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Phenthoate toxicity evaluation in root meristem of *Pisum sativum* L.

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Abstract. Phenthoate is an organothiophosphate insecticide. Effect of phenthoate on the cytogenetic alterations in root tip cells of *Pisum sativum* L., a multiuse crop was investigated in this study. *Pisum sativum* L. seeds were exposed to different concentrations of phenthoate (0.1, 0.2, 0.3, 0.4, and 0.5%) and were germinated at 24°C for 72 hours and cytogenetic alterations were assessed. Analysis of mitotic index revealed that phenthoate has cytotoxic attributes, and cell proliferation kinetics frequencies showed alterations in the kinetics of the mitotic process. Phenthoate treatment of 0.1% to 0.5% resulted in an increase in the metaphases, and a reduction in prophases, anaphases, and telophases ratio, dose dependently. The findings of the study reveal that, phenthoate reduced the percentage of seed germination, mitotic index, radicle length and increased chromosomal abnormalities dose dependently. Root tip cells of *Pisum sativum* L. seeds treated with phenthoate showed an increased occurrence of single and double bridges, fragments, stickiness, laggard, and vagrants.

Keywords: phenthoate, seed germination, radicle length, Mitotic Index, genotoxicity, cell proliferation kinetics, *Pisum sativum* L.

INTRODUCTION

Insecticides are used to kill harmful pests, precisely the insects which are most frequent pests of economically significant plants. All chemicals used in controlling plant or animal pests like fungicides, insecticides, rodenticides, and weedicides (herbicides) comes under the term pesticide. Pesticides are chemicals which are used to inhibit the reproduction of pests or to kill them. They are designed to eliminate undesirable organisms while protecting those that are required, for example, a weed killer or weedicide will eliminate weeds while protecting the crops that is grown for food. Sumitomo Corporation developed phenthoate, a broad-spectrum chiral organophosphate, sold widely in 1972, and used as racemate. Due to its acute toxicity to humans and non-target creatures (bees), crops (celery and eggplant), as well as its residue in environment (soil and water), this pesticide is of specific concern (Esturk et al. 2014; Nara et al. 2018). Phenthoate is phytotoxic to some plants. It has fast knockdown action and penetration. Phenthoate, an organophosphorus compound, is used generally because of its efficiency, high

solubility in water, and quick biodegradation (Nelson et al.1990). Nevertheless, a lot of these compounds and the byproducts of their breakdown are DNA alkylating agents (Bedford and Robinson 1972). Therefore, it is essential to look for any prospective genetic harm caused by minimal exposure with organophosphorus pesticides (Degraeve et al. 1984).

Since pesticides can lower agricultural products loss, and increase affordable production and food quality, they play a significant role in agriculture (Aktar et al. 2009; Strassemeyer et al. 2017; Taufeeq et al. 2021). Pesticides use increased in World War II (1939–45) due to the pressing need to improve and increase production of food and regulate insect-borne illnesses. After 1940, increased usage of synthetic chemicals for protection of crops allowed for an even greater surge in food production (Carvalho 2017). Furthermore, global production of pesticides increased annually at 11 percent from 0.0002 billion tons in 1950 to greater than 0.005 billion tons by 2000 (Chang et al. 2017). Only 1% of pesticides were utilized to efficiently protect target plants from insect pests, even though 3 billion kgs of pesticides are consumed annually worldwide (Carvalho 2017) and hence huge amounts of remaining pesticides continue to enter or affect environment and non-target plants. Pesticide contamination as a result has greatly damaged the ecosystem and had a negative effect on the health of human beings (Hernández 2013; Tudi et al. 2022; Abdel-Halim et al. 2020). Equipment required for applying pesticides effectively is essential (Lozier et al. 2013) to reduce loss of spraying solution, eradicate residual pesticides in environment and avert detrimental effects on the health of human beings from residues and over spraying. In addition to indirect exposure from food, air, soil, and water adulterated with pesticides (Kim et al. 2017; Tudi et al. 2022), humans are also directly exposed at workplace to pesticides (Macfarlane et al. 2013). The most common routes for pesticides to enter a human's body are through their skin, oral, and respiratory system (Damalas et al. 2011; Anderson et al. 2014). Globally, 3 million people gets poisoned by pesticides and 200,000 of them die, as per UNEP and WHO report (Yadav et al. 2015).

Although certain pesticides are developed to attack a specific set of targets, their toxic components will strike the entire organism (Castellanos et al. 2022). As per a study, methomyl induces genotoxicity in fishes (Afaf et al. 2022). Aquatic organisms like water spinach, coastal creatures, fishes, and Danio rerio have demonstrated methomyl toxicity (Jablonski et al. 2022; Camilo-Cotrim et al. 2022). DNA damage is a primary biotic phenomenon that can harm biotic assemblies and procedures, as well as induce genotoxic disorders linked with the growth of carcinogenic developments (Acar et al. 2022; Siddiqui and Sulaiman 2022 a and b; El-Houseiny et al. 2022). According to a recent study (Pesavento et al. 2018; Velazquez et al. 2022; Liman et al. 2022), several causes, together with DNA damage instigated by pesticides, stimulate carcinogenic growth in a wide array of species. For economic reasons, *P. sativum* (Fabaceae) is a widely consumed legume in diet for protein source. Till now limited studies are conducted on the impact of phenthoate on pea plant despite it being a multiuse crop (Sandhu et al. 1987; Somaiah et al. 2014; Dong et al. 2022). In this study, an effort was undertaken to evaluate the noxious effects of phenthoate on Pea plant.

METHODOLOGY

Procuring seeds and chemicals

Phenthoate was purchased from Sigma Chemicals Ltd., United States (CAS No. 16752-77-5). *P. sativum* L. (pea) seeds were bought from a registered trader in Abha, Saudi Arabia.

Exposure settings

Pisum sativum L. seeds of uniform size were picked, pre-soaked in distilled water for 12 h and divided into several groups of 30 seeds each. Seeds were then soaked in 250 mL solutions of phenthoate for 1 hour to expose them to different concentrations (0.1, 0.2, 0.3, 0.4, and 0.5%) of phenthoate. Seeds were soaked in double distilled water in control. The containers were repetitively shaken during the treatment phase to provide seeds with air. After treatment, to remove any traces of adhering phenthoate, seeds were carefully rinsed with double distilled water and kept in Petri dishes on moistened Whatman Filter Paper. The Petri dishes were maintained at 25±2°C in dark for the next 72 hours in a plant growth cabinet. By inspecting the formation of the radicle, it was possible to estimate the seed germination time. A millimeter ruler was used to measure the radicle length of germinated seed every 24 hours till 72 hours. Newly growing roots between one to two centimeters in length were used in the experiment. The complete experiment was done thrice with the same settings.

Analyzing genotoxicity and kinetics of mitosis

In the morning from 8 to 10 am, 1 to 2 cm newly grown roots were taken, submerged in a fixation solution

(ethanol: glacial acetic acid, 3:1) for 24 hours, moved to 70% ethanol and kept at 5°C till microscopic inspection. Ten roots were hydrolyzed in 1N HCl solution for ten minutes for every sample and root tips were dyed with 2% acetocarmine for ten minutes for the preparation of each slide. From root tips, chromosome preparations were done as in Qian et al. (1998) with slight alterations (described in Siddiqui and Suleiman 2022 b). 1000 cells from all samples including control were examined to determine the mitotic index. Cell proliferation kinetics frequencies were analyzed using the number of cells in every division phase to total number of mitotic cells. In a light microscope (100 x) in oil immersion, all the mitotic cells were examined. All the slides were investigated blind and coded.

Cytogenetic analysis

Cytogenetic evaluation was done on root tips of germinated seeds exposed to various concentrations of phenthoate. Chromosome preparations were done from root tips by applying the technique described by Qian (1998) having slight modifications. Root tips were cut, fixed for 24 hours in Carnoy's fixative (anhydrous alcohol:glacial acetic acid, 3:1), passed to 70% alcohol, and then kept in the fridge until needed. For 1 hour, 2% acetocarmine solution was used to stain the root tips after they had been hydrolyzed in 5 N HCl at room temperature for 20 minutes. Chromosome spreads were made by squash technique as described by Savaskan and Toker (1991). All slides were coded and observed blind. For the purpose of analyzing the mitotic index and expressing the results in percentage, 500 cells from every preparation were scored. At least 100 metaphase- anaphase plates were used to study several kinds of chromosomal anomalies like fragments, single bridges, double bridges, stickiness, vagrants and laggards.

Statistical analysis

To determine the significance of differences amongst variables, a one-way ANOVA test was performed using the GPIS 1.13 program (GRAPHPAD, California, USA). All outcomes were reported as mean ± standard error.

RESULT

Effect of phenthoate on seed germination

In the control, after 1 hour and 3 hours, 77.40% of seed germinated at 24 hours, 85.54% at 48 hours and 96.11% at 72 hours (Figure 1 A and B). As compared to control, at 24 hours treatment of *P. sativum* seeds with phenthoate concentrations ranging from 0.1 to 0.5% for 1 hour and 3 hours caused very significant inhibition of seed germination rate (SG) ($p<0.01$). Analogous trend in seed germination was recorded at 48 and 72 hours. After 1 hour and 3 hours of phenthoate treatment, the highest seed germination rate was recorded at 0.1% concentration at 24 hours (74.76%), (70.14%); at 48 hours (81.43%), (80.99%); and 72 hours (90.12%), (89.44%) and minimal seed germination was found at a concentration of 0.5%

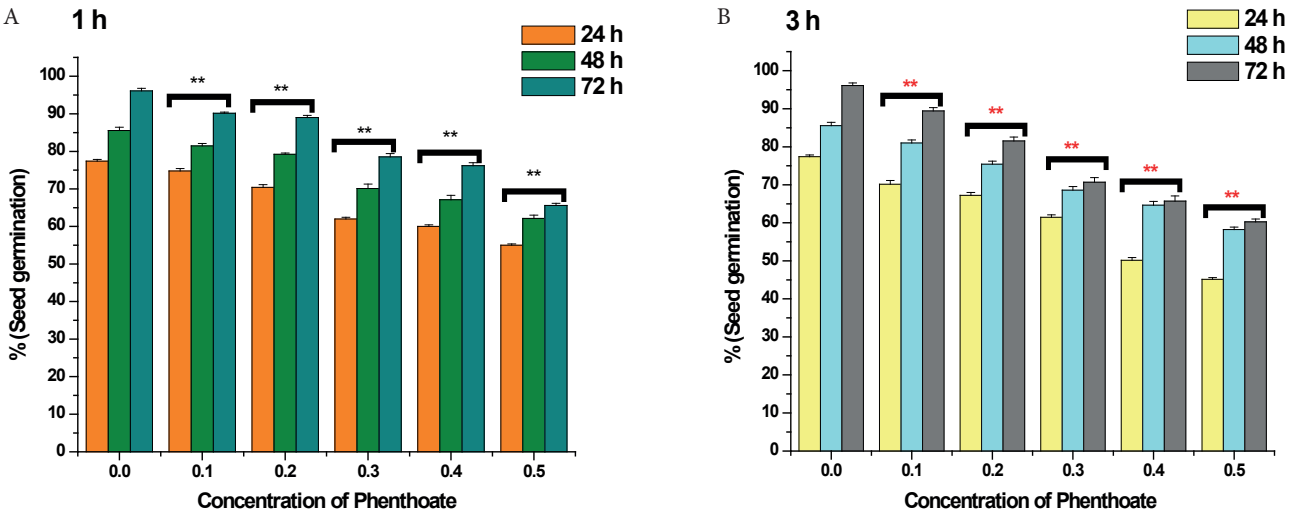

Figure 1. Effect of phenthoate on seed germination of *P. sativum* L. for 1 and 3 h. **p<0.01 compared to control group. Data are mean of three replicates ±SE, 0.0 = Control group.

Table 1. Effect of phenthoate on radicle length in *P. sativum* L for 1 h and 3 h.

Radicle length (cm)			
Conc. (%)	24 h	48 h	72 h
0.0	0.85 ± 0.03	1.67±0.09	2.20±0.09
1 h			
0.1	0.76 ± 0.02¥	1.25±0.07¥	1.98±0.07¥
0.2	0.54±0.02¥	0.98 ±0.02¥	1.69±0.07¥
0.3	0.51±0.04¥	0.78±0.04¥	1.32±0.06¥
0.4	0.46±0.01¥	0.84±0.05¥	1.10±0.12¥
0.5	0.39±0.02¥	0.50±0.04¥	0.64±0.02¥
3 h			
0.1	0.71±0.04$	1.26±0.07¥	1.95±0.05
0.2	0.62±0.03¥	1.12 ±0.14¥	1.50±0.06¥
0.3	0.54 ±0.05¥	0.65±0.04¥	1.32±0.05¥
0.4	0.46±0.06¥	0.58±0.05¥	0.98 ±0.12¥
0.5	0.35±0.03¥	0.53±0.04¥	0.78±0.05¥

$p<0.05; ¥p<0.01; compared to control group. Data are mean of three replicates ±SE, 0.0 = Control group, Conc. = concentration.

Table 2. Effect of phenthoate on cell proliferation kinetics in *P. sativum* L for 1 h and 3 h.

Conc. (%)	Prophases	Metaphase	Anaphases	Telophases
0.0	60.50±4.1	19.81±2.3	18.12±3.2	24.44±2.3
1 h				
0.1	52.00±3.6¥	22.51±1.5	16.23±2.1	21.56±3.0
0.2	50.70±2.2¥	24.31±3.2¥	14.11±1.4¥	19.34±1.3¥
0.3	48.20±2.0¥	26.23±3.5β	13.15± 1.6β	17.23±3.1¥
0.4	45.12±1.5¥	27.91±3.1β	12.45±1.4β	15.21±2.3¥
0.5	39.75±2.6¥	29.11±1.4β	00.23±1.3¥	13.43±3.2¥
3 h				
0.1	54.34±3.4¥	24.78±3.3¥	15.47±2.1	20.52±1.3$
0.2	52.12±2.7¥	27.34±4.5¥	12.28 ±3.2¥	18.22 ±2.7¥
0.3	50.43±3.2¥	29.33±3.5¥	10.78±2.2¥	16.32±3.2¥
0.4	43.42±1.2¥	25.91±2.2¥	9.12±2.15¥	13.42±3.4¥
0.5	35.75±2.8 ¥	23.12±2.2	8.23±2.20¥	10.57±3.5¥

$p<0.05; ¥p<0.01; βp<0.001 compared to control group. Data are mean of three replicates ±SE, 0.0 = Control group, Conc. = concentration.

at 24 hours (55%) (45.15%); 48 hours (62.15%), (58.22%); and 72 hours (65.6%), (60.27%) respectively in comparison to control (Figure 1A and B).

Effect of phenthoate on radicle length

The radicle length in the control group increased with time after treatment with double-distilled water for 1 h and 3 h: (0.85±0.03) at 24 h, (1.67±0.09) at 48 h, and (2.2±0.09) at 72 h (Table 1). In comparison to control, 0.1 to 0.5% phenthoate treatment for 1 hour and 3 hours caused a very significant reduction in radical length ($p<0.01$) and an analogous trend in the pattern of radical length was observed at 48 hours and 72 hours. Highest radicle length was found after treatment with phenthoate for 1 hour and 3 hours at 0.1% concentration at 24 hours (0.76±0.02), (0.71±0.04); 48 hours (1.25±0.07), (1.26±0.07); and 72 hours (1.98±0.07), (1.95±0.05) and the smallest radicle length was recorded at 0.5% concentration at 24 h (0.39±0.02), (0.35±0.03); 48 h (0.50±0.04), (0.48±0.04); and 72 h (0.64±0.02) (0.78±0.05) respectively, in comparison to control (Table 1).

Effect of phenthoate on cell proliferation kinetics

Cell proliferation kinetics, measured as the ratio of prophases, metaphases, anaphases, and telophases, demonstrated an increase in metaphase from 0.1 to 0.5% and a decrease in prophase, anaphase, and telophase as compared to the control (Table 2). At 1 hour, very significant reduction ($p<0.01$) was observed in prophase (0.1 to 0.5%); anaphase at 0.2% (14.11±1.4) and in telophase from 0.2 to 0.5%. Highly significant decrease ($p<0.001$) was reported in anaphase at 0.3% (13.15±1.6), 0.4% (12.45 ±1.4) and 0.5% (10.23±1.3). A very significant increase ($p<0.01$) was observed in metaphase at 0.2% (24.31±3.2) and highly significant increase ($p<0.001$) was reported at 0.3 to 0.5% in comparison to control. In case of 3 hours, very significant reduction ($p<0.01$) was observed in prophase; anaphase and telophase from 0.1 to 0.5% concentration and very significant increase ($p<0.01$) in metaphase was observed from 0.1% to 0.4% concentration in comparison to control (Table 2).

Effect of phenthoate on mitotic index

Figure 2 (A and B) depicts the effect of phenthoate on mitotic index of root tip cells in *P. sativum*. In the control, the mitotic index in case of seeds which were treated with double distilled water for 1 hour and 3 hours was 65.90%. Mitotic index decreased very significantly ($p<0.01$) in 0.1% to 0.5% phenthoate treated seeds for 1 hour and 3 hours, in a dose dependently as compared to control. Maximum mitotic index was reported at 0.1% for 1 hour and 3 hours (57.53%) (60.22%) and minimum mitotic index was reported at 0.5% (39.87%) (32%) respectively.

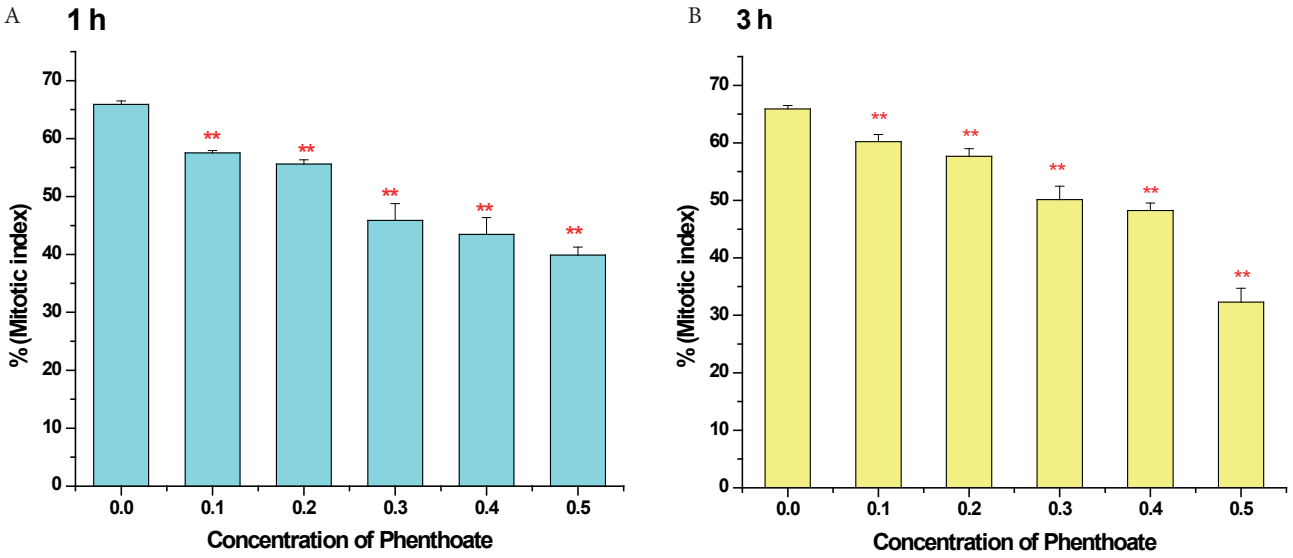

Figure 2. Effect of phenthoate on mitotic index of *P. sativum* L. for 1 h and 3 h. **p<0.01 compared to control group. Data are mean of three replicates ±SE, 0.0 = Control group.

Effect of phenthoate on chromosomal anomalies

In the control after 1 hour and 3 hours, the occurrence of chromosomal anomalies like fragment, single and double bridge, stickiness, vagrant and laggard in metaphase-anaphase plates was zero. Phenthoate treatment for 1 hour and 3 hours resulted in a dose dependent increase in chromosomal anomalies percentage like fragments, single and double bridges, stickiness, vagrants, and laggards, in metaphase-anaphase plates (Table 3, Figure 3). The treatment of seeds with 0.1% phenthoate for 1 and 3 hours caused 0% chromosomal anomalies like fragment, single and double bridge, stickiness, and vagrants. Laggards were found at 1 h (0.15%) and at 3 hours (0.3%).

Seeds treated with 0.2% phenthoate for 1 hour and 3 hours showed 0% chromosomal anomalies like fragment, stickiness, and vagrant for 1 hour and stickiness for 3 hours. However, single bridges (0.38%); double bridges (0.34%); and laggard (0.26%) were reported for 1 h at 0.2 % which were very significant (p<0.01) and fragments (0.12%); single bridges (0.42%); double bridges (0.55%); vagrant (0.25%) and laggards (0.29%) were

Table 3. Effect of phenthoate on chromosomal aberrations in *P. sativum* L root tip cells for 1 h and 3 h.

Conc. (%)	Fragment	Single bridge	Double Bridge	Stickiness	Vagrant	Laggard
0.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1 h						
0.1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.15±0.003¥
0.2	0.00±0.00	0.38±0.01¥	0.34±0.01¥	0.00±0.00	0.00±0.00	0.26±0.09¥
0.3	0.65±0.06¥	0.55±0.05¥	0.96 ± 0.05¥	0.25±0.03¥	0.35±0.03 ¥	0.46±0.03¥
0.4	0.97±0.05¥	0.76±0.02¥	1.66±0.63¥	0.98±0.10¥	0.68±0.05¥	0.66±0.05¥
0.5	1.24±0.20¥	0.98±0.10¥	1.50±0.99¥	0.97±0.23¥	0.91±0.23¥	0.86±0.11¥
3 h						
0.1	0.00±0.00	0.00 ± 0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.30±0.003¥
0.2	0.12±0.001	0.42±0.02¥	0.55±0.21¥	0.00±0.00	0.25±0.01	0.29±0.070¥
0.3	0.78±0.03¥	0.76±0.05¥	1.55±0.73¥	0.73±0.13¥	0.68±0.06¥	0.68±0.10¥
0.4	1.01±0.21¥	1.25±0.34¥	2.53±0.81¥	1.98±0.17¥	0.75±0.12¥	0.75±0.05¥
0.5	1.78±0.35¥	2.79±0.43¥	2.79±0.72¥	1.97±0.45¥	1.98 ±0.34¥	0.97±0.22¥

¥p<0.01 compared to control group. Data are mean of three replicates ±SE, 0.0 = Control group, Conc. = concentration.

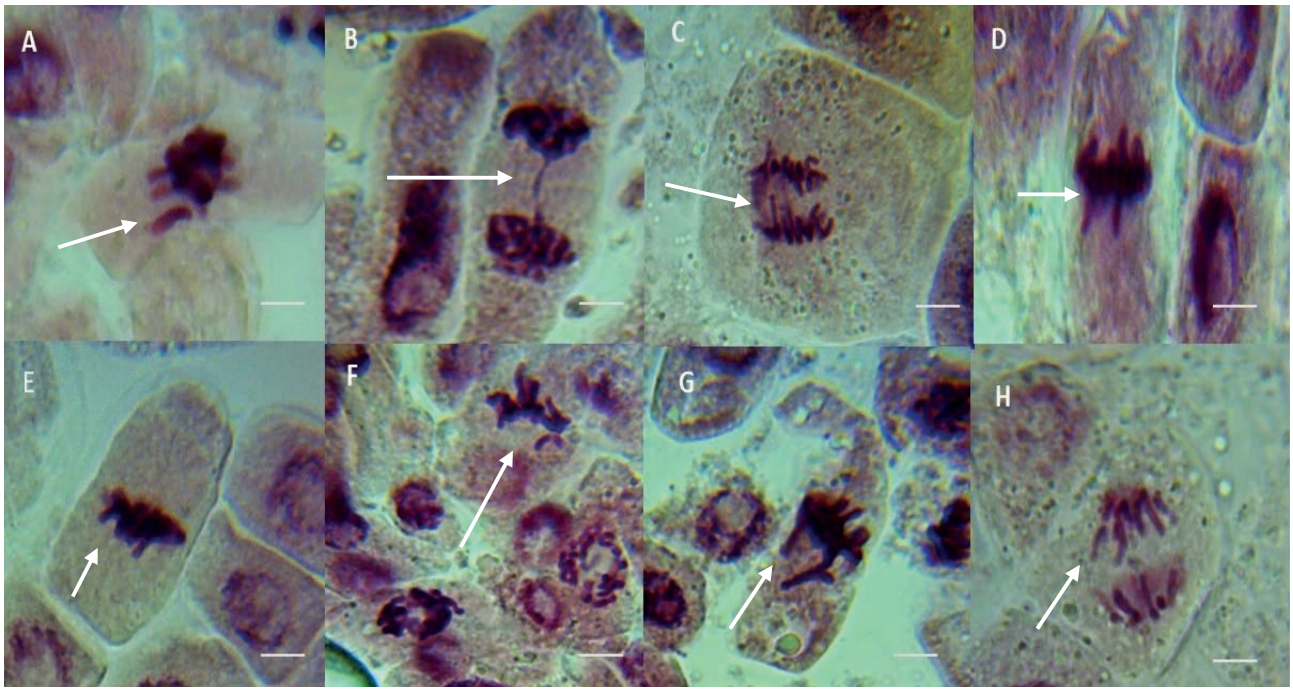

Figure 3. Chromosomal aberrations in phenthoate treated *P. sativum* L. root tip cells for 1 h and 3 h. A- Fragment. B- Single bridge., C- Double bridge., D-E- Stickiness., F-G-Vagrant., H- Laggard. Bar – 10 µm.

reported at 0.2 % for 3 h when compared to control. Seeds treated with 0.3% phenthoate for 1 hour and 3 hours caused very significant increase ($p<0.01$) in occurrence of chromosomal anomalies like fragments, single and double bridges, stickiness, vagrants, and laggards for 1 hour and fragments, single bridges, vagrants, and laggards for 3 hours as compared to control. Seeds treated with 0.4 to 0.5 % phenthoate for 1 and 3 hours caused very significant increase ($p<0.01$) in occurrence of chromosomal anomalies like fragments, single and double bridges, stickiness, vagrants, and laggards for 1 h and 3 h as compared to control.

DISCUSSION

The outcomes of the present study reveal that exposing seeds to increasing concentrations of phenthoate delays seed germination in *P. sativum*. The possibility of seeds to germinate is highly sensitive to environmental influences. Studies revealed that pesticide exposure considerably lowers seed germination rate (Siddiqui et al. 2008; Mahapatra et al. 2019; Bano et al. 2022). Endosulfan and Kitazin at higher doses inhibited seed germination in brinjal (*Solanum melongena* L.) (Sammaiah et al. 2011), *Capsicum annuum*, *Solanum lycopersicum, Solanum melongena, P. sativum, Zea mays, Brassica nigra* and *Typha latifolia* (Khan et al. 2021; Das et al. 2021). Pesticides in soil can prevent plant roots from absorbing essential nutrients, causing nutrient deficiency and retardation in growth (Sharma et al. 2019). Radicle lengthening is related to multiplication of cells. Nevertheless, phenthoate inhibited cell proliferation in this research study, as indicated by the mitotic index results. It could be due to variations in expression of certain genes that regulate the cell cycle. Methomyl and imbraclaobrid have previously been shown to be mutagenic in *P. sativum* and *Allium cepa* (Ozel et al. 2022; Sengupta et al. 2022; Siddiqui and Alrumman 2022 a and b). In our analysis, we found that phenthoate had an analogous effect on seeds of *P. sativum*. Significant inhibitory effect is shown by phenthoate on mitosis in *P. sativum* root tips, which could be attributed to its repressive action on spindle fibers (Barbara et al. 1991), DNA, RNA, and protein synthesis (Ogut et al. 2019; Kalefetoglu et al. 2021; Gogoi et al. 2021). Glyphosate halts cell cycle at G2-M stage by inhibiting activation of CDK1/cyclin (Marc et al. 2002; Das et al. 2021). Similar effects of organophosphates have been indicated by previous research on biological organisms (Ismail et al. 2009; Abdelsalam et al. 2022).

According to the results of the proportions of distribution of precise mitotic phases, phenthoate decreased the percentages of anaphase, prophase and telophase and augmented the metaphase percentage at all concen-

trations dose-dependently. These findings are consistent with the results of (Liman et al. 2010; Priya et al. 2014; Ozkul et al. 2016). Moreover, telophase percentage decreased as compared to the control. This suggests that decrease in telophase and hence mitotic index, could be caused by the arrest of one or more mitotic phases, or by a slowdown in the pace of cell development in mitosis (Ping et al. 2012).

Cytological anomalies in plants can be utilized to detect environmental pollutants that pose serious genetic concerns. Several types of chromosomal anomalies were observed in *P. sativum* after treatment with phenthoate comprising of single and double bridges, fragments, stickiness, laggards, and vagrants. The results prove that these substances, as previously described by other researchers (Siddiqui et al. 2012; Siddiqui and Al-Rumman 2020 a and b; Rahman et al. 2022; Siddiqui and Al-Rumman 2022 c), could produce mitotic anomalies. These pesticides have been linked to chromosomal abnormalities by blocking spindle proteins and inducing exchange of sister chromatids (Lukaszewicz et al. 2019; Khan et al. 2021; Siddiqui et al. 2021). Genetic instability is caused by free radicles in cells. Reactive oxygen is extremely unstable, causing cytoskeleton disruption, imbalance in energy metabolism, and DNA harm, ensuing chromosomal anomalies (Acar et al. 2021; Sengupta et al. 2022). DNA damage is a primary biotic incidence which can harm biotic assemblies and procedures, as well as induce genotoxic disorders linked with the growth of carcinogenic developments (Kaur et al. 2022; Ajermoun et al. 2022; Zhang et al. 2022). According to a recent study (Pesavento et al. 2018), several causes, together with DNA damage instigated by pesticides, stimulate carcinogenic growth in a wide array of species. The genotoxic influence of phenthoate observed in this research work might have been partly induced by the oxidative stress instigated by these substances. Various investigations have shown that these compounds alter redox status in plant cells, lending credence to this theory (Bonciu et al. 2018; Acar 2021; Acar et al. 2022). Phenthoate demonstrated a strong genotoxic effect on *P. sativum* plant in experimental conditions used in this investigation. Additional research on quality of crops derived from plants treated with phenthoate is required in relation to disease vulnerability, dietary value, and vulnerability to acclimatized stress.

CONCLUSION

The outcomes of the present study indicate that insecticides can be genotoxic to nontarget species like plants. Higher concentrations of phenthoate demonstrated detrimental effects on germination of seeds, radical length, frequency of cell kinetics, mitotic index, and chromosomal anomalies in *P. sativum* plant. Farmers are normally guided by dealers to use insecticides at twice the permitted level, which might have unfavorable cytogenetic consequences and limit plant growth. Hence, exceeding the recommended amount of insecticides should be prevented. Farmers and insecticide vendors should be educated regarding proper and optimum use of insecticides. The effect of insecticides on non-target host plants must be investigated further at the level of gene expression to identify the mechanism through which they cause harm to non-target plants.

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