

Research article

Determination of malathion's toxic effect on *Lens culinaris* Medik cell cycleSeir Antonio Salazar Mercado ^{a,*}, Jesús David Quintero Caleño ^{b,**}^a Department of Biology, Universidad Francisco de Paula Santander, Avenida Gran, Colombia No. 12E-96B Colsag, San José de Cúcuta, Colombia^b Department of Agricultural Sciences, Universidad Francisco de Paula Santander, Colombia**ARTICLE INFO****Keywords:**

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ABSTRACT

The present study aimed to determine the toxic effect of malathion pesticide on root growth, cell division and the chromosomal abnormalities frequency using the *L. culinaris* test. Initially, the lentil seeds were subjected to different doses of malathion (0.0 0.5, 1, 2.5, 5, 10, 15, 20, 25 and 30 mg L⁻¹) and during 24, 48, and 72 h, the root length was measured. Subsequently, at 72h, the mitotic index, mitotic inhibition, and cellular abnormalities were calculated for all treatments. According to the obtained results, it was visualized that the root growth was inversely proportional to the concentration of malathion at all times of exposure. After 72h of exposure, the lowest values of the mitotic index and inhibition were presented at malathion concentrations 20, 25 and 30 mg L⁻¹. Additionally, micronuclei cell abnormalities, metaphase sticky chromosomes, split chromosomes, nuclear lesions, irregular anaphase, anaphase bridges, binucleated cells, absence of nucleus and telophase bridge were observed. Finally, Malathion induced mitodepressive and cytotoxic effects in the meristematic cells of the *L. culinaris* root tip. A high frequency of abnormality was found in the micronuclei, which represented an indicator of a high degree of toxicity at the cellular level.

1. Introduction

Pesticides play an important role in pest control, in agriculture, and in non-agricultural settings. They are applied in high concentrations and their residues cause contamination to the environment, representing risks to organisms and human health (Ryberg and Gilliom, 2015; Willison et al., 2019; Elfikrie et al., 2020; Meftaul et al., 2020). Malathion (diethyl (dimethoxythiophosphorylthio) succinate or S-1,2-bis (ethoxycarbonyl) ethyl O, O-dimethyl phosphorodithioate) is one of the most widely used organophosphate pesticides worldwide (Singh and Roy, 2017), as an acaricide and insecticide, it is commonly applied to stored fruit, vegetable and grain crops (Climent et al., 2019). When ingested or inhaled, it quickly passes into the bloodstream, interfering with the nervous system by inhibiting the enzyme cholinesterase, whose function is to inactivate the acetylcholine neurotransmitter in synapses (Bavcon et al., 2003; Wu et al., 2011; Houbraken et al., 2017). Likewise, malathion has been found in the children's nutritional diet of Japan by 4% (Kawahara et al., 2007). Interviewing with several farmers, they described negative health symptoms after pesticide application, including vomiting, headache and eye irritation, and skin conditions (Sumon et al., 2016). Furthermore, according to Mendoza et al. (2015), exposure to Malathion could cause damage to

organs such as kidneys and heart. In rats, the mean lethal dose is 1200 mg/kg, via oral (Gallo and Lawryk, 1991), producing an increase in the activities of catalase, superoxide dismutase, as well as the concentration of malondialdehyde (MDA) in liver and erythrocytes (Akhgari et al., 2003).

According to Bujagić et al. (2019); Radović et al. (2015), there are high concentrations of malathion found in the sediments of Danube and Tisza rivers in Serbia at 5–10 cm depth (2.9 and 69 ng g⁻¹, respectively). The previous concentrations are significantly higher than the minimum levels known (6.7×10^{-3} ng g⁻¹), which negatively affects benthic organisms (Fisher et al., 1993). In surface waters they have found malathion doses of 0.193 µg·L⁻¹ and 25 ng/g, which represents a negative impact on aquatic ecosystems, especially fish, invertebrates and algae (Köck-Schulmeyer et al., 2013; Houbraken et al., 2017; Picó et al., 2018; Triassi et al., 2019). Additionally, it has been found in some amphibian tissues at doses of 0.68 ppm and in soils of 13.06 ppm, affecting the amphibian's liver metabolomics (Climent et al., 2019). Likewise, at malathion concentrations of 0.1 and 1.0 mg/L, larval activity decreases in anuran species (Relyea and Edwards, 2010). In tomato plants specifically in the fruit, they have found malathion doses higher than 1,000 µg/kg in the pericarp, in cabbage of 1000 ng/g and in Chinese cabbage of 330 ppb (Reiler et al., 2015; Wanwimolruk et al., 2015; Picó et al., 2018),

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which represents a high risk of consuming these foods without being washed and processed after harvest, particularly for children. In this sense, it is important to educate farmers to use less dangerous pesticides, in order to guarantee public and ecosystem health (Reiler et al., 2015).

Searching for bioindicators, plants that can detect toxic substances due to their high sensitivity to environmental changes have been used (Salazar and Maldonado, 2019). These have similar characteristics to mammalian chromosomes and are inexpensive to perform various toxicity tests (Abdelsalam et al., 2018; Salazar-Mercado et al., 2019). The plants mostly used as bioindicators in research related to cytotoxic and genotoxic tests are *Allium cepa* L. (Martins et al., 2016; Haq et al., 2017; de Souza et al., 2017; Silveira et al., 2017; Braga et al., 2018; Verma and Srivastava, 2018; Heikal et al., 2019; García-Medina et al., 2020; Salazar and Quintero, 2020), *Pisum sativum* L. (Salazar-Mercado et al., 2019), *Lactuca sativa* L. (Andrade-Vieira et al., 2018), *Zea mays* L. (Reynoso et al., 2015), *Triticum aestivum* L. (Abdelsalam et al., 2018) and in recent studies *Lens culinaris* Medik (Shahwar et al., 2019; Salazar and Maldonado, 2020; Salazar et al., 2020a).

Trials conducted by Singh and Roy (2017), who evaluated Malathion-induced cytogenetic effects using the *A. cepa* test. They found that, at malathion doses of 50, 125, 250, and 375 mgL⁻¹ at different exposure periods, it inhibited root growth, reduced the mitotic index, and produced different chromosomal aberrations. In the literature up till now, no research has been conducted on the malathion toxic effect on *L. culinaris* cells. As stated above, this study aims to determine the toxic effect of the malathion pesticide on root growth, cell division and the frequency of chromosomal abnormalities using the *L. culinaris* test.

2. Material and methods

2.1. Plant material and treatments

Malathion was diluted in distilled water at doses of 0.5, 1, 2.5, 5, 10, 15, 20, 25 and 30 mgL⁻¹ and control of distilled water. For *L. culinaris* seeds germination, it was carried out in Petri dishes with cotton and filter paper under controlled environmental conditions (26 ± 2 in the dark). The seeds were subjected to darkness for three days as described by Salazar and Botello (2018); Salazar et al. (2020b); Salazar et al. (2020c) and root growth was measured at 24, 48 and 72 h. Subsequently, the mitotic index and the frequency of cellular abnormalities were determined.

2.2. Mitotic index and inhibition

After 72 h, the root tips were cut 4 mm approximately, washed with running water and subjected to toluidine blue metachromatic dye for 30 min. Subsequently, the roots were taken to the slide, the coverslip was placed and the squash technique was performed, applying uniform pressure with the fingertip of the thumb to disperse the cells, making each phase of the cell cycle visible (Salazar and Maldonado, 2019). Samples were visualized under the Leica DME 500 compound microscope at 40X and 100X magnification. To determine the mitotic index, 1000 cells per repetition (5000 cells per treatment) were analyzed, using the formula Mitotic index (MI): number of dividing cells/number of total cells $\times 100$. Continuously, the mitosis inhibition was found according to Salazar-Mercado et al. (2019).

Cellular abnormalities are alterations that occur in the structure and number of chromosomes and depend on the concentration and type of cytotoxic substance (Fatma et al., 2018). Therefore, the following formula used by Salazar and Quintero (2020) was applied in this research: Frequency of chromosomal anomalies (%): Total number of abnormal cells/Total number of cells observed $\times 100$.

2.3. Statistical analysis

For root development, 25 *L. culinaris* roots were used per treatment with 5 replications. 1000 cells per repeat (5000 cells per treatment) were

analyzed for the mitotic index and cellular abnormalities. The obtained data were evaluated using the analysis of variance (ANOVA). Subsequently, the averages of each treatment were compared by applying the HSD multiple range test (Tukey's Honestly Significant Difference) ($P \leq 0.05$). Using the InfoStat program.

3. Results and discussion

3.1. Root length

Exposing lentil seeds to hydration stimulates cell growth, elongating their meristematic cells. Furthermore, when the roots are subjected to chemical substances, variations in their morphology and coloration occur (Khanna and Sharma, 2013). The degree of affection depends on the chemical substance and the time of exposure (Salazar-Mercado et al., 2019).

The results, as shown in Table 1, indicate that at 24, 48 and 72 h the highest root growth was achieved at the control treatment and the lowest growth was found at the 30 mgL⁻¹ dose, this proves that the root growth inhibition was greater, with the increase of the malathion concentration. At 24h, the doses of 20, 25 and 30 mgL⁻¹, root growth did not show statistically significant differences, whereas, at 48 and 72 h, the treatments at 25 and 30 mgL⁻¹ were homogeneous. However, at 24h (0.5, 1, 2.5 mgL⁻¹) and 48h (0.5, 1, 2.5, 5 mgL⁻¹) the doses of malathion did not differ from the control treatment. At 72h, only the control treatment was significantly related to the 0.5 mgL⁻¹ dose.

These results are similar to those reported by Singh and Roy (2017), where malathion had a negative effect on *A. cepa* root growth at all concentrations for 3 days. For this reason, root inhibition is a parameter that can be used to measure the toxicity of malathion. This agrees with studies carried on by Salazar and Maldonado (2019), who submitted different doses of chlorpyrifos organophosphate pesticide (0, 1, 3, 5, 7, 8, 10 and 15 mgL⁻¹) in *L. culinaris* seeds, affecting root growth significantly at 24, 48 and 72h of exposure. The inhibition of root growth caused by the malathion pesticide might be due to the disturbance of the Reactive oxygen species (ROS) in the plant since, according to Mhamdi and Van Breusegem (2018), when ROS homeostasis is altered, numerous processes are affected, from seed germination to root development, because ROS interact with hormones (auxin and cytokinin) associated with plant development.

3.2. Mitotic index and inhibition

In the evaluation of the mitotic index (5000 cells per treatment) after 72 h of exposure to malathion, it shows that the control had a higher MI with statistically significant differences with respect to the different doses

Table 1. *L. culinaris* root growth subjected to different doses of Malathion.

Dose: Malathion (mgL ⁻¹)	Root length (cm)		
	24h	48 h	72 h
T1: Control	2.36 ± 0.23 ^a	4 ± 0.7 ^a	5 ± 0.04 ^a
T2: 0.5	1.86 ± 0.18 ^{a,b,c}	3.66 ± 0.61 ^a	4.34 ± 0.42 ^{a,b}
T3: 1	2.0 ± 0.7 ^{a,b}	3.64 ± 0.65 ^a	4.04 ± 0.4 ^b
T4: 2.5	1.76 ± 0.11 ^{a,b,c}	3.22 ± 0.68 ^{a,b}	3.98 ± 0.04 ^{b,c}
T5: 5	1.52 ± 0.3 ^{b,c,d}	3.02 ± 0.17 ^{a,b,c}	3.64 ± 0.46 ^{b,c,d}
T6: 10	1.54 ± 0.35 ^{b,c,d}	2.54 ± 0.37 ^{b,c}	3.14 ± 0.49 ^{c,d,e}
T7: 15	1.38 ± 0.4 ^{b,c,d}	2.3 ± 0.44 ^{b,c}	2.92 ± 0.57 ^{d,e}
T8: 20	1.16 ± 0.15 ^{d,e}	2.14 ± 0.35 ^{b,c}	2.66 ± 0.46 ^{e,f}
T9: 25	0.94 ± 0.08 ^{d,e}	1.58 ± 0.34 ^{c,d}	2.22 ± 0.34 ^{f,g}
T10: 30	0.54 ± 0.23 ^e	1.04 ± 0.11 ^d	1.34 ± 0.39 ^g

The means ± SD values with different letter indicate statistically significant differences, according to Tukey ($P \leq 0.05$). SD: Standard deviation; cm: centimeter.

Table 2. Mitotic index and Percentage of mitosis inhibition of *L. culinaris* root tip cells, submitted to different doses of Malathion.

Dose: Malathion (mg L^{-1})	Mitotic index (MI: %)	Inhibition of mitosis (%)
	<i>L. culinaris</i>	<i>L. culinaris</i>
T1: Control	18.4 ± 1.5 ^a	—
T2: 0.5	14.2 ± 1.3 ^b	22.8
T3: 1	14.4 ± 1.1 ^b	21.7
T4: 2.5	14.6 ± 0.7 ^b	20.6
T5: 5	13.6 ± 1.1 ^{b,c}	26.1
T6: 10	13.2 ± 1.6 ^{b,c,d}	28.2
T7: 15	12.8 ± 0.8 ^{b,c,d}	30.4
T8: 20	11.4 ± 1.5 ^{b,d,e}	38
T9: 25	10.6 ± 10.6 ^{d,e}	42.3
T10: 30	8.8 ± 1 ^e	52.1

The means ± SD values with different letter indicate statistically significant differences, according to Tukey ($P \leq 0.05$). SD: Standard deviation. MI: Mitotic index.

of the pesticide (18.4%; **Table 2**). The lowest MI values were found at 20, 25 and 30 mg L^{-1} concentrations (11.4%, 10.6% and 8.8%, respectively). This indicates that the mitotic index decreased depending on the doses of malathion. According to results presented by Singh and Roy (2017), when subjecting *A. cepa* to 125 mg L^{-1} of malathion for 18h, they found a mitotic index of 8.77%. Likewise, in *Vicia faba* roots subjected to 320 mg L^{-1} of malathion, the mitotic index was 8.99% (Adam et al., 2014).

In a study, carried out by Srivastava and Singh (2020) in *A. cepa* root cells, exposed to different malathion concentrations (50, 130, 260, 390 and 520 mg L^{-1}) and exposure time (4, 8 and 18 h). They found that the application of malathion affected the MI as the concentration and the treatment periods increased, reducing the growth rate and cell division in the *A. cepa* roots. In the previous study, the 50 mg L^{-1} dose of malathion had an MI of approximately 12%. In contrast, in this research the concentration of 30 mg L^{-1} , the mitotic index was 8.8%. According to the above, the *L. culinari* method is more sensitive to malathion than the *A. cepa* test. This is corroborated by studies related to other pesticides,

where the mitotic index has been higher in *A. Cepa*, in relation to the *L. culinaris* test in equal doses (Salazar et al., 2020a). The mitotic index is a measurement to quantitatively evaluate the cell division of an individual (Lessa and Cariello, 2017). Therefore, if the MI is low compared to the control treatment, the pesticide is affecting mitosis. According to Rosales (2015), organophosphate pesticides cause damage to deoxyribonucleic acid (DNA) at any stage of the cell cycle.

Regarding mitotic inhibition, the concentration that achieved data greater than 50% cell cycle inhibition was 30 mg L^{-1} dose (52.1%: **Table 2**), evidencing the mitodepressive activity of malathion. In contrast, lower doses of mitotic inhibition were found at doses of 0.5, 1, and 2.5 mg L^{-1} (22.8%, 21.7% and 20.6%, respectively). These results differ from those reported by Salazar and Quintero (2020), where 30 mg L^{-1} of glyphosate in *A. cepa*, obtained a mitotic inhibition index of 90.8%. Likewise, in *L. culinaris* roots at 15 mg L^{-1} of chlorpyrifos, an inhibition of 72.41% was found for 72h (Salazar and Maldonado, 2019).

3.2.1. Cellular abnormalities and abnormality rate

As a reference indicator, **Figure 1** shows the normal phases of the cell cycle (interface, prophase, metaphase, anaphase and telophase) and the cellular anomalies found in meristematic cells from *L. culinaris* roots. Nine cellular abnormalities were found at all malathion concentrations, varying according to each treatment, except in control treatment (**Figure 1**; **Table 3**). Micronuclei, metaphase sticky chromosomes, split chromosomes, nuclear lesions, and irregular anaphase were visualized in all the doses that contained malathion, with the nuclear lesions being the mostly found at 30 mg L^{-1} dose (116.6 ± 16). Likewise, the anaphase bridges, binucleated cells, absence of nuclei and telophase bridges anomalies were not observed in some treatments (**Table 3**). In the same way, at 0.5 mg L^{-1} of malathion, fewer anomalies were found.

These results contrast with the study carried out by Singh and Roy (2017) in the *A. cepa* test, where 50, 125, 250 and 375 mg L^{-1} of malathion doses, produced various chromosomal aberrations (anaphase and telophase bridging, multipolarity, chromosomal breakage (sticky and lagging chromosomes, nuclear lesions and binucleated cells)), but no micronuclei were found in *A. cepa*. However, in *Vicia faba* roots subjected

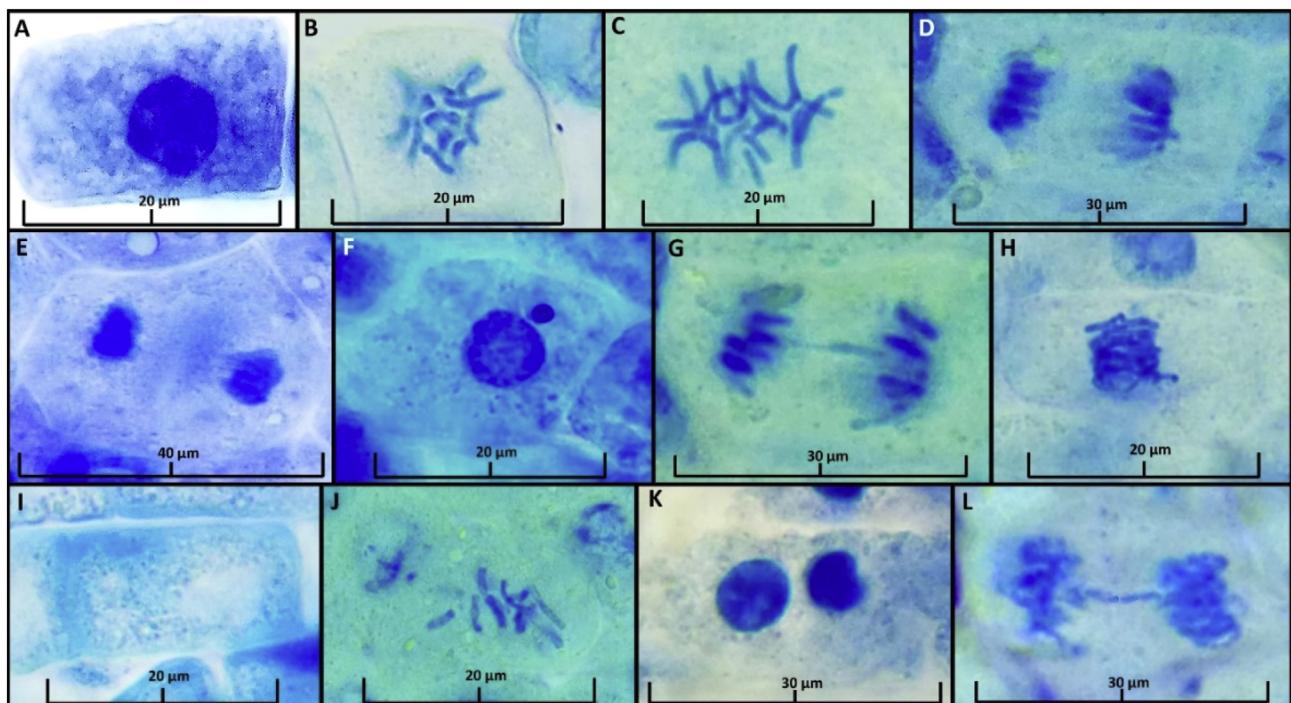


Figure 1. Cell cycle phases and cellular abnormalities in *L. culinaris* root cells treated with doses of Malathion. A = Interphase: T1. B = Prophase: T1. C = Metaphase: T1. D = Anaphase: T1. E = Telophase: T1. F = Micronuclei: T10 G = Anaphase bridge: T9. H = Sticky chromosomes at metaphase: T8. I = Absence of nucleus: T7. J = Chromosome break: T6. K = Binucleate cells: T6. L = Telophase bridge: T10.

Table 3. Frequency of chromosomal anomalies in *L. culinaris* treated with different doses of Malathion.

Dose: Malathion (mg L ⁻¹)	Frequency of Chromosomal Anomalies (Mean ± SD)								
	Mn	AB	SCM	AN	CB	BC	TB	NL	IA
T1: Control	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
T2: 0.5	1.2 ± 0.44 ^a	0 ^a	0.4 ± 0.1 ^a	0 ^a	0.6 ± 0.8 ^a	0 ^a	0 ^a	3.6 ± 0.8 ^a	0.2 ± 0.08 ^a
T3: 1	4.0 ± 1.2 ^{a,b}	0 ^a	1.8 ± 0.8 ^a	0.2 ± 0.1 ^a	3.0 ± 1.3 ^a	0 ^a	0 ^a	7 ± 1.5 ^a	0.2 ± 0.01 ^a
T4: 2.5	4.4 ± 1.5 ^a	0 ^a	3.8 ± 1.7 ^a	0.6 ± 0.8 ^a	3.2 ± 0.8 ^a	0 ^a	0 ^a	10 ± 0.9 ^a	0.2 ± 0.07 ^a
T5: 5	5.8 ± 0.8 ^{a,b}	0 ^a	4.2 ± 2.1 ^a	3.4 ± 1.1 ^a	3.6 ± 1.4 ^a	0 ^a	0 ^a	12.2 ± 1.9 ^a	0.4 ± 0.18 ^a
T6: 10	13 ± 1.3 ^{a,b}	0 ^a	4.4 ± 1.4 ^a	5.4 ± 0.8 ^a	5.8 ± 1.5 ^{a,b}	0.6 ± 0.25 ^{a,c}	0 ^a	15.8 ± 5.7 ^a	0.4 ± 0.14 ^a
T7: 15	13.6 ± 3.5 ^{a,b}	0.6 ± 0.1 ^a	4.8 ± 1.7 ^a	8.4 ± 1.1 ^a	8 ± 2.5 ^{a,b}	1 ± 0.4 ^a	0 ^a	20.2 ± 3.1 ^a	0.6 ± 0.12 ^a
T8: 20	16.8 ± 2.1 ^b	0.6 ± 0.12 ^a	5.8 ± 1.1 ^{a,c}	25.2 ± 3.2 ^b	16.6 ± 5.5	1.2 ± 0.43 ^{a,c,d}	0.2 ± 0.01 ^{a,b}	44.8 ± 14.7 ^b	0.6 ± 0.1 ^a
T9: 25	37.6 ± 9.8 ^c	2.6 ± 0.9 ^b	11 ± 6.2 ^{a,c}	35 ± 5.5 ^b	17.4 ± 5.9 ^b	1.2 ± 1.7 ^{a,c,d}	2.2 ± 1.7 ^b	87.4 ± 18 ^c	0.8 ± 0.2 ^a
T10: 30	47 ± 9.4 ^c	2.8 ± 0.8 ^b	16 ± 7.4 ^{a,c}	36 ± 5 ^b	24 ± 7.1	8.0 ± 3.1 ^{b,d}	2.2 ± 1.1 ^b	116.6 ± 16 ^d	2.6 ± 0.5 ^b

SD: Standard deviation. Mn: Micronuclei. AB: anaphase bridge. SCM: Sticky chromosomes at metaphase. AN: Absence of nucleus. CB: Chromosome break. BC: Binucleate cells. TB: Telophase bridge. NL: Nuclear lesions. IA: Irregular anaphase.

The means ± SD values with different letter indicate statistically significant differences, according to Tukey ($P \leq 0.05$).

to 80, 160 and 320 mg L⁻¹ of malathion, micronuclei were found in all treatments (Adam et al., 2014). According to Doherty et al. (2016), the micronuclei are fragments of DNA that are separated from the main nucleus and have originated from eccentric chromosomes or fragments of chromatic, can result from alterations of structural type (clastogenic effect) or numerical alterations (aneugenic effect). They are used as a high degree of toxicity parameter (Bhatia and Kumar, 2013). In this investigation, micronuclei were observed in all doses, being in greater quantity in 30 mg L⁻¹ concentration (47 ± 9.4; Table 3). Worrisome data since, it is evident that malathion induces genetic instability in *L. culinaris*, because it is a cytotoxic and genotoxic agent.

Table 4 compares the obtained results from the abnormality rate in treatments with malathion in *L. culinaris*. It is observed that at the concentration of 30 mg L⁻¹, a higher rate of relative abnormality (25.5) was found, indicating that the formation of abnormal cells in *L. culinaris* roots increases as the dose of malathion is increased. These results also agree with the observations made by Salazar and Maldonado (2019); Salazar et al. (2020a), by subjecting *L. culinaris* to different concentrations of chlорpyrifos and propanil. Likewise, according to Singh and Roy (2017), pesticides alter ROS homeostasis and cause intracellular oxidative stress (Wu et al., 2017). Consequently, the tubulin polymerization, the mitotic spindle, the assembly of the phragmoplast, the dynamics of the nuclear envelope, the separation and movement of the chromosomes, and the formation of cell plates are affected, delaying the stages of cell division (Livanos et al., 2012). Furthermore, according to Singh and Roy (2017), the toxic and genotoxic activity of malathion is possibly associated with an overproduction of pro-oxidant agents and oxidative stress due to the significant increase in concentrations of malondialdehyde (MDA) caused by malathion in the plant. These products of lipoperoxidation reactions are also associated with damage to lysosomal membranes and DNA structure (Cortesía et al., 2015).

Table 4. Relative abnormality rate for each doses of Paraquat.

Dose: Malathion (mg L ⁻¹)	Relative abnormality rate
T1: Control	0
T2: 0.5	0.6
T3: 1	1.66
T4: 2.5	2.36
T5: 5	2.9
T6: 10	4.8
T7: 15	5.56
T8: 20	11.1
T9: 25	19.38
T10: 30	25.5

4. Conclusion

To conclude, the present investigation determined the toxic effect of malathion on root growth, cell division and formation of cellular abnormalities, producing mitodepressive and cytotoxic effects on meristematic cells at *L. culinaris* root tip. The presence of changes at the cellular level and damage to the chromosomes was observed at 0.5 to 30 mg L⁻¹ of malathion; being the 30 mg L⁻¹ dose the one that produced a greater cellular inhibition and relative abnormality rate. In addition, a high frequency of abnormality was found in the micronuclei, which represents an indicator of a high degree of toxicity at the cellular level. Accordingly, the use of less toxic and environmentally friendly pesticides is suggested to farmers. According to the above, it is suggested that farmers use less toxic and environmentally friendly pesticides since the repeated and common use of this molecule can lead to problems in the performance and crop yield, thanks to physiological abnormalities that are harmful to development of the agricultural exploitation.

Declarations

Author contribution statement

Seir Antonio Salazar Mercado: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Jesús David Quintero Caleño: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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