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Methomyl has clastogenic and aneugenic effects and alters the mitotic kinetics in *Pisum sativum* L.

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Abstract. Methomyl is a carbamate pesticide that is frequently applied to crops all over the world. This research aims to evaluate the *Pisum sativum* L mitotic process and potential genotoxicity. The Cell Proliferation Kinetics (CPK) frequencies demonstrated changes in kinetics of mitotic process, and study of Mitotic Index (MI) demonstrated that methomyl had cytotoxic properties. In fact, the telophases ratio dropped at 0.1% to 0.5% methomyl treatment, while there was an increase in prophases, metaphases, and anaphases from 0.1% to 0.5% in a dose dependent manner. In terms of genotoxicity, methomyl cause an increase in the frequency of clastogenic and aneugenic chromosomal abnormalities at metaphase-anaphase at 0.1% to 0.5%. The effects on the mitotic spindle were further confirmed by an increase in the frequencies of c-mitosis from 0.1 to 0.5% methomyl treatment. The outcome of the current analysis indicates that regularly used insecticides methomyl has a considerable cytotoxic effect on mitotic cells of *Pisum sativum* L.

Keywords: methomyl, mitotic index, clastogenic, aneugenic, C- mitosis *Pisum sativum* L.

INTRODUCTION

In public areas, agricultural lands and gardens, pesticides are extensively used to eradicate weeds, undesirable pests, and diseases transmitted by vectors. Nevertheless, the prolonged usage of pesticides may leave behind toxic remains that, through surface drains, spray drift, runoff, spray leftovers, and leaching may pollute nearby surface water and ground natural water bodies (Mojiri et al. 2020; Chandra et al. 2021). The accumulation of residual pesticides in aquatic and marine organisms food chains can pose a risk to human health and have a detrimental effect on ecological systems (Lukaszewicz et al. 2019; Jing et al. 2022a; Abdel-Wahab et al. 2021).

From many decades, pesticides have been a crucial component in reducing crop loss and increasing output. Due to these advantages, farmers are spraying pesticides on crops more frequently and using modern techniques like drones (Nie et al. 2020). However, the propensity of pesticides to bio accumulate in edible goods may have an undesirable impact on human

health (Yu et al. 2016; Ramadan et al. 2020). Beyond their maximum residue limits (MRLs), pesticides in water and agricultural products have the potential to cause both acute and chronic illnesses in people (Li and Jennings 2018; Amaç and Liman 2021).

Carbamates are a diverse group of chemicals that are used as insecticides. The acetylcholinesterase (AChE) enzyme is selectively affected by carbamates, which results in a buildup of acetylcholine and overstimulation of the nervous system in both target and non-target species, including human beings (Eddleston et al. 2004). In the areas where onions, cucumbers, cabbage, and chili peppers are grown, methomyl, a carbamate insecticide, is frequently used (Ramadan et al. 2020). Acute poisoning may result from methomyl consumption by using contaminated agrifoods and water via occupational or non-occupational ways (Jing et al. 2022 b).

Due to its highly effective biological action in controlling pests and safeguarding the crops, methomyl (C5H10N2O2S), S-methyl-1-N- [(methyl carbamoyl)-oxy]-thioacetimidate, belongs to carbamate pesticide group that is commonly applied in various countries (Laicher et al. 2022; Pietrini et al. 2022). Several pesticides are designed to strike a particular group of targets, although their noxious constituents will affect the whole organism, both target and non-target (Castellanos et al. 2022). According to a study, methomyl causes genotoxic effects in fish (Afaf et al. 2022). Fish and aquatic creatures including Danio rerio, coastal aquatic system and water spinach have also shown toxicity to methomyl (Jablonski et al. 2022; Camilo-Cotrim et al. 2022).

DNA damage is a preliminary biotic phenomenon which could disrupt biological developments and structures and produce genotoxic disorders associated with carcinogenic complications (Acar et al. 2022; Siddiqui and Sulaiman 2022 a and b; El-Houseiny et al. 2022). As per a recent report, numerous species undergo carcinogenic progressions due to various causes, such as DNA damage instigated by chemical contaminants (Pesavento et al. 2018; Velázquez et al. 2022; Liman et al. 2022). This study aims to analyze the potential adverse effects of methomyl on mitotic processes and DNA integrity in the terrestrial plant *Pisum sativum* L.

MATERIAL AND METHODS

Purchasing of chemicals and seeds

Methomyl insecticide were bought from Sigma Chemicals Ltd., United States (CAS No. 16752-77-5). *Pisum sativum* L (Pea) seeds were procured from a licensed trader at a community market in Abha, Saudi Arabia.

Exposure conditions

Even sized *P. sativum* L seeds were chosen, presoaked for 12 hours in distilled water and then divided into various groups of 30 seeds each. After that, the seeds were exposed to various methomyl concentrations (0.1, 0.2, 0.3, 0.4, and 0.5%) for 1 h by soaking in 250 mL solutions of methomyl. Double-distilled water was used to soak the seeds in the control group. Throughout the treatment time, the containers were shaken repeatedly to make available ample aeration to the seeds. Following treatment, seeds were extensively rinsed with double distilled water (DDW) to eliminate any remaining traces of adhering methomyl and were placed in Petri dishes on moisturized Whatman Filter Paper. For the following 72 hours, the Petri dishes were kept in dark in a plant growth cabinet at 25±2°C. The experiment was conducted on newly emerging roots that were 1-2 cm long. The complete experiment was conducted thrice in identical conditions.

Evaluation of mitotic kinetics and genotoxicity

One to two cm long roots were collected between 8 to 10 am, soaked for 24 h in a fixation solution (ethanol: glacial acetic acid, 3:1), then transferred to 70% ethanol, maintained at 5°C till microscopic examination. For each sample, 10 roots were hydrolyzed in 1N HCl for 10 minutes, and with 2% acetocarmine solution, root tips were dyed for 10 minutes for preparing each slide. Chromosome preparation was done from root tips as stated by Qian et al. 1998 with minor modifications (Siddiqui and Suleiman 2022b). To calculate the MI, which is a proportion of dividing cells, 1000 cells from each sample were evaluated. The no. of cells in each division phase to all mitotic cells was used to compute CPK frequencies. All mitotic cells were studied in a light microscope under oil immersion (100 x). All slides were examined blind and coded.

Ratio of aberrant cells over 500 metaphase/anaphase cells per root tips were used to calculate the frequency of chromosomal aberrations. Chromosomal aberrations were categorized as per their origin in clastogenic (resulting in chromosomal breakage) or aneugenic (disrupting spindle function and leading to asynchronic chromosomal migration). Laggards and vagrants chromosomes have been scored with regards to aneugenic abnormalities. Single bridges, fragments, double bridges, and sticky chromosomes were taken into considera-

tion in clastogenic aberrations. C-mitosis as defined by Grant (1978) is an inactivation of spindle ensued by a haphazard scattering of chromosomes over the cell and is quantified and scored by assessing the frequency over 100 metaphases per root tips. All the aberrant and c-mitosis cells were studied in a light microscope under oil immersion (100x). All slides were examined blind and coded.

Statistical analysis

A one-way ANOVA test using GPIS 1.13 software (GRAPHPAD, California, USA) was applied to find significance of differences in variables. All results were articulated as mean ± standard error.

RESULTS

It is clear from the results that methomyl is toxic to MI, CPK, c- mitosis, aneugenic and clastogeneic aberrations. The observed MI, CPK, c-mitosis, aneugenic and clastogeneic aberrations are well represented in (Fig. 1, Table 1, Fig. 2, Fig. 3 and Fig. 4). The clastogenic abnormalities observed were single bridges, fragments, double bridges, sticky chromosome and aneugenic abnormalities were laggards and vagrants.

Effect of methomyl treatment on mitotic index of P. sativum L.

Fig. 1 shows how methomyl affected the MI of root tip cells in *P. sativum*. In control group, seeds treated with DDW for 1 hour had a MI of 9.3%. From 0.1 to 0.2% methomyl treated seeds, a non-significant decline ($p>0.05$) in MI was observed and at 0.3% concentration, there was a significant decrease ($p< 0.05$) in MI and at 0.4 to 0.5%, a very significant decrease ($p< 0.01$) in MI was reported in comparison to control for 1 hour. Overall, MI decreases dose dependently in all concentrations from 0.1 to 0.5%.

Effect of methomyl in Cell Proliferation Kinetics (CPK) of P. sativum L

Cell proliferation kinetics (CPK), assessed as the ratio of prophases, metaphases, anaphases and telophases revealed a rise in prophase, metaphase and anaphase from (0.1 to 0.5%) and a decrease in telophase at 0.1 to 0.5% of methomyl treated root tips in comparison to control (Table 1).

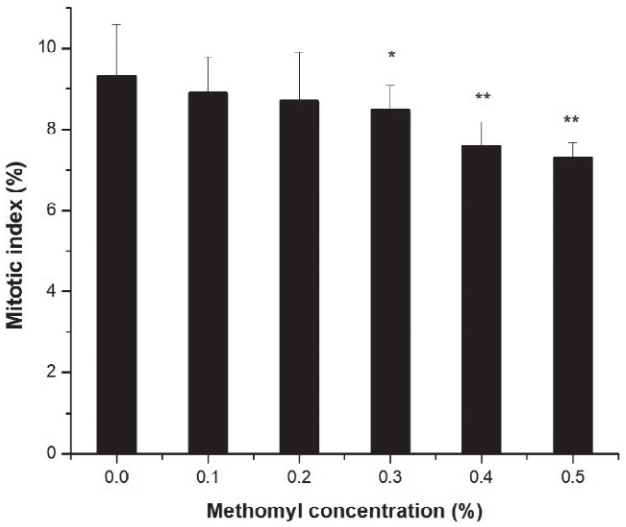

Figure 1. Effect of methomyl on mitotic index of *P. sativum* for 1 h. *p<0.05; compared to control group. Data are mean of three replicates ±SE, 0.0 = Control group.

Table 1. Effect of methomyl on cell proliferation kinetics in *P. sativum* L.

Concentration (%)	Prophases	Metaphases	Anaphases	Telophases
0.0	52.5±4.8	21.7± 2.7	18.5±3.4	23.12.8±2.3
0.1	50.7±4.6	23.5±1.7	20.4±2.4	21.5.6±3.0
0.2	50.4±2.4	27.4±3.5*	21.3±1.9*	20.9±1.30
0.3	55.7± 2.2	27.3.3±3.8**	23.1±1.7 **	19.12±3.6*
0.4	58.34±1.2*	28.45±3.6**	24.5±1.9 **	17.6±2.5**
0.5	60.12±2.6 **	29.40±1.2**	25.5± 1.6 **	16.80±3.6**

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

A significant increase ($p<0.05$) was reported in prophase at 0.4 % (58.34±1.2); metaphase at 0.2% (27.3±3.8), and anaphase at 0.2% (21.3±1.9) but a significant decrease ($p<0.05$) was observed in telophase at 0.3% (19.12 ±3.6) in comparison to control. Prophase at 0.5% (60.12±2.6); metaphase from 0.3 to 0.5% (27.4±3.5; 28.45±3.6; 29.40±1.2 respectively) and anaphase from 0.3 to 0.5% (23.1±1.7; 24.5±1.9; 25.5±1.6 respectively) resulted in a very significant increase ($p<0.01$) and telophase from 0.4 to 0.5% (17.6±2.5; 16.80±3.6) showed a very significant decrease ($p<0.01$) in comparison to control.

Effect of methomyl treatment on C-mitosis of P. sativum L

Fig. 2 demonstrates how methomyl affects c-mitosis in *P. sativum* root tips cells. Seedlings treated for 1h with

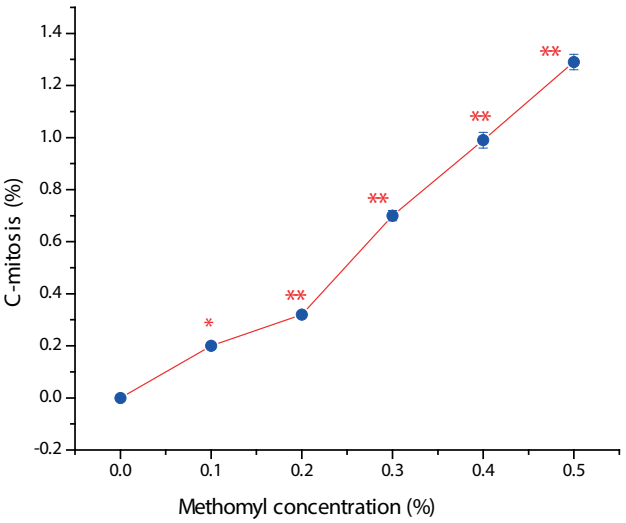

Figure 2. Effect of methomyl on c-mitosis in *P. sativum* for 1 h. *p<0.05; **p<0.01 compared to control group. Data are mean of three replicates ±SE, 0.0 = Control group.

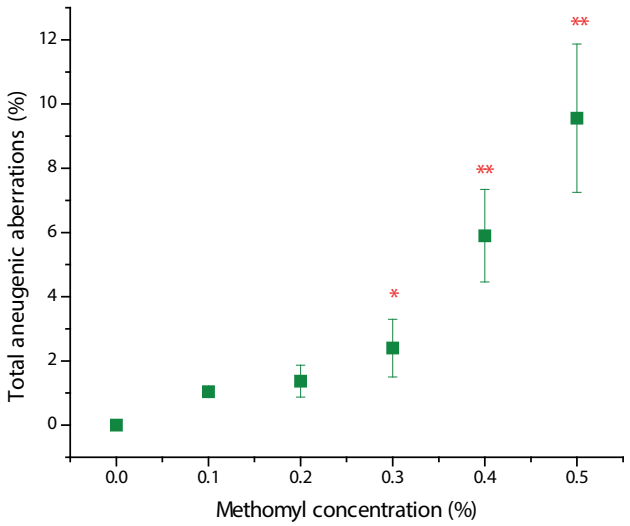

Figure 3. Effect of methomyl on total aneugenic aberrations in *P. sativum* for 1 h. *p<0.05; **p<0.01 compared to control group. Data are mean of three replicates ±SE, 0.0 = Control group.

DDW in the control group exhibited 0% c-mitosis. A significant increase (p<0.05) in the number of c-mitosis cells were seen in seeds treated with 0.1% methomyl for 1 hour and from 0.2 to 0.5%, there was a very significant increase (p<0.01) in c-mitosis cells in comparison to control for 1 hour. Overall, c-mitosis increases dose dependently in all concentrations ranging from 0.1 to 0.5%.

Effect of methomyl on aneugenic and clastogeneic aberration cells in P. sativum L

The incidence of aneugenic aberrations (laggards and vagrants) in metaphase-anaphase plates in the control group was zero. Percentage of aneugenic aberrations (laggards and vagrants) in the metaphase-anaphase plate dose dependently increased with methomyl treatment (Fig. 3 and Fig. 5). Seeds treatment with 0.1% methomyl resulted in a 1-fold increase and 0.2% treatment resulted in a 1.37-fold increase which was not significant and 0.3% methomyl treated seeds resulted in a 2.4-fold increase which was significant (p<0.05) in comparison to control. Further increase in concentration from 0.4 to 0.5% methomyl treated seeds resulted in a rise in incidence of aneugenic aberrations, 5.9-fold, and 9.56-fold respectively, which was very significant (p<0.01) in comparison to control.

The incidence of clastogeneic aberrations (single bridges, fragments, double bridges and sticky chromosome) at metaphase-anaphase plates in control group was zero (Fig. 4, and Fig. 5). Percentage of root tip cells with clastogeneic aberrations (single bridges, fragments,

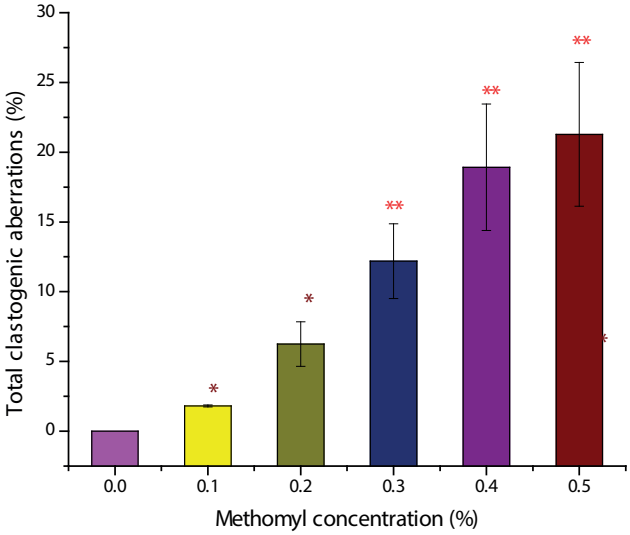

Figure 4. Effect of methomyl on total clastogenic aberrations in *P. sativum* for 1 h. *p<0.05; **p<0.01 compared to control group. Data are mean of three replicates ±SE, 0.0 = Control group.

double bridges and sticky chromosomes) at metaphase-anaphase plate increased dose dependently with methomyl treatment (Fig. 4). Treatment of seeds with 0.1% methomyl resulted in 1.81-fold increase which was non-significant as compared to control. However, from 0.2 to 0.5 % methomyl treated seeds resulted in 6.25-fold, 12.19-fold, 18.92-fold and 21.28-fold increase in clastogeneic aberrations respectively which was very significant (p<0.01) in comparison to control.

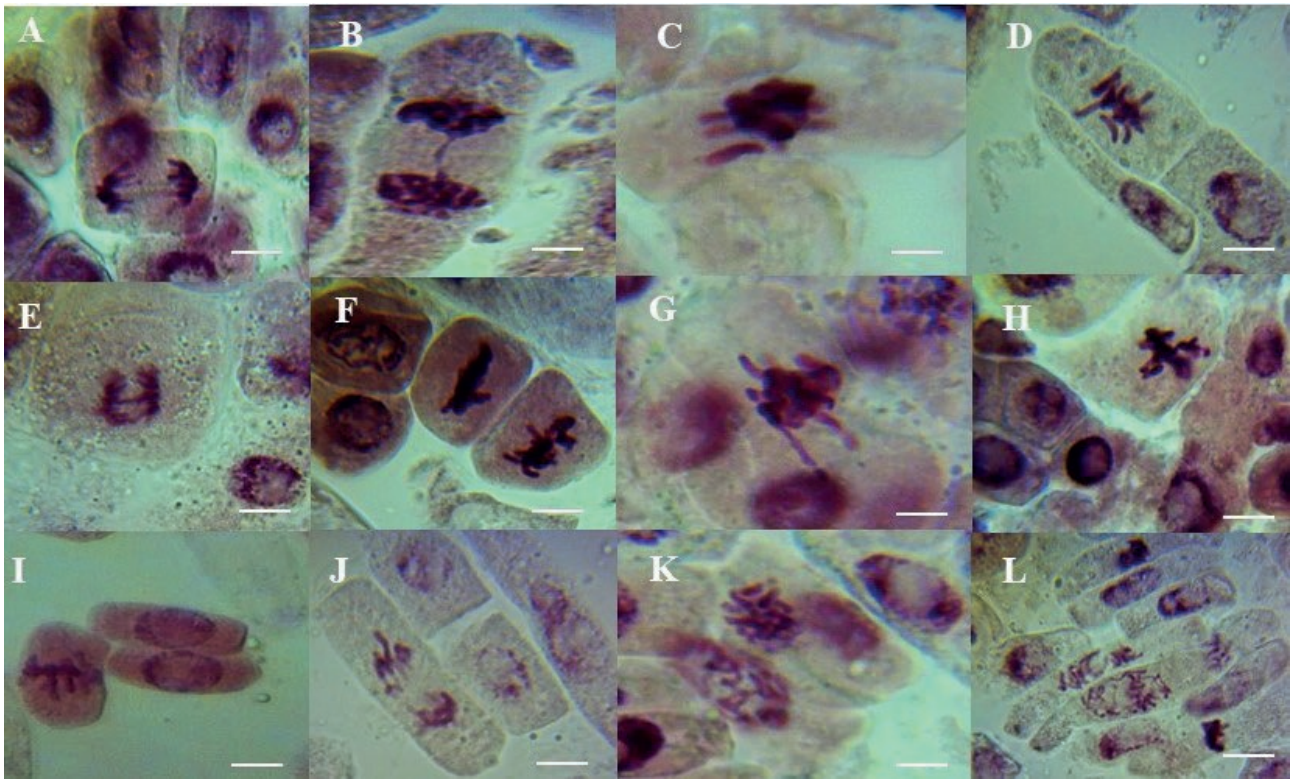

Figure 5. Clastogenic and aneugenic aberrations in methomyl treated *P. sativum* L root tip cells. Clastogenic aberrations A to F: A) Bridge in anaphase; B) Single bridge in telophase; C-D) Chromosome fragment in metaphase; E) Double bridge at anaphase; F) Sticky chromosome at metaphase. Aneugenic aberrations G to J: G-H-I) Chromosome vagrant at metaphase; J) Vagrant chromosome at anaphase; K –L) C-mitosis in metaphase; Bar - 10 µm.

DISCUSSION

This study shows that reduction in cell division indicates that tested methomyl have a mitodepressive potential. When mitotic activity is reduced, the amount of DNA also declines. This could be due to the blockage of cell cycle in the G2 phase or DNA synthesis inhibition or stopping the cell from starting mitosis (Siddiqui et al. 2007; Siddiqui et al. 2012; Siddiqui and Alrumman 2020 a and b). The significant decrease in mitotic index observed in this study might be the effect of methomyl interfering with the cell cycle by blocking G2 phase of cell cycle or DNA synthesis inhibition, or it could be the outcome of a rise in the frequency of chromosomal anomalies with analogous increase in methomyl concentration. These findings are also consistent with the outcomes of several research teams which have stated the cytotoxic effects of ethephon (Ayşe and Kılıç 2017; Bonciu et al. 2022), various synthetic plant growth regulators (Singh et al. 2022; Asif et al. 2022), and various pesticides (Lukaszewicz et al. 2019; Siddiqui and Alrumman 2022 a and b; Omeiri et al. 2022; Hafez et al. 2022; Bandopadhyay et al. 2022).

In this study, Methomyl raised the percentage of metaphase, prophase and anaphase and reduced the percentage of telophase in all concentrations in a dose dependent manner, as per the outcomes of proportions of distribution of specific mitotic stages. There is an increase at all concentrations of metaphase, prophase and anaphase phases. The outcomes are consistent with the findings of Liman et al. (2010), Priya et al. (2014), and Ozkul et al. 2016). Furthermore, the percentage of telophase stage decreased in comparison to control. These findings suggest that decline in telophase stages and henceforth MI might be due to arrest of one or more mitotic stages or due to a slowdown in the rate of cell development during mitosis (Ping et al. 2012).

C-mitosis was found in the present study. C-mitosis was created by unstable microtubules (Odeigah et al. 1997) or disruptions in the development of spindle fibers (Shimoi et al. 2019; Haliem 1990). The incidence of c-mitosis in root tip cells of Pisum sativum shows that spindle formation was harmfully affected (El-Ghamery et al. 2000). Considerable numbers of c-mitosis detected in this study implies that methomyl is a strong spindle

inhibitor. C-mitosis is also an indication of spindle poisoning, as per Rank (2003). The cause of the generation of c-mitosis might be due to disruptions in spindle formation, affected by methomyl.

In relation to genotoxicity, methomyl enhanced the incidence of clastogenic as well as aneugenic anomalies at the metaphase-anaphase plate. Single bridges, fragments, double bridges and stickiness, were the clastogenic anomalies whereas vagrants and laggards were the aneugenic anomalies observed in the present study. In treated seeds, a number of bridges were created in anaphases I and II plate. Bridges were most likely formed by breakage and combining of chromosomal bridges, which got enhanced with methomyl treatment. Chromosome stickiness and subsequent failure of free anaphase division or irregular translocation or inversion of chromosomal fragments can all lead to the creation of chromosomal bridges (Jing et al. 2022[a]; Honles et al. 2022). The fusion of broken chromosomes was the primary cause of the formation of bridges as per Rosculet et al (2019; Honles et al. 2022).

Increases in methomyl concentrations were associated with stickiness. Stickiness may result from partial detachment of nuclear proteins and alterations in their association design or from partial detachment of nucleoproteins and alterations in their association design or due to nucleic acid depolymerization activated by methomyl treatment. Disruptions in cytochemical balance reaction may lead to stickness (Dewitte et al. 2010; Rosculet et al. 2019). Nucleic acid depolymerization because of herbicidal treatment or by partial detachment of nucleoproteins (Kaufman et al. 1955) or by incomplete separation of nucleoprotein variation in their organization design (Evans 1962) might cause stickness.

The fragments formed from chromatid and chromosomal break imply its mutagenic events within the cell. In a previous study, Siddiqui et al. (2020 a,b) had reported that pesticides cause various chromosomal anomalies. Generation of giant cells having diverse chromosomal anomalies had been reported in a previous study by food colorants (Prajitha and Thoppil 2016).

The laggards observed during the current study may result from failure of chromosome movement or from deferred ending of stickiness of ends of chromosomes. At metaphase I, chromosome lagging could result from disturbances in bivalents motion to equatorial plate. Single univalent lagging was the most common incidence (Zeyad et al. 2019). Laggards and bridges could be created due to deferred ending of stickiness of ends of chromosomes (Kaur and Grover 1985). Laggards are responsible for the formation of micronuclei at telophase I. Acentric fragments or laggards are liable for micronuclei generation at telophase II and hence it leads to the changes in size and number of pollen grains arising from mother cells.

The other frequent aneugenic form of anomaly observed in dividing cells was vagrant chromosomes. As per Rank (2003), vagrant chromosomes are pointers of spindle poisoning. These aberrations might have developed as a result of the disruption in spindle formation, which was affected by methomyl treatment.

Genotoxic stress or genomic instability caused by DNA damage may result in illnesses, senescence, alterations in gene expression or cellular aging (Bonciu et al. 2018; Iturburu et al. 2018; Shabbir et al. 2021; Omeiri et al. 2022; Castellanos et al. 2022). In both plants and animals, a rise in genomic instability has been advocated as a basis for the decline in population fitness. Genotoxicity biomarkers must be taken into consideration when assessing potential noxious effects in aquatic organisms since genotoxic substances have the potential to cause damage that extends beyond the individual and can be seen over several generations (Frenzilli et al. 2009; Fioresi et al. 2020; Ergin et al. 2020 Amac and Liman 2021; Menzyanova et al. 2022).

CONCLUSION

According to the findings of the current study, methomyl can alter kinetics of mitotic cell process in root tip cells and can have genotoxic effects on *P. sativum* L through aneugenic and clastogenic processes. These findings raise concern about noxious effects of pesticides on non-target organisms. For the benefit of human welfare, additional genotoxicological and risk assessment studies needs to be conducted on various eukaryotic test systems.

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