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CYTOTOXICITY ASSAY OF INSECTICIDE CORAGEN WITH REFERENCE TO EFFECT ON GROWTH AND CYTOLOGICAL PARAMETERS IN *HORDEUM VULGARE* L.

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ABSTRACT:
Insecticides have revolutionized to fight against endemic diseases in developing countries. Secondly, there is a reverse side to this synthetic pesticide; these agents pose a potential risk to humans and animals due to presence of their residues in the food. These synthetic insecticides are not only effective against the insect but also found to affect non target animals adversely. To study their effects on living organism, in present attempt Barley (*Hordeum vulgare*) is used as a model plant. Effect of different concentrations of synthetic insecticide Coragen on germination, seedling growth and cytology of Barley seeds was studied. We have been found that Coragen does not affect germination, it affect seedling growth with respect to doses and also induce mitotic abnormalities in higher concentrations. Increase in mitotic abnormalities with increase in concentration of Coragen implies that the insecticide affects normal cellular division process. We can conclude that most of these divisional irregularities were repaired and also germination, seedling growth were not affected adversely. hence, Coragen may be the safer sythetic insecticides which can be used to control insect population.

Keywords: *Coragen, Hordeum vulgare, Seed germination, Seedling growth, Mitotic abnormalities etc.*

INTRODUCTION:

Evolution in genetic trends of insects and pests open up wide doors for discovery of synthetic insecticides and pesticides. These insecticides and pesticides are mostly effective against specific insects but also extremely cytotoxic to non-target organisms. Coragen is one of the insecticides which primarily used as a larvicide. It is most effective against lepidopteran insect pests. Coragen is an anthranilic diamide insecticide in the form of a suspension concentrate. Chemically FMC Coragen® consist of Rynaxypyr as an important ingredient which possesses unique mode of action. It not only controls pests those are resistant to other insecticides, but also avoids any lethal effect on non-target arthropods conserving natural parasitoids, predators and pollinators (FMC, 2020). There is need to evaluate possible cytotoxic effects of Coragen at cellular level.

In the present study *Hordeum vulgare* (2n=14) is used as a test system. It is diploid nature, has low chromosome number ($2n$ = 14) with high degree of self-fertility and relatively large chromosomes, which allow easy detection of mitotic phases and chromosome aberrations. Barley is enough sensitive to cytotoxicity and genotoxicity assay which is effectively used to evaluate toxic activities of certain chemical compounds. Hence, present investigation aimed at evaluation of cytotoxicity and genotoxicity of Coragen against seedling growth and mitotic chromosomes on Barley.

MATERIAL & METHODS:

For evaluation of cytotoxicity of Coragen against Barley (*Hordeum vulgare)* seeds, following procedure was carried out -

1. **Selection of Material**

The barley (*Hordeum vulgare)* seeds were procured from the Sadhana Vidnyan Kendra Durgapur, Badnera. Total 600 seeds were used in four treatments (150 seeds each), three replication for each treatment.

2. **Treatment of Insecticide**

FMC Coragen® was bought from Krishi Seva Kendra, Amravati. Doses of 0.1%, 0.2% and 0.3% concentration were prepared by using amount of Coragen in distilled water. Physiologically similar seeds were directly soaked in insecticide solution and kept in Remi Orbital Shaking incubator at 110rpm for 18 Hrs (Plate I: a). All treatments were carried out in triplicates at Cytogenetics and Molecular Biology Laboratory, Department of Botany, Government Vidarbha Institute of Science and Humanities, Amravati.

3. **Seedling growth parameters**

a. Seed Germination: For germination study, 25 seeds were kept on moist blotting paper. Actively emerging radicals were considered as criteria for germination. Seed germination data was recorded after 3 days (Plate I: b).

b. Seedling Height: Seedling height was measured following the procedure of More and Malode (2018). Twenty-five seeds were arrange in slots of blotting paper kept in trays for seedling height determination (Plate I: c). Data of seedling height i.e. shoot length, root length and seedling survival recorded after 7 days. Seedling vigour index was calculated by following formula –

$$Seedling\ Vigour\ Index = \frac{Seed\ Germination\ percent}{Seedling\ height} \times 100$$

Seedling height measurements were statistically analyzed by using SPSS software provided by Department of Statistics, Government Vidarbha Institute of Science and Humanities, Amravati. Standard error was calculated for each of control and different concentrations. Paired sample t-test was conducted for randomly selected 15 samples to calculate significance of treatments comparing to control.

4. **Cytological Study**

For cytological study germinated seeds with radical length ranging from 0.5 to 0.7cm were preserved in Cornoy's fixative (Alcohol: Glacial Acetic acid in 3:1 proportion) for 24Hrs. and then transferred to 70% alcohol till their use in cytology. For cytological preparation, root tips were first hydrolysed in 1N HCl for 10 minutes at 65^0C. After hydrolysis, root tip are transferred to cavity block containing 2% Aceto-carmine for staining for 15 min. After staining slides were prepared as per standard procedure. Semi-permanent slides observed under high magnification microscope and total no. dividing cells, number of mitotic abnormalities recorded. Mitotic index and percent chromosomal aberration (Abnormality %) calculated by following formulas -

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

$$\text{Chromosomal aberration \%} = \frac{\text{Number of aberrant cells}}{\text{Total number of dividing cells}} \times 100$$

RESULT & DISCUSSION:

Effects of Insecticide Coragen on various parameters like seed germination, seedling growth, cytology was evaluated (Table 1).

Seed Germination Percentage

Seed is a package of heredity. Its growth after the period of dormancy starts with imbibition of water which in turn causes embryo expansion, cell expansion, cell wall synthesis and activation of metabolic signaling resulting into radical protruding out of seed. Overall process is called as germination. This process is a preliminary stage of every seed propagating plant (Barroco *et al.,* 2005).

Data on effect of different concentrations of 18Hrs Coragen treatment on seed germination in *Hordeum vulgare* are tabulated in Table 1. All treatments showed 100% germination followed by control implying the fact that insecticide does not affect germination rate of barley. These may be due to active repair system which instantly repairs every error caused in metabolic pathways required for normal seed germination.

The percent germination in seeds depends on the nature of the Insecticide and its dose of treatment. Most of the insecticides also act as a mutagen or impose teratogenic effect on plant system. Many of these insecticides have clastogenic (chromosome damaging) effects on plants via reactive oxygen derived radicals (Yuan, 1993).

Seedling Height and Seedling Survival

Data on effects of different concentration of 18Hrs. Coragen treatment on seedling height in *Hordeum vulgare* are tabulated in Table 1, Figure 1. All treatments show seedling height increased over the control (23.1cm) with maximum seedling in height (27.10 cm) recorded in 0.2% concentration of Coragen. 0.1% and 0.3% concentrations shows 23.17 and 23.73 cm seedling height respectively, which are less than seedling height observed in 0.2% concentration but increased over the control. Hypothetical (tabulated) t-value at 0.05 probability ($t_{.05}$) is 1.701 at df (degree of freedom) = 28. All the t-value calculated for different treatments showed increase over tabulated value (i.e. > 1.701). Hence null hypothesis is rejected. Treatments found to be significant and effective.

Germination result not affected in initial phase of treatment; further growth of seedling get arrested due to effect of insecticide due to micro mutations called in the late stage of development. Seedling survival is 100% in all treatments followed by control. This implies that Seedling survival is not affected by treatments; while seedling growth is increased over control in all treatments. This might be attributed to the fact that insecticide may affect the normal cell division and increased the rate of division process which may be caused by increased synthesis of regulatory proteins required for cell division.

Seedling growth and cytological study is an effective tool to evaluate cytotoxic and genotoxic effects of certain chemical extracts. This method is effective, employed to check hazardous effects of certain plant extracts, insecticides, pesticides, herbicides, other chemical compounds and even heavy drugs used to treat certain devastating diseases. This inhibitory or stimulatory effect of insecticides resembles with mutagenic effects of different chemical or physical mutagen. Mutagenic effects reported by many coworkers on different crops like Nilan *et al.*, (1973) in barley, Awan *et al.*, (1980) in rice, Bhat *et al.*, (2007) in *Vicia faba* L. etc.

Cytological Study

Data on effect of different concentration of 18Hrs Coragen treatments on mitotic index and frequencies of mitotic abnormality tabulated in Table 1, Figure 1. Mitotic index found to be increasing with increasing concentration of insecticide. Maximum mitotic index found in

0.03% concentration (26.80); while 0.1% and 0.2% concentrations shows intermediate values of mitotic index 24.61 and 25.26 % respectively. Control showed MI 22.97%. Mitotic index reported to be a good indicator to access the cytotoxic level and to test mutagenicity of chemicals in cells (Akinboro *et al.,* 2001a; Fiskesjo, 1985; Leme and Marin-Morales, 2009). In Control, only normal mitotic phases were observed and no abnormality recorded (Plate I: d to g); while all treatments shows increasing percent of mitotic abnormalities in nonlinear manner. Maximum abnormality percent observed in 0.2% concentration of Coragen treatment (10.99%) while 0.1% and 0.3% concentrations show 7.03% and 10.28% abnormality percent respectively. Mitotic abnormalities were observed in the form of chromosomal bridges, laggards, distorted metaphase, sticky chromosomes, distorted prophase, non-orientation of chromosomes and unequal distribution of chromosomes etc. (Plate I: h to o).

Lagging of chromosomes may be attributed to chromosome attachment to very weak spindle fiber or no attachment at all. Lagging chromosomes and fragments are observed due to the formation of acentric chromosomal fragment during exchange or chromosomal breaks (Verma *et al.* 2012). Distortion of chromosome in prophase and metaphase may be due to loss of polarity. Non-orientation of bivalents caused by abnormal spindle function and clumping of chromosomes may result in unequal separation (Bhat and Wani, 2017). Chromosome stickiness reflects toxic effects of insecticide, usually of an irreversible type and probably leading to death.

Akhter *et al.* in 2015 reported effect of Morter-48_{EC} and Safathrin-10_{EC} on morphological and cytological characters of barley. He found linear relationship among increasing abnormality percent and increasing dose of insecticides. Friesen *et al.* in 1964 observed that Dicamba (2-methoxy, 3, 6-dichlorobenzoic acid) when applied to barley during the period of high meristematic activity, disrupted normal growth and induced morphological and cytological aberrations. Root tips of barley seedlings germinated in different concentrations of dicamba, showed a sharp reduction in number of dividing cells and there were much evidences of chromosome clumping and the formation of multinucleate cells.

CONCLUSION:

Present investigation aimed at evaluating cytotoxic and genotoxic effect of well-known insecticide - FMC Coragen on growth and cytological parameters of barley (*Hordeum vulgare*). Insecticide does not found to affect seed germination or seedling survival and hence not found to have any lethal effect on seedlings of barley. But increase in seedling height over the control may be attributed to stimulatory effect of insecticide on regulatory proteins. Mitotic index value was found to be increased with increasing concentration of Coragen. Control does not show presence of any abnormalities; while all treatments show presence of mitotic abnormalities. Increase in mitotic abnormalities with increase in concentration of Coragen implies that the insecticide affects normal cellular division process. But as most of these divisional irregularities were repaired autonomously due to which germination and seedling growth were not affected adversely. Hence, Coragen found to be safer synthetic insecticides which can be used to control insect population.

REFERENCES:

Akhter, R., Razu M. H., Mazid M. H., Hamidur M., Arbia L., Kabir G. and Zaman (2015): Cytomorphological Effects of Two Insecticides on Mitotic and Meiotic Cells of Barley (*Hordeum vulgare* L.). Plant Environment Development 4: Pp.14-19.

Akinboro, A. and Bakare A. A. (2007): Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. Journal of Ethnopharmacology 112 (3): Pp. 470-475.

Awan, M. A., Konzak C. F., Rutger J. N. and Nilan R. A. (1980): Mutagenic Effects of Sodium Azide in Rice1. Crop Sci. 20: Pp. 663-668.

Barroco, R. M., Poucke C. V., Bergevoet J. H. W., Veylder L. de, Groot S. P. C. and Inze D. (2005): The role of the cell cycle machinery in resumption of postembryonic development. Plant Physiol. (137): Pp. 127-140.

Bhat, T. A., Sharma M., and Anis M. (2007): Comparative analysis of meiotic aberrations induced by diethylsulphate and sodium azide in broad bean (*Vicia faba* L.). Asian Jour. Plant Sci. 6 (7): Pp. 1051-1055.

Bhat, T. A. and Wani A. A. (2017): Mutagenic Effects on Meiosis in Legumes and a Practical Case Study of *Vicia faba* L. *In* chromosome structure and aberrations: Pp. 219 -240.

Fiskesjo, G. (1985): The Allium test as a standard in environmental monitoring. Hereditas 102: Pp. 99-112.

FMC. (2020): Coragen an excellent tool for Integrated Pest Management. Online available at: http://www.fmc.in/Agricultural-Solutions/Insecticides /Coragen. [Accessed March 20, 2020].

Friesen, H., Baenzig H. and Keys C. H. (1964): Morphological and Cytological Effects of Dicamba on Wheat and Barley. Canadian Journal of Plant Science 44(3): Pp. 288-294.

Leme, D. and Marin-Morales M. (2009): *Allium cepa* test in environmental monitoring: A review on its application. Mutation research 682: Pp. 71-81.

More, U. A. and Malode S. N. (2018): New improved slot holder assembly for measurement of seedling growth: a rapid analysis technique for mutation breeders. Proceedings of UGC and DST sponsored International Conference on Recent Trends in Science and Technology: Pp. 305-307.

Nilan, R. A., Sideris E. G., Kleinhofs A., Sander C. and Konzak C. F. (1973): Azide-a potent mutagen. Muta. Res. 17: Pp. 142-144.

Verma, A. K., Singh R. R. and Singh S. (2012): Cytogenetic effect of EMS on root meristem cells of *Catharanthus roseus* (L.) G. Donvar. Nirmal, *IJPBS* 2 (1): Pp. 20-24.

Wikipedia. (2020): Barley. Online available at: https://en.wikipedia. org/wiki/Barley. [Accessed March 20, 2020]

Wikipedia. (2020): List of countries by barley production. Online available at: https://en.wikipedia.org/ wiki/List_of_countries_by_barley_ production. [Accessed March 20, 2020]

Yuan, H.Y. and Zhang Z. (1993): Effect of free radicals and temperature on sister chromatids exchanges. In *Hordeum vulgare* L. Acta Bot Sin. 35: Pp. 20-26.

Table 1: Effect of different concentrations of Insecticide Coragen on Seed germination and Seedling Height of *Hordeum vulgare*

Sr. No.	Conc./ Dose	SG %	Seedling Parameters			t-value	SVI	SS%	TCO	NDC	TNCA	MI	AB%
			S.L.	R.L.	SH								
1	Control	100	14.77±0.91	8.33±0.95	23.1	-	2310	100	640	147	0	22.97	0
2	0.1%	100	14.41±1.86	8.76±1.66	23.17	2.001	2317	100	768	189	33	24.61	7.03
3	**0.2%**	**100**	**17.03±1.16**	**10.4±6.59**	**27.43**	**4.82**	**2743**	**100**	**760**	**192**	**53**	**25.26**	**10.9**
4	0.3%	100	16.23±1.22	8.50±1.76	24.73	3.61	2473	100	750	201	51	26.80	10.28

SG% - Seed Germination percent, S.L. - Shoot Length, R.L. - Root Length, S.H. - Seedling Height,
SVI - Seedling Vigor Index, SS% - Seedling Survival percent, TCO - Total Cells Observed,
NDC - Number of Dividing Cells, TNCA – Total no. of chromosomal abnormality MI - Mitotic Index,
AB% - Abnormality percent

Fig. 1: Effect of different concentrations of Insecticide Coragen on Seed germination, Seedling Height, Mitotic index and Abnormality percent of *Hordeum vulgare*

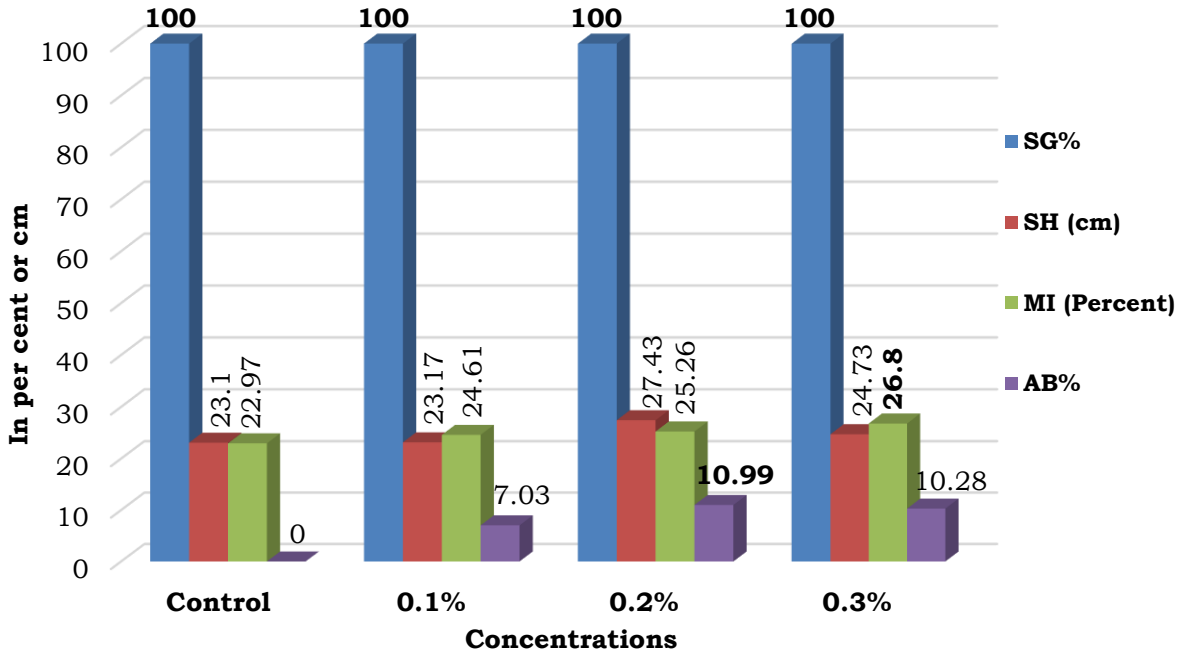
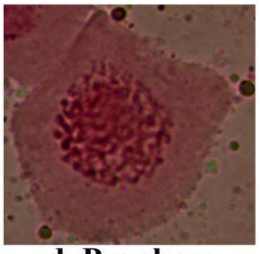


PLATE I: PHOTOMICROGRAPH SHOWING METHODOLOGY OF EXPERIMENT, NORMAL MITOTIC PHASES AND MITOTIC ABNORMALITIES

a. Insecticide treatment

b. Germination count

c. Seedling after 7 days



d. Prophase

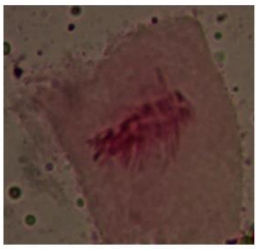
e. Metaphase

f. Anaphase

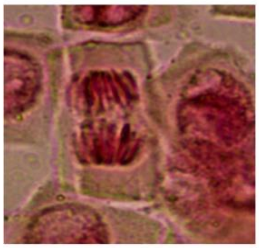
g. Telophase

NORMAL MITOTIC PHASES

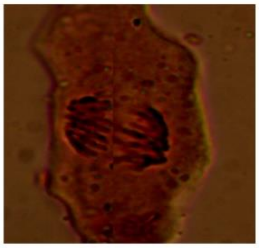
h. Distorted prophase



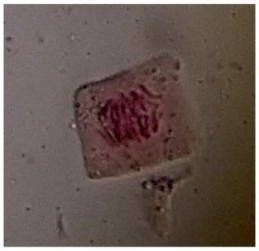
i. Distorted metaphase



j. Chromosome bridges

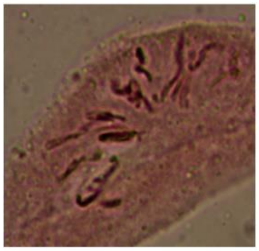


k. Chromosome bridges

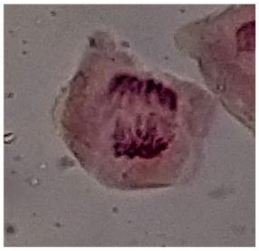


l. Sticky anaphase

m. Laggard



n - Non - orientation of chromosome



o. Unequal chromosome seperation

MITOTIC ABNORMALITIES