

CYTOTOXICITY AND GENOTOXICITY OF INSTANT NOODLE SEASONING EXTRACT ON *Allium cepa* ROOT

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Abstract

The increasing consumption of instant noodles has raised concerns about their potential health and environmental impacts. This study aimed to investigate the effects of instant noodle seasoning extract on the mitotic index of *Allium cepa* root tip. Using *A. cepa* as a model organism, the study compared the mitotic index under different treatments: distilled water (negative control), malathion (0.9 g/L) as (positive control), and instant noodle seasoning (73.3 g/L). The onion root tips were exposed to the treatments for 24 and 48 hours. Roots were fixed with ethanol-acetic acid and stained with 2% acetic-orcein for microscopic analysis. The mitotic index was calculated by counting the number of cells in mitosis out of the total observed cells. The results showed that instant noodle seasoning exhibited an initial mitotic index of $14.6\% \pm 0.04$ in 24 hours, which significantly dropped to $7.4\% \pm 0.01$ after 48 hours ($p = 0.0018$), meanwhile distilled water maintained a high mitotic index of $9.6\% \pm 0.01$ at 24 hours, which increased to $15.2\% \pm 0.03$ at 48 hours with $p = 0.014$. In contrast, malathion had the lowest mitotic index, starting at $7.6\% \pm 0.04$ in 24 hours and decreased to $4.9\% \pm 0.01$ at 48 hours but not significant. Furthermore, both malathion and instant noodle seasoning treatments resulted in various chromosomal aberrations, such as lagging chromosomes and chromosomal stickiness. Hence, these findings highlighted that instant noodle seasoning possesses cytotoxic properties that disrupt normal cell division, with more pronounced effects observed after 48 hours ($p < 0.05$). This study shows the utility of *Allium cepa* as a sensitive model for evaluating the genotoxicity of food additives, offering critical insights into their potential impact on health. Further research is recommended to explore the specific mechanisms of toxicity, including long-term effects of chronic exposure and identification of the active components responsible for the observed cytotoxicity.

Keywords: cytotoxicity, genotoxicity, instant noodle, mitotic index, *A. cepa* root

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Introduction

Instant noodles have become a staple food in most Asian countries due to their affordability, convenience, and especially their flavorful seasoning, which greatly contributes to their widespread (Gulia et al., 2014). To enhance the taste and prolonged shelf life, various food additives are incorporated into the seasoning, such as preservatives and flavors enhancers. Among these, monosodium glutamate (MSG) is commonly used to intensify flavors and has been linked to oxidative stress and mitotic spindle damage, necessitating detailed investigations of their impact on health (Sikander et al., 2017). To evaluate the cytotoxicity and genotoxicity of instant noodle seasoning, onion (*Allium cepa* L.) root meristem cells were employed as a biological model. These roots are highly sensitive to genetic damage caused by

chemicals and are ideal for tests involving chromosomal aberration and mitotic index (Firbas & Amon, 2014). Healthy *A. cepa* have 16 chromosomes ($2n = 16$), and their relative size makes them suitable for detecting morphological changes (Kubalová et al., 2023). The complete cell division process starting from interphase, prophase, metaphase, anaphase, to telophase can be observed in meristem cells. Metaphase chromosomes, with the most condensed chromatin, are ideal studied with an optical microscope (Kubalová et al., 2023). Chromosomal aberrations in *A. cepa* root tips can take various forms, including stickiness, anaphase bridges, and multipolar anaphases (Sabeen et al., 2020).

Hence, this study aims to evaluate the impact of instant noodle seasoning on chromosomes by monitoring damage to *Allium cepa* root meristem cells in terms of genotoxicity and cytotoxicity. The results obtained from this observation can serve as biomarkers for various toxins found in instant noodles seasoning. The toxicity evaluation of instant noodle seasoning extract is of utmost importance, as it may cause several negative consequences for human health. Prolonged and frequent consumption of instant noodles with their seasoning may yield deleterious consequences, including but not limited to the manifestation of severe health issues such as stomach cancer and metabolic syndrome. These health problems illustrate the importance of longitudinal observation, as the negative effects may not be apparent until a considerable amount of time has passed and a significant number of instant noodles have been consumed. Consequently, this research helps to show the health risks that are associated with the dietary habits of eating instant noodles for human well-being. To visually elucidate the impact of instant noodle extract, this study plays a pivotal role in illuminating the effects on the meristem cells of *Allium cepa* L. By employing this renowned biological model, current research aims to determine the chromosomal abnormalities, offering an important insight into the potential hazards posed by instant noodle ingredients.

Methods

Materials and apparatus

The materials used in this research include malathion, ice-cold water, ethanol-acetic acid (3:1), double-distilled water, hydrogen chloride, 2% aceto-orcein, and acetic acid. The apparatus included were beakers, scalpels, forceps, vials, slides, slide covers, needles, a Bunsen burner, a light microscope (Olympus CX-21), and a DINO-EYE camera.

Sample preparation

Onion bulbs were prepared by growing their roots for two weeks in small bottles with sufficient water to cover the roots. These roots were then submerged in three different solutions: malathion (positive control), distilled water (negative control), and seasoning solution for 24 and 48 hours. Malathion was diluted by mixing 0.9 g with one liter of distilled water, while one packet of seasoning (22 g) was dissolved in 300 ml of hot water making up for 73.3 g/L. The concentration of 0.9 g/L for malathion was selected based on its established cytotoxic profile in similar studies (Srivastava & Singh, 2020).

Cytological analysis

Cytological analysis was conducted using the squash method to study chromosome numbers, morphology, and cell division mechanisms. Roots from each treatment were collected and fixed in ethanol-acetic acid (3:1) for 12 hours at room temperature to preserve cell division stages (Gupta, 2023). The roots were then washed with double distilled water and hydrolyzed in 1M hydrogen chloride at 45 °C for two hours before being washed again with double distilled water. For staining, roots were soaked in 2% aceto-orcein for five hours, after which the root meristem was squashed on a clean slide and broken into fine pieces with a needle (Syakhril et al., 2019). Slides were observed under a compound light microscope equipped with a DINO-EYE camera using magnifications ranging from 4X to 100X. For each treatment, three independent root tips (biological replicates) were prepared and squashed onto slides, and at least 100 cells per slide were counted to ensure robust data. The mitotic index was calculated using the formula $MI = (\text{Number of Dividing Cells} / \text{Total Number of Cells}) \times 100$. Division stages (prophase, metaphase, anaphase, telophase) were identified and counted across five fields of view to obtain accurate results.

Chromosome numbers ($2n = 16$ for *Allium cepa*) were checked for variations, such as monosomy

(reduction) or trisomy (addition). Structural aberrations, like the loss of chromosome arms (p or q), were also noted. Chromosomes were primarily examined during metaphase due to their high visibility. A two-way analysis of variance (ANOVA) was conducted to examine the effects of treatment (distilled water, seasoning extract, and malathion) and exposure time (24 h and 48 h) on the mitotic index of *Allium cepa* root cells. Bonferroni's post hoc multiple comparisons test was applied to identify specific group differences. The significance level of $p < 0.05$ was considered statistically significant. All statistical analyses were performed using GraphPad Prism version 10.3.0.

Results and Discussion

Cytotoxic Effects: Mitotic Index

The mitotic index (MI) is a critical parameter to evaluate the cytotoxic effects, as it reflects the rate of cell proliferation. A two-way ANOVA revealed a significant interaction between treatment and exposure time ($F(2,18) = 13.95$, $p = 0.0002$), indicating that the effect of treatment on MI is dependent on the duration of exposure. A significant main effect of treatment was also observed ($F(2,18) = 13.82$, $p = 0.0002$), whereas exposure time alone did not significantly affect the MI ($F(1,18) = 2.12$, $p = 0.1626$). From Table 1, Bonferroni's post hoc test revealed that MI significantly increased in the distilled water (ddH₂O) group from 24 to 48 hours ($p = 0.0148$), suggesting a normal progression of cell division under non-toxic conditions. In contrast, a significant decrease in MI was observed in the seasoning extract group ($p = 0.0018$), implying time-dependent cytotoxic effects. No significant difference was detected in the malathion-treated group ($p = 0.4057$), suggesting a consistent level of mitotic inhibition across time points. Among all treatments, distilled water maintained the highest MI ($15.2\% \pm 0.03$ at 48 hours), indicating optimal conditions for root cell proliferation. The seasoning treatment initially showed a high MI ($14.6\% \pm 0.04$ at 24 hours) but dropped significantly to $7.4\% \pm 0.01$ at 48 hours, suggesting prolonged exposure induces cytotoxicity. This reduction may be attributed to interference with mitotic spindle dynamics, possibly caused by additives such as monosodium glutamate (MSG), as suggested by Khezerlou et al., (2022).

Table 1. Mitotic index of *Allium cepa* root tip cells under different treatments and exposure times.

Time (hours)	Treatments					
	Distilled water		Seasoning		Malathion	
	mean	Std.dev	mean	Std.dev	mean	Std.dev
24	9.6	0.01	14.65	0.04	7.64	0.04
48	15.2*	0.03	7.44*	0.01	4.93	0.01

Values are expressed as mean $\pm$ standard deviation ($n = 3$). *Indicates significant difference compared to 24 h within the same treatment group ($p < 0.05$, Bonferroni post hoc test).

Genotoxic Effects: Chromosomal Aberrations Numerical Aberration

Figure 1 shows the chromosomal of *Allium cepa* root cell in the treatment of double distilled water for 24 hours that are in the anaphase stage. The centromeres are split, and the spindle fibers are pulled, the sister chromatids are pull towards the opposite poles of the cell. Each chromatid, now an individual chromosome, moved to the opposite pole (Ligasová et al., 2023). In the current study, precise chromosome counts during certain stages of mitosis were not feasible due to overlapping chromosomes, especially in metaphase and anaphase stages. This limitation hindered the accurate determination of numerical chromosomal aberrations such as aneuploidy (e.g., monosomy or trisomy). Despite this, qualitative assessments were performed based on chromosomal morphology and organization. For instance, in the malathion-treated group, cells exhibited metaphase misalignment and random chromosomal dispersion suggestive of potential numerical loss (Figure 2).

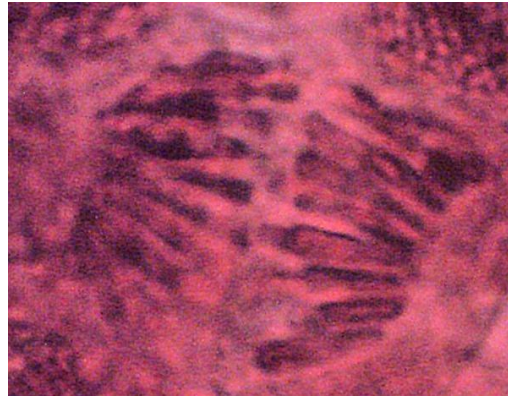


Figure 1. Anaphase stage of mitosis in *Allium cepa* root tip cells treated with distilled water, observed under 40× magnification. Sister chromatids are seen separating equally toward opposite poles, indicating normal chromosomal segregation. However, accurate chromosome counting could not be performed due to overlapping chromosomes.

Figure 2 shows the chromosomal of *Allium cepa* root in malathion for 24 hours, in the disturbed metaphase stage. This cell was observed to have a smaller number of chromosomes compared to the control. It might lose its chromosome due to mutation. However, chromosome counting at these images was unable to be performed due to overlapping chromosomes. From a similar image, the chromosomes are not aligned at the metaphase plate and are distributed randomly in the cell. A mutation might result in half the number of chromosomes, leading to haploid cells, which can arise from errors during cell division (Karadima et al., 1998). In the haploid state, a cell has only one set of chromosomes (n), instead of the usual diploid number (2n) present in the somatic cells of eukaryotic organisms. Somatic cells with haploidy often lead to developmental problems (Mayrose & Lysak, 2021). Malathion can cause cell cycle arrest in metaphase or anaphase by interfering with the development and operation of the mitotic spindle, which is essential for chromosomal segregation (Haschka et al., 2018).

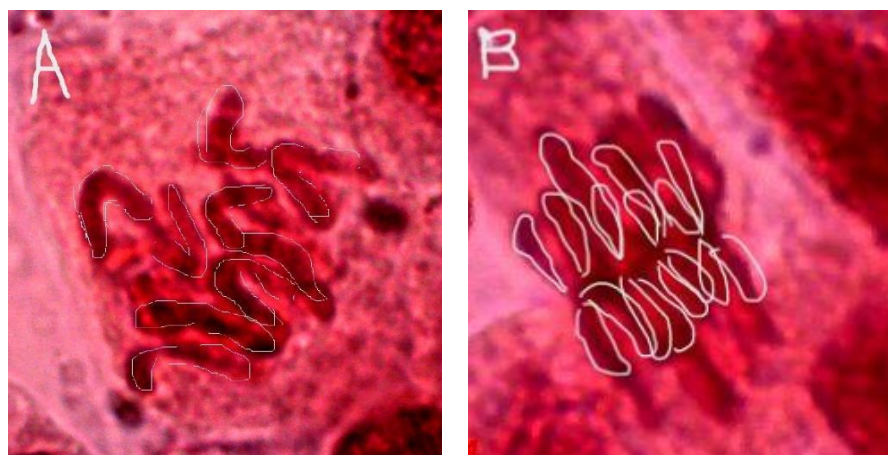


Figure 2. (A) Disturbed metaphase in *Allium cepa* root tip cells treated with malathion for 24 hours, showing disorganized chromosomes and potential numerical reduction due to improper alignment and segregation and (B) Normal metaphase in root tip cells treated with distilled water, with chromosomes properly aligned at the metaphase plate. Images captured under 40× magnification.

Similarly, telophase cells in the seasoning-treated group showed a visibly reduced chromosome count (approximately 13), indicating possible chromosomal loss due to treatment. These observations suggest that instant noodle seasoning and malathion may induce chromosomal mis-segregation or spindle apparatus disruption, even though direct numerical quantification could not be confirmed microscopically (Figure 3). There is chromosomal breakage, and physical breaking of the chromosome can occur due to

chemical substances in seasoning. Telophase is the final stage in mitosis before cytokinesis, during this stage, the chromosomes reach the poles and begin to decondense back into chromatin. A new nuclear envelope forms around each set of chromosomes, resulting in two separate nuclei within the cell (Ayra-Plasencia & Machín, 2019). Under normal conditions, the number of chromosomes in each daughter cell is identical to that of the original parent cell (Wu & Palazzo, 1999). Moreover, misaligned or wrongly connected chromosomes during metaphase can lead to wrong segregation. This is because of problems with the mitotic spindle apparatus (Potapova & Gorbsky, 2017). Prior to the onset of anaphase, the spindle checkpoint makes sure that every chromosome is correctly connected to the genome. Chromosome segregation might go awry if this checkpoint is lost (London & Biggins, 2014). The numerous chemical components of instant noodle seasoning and their possibly harmful effects on cells can result in chromosomal abnormalities, including an inadequate number of chromosomes (Eteng et al., 2023). Seasoning ingredients and preservatives have the potential to be hazardous to cells, disrupting regular cellular functions and harming proteins and DNA involved in chromosome segregation (Granic & Potter, 2013).

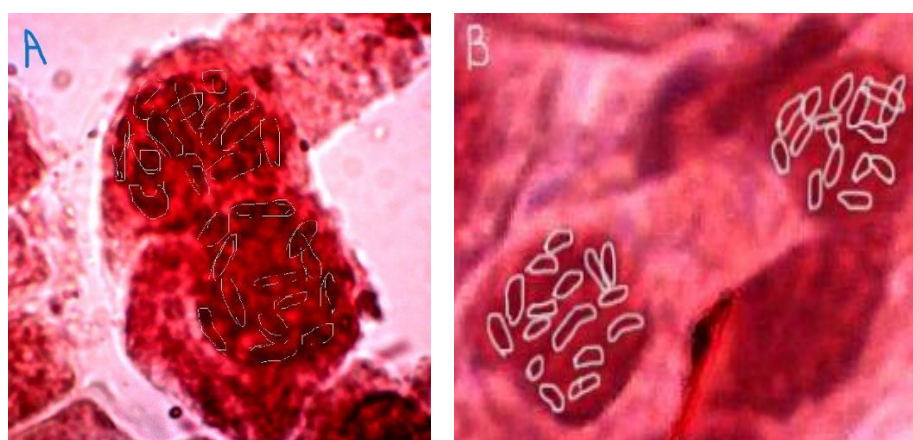


Figure 3. (A) Telophase stage in *Allium cepa* root tip cells treated with instant noodle seasoning, showing an apparently reduced chromosome number (approximately 13), suggesting possible chromosome loss due to chemical exposure and (B) Telophase stage in root tip cells treated with distilled water, showing a typical diploid chromosome number ($2n = 16$). Chromosome counts were estimated based on visible structures under $40\times$ magnification and may be affected by overlapping chromosomes.

Structural Chromosomal Aberrations (SCA)

Due to the low frequency of mitotic cells and the predominance of interphase stages in the treated root tips, quantitative scoring of chromosomal aberrations was not feasible. Therefore, the analysis was conducted qualitatively based on observable chromosomal aberration patterns in available metaphase cells. Figure 4 shows a chromosomes fragmentation of *Allium cepa* root tip cell after 24 hours of submersion in instant noodle seasoning.

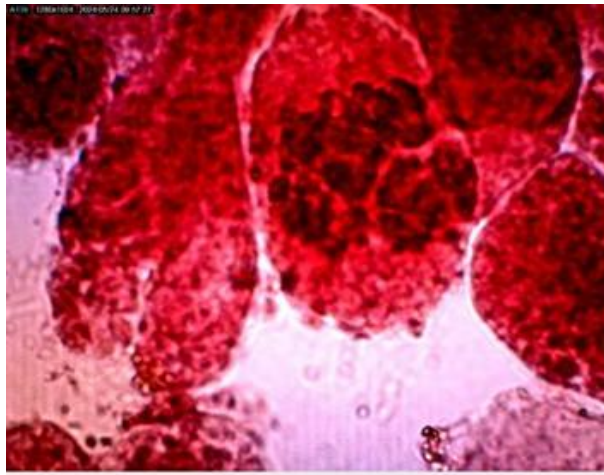


Figure 4. Chromosome fragmentation observed during metaphase in *Allium cepa* root tip cells after 24 hours of seasoning treatment at 40× magnification. Fragmented chromosomes, indicated by unevenly dispersed or scattered chromatin, suggest the presence of genotoxic stress.

The genotoxic effects of instant noodle seasoning in *Allium cepa* root cells were further investigated through the observation of various structural chromosomal abnormalities (SCA), such as chromosomal fragmentation and laggard chromosome. In addition to these microscopic changes, a noticeable cytotoxic effect was observed macroscopically, as the root tip appeared severely dehydrated following treatment (images not shown). This dehydration may be attributed to osmotic stress induced by the seasoning components, which can interfere with water uptake and disrupt normal cellular functions, ultimately inhibiting cell division (Skirycz et al., 2011). Figure 5 shows that chromosome fragmentation during metaphase is a potential indicator for assessing the genotoxic effect of instant noodle seasoning. The cells vary in size and are closely packed together. The chromosomes are long, condensed and scattered in the cytoplasm. The chromosome fragments are much smaller than the rest of the chromosomes (Alberts et al., 2002).

Nevertheless, chromosomal stickiness, as observed in Figure 5(B), may result from the partial breakdown of nucleoproteins, DNA depolymerization, or enhanced chromosomal contraction or condensation. Additionally, the absence of telomeres can cause chromosomes to become ‘sticky,’ leading them to join with other fragmented chromosomal ends. This phenomenon, which reflects harmful effects on cellular integrity, often indicates a failure of chromosomal arm separation, a hallmark of genotoxic stress induced by chemical mutagens and, while typically reversible, can lead to cell death (Bailey & Murnane, 2006; Elmore, 2007). Figure 5(C) illustrates a polar deviation at metaphase. This condition occurs due to the oscillatory movements of chromosomes towards and away from the spindle poles, influenced by forces such as the polar ejection force (PEF) and the pulling forces exerted by microtubules attached to kinetochores (Armond et al., 2015; Sigmund et al., 2024). These oscillations ensure proper alignment and tension distribution before the cell proceeds to anaphase, where sister chromatids are separated and pulled towards opposite poles. Moreover, prolonged prophase as observed in Figure 5(D) is because of disruptions in the normal progression of cell division. These disruptions can be caused by various factors, including genetic mutations that affect cell cycle regulation, exposure to chemical inhibitors that interfere with spindle formation or chromatin condensation, and cellular stress from environmental factors. Such conditions can lead to extended durations of chromatin condensation, delayed nuclear envelope breakdown, and inefficient spindle assembly, ultimately causing the cell to remain in prophase longer than usual (references).

Furthermore, the current investigation demonstrated the presence of lagging chromosomes in Figure 5(E), which occurs when a chromosome fails to remain attached to the spindle fiber and may migrate to either pole (Sabeen et al., 2020). During the important metaphase phase, chromosomes line up at the metaphase

plate, and proper chromosome segregation depends on the correct attachment to the spindle apparatus (Gorbsky, 2015). Many preservatives and chemicals such as monosodium glutamate, sodium benzoate, tartrazine, and silicon dioxide, are included in seasoning. These substances are harmful to the cells and can disrupt the process of microtubule polymerization or depolymerization, which is necessary for healthy spindle activity (Tanabe, 2017). Consequently, the structural chromosomal abnormalities detected in the onion root tip cells may be attributed to the extended exposure to or ingestion of instant noodle seasoning.

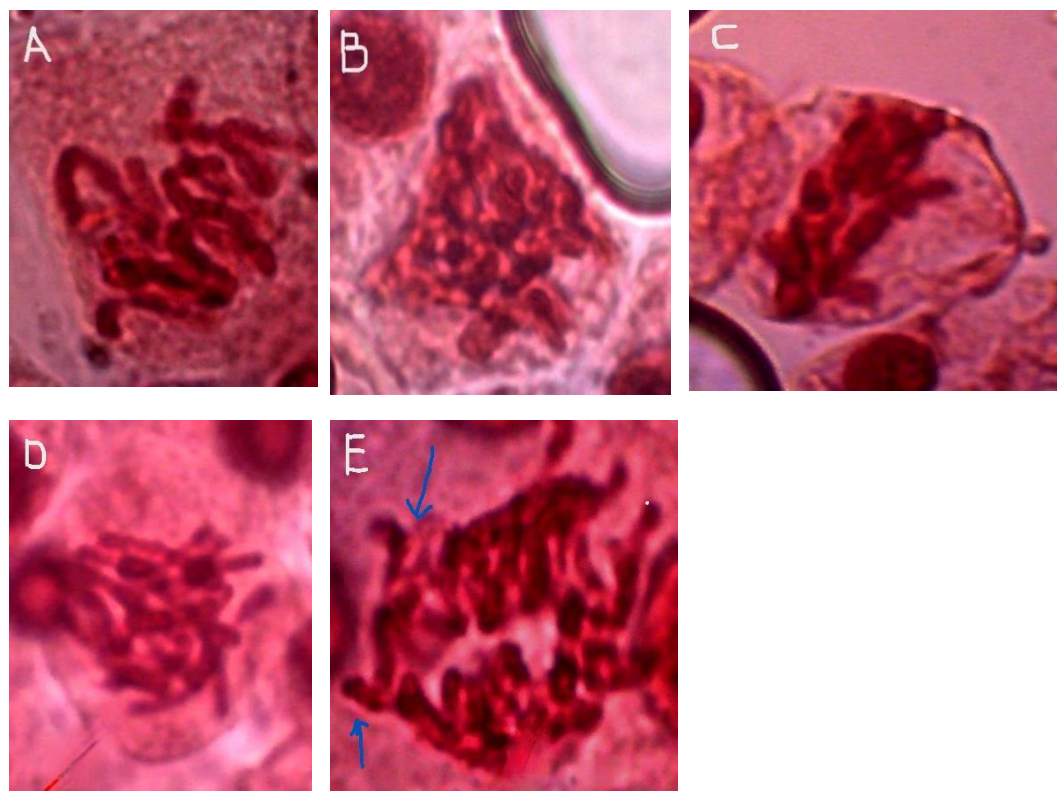


Figure 5. Various types of chromosomal aberrations observed in *Allium cepa* root tip cells after 48 hours of malathion treatment at 40× magnification. (A) Disturbed metaphase, (B) chromosome stickiness at metaphase, (C) polar deviation at metaphase, (D) prolonged prophase, and (E) laggard chromosomes (indicated by arrows) observed during metaphase/anaphase.

Comparative Analysis

While malathion exhibited significant cytotoxic and genotoxic effects, the instant noodle seasoning, typically considered safe, also demonstrated a notable impact on cellular health in *A. cepa*. Statistical analysis using two-way ANOVA revealed that both the treatment type and exposure time significantly influenced the mitotic index as stated earlier. Specifically, while the mitotic index increased in the ddH₂O group over time, the seasoning extract caused a significant decrease in mitotic index, suggesting a potential disruption in cell division. No significant change was observed in the malathion group. These results, supported by statistical tests, highlight the importance of reassessing the safety of food additives and their prolonged exposure effects on cellular functions.

Conclusion

This study examined the cytotoxic and genotoxic effects of instant noodle seasoning on *Allium cepa* root meristem cells, with distilled water and malathion serving as controls. The findings revealed that instant noodle seasoning significantly reduced the mitotic index and induced chromosomal aberrations, particularly after prolonged exposure. While malathion demonstrated the most severe cytogenetic effects, the results highlight that the chemical additives in instant noodle seasoning also pose potential risks to cellular health. These findings emphasize the need for greater awareness regarding the potential adverse

effects of food additives and preservatives on human health and the environment. Future research should aim to isolate the active toxic components of instant noodle seasoning, elucidate the molecular mechanisms underlying their effects, and assess their impacts in mammalian models to validate these findings. Additionally, studies should explore the long-term consequences of chronic exposure and evaluate alternative, safer food processing methods. Advancing our understanding of the cytotoxicity and genotoxicity of widely consumed food products will contribute significantly to the broader discourse on food safety and public health.

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Author Contribution

NA Su Reply conceptualization and data curation; SF Syed Mohamad supervision, writing, review and editing the manuscript. Both authors have read and approved the final version of the manuscript for submission.

Conflict of Interest

Authors declare no conflict of interest.

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