***

* Values Significant at P< 0.05

Fig. Comparative effects of Rogor and Malathion on total mitotic abnormalities (%) in root tip cells of Onion



Conclusion

The effects of two insecticides (Rogor and Malathion) were studied on cytological parameters in onion. The cytological characters studied were Mitotic index, % of different stages of mitosis and different mitotic abnormalities (%) in root tip cells of onion.

Among total mitotic abnormalities (%), were invariably observed in onion after Rogor and malathion treatments separately. Whereas their effects on mitotic index (MI), both insecticides acted as mitotic inhibitor. MI and (%) of cells of different stages of mitosis gradually decreased in root tip cells of onion with increased doses of both Rogor and Malathion.

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