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REVIEW ARTICLE

Plant Bioassay in Cytogenetic Monitoring for the Review of the Environmental Toxins

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ABSTRACT

Throughout history, human beings have caused irreversible changes to the environment. However, with the emergence of modern science in recent decades, scientists have been able to quantify the extent of these changes. It is therefore crucial to monitor the environment comprehensively in order to establish laws and standards that ensure its cleanliness. Traditional chemical analysis methods often do not adhere to the twelve principles of Green Chemistry, are costly, and involve large amounts of toxic and harmful solvents that can harm the environment. As a result, it is essential to develop reliable analytical techniques that meet the requirements of Green Analytical Chemistry, complement, or replace traditional classical methods, and are environmentally friendly. Bioassays offer a potential alternative. It has long been acknowledged that high plants, particularly *Vicia faba*, are excellent genetic models for identifying mutagenic and cytogenetic agents and are commonly used in environmental monitoring studies. *V. faba*-based bioassays have been employed to investigate the DNA damage caused by various substances, such as metal compounds, pesticides, complex mixtures, petroleum derivatives, toxins, nanoparticles, and industrial effluents, resulting in chromosomal and nuclear errors. *V. faba*'s test system is widely used to assess toxic agents due to its numerous advantages, and it has become a crucial bioassay for ecotoxicological studies. The aim of this study was to demonstrate how *V. faba* bioassays can be complementary alternatives to traditional analysis methods, satisfy Green Analytical Chemistry criteria, and comply with environmental monitoring laws and regulations.

Key words: *Vicia faba*, Environmental monitoring, DNA damage, pesticides, Cytogenetics, Plants.

INTRODUCTION

The environment consists of interconnected biotic and abiotic components that continuously exchange matter and energy as part of an intricate system of processes (Zafar et al., 2022a; Chowdhary *et al.*, 2020). An ideal state of balance between these processes is what is known as homeostasis (Nieves *et al.*, 2022; Liu *et al.*, 2023). Xenobiotic compounds, which are often released into the environment in large quantities outside the confines of the law, can disrupt the delicate balance of the environment and accumulate along the food chain as they are not easily degraded by indigenous microfauna and flora (Haroon et al., 2022; Zegzouti *et al.*, 2020; Bhat *et al.*, 2019).

Nearly all human activities can cause environmental pollution, but some of them have a significant impact on anthropogenic impact on the environment (Rascio *et al.*, 2022; Zafar et al., 2021). The noted examples of industries that contribute to environmental pollution: The petrochemical industry, which includes the extraction and refining of petroleum, as well as the production of petrochemicals such as plastics and fertilizers. The mining of precious metals and stones, which includes the extraction of metals such as gold, silver, and copper from the earth, as well as the extraction of precious stones such as diamonds and rubies. Tanneries, which are facilities where animal hides are processed into leather. The process of tanning can involve the use of harsh chemicals and can produce large amounts of wastewater that can contain pollutants such as chromium (Yanamandra *et al.*, 2022). The lead battery industry includes the production of lead-acid batteries used in vehicles and other devices (Nouairi *et al.*, 2019).

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The manufacturing process can involve the use of lead and other harmful chemicals, which can be released into the environment if not properly managed. Industrial and municipal discharges, which include the release of pollutants into the environment from various industrial and municipal sources, such as factories, power plants, and sewage treatment facilities (Al-Ahmadi, 2015; Buitrago *et al*., 2013). There are three categories of indicators for monitoring environmental pollution: physical indicators (e.g. odors, colors, tastes, porosities, temperatures, conductivity, and aggregate stability), chemical indicators (e.g. redox potential, salinity, and biological, and chemical oxygen demand), and biological indicators (e.g. microbes, plants, and animals) (Guimarães *et al*., 2019; Tang *et al*., 2020). Bioassays that utilize living organisms as indicators are commonly used to analyze the presence and concentration of chemicals in water, sediment, and soil samples. These bioassays can also be used to compare the relative toxicity of chemicals in different environmental matrices by observing the effects on microorganisms, plants, and animals (Hassan *et al*., 2019; Mustapha *et al*., 2019; Zafar et al., 2022c). Bioassays offer a greater sensitivity and reproducibility level compared to other physical or chemical methods, making them the preferred method to detect pollutants (Luo *et al*., 2020; Zafar et al., 2020; Kamal et al., 2019b). As it turns out, the *V. faba* plant has a number of applications, including studies of cytology, physiology, radiobiology, and toxicology evaluation (Gregušková and Mičieta 2013). V. faba has several advantages, including year-round availability, affordability, ease of cultivation, and handling, and not requiring sterile conditions or expensive materials. Additionally, it has a fast cell division rate and its chromosomes are easily assessed for genetic damage (Khazaei *et al*., 2018; Getahun and Haile 2018; Anwar *et al*., 2011; Adam and El-Ashry 2010). The major objective of this study is to showcase how V. faba bioassays can serve as supplementary alternatives to conventional analytical methods. These bioassays fulfill the criteria of Green Analytical Chemistry and align with laws and regulations for environmental monitoring.

The Combined Effect of the Interplay of Biotic and Abiotic Stresses

In real-world agricultural conditions, plants are exposed to various environmental stresses that can severely impact growth and yield. Factors such as drought, extreme temperatures, high salt levels in soil, cold snaps, and disease infections can disrupt normal plant development and dramatically reduce production (Atkinson *et al*., 2013; Haroon et al., 2022b; Farooq et al., 2022). Typically, researchers examine the effects of these stressors on plants in controlled laboratory environments (Jackson, 2018). Real-world field conditions differ substantially from the controlled settings of laboratory studies. In the field, plants are often exposed to multiple stressful conditions at once, rather than single factors in isolation. For example, plants may experience the combined stresses of high salinity and high temperatures, drought and heat waves, or major abiotic stress along with pathogen infection (Mittler, 2006). This work will explore the interconnected nature of plant stress responses and how those integrated responses impact plant growth and function (Narsai *et al*., 2013). Studies have revealed that plants respond to combinations of stresses in unique ways that cannot be predicted based solely on their individual responses to each stress in isolation. The collective effect of multiple simultaneous stresses on plants produces emergent effects that are distinct from the sum of how plants respond to each stress on their own (Oldenburg *et al*., 2009).

Furthermore, many stresses can occur at the same time, which may contribute to the high level of complexity in plant responses (Kamal et al., 2019a; Zafar et al., 2022b). These responses are mostly governed by various, sometimes conflicting, signaling pathways that may interact and counteract each other (Bethenod *et al*., 2001). Plants exhibit unique responses to combinations of multiple stressors, which cannot be predicted by observing their reactions to individual stressors, according to recent research (González-Dugo *et al*., 2006). The simultaneous occurrence of multiple stresses creates a high level of complexity in plants' responses. Plants must coordinate various signaling pathways to respond to the combination of stresses, but these pathways can conflict with or inhibit one another. As a result, plants' responses to multiple, concurrent stresses involve intricate interactions between complex signaling networks (Fig. 1) (Blum, 2009; Surówka *et al*., 2020; Lohani *et al*., 2020; Rejeb *et al*., 2014; Anderson *et al*., 2004; Diourte *et al*., 1995).

Plants Provide Cues to the Environmental Conditions

Plants are sessile and therefore cannot avoid biotic and abiotic environmental cues.In response to biotic and abiotic threats, plants have evolved a variety of adaptations and mechanisms to overcome these challenges to their survival. In response to detecting an environmental stressor, certain biological systems become activated. This activation then triggers the necessary physiological responses and reactions to cope with the stressor (Brown and Donnelly 1984). Cells convert external signals into changes in gene expression via multiple signaling pathways. These pathways transmit signals from sensors on the cell surface or in the cytoplasm to the cellular components in the nucleus that control transcription. Through these signaling pathways, the cell is able to relay information about its external and internal state to the nucleus, which can then alter gene expression in response (Cooman *et al*., 2003). Through changes in gene expression, the plant develops increased tolerance to stress (Cotelle *et al*., 2012). Cellular signaling pathways provide the mechanism by which cells sense and respond to stressful conditions. These pathways detect signals from the cellular environment indicating stress or

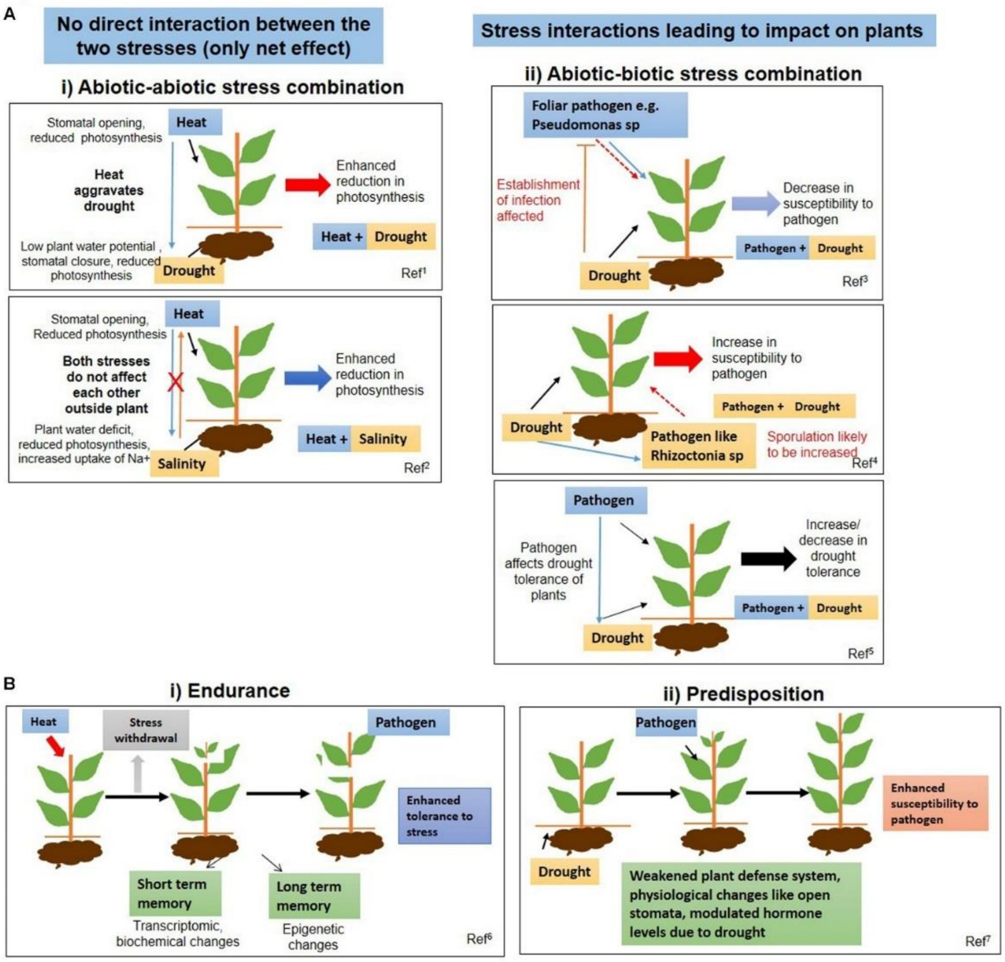

Fig. 1: Schematic representation of the effect of stress combination on plants. (A) Using examples from heat and drought (abiotic–abiotic stress) and drought and pathogen stress (abiotic–biotic stress) combinations, we explain how the effects of combined stress on plants can be explained. (B) Examples of a combination of heat and pathogen stress or drought and pathogen stress have also been shown to prime or predispose plants to subsequent stresses, based on examples of heat and pathogen stress combinations (Pandey *et al.*, 2017)

damage and then trigger an appropriate biochemical and physiological response to that stress (Iqbal *et al.*, 2019; Zafar et al., 2022d). As people gain a deeper understanding of environmental systems and chemical compounds, a comprehensive study of environmental pollution must account for the physical, chemical and biological impacts that may arise (Zhitkovich, 2011; Farooq et al., 2021). A thorough analysis of pollution should consider the full range of potential effects on the environment, incorporating the latest knowledge about environmental processes and chemical substances. Observing bioindicators, bio-monitors, and species of organisms with a restricted ecological tolerance for xenobiotics are all part of in situ biomonitoring (Fernandes *et al.*, 2007). The degradation of the environment may be indicated by changes that are brought about in the behavior, function, or overall population of bioindicators (Zaghloul *et al.*, 2020). Advanced bio-monitoring techniques can provide a deeper level of insight. By employing appropriate sensors and measurement devices, as well as utilizing multiple methods and protocols, the biological characteristics of an environment can be closely tracked and evaluated. Environmental samples can be collected and analyzed outside the body using bioanalytical techniques such as biosensors and bioassays (Haroon et al., 2023). These instruments allow the samples to be tested for biological markers of interest. A fast-growing area of environmental sciences is bioanalysis (Dong and Zhang 2010; Patra *et al.*, 2004; El Hajjouji *et al.*, 2007). Since the early 20th century, it has evolved and proven effective in monitoring and evaluating environmental quality. Today, diverse bioanalytical techniques, such as bioassays, are utilized for this purpose (Table 1) (Grant, 1994; Clague *et al.*, 2015).

Why *choose the Vicia faba* Model System for Environmental Monitoring?

The faba plant is commonly known by several names, including *Vicia faba*, faba bean, fava bean, and horse bean (Anwar *et al.*, 2011). The faba bean is an annual crop grown around the world. Because of its ability to withstand cold temperatures and high salt levels, the faba bean is renowned for its resilience in harsh, stressful environmental conditions with limited water or nutrients (Yanamandra *et al.*, 2022). The

Table 1: Using plants to assess environmental quality: Bioassays that evaluate the effects of contaminants and pollutants

Bioassay – production company	Species of the test organism	Toxicity/measure of toxic effects, observed endpoint	Duration of test	Application/sample type	Recommending organization	Advantages
Allium cepa	*Allium cepa* (onion)	Tests indicate that the substance induces genetic damage and mutations. Specifically, it causes breaks and rearrangements in chromosomes (clastogenic effects) as well as abnormal numbers or structures of chromosomes (chromosome aberrations). It also causes abnormalities in the nucleus of cells, such as micronuclei	Depending on sample incubation and purpose of the study	Various environmental pollutants and contaminants such as pesticides, herbicides, radiofrequency radiation, electromagnetic fields, coal fly ash, contaminated soils, sewage sludge, industrially polluted river water, metals, dyes, and contaminated drinking water can have harmful effects on human exposure and health (deKergommeaux et al., 1983).	OECD (Van Beuzekom, & Arundel, 2006).	- Low cost and simplicity of the procedure. The test is easy to perform and does not require complex sample preparation. - Direct exposure of bacteria to the test sample, eliminating the need for extensive sample processing. - The bacteria have oxidase enzymes, allowing them to activate pro-mutagens. Therefore, the Ames test does not require the addition of an S9 mixture to detect pro-mutagens.
Zea mays	*Zea mays* (maize)	Tests indicate that the substance induces genetic damage and mutations. Specifically, it causes breaks and rearrangements in chromosomes (clastogenic effects) as well as abnormal numbers or structures of chromosomes (chromosome aberrations) and change in phenotype	The sample incubation period and intended goals of the study will determine the appropriate approach	According to J. P. Anderson *et al.* (2004), the following were mentioned: soil that is contaminated, wastewater that is polluted, pure chemicals, and herbicides	OECD	The test organism is inexpensive and straightforward to work with. When subjected to stress, it emits volatile organic compounds that can be readily detected and analyzed[33]
Vicia faba	*Vicia faba*] (faba bean)	Tests indicate that the substance induces genetic damage and mutations. Specifically, it causes breaks and rearrangements in chromosomes (clastogenic effects) as well as abnormal numbers or structures of chromosomes (chromosome aberrations)	The sample incubation period and intended goals of the study will determine the appropriate approach	Water, wastewater, sediment, contaminated soil, river water, model compounds, heavy metals, radiation effect (Nouairi et al., 2019)	ISO	- Can detect mutagenic compounds - Can evaluate various endpoints, ranging from point mutations to chromosomal aberrations - Available for research throughout the year - Inexpensive, easy to cultivate and manage - High rate of cell division with chromosomes readily exposed to mutagenic agents[9].
Tradescantia sp.	*Tradescantia sp.* (spiderworts)	Tests indicate that the substance induces genetic damage and mutations. Specifically, it causes breaks and rearrangements in chromosomes (clastogenic effects) as well as abnormal numbers or structures of chromosome (chromosome aberrations). It also causes abnormalities in the nucleus of cells, such as micronuclei	Depending on sample incubation and purpose of the study	Contaminated soils, wastewater, chemicals, polluted air (Gregušková and Mičieta 2013)	Lack of recommending organizations	- Can detect mutagenic compounds - Can evaluate various endpoints, ranging from point mutations to chromosomal aberrations - Available for research throughout the year - Inexpensive, easy to cultivate and manage - High rate of cell division with chromosomes readily exposed to mutagenic agents[38]

LemnaTest – LemnaTec GmbH, Germany	*Lemna minor, Lemna gibba* (duckweed)	Short-term and intermediate-term exposure: Suppression of growth.	7 days	Chemicals, pesticides, aqueous samples, substances soluble in water (Gregušková and Mičieta 2013).	SIS, AFNOR, ASTM, US EPA, OECD.	- Low cost and simplicity of the procedure. The test is easy to perform and does not require complex sample preparation. - Direct exposure of bacteria to the test sample, eliminating the need for extensive sample processing. - The bacteria have oxidase enzymes, allowing them to activate pro-mutagens. Therefore, the Ames test does not require the addition of an S9 mixture to detect pro-mutagens[45]
PHYTOTOXKIT F™ - MicroBioTests Inc., Belgium	*Monocot plants Sorghum saccharatum* (sorghum) and dicot plants *Lepidium sativum* (cress), *Sinapis alba* (mustard)	Short-term and intermediate-term exposure: Suppression of growth..	3 days	Soil, sediments, sewage sludge, compost, wastewaters used for irrigation, chemicals and biocides, composts (Cavusoglu et al., 2010)	Lack of recommending organizations	Soil, sediments, sewage sludge, compost, wastewaters used for irrigation, chemicals and biocides, composts[63]

cultivation of this crop begins in early fall, around October. The harvest of its pods occurs the following spring, around May (Adam and El-Ashry 2010). Because legumes can fix nitrogen in the soil and enrich it, they should be cultivated before strategic crops like cotton in order to enhance the soil's fertility and the cotton plants' growth (Arya and Mukherjee 2014).

The germination rate of *Vicia faba* seeds after being treated with the tested material shows that it is phytotoxic (Béraud *et al.*, 2007). Studies of the mitotic apparatus have shown that certain measures can indicate the cytotoxic, mutagenic, and genotoxic effects of treatment. These measures include the mitotic index (the percentage of cells undergoing mitosis), the phase index (the relative distribution of cells across the phases of mitosis), the percentage of abnormal mitoses, the types of mitotic abnormalities observed, and the frequency of micronuclei. Elevations or changes in these parameters suggest that the treatment has disrupted normal somatic cell division and plant growth (Bhat *et al.*, 2019). The Vicia faba plant is an ideal model system for cytogenetic studies evaluating the effects of toxicants, pollutants, chemicals, heavy metals, and novel mixtures. Its various attributes, including fast growth, large chromosomes, and sensitivity to DNA damage, make it well-suited for assessing the concentrations and impacts of these agents (Büyükkeskin *et al.*, 2015).

Vicia faba Cytogenetic Tests for Environmental Mutagens

Vicia faba has been utilized since the 1950s to investigate the impact of radiation on chromosome breakage and aberrations, owing to its possession of 6 pairs of comparatively large chromosomes. Beginning in the early 1960s, this technique was widely adopted to investigate chromosome damage induced by chemical agents (deKergommeaux *et al.*, 1983). Chromosome studies using newly germinated roots can be conducted continuously throughout the year in a small space with minimal resources. This is because only the roots of recently germinated plants are required for these experiments (Do Céu Silva *et al.*, 2003). Scientists have extensively studied around 80 chemicals for their ability to induce mutations by measuring how frequently these chemicals cause chromosomal or chromatid abnormalities. The frequency of such aberrations serves as a measure of a chemical's mutagenic potential (El-Shahaby *et al.*, 2002). The Giemsa stain sister-chromatid exchange test, commonly used today to assess mutagenicity in mammalian cells, was originally developed in the 1960s and 1970s for plants. Scientists first applied this test to *Vicia faba*, or the common vetch, to evaluate the mutagenic effects of substances (El-Shazly and El-Sheikh 2000). By further refining the sister-chromatid exchange assay in *Vicia faba,* the test could be made more sensitive and precise. This would enable it to be more widely applied as a screening technique to assess the mutagenic potential of chemicals (Zhitkovich, 2011).

Examples of the Cytogenetic Studies Carried out on the *Vicia faba* Plant to Test Pesticides

Modern agricultural practices that utilize pesticides have introduced numerous toxic chemicals into the environment, exposing it to harmful pollution. Over the past 20 years, the use of pesticides and chemicals in disease and pest control has grown at an extremely rapid rate (Leme and Morales 2009). While certain industrial chemicals have become necessary for manufacturing processes, inadequate attention has been paid to their potential harmful effects on the environment and human health. These chemicals and their ingredients have been shown to cause acute toxicity in plants, animals, and humans, but the full extent of their negative impacts has not been sufficiently considered (White and Claxton 2004).

Table 2: Some cytogenetic studies concerned the effects of chemical pollutants (abiotic stress) carried on the *Vicia faba* plant as a model system.

Chemical pollutants	Reference
Acrylonitrile vinyl cyanide (CAN)	Lambotte-Vandepaer and Bogaert (1984)
Di ethyl amino ethanol	El-Ashry and Mohamed (2012)
Potassium dichromate	Mohamed (2013)
Crude oil contamination	Alavi et al., (2022)
Heavy metals	Mustapha et al., (2019)
lead acetate	Bethenod et al. (2001)
potassium dichromate	Jackson (1980)
Cadmium	Nouairi et al. (2019)

Table 3: The results of exposing Vicia faba plant seeds to varying concentrations of light crude oil.

Traits	Light Crude Oil Concentration in Soil (%)			
	0% (Control)	1%	2%	4%
Root Length (cm)	92.59±1.5^{a}	81.47±3.2^{b}	70.37±4.2^{c}	50.92±1.5^{d}
Seed Germination (%)	2.86±0.30^{a}	2.23±0.25^{b}	1.43±0.11^{c}	1.10±0.17^{d}

The statistical calculations incorporate mean values. Means that do not share a letter are significant. α = 0.5 and ± points to the standard deviation among the samples

Table 4: Effects of Different Concentrations of Light Crude Oil on Cell Indices in Soil

Cell Indices	Light Crude Oil Concentration in Soil (%)			
	0% (Control)	1%	2%	4%
M.N. (%)	3.33±0.57^{c}	5.66±0.57^{c}	14.66±1.5^{b}	18.66±2.0^{a}
C.A. (%)	0.69±0.04^{c}	2.00±0.20^{b}	2.48±0.33^{a}	1.81±0.22^{b}
MI (%)	2.73±0.68^{d}	8.70±0.73^{a}	6.23±0.30^{b}	4.76±0.60^{c}

MN: micronucleus, CA: chromosome aberration, MI: mitotic index; Means that do not share a letter are significant. α = 0.5 ± is the standard deviation among samples

Pesticide use can harm public and environmental health in multiple ways. Not only are pesticides directly applied to crops and enter the food supply, but they can also accumulate and persist in the food supply and the environment. As pesticides build up to toxic levels, they pose risks to human and ecosystem health (Béraud *et al.*, 2007). Cytological analyses have investigated the detrimental impacts of agricultural chemicals on various crop plants. Multiple studies have examined the cellular-level effects of pesticides and other agricultural chemicals on crops (Zafar et al., 2020). Studies of the mutagenicity of both insecticides found that increasing concentrations of the insecticides induced significantly higher percentages of abnormal cell division in *Vicia faba* root meristems (Ren et al., 2019). The pesticides Malathion and Phosphine induced high percentages of abnormal cell divisions (mitoses) at the tested concentrations. Malathion at 1% concentration led to 13.10% abnormal mitoses, while Phosphine led to 21.25% abnormal mitoses, indicating that these pesticides were highly damaging to cells. The insecticides induced DNA and chromatin abnormalities in the insects, including chromosome stickiness and sticky bridges, which affected up to 15.13% of cells after exposure to 4EC of phosphine. Cells with chromosomes that exhibited excessive stickiness tended to dominate the population. The presence of chromosomal stickiness indicated irreversible cellular damage that could lead to cell death (White and Claxton 2004). The stickiness observed between chromosomes has been attributed to several molecular level effects, including the breakdown of DNA polymers into shorter segments, the partial unravelling of nucleoproteins, the fracturing and rejoining of the fundamental folded structural units that make up chromatids, and the removal of proteins that coat the DNA within chromosomes (Mattar *et al.*, 2014) (Fig. 2; Table 2).

Light Crude Oil in Soil

The research investigated how crude oil exposure impacted the plant's cells, tissues, and DNA (i.e. phytotoxic, cytotoxic, and genotoxic effects). Crude oil is a widely used fossil fuel for energy in homes and industry (Balota *et al.*, 2005). Pollution from crude oil alters the structure and acidity level (pH) of soil. These changes to the soil then impact the plants growing in the soil [58]. The phytotoxic effects of light crude oil on soils were assessed by measuring the germination of seeds and the length of roots that developed (Daami-Remadi *et al.*, 2009). A decline in root length and seed germination was noticed when vicia faba plant seeds were exposed to various concentrations of light crude oil (Table 3).

According to (Table 4), root tips of Vicia faba L. that come into contact with crude oil are susceptible to cellular abnormalities and micronucleus formation. In addition to the amount of crude oil present in California soil, various factors can influence the concentrations of crude oil and impact the risk of adverse effects on Vicia faba L. root tips (Foltête *et al.*, 2012). The highest levels of chromosomal aberrations were found in seeds contaminated with 2% oil. Even though the contaminated seeds contained only about half as much oil as the control seeds, the levels of C.A. in the contaminated seeds were approximately 65 percentage points higher. There was no statistically significant difference between the levels of soil contamination at 1 and 4 percent ($p > 0.05$). There is no significant difference between these values.

Nano Fertilizers (nano zinc, nano Phosphorus)

To ensure healthy growth of plants, it is essential to fertilize the soil (Foltête *et al.*, 2012; Ahmed et al., 2023). The use of these fertilizers can pose an environmental problem and potentially suffocate the soil with their application (Dhyèvre *et al.*, 2014). The development of nanotechnology-based fertilizers helped address this problem. Nanoparticle fertilizers are able to be fully absorbed by root hairs due to their small size, enabling more efficient uptake of nutrients. The nano-scale of these fertilizers guarantees that they can be readily absorbed and utilized by plants (Cavusoglu *et al.*, 2010). The high

Fig. 2: Abnormal mitotic cells at different mitotic phases captured in V. faba plant's root-tip meristem cells, germinated from seeds dusted with Malathion 1% powder and other fumigated with Phosphine in 400X shows the chromo-toxic (DNA liquefaction), turbo-genic effect (spindle fiber alteration) and mutagenic effect (micronucleus formation). (A) Stickiness in interphase, (B) Stickiness in prophase, (C) Chromosome breaks, (D) Chromosome breaks and chromosome bridge, (E) Chromosome bridge and micronuclei, (F) Chromatin bridge, (G) Lagging chromosome and chromosome bridge in anaphase, (H) Lagging chromosome in metaphase, (I) Micronuclei (1/10 from the size of the mean nucleus), (J) Micronuclei (1/3 from the size of the mean nucleus), (K) Multinuclei cell, (L) Unequal distribution, (M) multipolar cell and chromosome bridge, (N) C-metaphase, (O) C-anaphase.

Table 5: Types or number of abnormalities recorded in the mitoses of *Vicia faba* root-tip meristems

Treatment	Concentrations	% of different types of abnormal mitoses/scored number		
		Chromatin material Liquefaction	Chromosomal kinetic	Chromosome structural
PVP+ZnO	0.50%	20.76	71.69	5.67
	1.00%	33.33	66.66	0.00
	2.00%	27.59	68.97	3.45
Pvp+Glycerol+H_3PO_4	0.50%	33.78	54.05	12.16
	1.00%	18.87	58.49	22.65
	2.00%	24.29	74.29	1.43
PVP+ Glycerol + ZnO+ H_3PO_4	0.50%	4.31	54.9	19.61
	1.00%	16.36	78.17	5.46
	2.00%	28.07	67.54	3.5
Control		30.00	70.00	0.00

Treatment with three prepared nanoparticles stabilized PVP, prepared via gamma irradiation: PVP= Polyvinylpyrrolidone; ZnO= Zinc oxide; H3PO4= Phosphoric acid.

surface area of nanoparticles magnifies the benefits to plants. However, the direct effects of nanotechnology-based fertilizers on plants, both beneficial and adverse, must be thoroughly studied and evaluated before they are widely applied (Beig *et al.*, 2022).

The three nano-scale chemical fertilizers induced different types of chromosomal abnormalities. These abnormalities fell into three classes: those caused by heat (thermogenic), those caused by breakage (clastogenic), and those caused by toxicity to chromosomes (chromo-toxic). The three nano preparations primarily disrupted the spindle apparatus, which is composed of microtubules, and affected chromosome movement. This led to abnormalities in cell division, including disrupted prometaphase progression and clumped chromosomes, in a percentage of cell (Jain *et al*., 2004). The toxic effects on chromosomes were the second major impact; the changes caused chromosomes' genetic material to become more viscous, leading chromosomes to physically stick together. This adhesion prevented the proper separation and division of the full set of chromosomes between daughter cells during cell division. The clastogenic effect directly impacts the histone proteins that comprise the core of chromosomes, but this effect accounted for the smallest percentage of effects in the study. At lower tested concentrations, zinc oxide nanoparticles accelerated mitosis in meristematic root cells. However, they also induced mitotic abnormalities, including chromosomal misorientation, lagging chromosomes, chromosome bridges, and chromosome stickiness[3]. The study suggested that, although the usage of low concentration of zinc nanoparticles gave us hope for its non-toxicity (Bolsunovsky *et al*., 2019). Although zinc nanoparticles may increase cell division and chromosome abnormalities when used at high concentrations, using them at low concentrations can enhance plant growth without significantly impacting chromosome structure (Table 5). This study identified an approach to produce zinc nanoparticles in an environmentally friendly way that minimizes risks of toxicity and genetic damage (Mustapha *et al*., 2019).

Conclusion

Higher plants are valuable genetic models for detecting environmental mutagens. They are frequently utilized in monitoring studies to assess DNA damage and other genetic effects from environmental agents. One of the most widely used plants for this purpose is Vicia faba, the common vetch. The *V. faba* bioassay test enables the evaluation of multiple types of genetic damage, including chromosome aberrations and disturbances in the mitotic cycle. As this review highlights, the V. faba test is a fast and sensitive method for detecting genotoxins and mutagens in the environment under various stress conditions. Beyond its ability to assess multiple genetic endpoints, the V. faba test can also provide insights into the mechanism of action of tested agents on the DNA of exposed organisms. By elucidating how environmental contaminants interact with and damage DNA, the V. faba bioassay can help determine the effectiveness and severity of the contamination. Therefore, this plant-based test system serves as a useful screening tool for environmental monitoring. Its results can alert researchers to conduct additional testing to fully characterize and evaluate the contamination that is detected. Overall, the V. faba bioassay test is a valuable technique for assessing genetic damage from environmental pollution and warning of potential risks to other species.

Significance Statement

The significance of this study is the comprehensive monitoring of the environment to establish laws and standards that ensure its cleanliness. Traditional chemical analysis methods, which are costly, involve large amounts of toxic and harmful solvents, and often do not adhere to the principles of Green Chemistry, need to be replaced or complemented by reliable and environmentally-friendly analytical techniques that meet Green Analytical Chemistry requirements. This study demonstrates the use of *V. faba* bioassays as complementary alternatives to traditional analysis methods that satisfy Green Analytical Chemistry criteria and comply with environmental monitoring laws and regulations. The aim is to provide a potential solution to environmental monitoring through bioassays that complement or replace traditional techniques, ultimately contributing to a cleaner and safer environment.

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Retraction Note: We regret to inform you that this article has been retracted at the request of the authors. This decision came in light of the discovery of undisclosed conflicts of interest (authorship, competing interests) that compromise the integrity and objectivity of the research findings presented in the article. After thorough examination and discussion with the authors, it became apparent that certain conflicts of interest existed which were not disclosed at the time of publication. As this journal is committed to upholding the highest standards of transparency and ethical conduct in scholarly publishing, we take such matters very seriously.