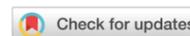




Toxic effect of 2,4-D on cytology of *Vigna radiata* (L.) Wilczek

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Abstract: Farmers' preference for cultivation of Mung bean (*Vigna radiata*) especially in the activity of sprout germination can exhibit zestful features while in bio-physiological and metabolical actions. Desiring for improved use of this habitually consumed crop for its ranges of medicinal values which possesses anti-bacterial, anti-oxidant, anti-hyperglycemic, anti-hypertensive, anti-inflammatory, anti-diabetic, metabolic accommodation of lipid, and antitumor effect, and many more scientists are working on it in order gather more evidences. The current study utilised concentrations of 50, 100, 500, and 1000 ppm in mung bean somatic cells to determine the toxicity of 2,4-D. Each concentration is treated for a length of 24, 48, 72, and 96 hours. Low concentration (50 ppm) pollutants were exposed for 24 hours in Mung bean, and the mitotic index decreased with the increased exposure times of 24, 48, 72, and 96 hours. Mitotic indexes dropped, whereas chromosomal abnormalities rose. The mitotic index had a propensity to decline as pollution concentrations rose concurrently. Some of the common anomalies seen across all treatments include C-metaphase, star shaped, binucleate, micronuclei, sticky anaphase erosion, chromosomal distributional error, chromosomal clumping, and failure of cell plate formation. Chromosome stickiness at 50 ppm, increased cell size at 100 ppm, chromosomal compression at 500 ppm, and chromosomal disintegration at 1000 ppm are relatively prevalent among all the treatments. For such crops, which have numerous uses, biomonitoring is necessary. This study can provide guidelines for determining the proper pesticide dose for extensive farming.

Introduction

The amounts of pesticides used and the indications and symptoms of sickness among farmers as a result of exposure are directly correlated (Kishi et al., 1995). The use of pesticides represents a new era in the application of man-made chemicals in the management of pests, increasing food production, increasing farmer income, and aiding in the elimination of illnesses. For upcoming discussions on *Vigna radiata* (L.) Wilczek and the safety of consuming this crop, knowledge of the effects of the common herbicide 2,4-D (2,4 dichlorophenoxyacetic acid) would be quite helpful. Among the group of plant growth regulators known as synthetic auxins is 2,4-D. It enters the plant's meristems via the leaves and is then transported there. Growth that is out of control and unsustainable results curling in stem, withering leaves,

and eventually plant death. Herbicide is employed to control weeds in broadleaf in the habitat ranges from aquatic environments, lawn, sand woods, as one of the most suitable herbicide. In the world one of the herbicides 2,4-D is most widely utilised in agricultural practices. Low to moderate acute toxicity applies to 2,4-D. Due to the acidic nature of 2,4-D, high doses can produce nausea, vomiting, burning in the mouth, and other unpleasant side effects (Freisthler et al., 2022). 2,4-D primarily destroy plants by changing the flexibility of their cell walls, interfering with protein synthesis, and upping ethylene production as suggested by the Environmental Protection Agency/EPA, U.S. At the recommended levels, 2,4-D induces unchecked and unfeasible growth in plants, which results curling in stem, withering leaves, and finally death of plant life (Song,

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2014). In order to treat citrus fruits during packing line processing and prevent stem-end rots caused by *L. theobromae* and other fungal diseases during long-distance shipping or lengthy storage, 2,4-D has frequently been commercially included into wax compositions at 500 mg L⁻¹ (Zhang, 2014). High 2,4-D dosages can enhance the levels of lipid peroxidation and oxidative stress in plants and may have an adverse impact on the antioxidant enzymes' protective enzyme activities (Damayanti et al., 2020).

In terms of herbicide, 2,4-D application in agricultural use can cause adverse effect on human population and ecosystem. Biomonitoring research on 2,4-D containing urine can show that we might also be concentrate on gathering appropriate data pertinent to the administration (Freisthler et al., 2022). The Environmental Protection Agency of the United States (2005), and Health Canada (2008) have reregistered several herbicides, and the European Union is currently reevaluating them for their intended use. Biomonitoring studies of 2,4-D applicators and their wives showed harmful effect (Alexander et al., 2007). Studies on cancer and 2,4-D in the lymphatic system have historically focused mostly on Non-Hodgkin lymphoma (Mills et al., 2005; Boers et al., 2010). Multiple myeloma, Leukemia, and Hodgkin lymphoma were also investigated, but no association with 2,4-D exposure was found (Burns et al., 2011).

Mung beans, often known as green gram, are a source of protein for humans. It is a fabaceae family legume plant. The mung bean's capacity to support nitrogen-fixing bacteria enhances soil fertility and lowers the emission of greenhouse gases (Nair et al., 2012; Islam et al., 2021). It has the enhancing quality of nitrogen and carbon in the soil for the subsequent crops growing beside (Wang et al., 2022). The crop has expanded globally in a balanced manner, particularly in emerging nations. Due to its high amounts of folate and iron and excellent protein content, mung bean is highly desired and has expensive market value, which makes the crop growers happy and content. Mung bean sprouts are also frequently consumed in Western nations, South East Asia, India, and Bangladesh (Tang et al., 2014). Some people eat it as a main diet while others consume it as a salad of garden vegetables (Lambrides, 2007). The antibacterial, antioxidant, anti-diabetic, anticancer and anti-inflammatory properties in Mung beans are assumed to be primarily attributed to the high quantities of polyphenols, amino acids, oligosaccharides, and proteins found in this diet (Kannatt et al., 2011). This substance are also connected to the regulation of lipoprotein metabolism and possesses different nutritional form

(Vanamala et al., 2006; Davidson, 2023). Seeds can be dried, roasted, crushed into flour, milled before being consumed split or whole (Naik, 2020). Moreover, dishes including soups, oatmeal, alcoholic beverages desserts, and curries, can be prepared by using mung beans seeds. Mung beans pertain a handful of microelements and high protein, different enzymes with low calorie content.

In the current study, an effort was made to ascertain any prospects of harmful effects of 2,4-D on mung bean somatic cells. In order to assess the cytotoxicity and genotoxicity of 2,4-D, the root meristem was employed as an in situ experimental setup.

Material and Methods

Market-purchased seeds were planted on moist filter paper and set to germinate at 20°C in the dark. Root tips are removed from the plant between 6.45 and 7.00 am for cytological research. Intensively growing root tips of 1-2 cm long were removed from germination seeds and processed with a p-dichlorobenzene (PDB) aqueous solution for three hours at standard temperature (ST) thus pretreatment was done. Fixation of the root tips were carried out in newly made acetic alcohol of 1:3 ratio for at least 24 hours before being kept in 70% alcohol until they were crushed. After pretreatment, root tips are carefully cleaned with distilled water unless the smell of PDB has vanished. For 1 hour, root tips were hydrolyzed in 5N HCl at room temperature. The root tip is stained with 2% acetoorcein, which is then mashed up in 45% acetic acid (Hore and Tanti, 2014; Sarma et al., 2017). Root tip portion is prepared by being cut out and squashed (Sarma and Tanti, 2015). Young, healthy roots that were 1 cm or shorter in length and began to emerge after one to two days were adequately cleansed in water before being taken for treatment.

Setting up chemical

2,4-D was the pesticide employed in this study. Healthy, uniform-sized mung bean seeds were exposed to 2,4-D at four different saturations during 24, 48, 72, and 96 hours, respectively and allowed to germinate in moist filter paper. The measured concentrations were 50 ppm, 100 ppm, 500 ppm, and 1000 ppm and each dosage employed with above mentioned four hours individually. As a measure of control, certain roots are gathered and kept in distilled water for 24 hours and then stored in 70% alcohol. Roots were taken out of the treated mung bean seeds while keeping the necessary exposure period.

Slides preparation and data evaluation

Slides scoring

Cells undergoing mitosis were recorded and microscopic observations were conducted by gently tamping on the glass slide and stapling it to

disperse the cells. Photographs were taken of the illustrative slides for each physical chromosomal aberration.

Evaluation of Mitotic index (MI)

The mitotic index was calculated by dividing the number of cells undergoing mitosis by the total number of cells seen for each dose (Balog, 1982). The information gathered was examined to ascertain the impact of the treatments (both concentration and treatment period) on the mitotic activity of *V. radiata* root tip cells. Each concentration was given a mean value and standard deviation. For each treatment 1000 cells were evaluated on one slide (Sarma et al., 2017). The number of dividing cells per total number of cells assessed is how the mitotic index (MI) was calculated:

Mitotic index (MI) = (Total number of dividing cells/Total number of cells examined) × 100

Toxic effect on Cytology

It was determined whether the treated cells had a higher mitotic index than the untreated cell.

Genotoxicity

Each 2,4-D concentration was evaluated and chromosomal aberration per dose was photographed as per possible. The aberrations of cells taking a small portion was measured with each dosage of 2,4-D and correlated with that of the control or untreated cells employing the equation:

Chromosomal aberration frequency (CF) = Total number of abnormal cells/Total number of normal cells

Results

Mitotic index (MI)

The 2,4-D treatment findings are shown in Table 1 and Figure 1, and it appeared that after a 24 hour administration period, there is huge reduction of MI from 50 ppm and 1000 ppm. With a rise in 2,4-D concentration, the mitotic index has decreased. The percentage of anomalies, however, revealed a different pattern. Depending on the 2,4-D concentration and exposure time, different percentages of cell division and abnormalities were generated. Maximum effect of 2,4-D was obtained in roots treated with 1000 ppm solution, while roots treated with 50 ppm solution showed the least amount of cytotoxicity.

Chromosomal aberrations (CA)

Comparing 2,4-D treated cells to control sets, there was remarkable declining trend in the mitotic index for each dosage and various time periods were all recorded. At higher dose and duration, this divisional frequency decreased. Stickiness (50 ppm), c-mitosis, cell size elongation (100 ppm), unequal or unoriented chromosomes distribution, fragments and bridges (primarily in anaphase) (500 ppm), chromosomal splits and chromosomes shrinkage, lagging or disjunction chromosomes, and multipolarity were the abnormalities found at the ranged concentrations of 2,4-D herbicides treated (1000 ppm). Moreover, various concentrations showed star-shaped chromosomal structure and the failure of cell plate development were noticed. In each

concentration, there was chromosome disruption. At 24 hours, sticky anaphase, telophase spiralling, regular metaphase breaking, and disruptive nuclei were all seen. Chromosome fragmentation into short and thick pieces, disorganized anaphase, the disappearance of the nucleolus, sticky anaphase, chromosome laggards, adherent chromosomes, and distortion of the pole in anaphase were all observed after a 48-hour exposure to various concentrations. Chromosome aberrations include chromosomal bridges, lagging chromosomes, uneven nucleus distribution, chromosome disruption, aberrantly uncoiled chromosomes, etc. were identified throughout the 72-hour dosing interval (Fig. 1) At 96 hours during exposure time, binucleate cells, the inhibition of cell plate formation, sticky metaphase, dead cells, disoriented anaphase, clumped metaphase chromosomes in, sticky anaphase, bridge, and inflated chromosomes were more frequently seen. The induced chromosomal aberration was dependent on dosage and duration.

A-Sticky metaphase, B-Cell elongation with chromosomal disturbance, C-c-metaphase, D-Disorientation of metaphase-cell elongation, E-Chromosome disturbance in metaphase, F-Clumped metaphase, G-Clumped Chromosome, H-Binucleate, I-Binucleate, J,K- Breakage and Bridge, L-Bridge in anaphase, M-Stickiness with star anaphase, N-Chromosome disturbance in anaphase, O-Late telophase with nuclear lesion, P-Nuclear lesion and fragmentation Q-Micro-nuclei, R-Lagging Chromosome.

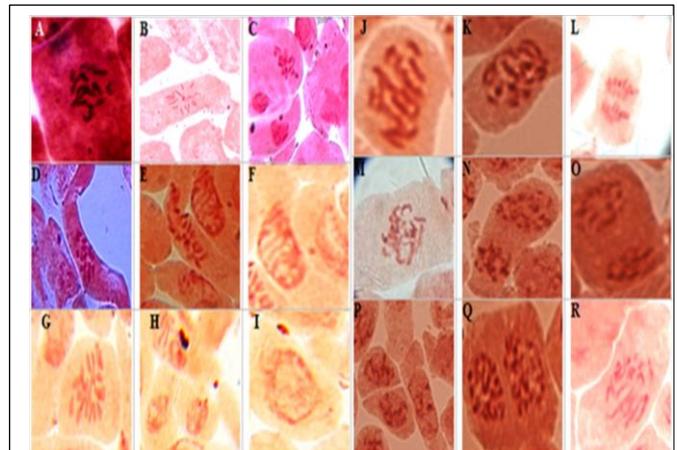


Figure 1. Chromosomal variation under 100x magnifications due to effect of dosage of 2,4-D.

Discussion

Several forms of chromosomal abnormalities are brought on by 2,4-D treatments at various doses. Salts, esters, and acids are only a few of the chemical forms that 2,4-D can take. There may be numerous ways that the herbicides prevent cell division (Lehnen and Vaughn, 1992; Galloway et al., 1998). Certain herbicides affect the primary crop after coming into touch with the soil and causing anomalous physiological, morphological and

cytological and biochemical activities (Yoshida et al., 1983). According to Patil and Bhat (1992), stickiness might result from the physical adherence of chromosomal proteins, from problems with the cell's metabolism of nucleic acids, or from the breakdown of proteins that

to wheat seedlings for 72 hours at room temperature. Over control, twelve different anomalies in chromosomal structure were found in the mitotic cells. Sticky metaphase, C-metaphase, dead cells, irregular binucleate cells, disoriented anaphase with discontinuous laggards,

Table 1. Visual chromosomal aberration and Mitotic indices in *Vigna radiata* root meristem with 2,4-D dosage.

Concentration of 2,4-D (ppm)	Duration	Analysed cells in count	Dividing cells in count	No. of aberrant cells	Mitotic indices	Chromosome aberrations
Control	24	777	543	0	69.8	14±2.13
50	24	745	387	15	51.9	19±1.80
	48	742	380	19	51.2	22±0.43
	72	740	378	26	51.08	26±0.74
	96	739	373	29	50.47	27±1.40 29±0.96
100	24	735	356	30	48.43	30±3.12
	48	732	354	35	49.36	31±0.54
	72	730	344	36	47.12	32±1.34
	96	729	320	42	43.89	34±1.28
500	24	725	304	50	41.93	36±2.34
	48	724	298	57	41.16	37±1.88
	72	720	283	59	39.30	43±2.34
	96	719	272	63	37.83	46±3.45
1000	24	715	257	67	35.94	50±0.87
	48	711	229	72	32.20	53±2.33
	72	710	209	76	29.43	56±1.90
	96	704	196	80	27.84	60±3.54

cover the DNA in chromosomes. It is possible to record a sizeable number of nuclear lesions, the majority of which may result from the pesticides' effect degrading a component of the nuclear material (Omanakumari et al., 2006). Higher concentrations and longer treatment times using castor seed extract, according to Borah and Talukdar (2002), significantly reduced the mitotic index. The evaluated herbicides may offer certain risk to public health and the ecosystem if it gets exposed for an extended period of time, according to cytological drawings (Goravaya and Klinkina, 2002). To conduct genomic research on legumes, mungbean possesses all the necessary characteristics to serve as a model plant system (Chakraborty et al., 2022). To analyse the pollutants of herbicides or their derivatives that may be the source of the herbicides' chromotoxicity, mutagenicity, carcinogenicity, or turbogenicity in organisms, a sufficiently scientific, sensitive, and molecular technique is required. The plant may have diverged from its initial parental line due to chromosomal aberrations that revealed the herbicides' chromotoxicity (Ghersa et al., 1994; Yemets et al., 2008). In *Triticum aestivum* L. mitotic cells, 2,4-D and isoproturon have different impacts on the chromosomal morphology, according to Kumar (2010). A variety of 2,4-D and isoproturon concentrations (50–1200 ppm) were applied

clumped chromosomes in metaphase, sticky anaphase, spiral telophase cells with bridge, and swollen chromosomes were more frequently seen in the 72 and 96 hours of exposure time (Tanti et al., 2012).

The application of 2,4-D as herbicide in the current investigation revealed root tip protuberances at 100 ppm and overall root tip growth inhibition at 1000 ppm. This unusual mass protuberance may indicate widened root tips and is likely to encourage larger cells. With herbicides treated at the 50ppm concentration, chromosome stickiness was quite common. The observed variations from typical mitosis under 100x magnification for all figures (Fig. 1) were documented and photographed. Records were made of the various chromosomal malformations. The abnormalities were stickiness (50 ppm), c-mitosis, cell size increased (100 ppm), distributional error or unoriented chromosomes, micronuclei, chromosomal splits, chromosomal compression (500 ppm), fragments and bridges, lagging or disjunction chromosomes, and star-shaped chromosome structure (1000 ppm).

Conclusion

Offering consumers high-quality crops is one of agriculture's main goals. Due to the significant economic worth of *Vigna's* species, cytogenetic research, particularly the dosage application of herbicide has been

a topic of considerable relevance. It is abundantly obvious from the above explanation that *Vigna radiata* undergoes chromosomal regression and general chromosomal aberrations, with the increasing trend of dose and duration of 2,4-D which can be directly associated with their karyological malformation. From the aforementioned results, it can be suggested that modest dosages of 2,4-D, such as 50 ppm, have caused the fewest chromosomal abnormalities. Additionally, the mitotic index is maximum at this concentration when compared to other 2,4-D concentrations. At 1000 ppm, significant anomalies were detected and recorded; typically, dead cells are discovered. As a result of the aforementioned facts, we can draw the conclusion that large dosages of 2,4-D introduced into agricultural practices may be harmful to both plant life and human consumption.

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