Alqalam Journal of Medical and Applied Sciences. 2025;8(4):2808-2813
https://doi.org/10.54361/ajmas.2584105

Original article

Dose –Response Analysis of *Moringa oleifera* (From Jalu City, Libya) Leaf Aqueous Extracts on Mitotic Indices and Chromosomal Aberrations in *Allium cepa* Bioassay

Salem El shatshat*, Fawzia Al Jazwi, Salma Hamad Idris

Department of Botany, Faculty of Science, University of Benghazi, Benghazi, Libya
Corresponding author. salem.elshatshat@uob.edu.ly

Abstract
This study used the *Allium cepa* bioassay to investigate the cytotoxicity and genotoxicity effects of water leaf extracts from Moringa trees, a plant recently introduced to Libya. Root tips were treated with different extract concentrations (0%, 2.5%, 5%, 10%) for various durations. The findings revealed a complex dual effect. Higher concentrations and longer exposure times significantly stimulated cell division, dramatically increasing the mitotic index by over threefold in some cases. However, the extracts were also clastogenic, including chromosomal abnormalities like sticky chromosomes and C-metaphase. Notably, the highest percentage of abnormal cells (25.65%) occurred at a moderate concentration (5%) after only 3 hours, suggesting potent genotoxicity at certain exposure levels. The results indicate that *M. oleifera* leaf extracts can act as both a mitotic stimulant and a genotoxic agent, with effects critically dependent on concentration in traditional medicinal use.
Keywords. Libya, *Moringa Oleifera,* Cytotoxicity, Chromosomal Abnormalities, Aqueous Extracts.

Introduction

Moringa oleifera is a native tree to the northern Indian subcontinent and has been cultivated across diverse climates worldwide [1], including the Red Sea regions, parts of Europe, and much of Asia, Africa, and the Latin Americas (Figure 1). It is known by several common names, including the drumstick tree, horse radish tree (a name derived from the taste of its roots), and the ben oil tree [2].

The Moringa tree is primarily cultivated in semiarid, tropical, and subtropical climates. It can adapt to a wide range of soil types but grows best in well-drained, sandy or loamy soils with a neutral to slightly acidic pH [3]. Its drought resistance makes it particularly suitable for dry regions, as it can often be cultivated using only rainwater without relying on advanced irrigation techniques. The tree is widely grown for its young seed pods and leaves, which are consumed as vegetables and used in traditional herbal medicine [4]. While officially categorized as an invasive species in some countries, current evidence suggests that Moringa has not been observed invading intact ecosystems or displacing native plants. therefore, it should currently be regarded as a widely cultivated species with low invasive potential.

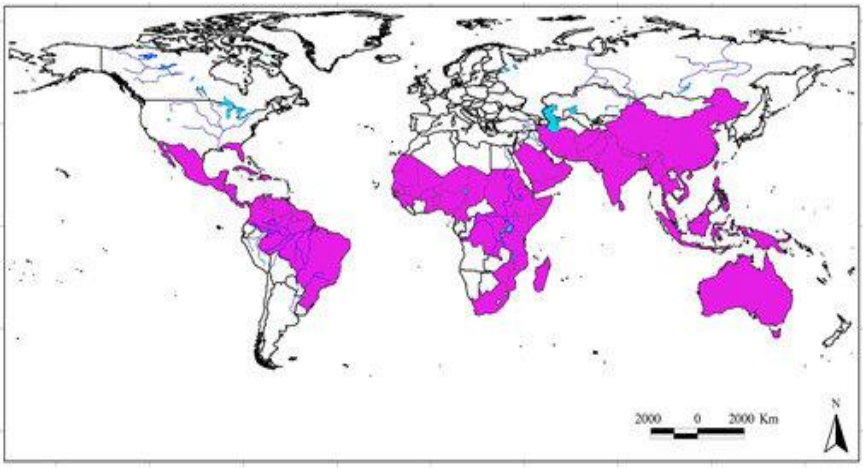


Figure 1. The distribution of Moringa oleifera in the world according to Navie and Csurhes [5]. Note, it was not marked in Libya until this time.

In Libya, the Moringa plant was known from 2018, and till the present, it has not been recorded in the Libyan flora. Recently, the species has spread to different regions and areas of the southeastern parts of the country. The date and method of introduction of this plant to Libya is uncertain unclear enough but may have been relatively recent (2015). The species is thought to have been introduced from neighboring countries, especially Sudan, Tunisia, and Egypt, or might have been imported as an ornamental plant by some farmers. The historical use of Moringa in ancient medicinal practices is well-established. As noted by Farooq et al. [6], an ancient culture as early as 150 B.C. incorporated it into their diets for benefits like healthy skin and mental alertness. This long-standing traditional use is supported by the plant's

Received*: 10-10-2025 -* ***Accepted****: 08-12-2025 -* ***Published****: 15-12-2025*

composition, which contains over 40 natural antioxidants, vitamins, minerals, and more, highlighting its exceptional medicinal properties and its ability to fulfill critical healthcare roles.

Like any other medicinal plant, Moringa is becoming widely used in Folk medicine and for nutritional purposes in Libya, even though it appeared recently in the country, without any information about its genotoxic or phytotoxic effects when it grows under Libyan environmental conditions. This required more investigation and research. For nearly a century (from 1938), scientists have used the common onion (*Allium cepa*) as a simple, powerful tool to test whether chemicals, radiation, medicinal plant extracts, or other agents can damage the genetic material [7; 14]. This process, known as the Allium test, works by examining the onions' chromosomes for abnormalities after exposure. The onion's roots are sensitive, and when exposed to something harmful, its cells show clear, visible signs of stress under a microscope. These signs can include stunted root growth or misshapen chromosomes, which can even lead to cell death. Onions are cheap, easy to work with year-round, and have large, easy-to-count chromosomes [15; 16]. This method is so reliable that it is officially endorsed by international organizations like WHO and UNEP.

For safe use, it is best to adhere to recommended dosages and consult with a healthcare professional. While some studies reported that dosage is critical and noted potential toxicological effects, including genotoxicity, at very high, unrealistic doses. This underscores the importance of dosage and that a "natural" dose automatically means safe at any amount. Because of scares of information about this plant in Libya and its effects on genotoxicity and cytotoxicity levels, this study was aimed and designed to evaluate the cytotoxic and antimitotic activities of different leaf aqueous extract concentrations of *Moringa oleifera* by using *Allium cepa* bioassay.

Methods

Plant material

Leaves of *Moringa oleifera* collected from Jalu city, which is located in the southeast of Libya, and it is far around 400 kilometers away from Benghazi at the end of 2023. Climatically, the study area is located in the arid zone, with a dry season of 11 months per year. The plant was classified and identified according to the herbarium of the Botany department at Benghazi University.

Preparation of aqueous extracts

Leaves were transported to the laboratory, cleaned, and left at room temperature (22°C ±2) in a place protected from sunlight for 7 days (air drying). After drying, the leaves were ground using a blender to a fine powder and weighed using a balance as follows: (2.5g, 5g, and 10g). The dried powdered material was mixed in 100 mL of distilled water, and the mixtures were homogenized for 2 h in a shaker, then, were filtered through Whatman filter paper. Distilled water was used as a control (0%).

Onion bulb germination and treatment

The protocol followed for the *Allium cepa* L. test was that of Fiskesjö [17] with modifications made by Shweta *et al.* [18]. The equal-sized *Allium cepa* bulbs were collected from the local market in Benghazi city. The bulbs were placed in small glass (50ml) containers filled with distilled water and allowed to sprout roots for approximately 3 days at room temperature (22±2°C) in the darkness. After bulb sprouting when the primary root tips are 0.5-1 cm long, the bulbs were transferred to clean and dried containers, including the treatments with a series of concentrations of leaf extract (0%, 2.5%, 5% and 10%) for different time intervals, 3, 6, 12, and 24h.

Fixation and slide preparation

After 3, 6, 12, and 24h of treatment, six roots from each treatment and control bulb were collected. For cytological preparation as described by Fiskesjö [19], the root tips were fixed in ethanol /glacial acetic acid (3:1 v/v) for 24h. at 5°C. The samples were then stored in glass bottles in the preservative 70% ethanol for later use. Slides were prepared using the aceto-carmine squash technique, by hydrolyzing the root tips in 1N HCl at 60°C for 12 min. and staining with aceto-carmine stain for 24h. The meristematic region of the root was removed and washed with 45% glacial acetic acid on a glass slide, squashed in Glycerol, mounted with a cover slip, and temporarily sealed with clear fingernail polish. Slides from each treatment were examined by light microscope at 400X magnification to determine the mitotic index and mitotic aberrations for each concentration and treatment interval time. Types and percentages of abnormalities were also recorded.

Statistical analysis

The data were analyzed by SPSS (two-way ANOVA). The sample number was 6 treated root tips with different concentrations. (LSD) were presented as means with standard deviation (SD). The statistical significance by analysis of mitotic index (MI) and chromosomal aberrations differences at p value <0.05 was considered.

Received*: 10-10-2025 -* ***Accepted****: 08-12-2025 -* ***Published****: 15-12-2025*

https://doi.org/10.54361/ajmas.2584105

Results

Effect on Cell Division (Mitotic Index MI)

The extract significantly altered the progression of cell division. With increasing concentration and exposure time, there was a marked accumulation of cells in the prophase stage (Table 1). This effect was most extreme at the 5% concentration, where 100% of the dividing cells were arrested in prophase at both the 12 and 24-hour time points, indicating a potent disruption of the cell cycle (Table 1).

Table 1. Mean of mitotic index and percentage of phases in the root tip of Allium cepa exposed to the aqueous extract of Moringa oleifera leaves (*)

T (h)	Conc. (g/100ml)	Total cells	Dcs	x MI ±SD	Pro%	M%	An%	T%
3	Control	4490	334	7.438 ± 1.23	82.34	4.79	7.19	5.68
	2.5	9877	600	6.07 ± 0.42	66.50	16.33	9.17	8
	5	9492	1104	11.6 ± 0.60	82.07	10.69	3.98	3.26
	10	3477	739	21.25 ± 2.19	93.23	4.34	1.08	1.35
6	Control	3468	194	5.59 ± 0.33	64.44	14.95	9.79	10.82
	2.5	5692	398	6.99 ± 0.52	90.20	5.28	1.26	3.26
	5	7002	359	5.127 ± 0.69	96.94	2.50	0.28	0.28
	10	4499	600	13.34 ± 0.34	93.00	2.84	2.16	2
12	Control	3332	206	6.180 ± 0.10	67.96	10.68	12.62	8.74
	2.5	4900	545	11.12 ± 1.38	94.13	1.47	1.65	2.75
	5	4939	1314	26.6 ± 0.29	100	0	0	0
	10	9895	2519	25.457 ± 0.71	98.64	0.65	0.04	0.67
24	Control	5549	1050	18.92 ± 0.67	87.17	5.79	2.14	4.90
	2.5	4483	2277	50.79 ± 1.62	99.82	0.04	0.05	0.09
	5	3859	2471	64.03 ± 1.13	100	0	0	0
	10	5527	2643	47.82± 0.89	99.96	0	0	0.04

** T(h)=Treatment Time, Conc=Concentration, Dcs=Dividing cells, xMI= Mean of Mitotic index, SD=Standard deviation, Pro%=prophase, M%=Metaphase, Ana%=Anaphase. T%=Telophase.*

Effect of Exposure Time and Dose on Cell Division:

The extract had a highly significant and time- concentration -dependent effect on the mitotic index (Figure 2). Contrary to a simple effect, higher concentrations (5% and 10%) and longer exposure times (12h and 24h) generally stimulated cell division, leading to a dramatic increase in MI compared to the control. At 24h after treatment, the MI of 5% was 64.05, which was over three times the control value of 18.77. The only indication of mitotic inhibition was observed at the 6h time for the 5% concentration, which showed a slight decrease (Figure 2).

Induction of Chromosomal Abnormalities

The extract was clastogenic, meaning it induced various structural chromosomal abnormalities. The type and frequency of aberrations were highly dependent on concentration and exposure time (Figures 3 and 4). The most common abnormalities observed were condensed prophase and C- C-metaphase (a failure of the spindle apparatus), which were prevalent across many treatment groups. Other observed abnormalities included sticky anaphase, micronuclei, laggard chromosomes, and chromosomal fragments (Table 2). A notable finding was that the highest percentage of abnormal cells (25.6%) was recorded at the 3-hour time point with the 5% concentration (Figure 3), suggesting that acute exposure to moderate concentrations may be more genotoxic than longer exposures.

Received*: 10-10-2025 -* ***Accepted****: 08-12-2025 -* ***Published****: 15-12-2025*

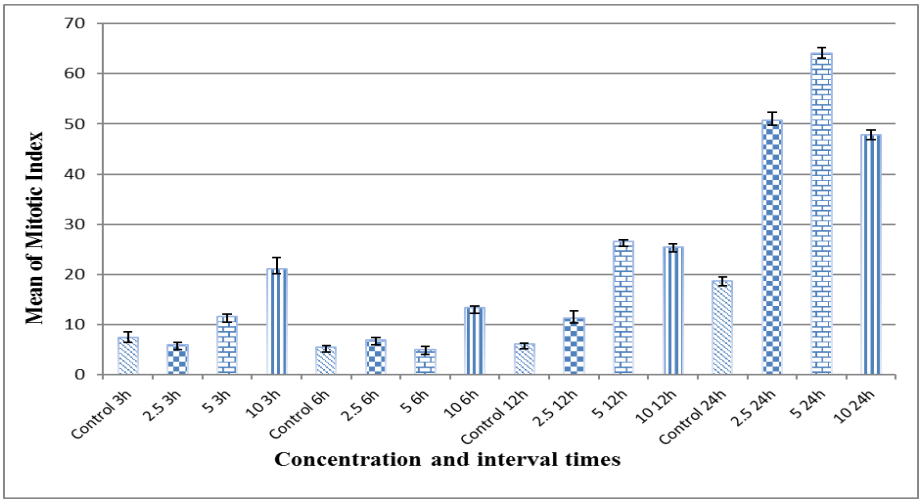


Figure 2. Mean of mitotic index in the root tips of Allium cepa exposed to the aqueous extract of Moringa oleifera leaves

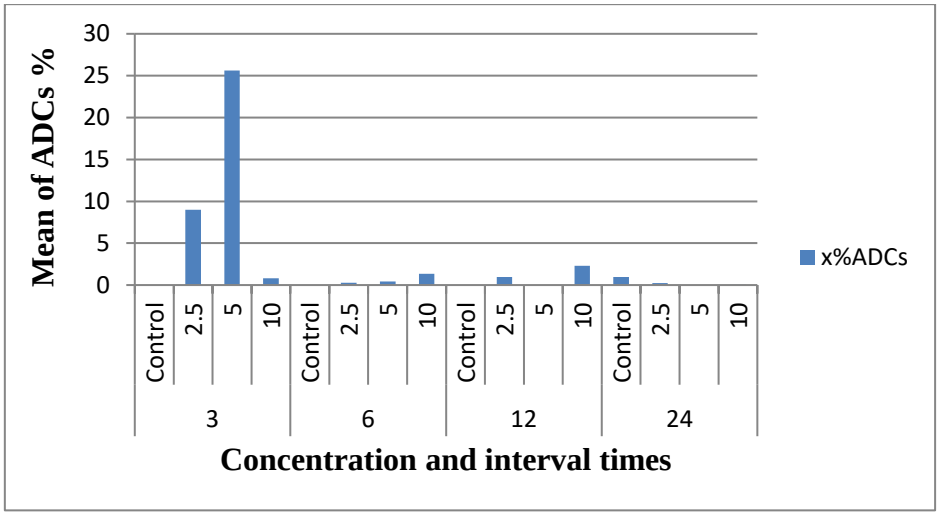
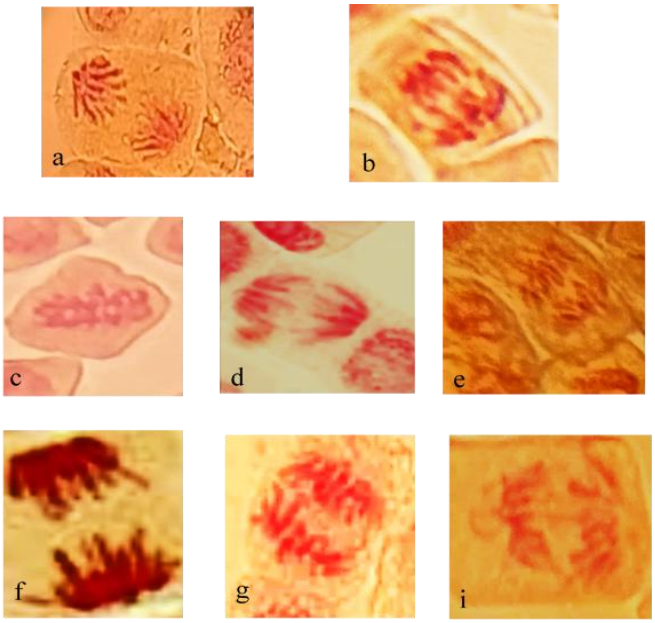


Figure 3. Mean of chromosomal abnormalities in the root tips of Allium cepa exposed to the aqueous extract of Moringa oleifera leaves for different times.



Figures 4. Various types of chromosomal aberrations in metaphase under the influence of aqueous extracts of Moringa oleifera leaves at 400X magnification. (a) normal Anaphase, (b) Multi-bridge Anaphase, (c) Sticky Anaphase, (d) Anaphase with micronucleus, (e) Multibridge and multipolar anaphase, (f) Fragment Anaphase, (g) Bridge and multipolar anaphase, (i) Bridge Anaphase

Received*: 10-10-2025 -* ***Accepted****: 08-12-2025 -* ***Published****: 15-12-2025*

Discussion

The *Allium cepa* test is one of the established plant test systems for studying such mitotic abnormalities and is validated by the UNEP and WHO. The results of this study were consistent with a number of studies that used the above test [7; 10; 14; 15]. The key findings reveal a dual role for the extracts, as they can significantly stimulate cell division but also induce genetic abnormalities, with the effects being highly dependent on concentration and exposure time.

Most concentrations, particularly the higher ones, caused a statistically significant increase in the mitotic index compared to the untreated control group. A direct concentration-dependent relationship was observed, where higher concentrations led to greater cell division activity. This effect is likely attributed to the extract's rich content of plant growth hormones, such as auxins, gibberellins, and cytokinins, as well as various phytochemicals, which are known to promote the cellular processes required for division. These findings align with previous studies that reported a higher mitotic index following Moringa treatment [8; 20; 21; 22; 23].

The extract also disrupted the normal progression of mitosis. A significant increase was observed in the prophase index, while corresponding with a decrease in metaphase cells. This suggests that the leaf extract may cause a delay or arrest in the cell cycle at the prophase stage. The indices for the later anaphase and telophase stages significantly decreased. These results were also reported in a number of studies [24- 26].

Despite stimulating division, the *Moringa* leaf extracts were found to be genotoxic, causing various structural and numerical chromosomal abnormalities (Figure 4). Common aberrations included C-metaphase (failure of chromosome alignment), sticky chromosomes, chromosomal bridges, lagging chromosomes, and micronuclei, which appeared as types of abnormalities. The frequency of these abnormalities was highest at specific lower concentrations (e.g., 2.5% and 5%) and shorter exposure times (3hours). The study suggests that at these levels, the leaf extract's phytochemicals are bioavailable enough to cause genetic damage without immediately killing the cells [27]. At higher concentrations (10%), the number of observed abnormalities decreased. This is interpreted as a sign of heightened cytotoxicity [28], where the extract is so toxic that it strongly inhibits cell division altogether, leaving fewer dividing cells in which to observe aberrations.

Conclusion

In conclusion, the Allium cepa assay confirmed that Moringa leaf extracts exert a dual biological effect: they enhance mitotic activity through the action of plant growth hormones and phytochemicals, yet simultaneously disrupt normal cell cycle progression and induce genotoxic abnormalities. The outcomes demonstrate a clear concentration-dependent relationship, with moderate doses promoting division but also increasing chromosomal aberrations, while higher doses suppress mitosis altogether due to cytotoxicity. These findings underscore both the potential and the risks of plant-derived bioactive compounds, highlighting the need for careful dose consideration and further mechanistic studies to balance their stimulatory properties with their genotoxic effects.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Singh AK, Rana HK, Tshabalala T, Kumar R, Gupta A, Ndhlala AR, Pandey AK. Phytochemical, nutraceutical, and pharmacological attributes of a functional crop Moringa oleifera Lam: an overview. S Afr J Bot. 2019;129:209–20.
2. Olson ME; Flora of North America Committee, editors. eFlora summary: Moringaceae: Drumstick Family. Flora of North America North of Mexico. Vol. 7. New York: Oxford University Press; 2010. p.167–9.
3. Radovich T, Elevitch CR, editors. Farm and forestry production and marketing profile for Moringa. In: Specialty Crops for Pacific Island Agroforestry. 2011.
4. Kalibbala HM, Wahlberg O, Hawumba TJ. The impact of Moringa oleifera as a coagulant aid on the removal of trihalomethane (THM) precursors and iron from drinking water. Water Sci Technol Water Supply. 2009;9(6):707–14.
5. Navie S, Csurhes S. Weed risk assessment: Horseradish tree, Moringa oleifera. Biosecurity Queensland, Department of Employment, Economic Development and Innovation, Queensland Government. 2010. 26 p.
6. Farooq F, Rai M, Tiwari A, Khan A, Shaila. Medicinal properties of Moringa oleifera: an overview of a promising healer. J Med Plants Res. 2012;6:4368–74.
7. Andrade LF, Campos JMS, Davide LC. Cytogenetic alterations induced by SPL (spent pot liners) in meristematic cells of plant bioassays. Ecotoxicol Environ Saf. 2008;71:706–10.
8. Bakare AA, Adeyemi AO, Adeyemi A, Alabi OA, Osibanjo O. Cytogenotoxic effects of electronic waste leachate in Allium cepa. Caryologia. 2012;65:94–100.
9. Frescura VD, Laughinghouse SIHDV, Tedesco SB. Antiproliferative effect of the tree and medicinal species Luehea divaricata on the Allium cepa cell cycle. Caryologia. 2012;65:27–33.

Received*: 10-10-2025 -* ***Accepted****: 08-12-2025 -* ***Published****: 15-12-2025*

10. Ray S, Kundu LM, Goswami S, Roy GC, Chatterjee S, Dutta S, Chaudhuri A, Chakrabarti CS. Metaphase arrest and delay in cell cycle kinetics of root apical meristems and mouse bone marrow cells treated with leaf aqueous extract of Clerodendrum viscosum Vent. Cell Prolif. 2013;46:109–17.
11. Chaudhuri A, Ray S. Antiproliferative activity of phytochemicals present in aqueous extract of Ampelocissus latifolia (Roxb.) Planch. on apical meristem cells. Int J Pharm Biol Sci. 2015;6:99–108.
12. Barman M, Roy S, Ray S. Colchicine-like metaphase and cell cycle delay inducing effects of leaf aqueous extract of Clerodendrum inerme (L.) Gaertn. in Allium cepa root apical meristem cells. Cytologia. 2020;85:197–201.
13. Firbas P, Amon T. Combined chemical analysis, fish micronuclei, and onion chromosome damage for assessing the cleaning effect in the WWTP central Domžale-Kamnik and the quality of Kamniška Bistrica River. Cepal Rev. 2017;121:2825–42.
14. Dimitry S, Pesnya A, Romanovsky V. Comparison of cytotoxic and genotoxic effects of plutonium-239 alpha particles and mobile phone GSM 900 radiation in the Allium cepa test. Mutat Res. 2013;750:27–33.
15. Cabrera GL, Rodriguez DMG. Genotoxicity of soil from farmland irrigated with wastewater using three plant bioassays. Mutat Res. 1999;426(2):211–4.
16. Gomes KM, Oliveira MV, Carvalho FR, Menezes CC, Peron AP. Cytotoxicity of food dyes sunset yellow (E110), bordeaux red (E-123), and tartrazine yellow (E-102) on Allium cepa L. root meristematic cells. Food Sci Technol. 2013;33:218–23.
17. Fiskesj G. The Allium-test as a standard in environmental monitoring. Hereditas. 1985;102:99–112.
18. Shweta S, Khadabadi S, Ganesh TG. In vitro antimitotic, antiproliferative, DNA fragmentation, and anticancer activity of chloroform and ethanol extract of Revia hypocrateriformis. Asian Pac J Trop Dis. 2012;S:503–8.
19. Fiskesj G. The Allium test in wastewater monitoring. Environ Toxicol Water Qual. 1993;8(3):291–8.
20. El-Awadi H. The mutagenic effect of some chemical fertilizers on Allium cepa and Vicia faba plants [dissertation]. Cairo: Ain-Shams University; 1996.
21. Fuglie LJ. New uses of Moringa studied in Nicaragua. ECHO Dev Notes. 2000;68:1–2.
22. Abdalla MM. The potential of Moringa oleifera extract as a biostimulant in enhancing the growth, biochemical, and hormonal contents in rocket (Eruca vesicaria subsp. sativa) plants. Int J Plant Physiol Biochem. 2013;5(3):42–9.
23. Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive components in Moringa oleifera leaves protect against chronic disease. Antioxidants (Basel). 2017;6(4):91.
24. El-Bayoumi AS, Kabarity A, Habib A. Cytological effects of papaverin hydrochloride on root tips of Allium cepa L. Cytologia. 1979;44:745–55.
25. Soliman MI. Genotoxicity testing of neem plant (Azadirachta indica Juss) using the Allium cepa chromosome aberration assay. J Biol Sci. 2001;1:1021–7.
26. Irana K. Synthetic and natural coumarins as cytotoxic agents. Curr Med Chem Anticancer Agents. 2005;5:29–46.
27. Phan TT, Wang L, See P, Grayer RJ, Chan SY, Lee ST. Phenolic compounds of Chromolaena odorata protect cultured skin cells from oxidative damage: implications for cutaneous wound healing. Biol Pharm Bull. 2001;24:1373–9.
28. Çavuşoğlu K, Macar TK, Macar O, Çavuşoğlu D, Yalçın E. Comparative investigation of toxicity induced by UV-A and UV-C radiation using the Allium test. Environ Sci Pollut Res. 2022;29:33988–98. doi:10.1007/s11356-021-18147.

Received*: 10-10-2025 -* ***Accepted****: 08-12-2025 -* ***Published****: 15-12-2025*