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Evaluation of cytogenetic effect of pesticides in onion root meristem

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ABSTRACT

Farmers use chemical insecticides, fungicides and herbicides to control insect pests, diseases and weeds. Extensive use of chemical pesticides is becoming hazardous to the environment and human and animal health. Biopesticides have been used as a safer alternative. Cytogenetic analysis is needed to determine the effects of chemical and biopesticides in plants if these are used frequently in an improper concentration. This investigation assessed the effects of a chemical systemic insecticide 'Actara 25WG' and a bioinsecticide 'Bio Sona' for seed germination and on root mitosis of five onion varieties. Three concentrations of Actara 25WG were used as seed treatment, *viz*., 25 g, 50g (recommended) and 75 g/kg seed. Similarly, Bio Sona was applied @ 2 %, 5 % (recommended) and 8 % concentrations. Depressing effects of the two pesticides were recorded in seed germination percentage except for Bio Sona 2% in variety Bhima Shakti. Actara 25WG and Bio Sona showed either mitoenhancing or cytotoxic effects depending on dose and variety. These pesticides also induced genotoxicity at recommended and higher doses on root tip cells as indicated by the frequency of total chromosomal aberrations like binucleate cells, clumps, stickiness, disturbed stage, laggards, bridges and fragments. Bio Sona at higher concentrations showed more aberrations than Actara 25WG. There were differential responses of the varieties to the pesticide treatment. More research must be done on the effects of chemicals and biopesticides in plants to assess their cytogenotoxicity.

Keywords: *Allium cepa* L., Cytotoxicity, Genotoxicity, Total abnormality percentage, Mitotic index.

INTRODUCTION

Pesticides have been used in crop protection to increase agricultural production, thus playing an important role in food security. However, excessive use of chemical pesticides in modern agriculture has negatively impacted the environment and living organisms. Several authors have reported (Amer and Farah, 3; Adesuyi *et al*., 2; Fatma *et al*., 4 and Popova *et al*., 13) about cytogenetic effects of various chemical pesticides in plants causing changes in the mitotic index, inducing various chromosomal aberrations. Biopesticides have been developed and used as safer alternatives for crop pest management. Studies have revealed that biopesticides can reduce the negative environmental impact. Despite the benefits of these biopesticides, it is important to know the negative effect of using them in improper concentration for a long period. Research is in progress on the cytogenetic effect of biopesticides in plants. Adegbite *et al.* (1) found the mutagenic effect of neem leaf extract in onion root mitosis. Therefore, more study is necessary to determine the cytotoxicity and genotoxicity of chemical and biopesticides. The present investigation was carried out to assess the cytogenetic effects of two pesticides- 'Actara 25 WG' (chemical insecticide) and 'Bio Sona' (bioinsecticide) on root mitosis in onions.

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MATERIALS AND METHODS

The study was carried out on five varieties of onion. Four varieties (Bhima Red, Bhima Kiran, Bhima Light Red and Bhima Shakti) were collected from the Directorate of Onion and Garlic Research, Pune and Nasik Red was obtained at the local market of Jorhat, Assam (Durga seeds). Three concentrations of each of the two pesticides were used: Actara 25WG (25 g, 50 g (Recommended) and 75 g/kg seed) and Bio Sona (2 %, 5 % (Recommended) and 8 %). Actara 25 WG (Syngenta) is a systemic insecticide containing thiamethoxam 25 % as an active ingredient mainly used against sucking pests in vegetable crops such as aphids, thrips, etc. The bioinsecticide Bio Sona (Department of Plant Pathology, AAU, Jorhat) is a talc-based formulation of *Beauveria bassiana* used to control sucking insect pests in vegetables. Germination tests and cytogenetic analysis were done in the laboratory of the Department of Plant Breeding and Genetics, AAU, Jorhat (2020-2021) in a completely randomized design with three replications. Seeds of each variety were treated for 24 hrs with three concentrations each of Actara 25WG and Bio Sona as mentioned along with an untreated control.

Germination test was carried out by paper method in petri plates. After 24 hours of pesticide seed treatment, seeds were appropriately washed with distilled water. Twenty seeds were placed in each petri dish on Whatman's filter paper, moistened

with distilled water and kept at room temperature. The germination percentage was recorded on the 12th day.

Root tips (0.5 - 1.0 cm) were collected from the germinated seedlings in each treatment for cytogenetic analysis. Tips were then fixed in Carnoy's fixative (3:1 ratio of ethanol and glacial acetic acid), hydrolyzed (1 N HCl) at 60°C for 3 min, and washed in distilled water. Finally, tips were stained with 2% acetocarmine, and tapping was done for even squashing the sample. Cells in the sampled slide were observed under 400x magnification in a compound microscope (Coslab). Observations were noted on the mitotic index and total abnormality percentage (chromosomal aberrations). Cytogenetic parameters were calculated with the following formulae:

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

(Yekeen and Adeboye, 17)

$$\text{Total abnormality percentage} = \frac{\text{Number of abnormal cells}}{\text{Total number of cells}} \times 100$$

(Kumar and Dwivedi, 9)

Analysis of variance was done for each character variety-wise based on mean values in a completely randomized design using standard statistical procedure following Gomez and Gomez (6). Then pooled analysis was performed for each character over the varieties. Finally, the significance of the difference between means, among treatments and varieties was tested by critical difference at 5% and 1% probability levels. All computations were done in MS Excel 2007.

RESULTS AND DISCUSSION

Marked differences were observed for germination percentage, mitotic index and total abnormal percentage due to treatment with Actara 25WG and Bio Sona. Results showed a differential response of each onion variety to each treatment. The pooled ANOVA over the five varieties showed significant effects of varieties, pesticides, Actara 25WG and Bio Sona on per cent germination (Table 1). The difference between Actara 25WG and Bio Sona and pesticides vs. control was significant. The interaction effects of varieties with pesticides and biopesticides were also significant. The effects of both pesticides on each variety were indicated by their mean germination percentage values (Table 2). Both the pesticides (all concentrations) significantly suppressed germination percentage in the variety Bhima Red. Bhima Kiran showed reduced germination for all three concentrations of Bio Sona. A greater decrease in Bhima Light Red was recorded due to Actara 25WG @ 75 g kg^{-1} seed and all concentrations of Bio Sona. Germination percentage was enhanced and reduced in Bhima Shakti due to Bio Sona treatments (2% and 8%, respectively). In Nasik Red, Actara 25WG @ 50g and 75 g kg^{-1} seed and Bio Sona (all doses) resulted in a depressed germination percentage. Reduction of germination percentage was observed with increasing doses of pesticides (Actara 25WG and Bio Sona), in accordance with the findings of other authors (Reddy and Rao, 14 and Xuan *et al*., 16). Russo et al. (15) reported a similar enhancement in seed germination in corn seeds.

Significant effects of varieties, pesticides, Actara 25WG and Bio Sona were observed on the mitotic index (Table 1). The difference between the effects of Actara 25WG and Bio Sona was significant. All the interaction effects were also significant. The variety-wise mean mitotic index has been recorded, as shown in Table 3. In the variety Bhima Red, Actara 25WG

Table 1. Analysis of variance (mean squares) for germination percentage, mitotic index and total abnormality percentage in onion pooled over varieties.

Source	df	Germination (%)	Mitotic Index (%)	Total abnormality percentage
Varieties	4	2371.79**	2989.22**	1.107**
Pesticides	5	543.11**	1613.78**	1.423**
Actara 25 WG	2	137.22*	2874.28**	1.929**
Bio Sona	2	240.56**	128.50**	0.097**
Actara 25WG vs. Bio Sona	1	1960.00**	2063.34**	3.061**
Pesticides vs. Control	1	2210.16**	52.45	0.486**
Varieties × Pesticides	20	238.14**	340.75**	0.411**
Varieties × Actara 25WG	8	5.97	414.74**	0.206**
Varieties × Bio Sona	8	104.44*	54.79**	0.223**
Error	70	42.86	14.59	0.005
CV%		10.06	9.20	5.43

*Significant at P ≤ 5%, ** Significant at P ≤ 1%

Table 2. Mean seed germination percentages in five varieties of onion due to pesticide treatments.

Pesticides	Treatment	Bhima Red	Bhima Kiran	Bhima Light Red	Bhima Shakti	Nasik Red	Treatment mean
	25 g/kg seed	51.67	93.33	60.00	81.67	66.67	70.67
Actara 25 WG	50 g/kg seed	48.33	90.00	60.00	80.00	63.33	68.33
	75 g/kg seed	46.67	86.67	51.67	76.67	61.67	64.67
	(2%)	53.33	58.33	56.67	85.00	60.00	62.67
Bio Sona	(5%)	50.00	56.67	51.67	78.33	55.00	58.33
	(8%)	41.67	58.33	56.67	60.00	56.67	54.67
	Control	76.67	88.33	70.00	71.67	75.00	76.33
	Variety means	52.62	75.95	58.10	76.19	62.62	65.10

	CD 5%	CD 1%
Between two means	10.69	14.22
Between treatments	4.78	6.36
Between varieties	4.04	5.37

@ 25 g and 50 g/kg seed, and all three doses of Bio Sona resulted in a significant increase in mitotic index. Bhima Kiran showed an increased mitotic index due to Actara 25WG (lower and recommended) and Bio Sona @ 5% concentration. However, a significant increase in the mitotic index was observed in Bhima Light Red only due to treatment with Actara 25WG @ 25 and 50 g kg^{-1} seed compared to the control. A greater reduction was observed in Bhima Shakti due to treatment with 75 g kg^{-1} seed of Actara 25 WG and Bio Sona at all three concentrations, but a lower dose of Actara promoted mitotic index. Both pesticides resulted in the suppressed mitotic index in the variety Nasik Red. The mitotic index was either enhanced or depressed by Actara and Bio Sona. Karaismailoglu (8) also recorded a reduction in the mitotic index, whereas Kumar and Naseem (10) found a positive impact of biofertilizer in *Trigonella*. A decrease in the mitotic index could be due to the inhibition of DNA synthesis in the S-phase as a result of which cells could not enter mitosis (Yekeen and Adeboye, 17).

The frequency of chromosomal aberrations was indicated by the total abnormality percentage. Table 1 showed significant effects of varieties, pesticides, Actara 25 WG and Bio Sona for total abnormality percentage. The differences between Actara 25WG vs. Bio Sona and pesticides vs. control were significant. Data on total abnormality percentage (Table 4)

Table 3. Mean mitotic index (%) in five varieties of onion due to pesticide treatments.

Pesticides	Treatment	Bhima Red	Bhima Kiran	Bhima Light Red	Bhima Shakti	Nasik Red	Treatment mean
Actara 25 WG	25 g kg^{-1} seed	61.16	73.58	78.22	74.12	18.10	61.04
	50 g kg^{-1} seed	48.74	58.21	45.09	52.84	21.89	45.35
	75 g kg^{-1} seed	46.28	37.79	35.36	24.74	23.01	33.44
Bio Sona	2%	60.41	43.32	41.25	34.02	19.70	39.74
	5%	55.49	53.06	32.77	24.22	21.60	37.43
	8%	50.54	43.20	35.07	22.89	17.94	33.93
	Control	40.15	40.64	37.83	49.47	30.91	39.80
	Variety mean	51.82	49.97	43.65	40.33	21.88	41.53

	CD 5%	CD 1%
Between two means	6.24	8.30
Between treatments	2.79	3.71
Between varieties	2.36	3.14

Table 4. Mean total abnormality percentage due to pesticide treatments in five onion varieties and types of chromosomal aberrations.

Varieties	Pesticides	Treatments	Total abnormality percentage	Types of chromosomal abnormalities								
				Laggard (%)	Clump (%)	Stickiness (%)	Disturbed stage (%)	Bridge (%)	Binucleate cell (%)	C-mitosis (%)	Breaks (%)	Fragment (%)
Bhima Red	Actara 25 WG	25 g kg^{-1}seed	0.00 (0.71)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		50 g kg^{-1}seed	0.53 (0.99)	0.16	0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		75 g kg^{-1}seed	0.87 (1.13)	0.00	0.35	0.00	0.00	0.00	0.52	0.00	0.00	0.00
	Bio Sona 25 wG	2%	4.93 (2.35)	0.19	1.21	0.19	0.78	0.52	2.04	0.00	0.00	0.00
		5%	3.26 (1.78)	0.15	0.56	0.14	0.00	0.42	1.99	0.00	0.00	0.00
		8%	4.10 (1.93)	0.29	1.43	0.40	0.29	0.40	1.29	0.00	0.00	0.00
		Control	1.95 (1.47)	0.00	0.44	0.00	0.00	0.00	1.51	0.00	0.00	0.00
Bhima Kiran	Actara 25 WG	25 g kg^{-1}seed	0.00 (0.71)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		50 g kg^{-1}seed	2.42 (1.69)	0.36	0.45	0.00	0.43	0.00	1.18	0.00	0.00	0.00
		75 g kg^{-1}seed	3.24 (1.93)	0.51	0.75	0.79	0.52	0.00	0.51	0.00	0.00	0.16
	Bio Sona 25 wG	2%	3.24 (1.89)	0.20	0.42	0.18	0.40	0.00	1.65	0.00	0.00	0.39
		5%	2.59 (1.74)	0.14	0.28	0.28	0.22	0.14	1.25	0.00	0.00	0.28
		8%	3.35 (1.93)	0.20	1.53	0.00	0.40	0.00	1.01	0.00	0.00	0.21
		Control	0.44 (0.95)	0.00	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bhima Light Red	Actara 25 WG	25 g kg^{-1}seed	0.00 (0.71)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		50 g kg^{-1}seed	0.51 (0.98)	0.17	0.17	0.17	0.00	0.00	0.00	0.00	0.00	0.00
		75 g kg^{-1}seed	1.36 (1.34)	0.00	0.18	0.16	1.02	0.00	0.00	0.00	0.00	0.00
	Bio Sona 25 wG	2%	0.88 (1.16)	0.00	0.24	0.00	0.30	0.00	0.34	0.00	0.00	0.00
		5%	2.99 (1.85)	0.18	0.97	0.18	0.17	0.00	1.28	0.00	0.00	0.21
		8%	1.94 (1.55)	0.00	1.08	0.00	0.00	0.00	0.86	0.00	0.00	0.00
		Control	0.92 (1.13)	0.00	0.18	0.00	0.00	0.18	0.56	0.00	0.00	0.00
Bhima Shakti	Actara 25 WG	25 g kg^{-1}seed	0.00 (0.71)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		50 g kg^{-1}seed	1.36 (1.35)	0.00	0.74	0.00	0.31	0.00	0.31	0.00	0.00	0.00
		75 g kg^{-1}seed	0.28 (0.86)	0.00	0.14	0.14	0.00	0.00	0.00	0.00	0.00	0.00
	Bio Sona 25 wG	2%	0.85 (1.16)	0.00	0.00	0.00	0.43	0.00	0.42	0.00	0.00	0.00
		5%	0.00 (0.71)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		8%	1.36 (1.36)	0.00	0.99	0.00	0.18	0.19	0.00	0.00	0.00	0.00
		Control	1.37 (1.30)	0.00	0.00	0.00	0.00	0.00	1.37	0.00	0.00	0.00
Nasik Red	Actara 25 WG	25 g kg^{-1}seed	0.00 (0.71)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		50 g kg^{-1}seed	2.31 (1.67)	0.00	1.91	0.00	0.40	0.00	0.00	0.00	0.00	0.00
		75 g kg^{-1}seed	1.31 (1.34)	0.29	0.58	0.29	0.15	0.00	0.00	0.00	0.00	0.00
	Bio Sona 25 wG	2%	0.68 (1.00)	0.00	0.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5%	0.40 (0.93)	0.00	0.00	0.21	0.19	0.00	0.00	0.00	0.00	0.00
		8%	0.59 (1.01)	0.00	0.16	0.00	0.43	0.00	0.00	0.00	0.00	0.00
		Control	0.00 (0.71)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Mean within parenthesis are transformed data

	CD 5%	CD 1%
Between two means	0.11	0.15
Between treatments	0.05	0.07
Between varieties	0.04	0.06

was affected by the pesticides in each variety and was categorized into different types of aberrations. In the case of Bhima Red, Bio Sona caused a significant increase in the per cent total aberrations than control. Similarly, Actara 25WG (at recommended and higher dose) and Bio Sona (all concentrations) resulted in an increased percentage of total aberrations in Bhima Kiran. Bhima Light Red showed increased per cent aberrations at higher concentrations of both pesticides than the control. All treatments significantly affected the total abnormality percentage except for Actara @ 25 g kg^{-1} seed in Nasik Red. Increased total abnormality percentage was also recorded by Popova *et al*. (13). Several aberrations were observed (Fig. 1) at varying frequencies depending on concentration and genotype (binucleate cells, bridge, stickiness, clumps, fragments, laggards and disturbed stages). Binucleate cells could be produced due to inhibition of cytokinesis during the cell cycle (Ozkara *et al*., 12). Stickiness could be caused by a sub-chromatid linkage between chromosomes or the inability of chromosomes to move to the poles and get stuck at any place inside the cell (Fatma *et al*., 4). Gantayat *et al*. (5) observed clumps and disturbed stages in onions. Disturbance in the formation of the spindle apparatus might lead to disturbed stages. Laggards were produced due to spindle inhibition (Karaismailoglu, 8). The occurrence of bridge might be due to breakage and reunion of chromosomes or chromosome stickiness (Kumar and Srivastava, 11), and chromosome fragments might result from multiple breaks (Grant, 7).

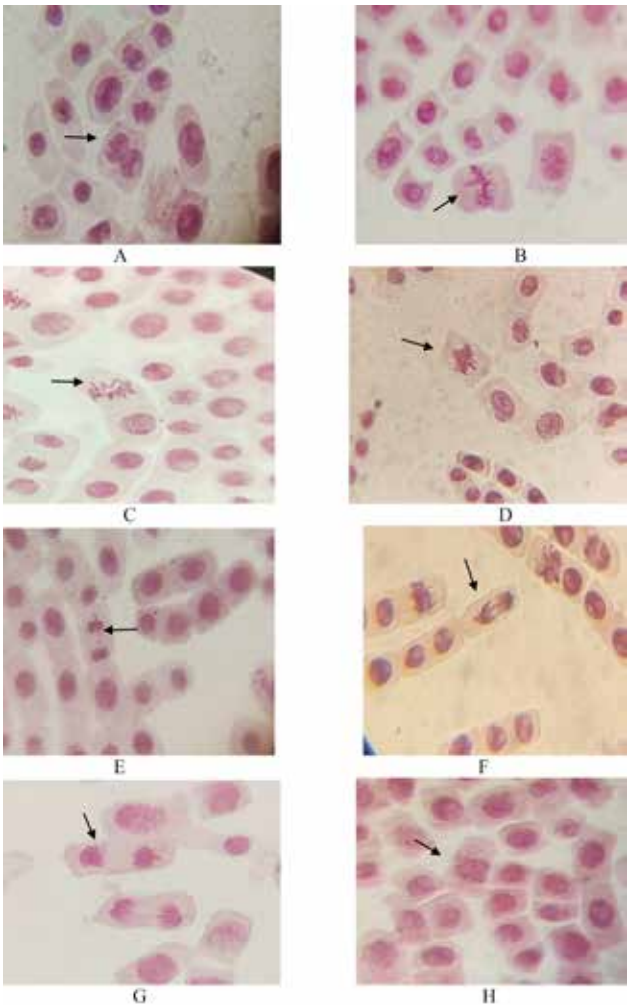

Fig. 1. Types of chromosomal aberrations – (A) Binucleate cell (B) Stickiness (C) Disturbed stage (D) Clumping (E) Laggard (F) Bridge and (G)-(H) Fragments.

This study shows that the effect of both the pesticides on seed germination, mitotic index and chromosomal aberrations depends on concentration and genotype. Actara 25 WG and Bio Sona inhibited seed germination in most of the onion varieties under study. The pesticides either promoted mitotic index or induced cytotoxicity at lower, recommended and higher concentrations, depending on the genotype. These pesticides were found to be a potent source of genotoxicity, thus indicating their harmful effect on living organisms at a genetic level. Bio Sona at a higher dose was more genotoxic than Actara 25WG. Biopesticides might also have genotoxic effects if applied indiscriminately at a higher dose. So, proper monitoring of pesticide concentration and selection of suitable genotype is very important to minimize their negative environmental impact. Future study is needed for further research on the effects of chemical and biopesticide in inducing genetic variation in plants both in positive and negative direction.

AUTHORS' CONTRIBUTION

Conceptualization of research (RC, GS and PKB); Designing of the experiments (RC, GS and PKB); Contribution of experimental materials (RC, GS and PKB); Execution of lab experiments and data collection (RC); Analysis of data and interpretation (RC, GS and PKB); Preparation of the manuscript (RC, GS and PKB).

DECLARATION

The authors declare no conflict of interest.

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