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Differential sensitivity of barley (*Hordeum vulgare* L.) to chlorpyrifos and propiconazole: Morphology, cytogenetic assay and photosynthetic pigments

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ABSTRACT

The present investigation was performed to evaluate the effects of an insecticide and fungicide, namely, chlorpyrifos (CP) and propiconazole (PZ) on barley (*Hordeum vulgare* L. variety Karan-16). The seeds were treated with three concentrations of CP and PZ, i.e., 0.05%, 0.1% and 0.5% for 6 hours after different pre-soaking durations of 7, 17 and 27 hours. Different pre-soaking durations (7, 17 and 27 h) represent three phases of the cell cycle i.e., G_1, S and G_2, respectively. Double distilled water and ethyl methane sulfonate were used as negative and positive controls, respectively. As compared to their respective controls, treated root tip meristematic cells of barley showed significant reductions in the germination percentage, seedling height, mitotic index and comparative increase in chromosomal aberrations against both the pesticides, and the magnitude was higher in CP. After treatment with the pesticides, chlorophyll and carotenoid contents increased up to 0.1% but reduced at 0.5% and the decrease was more prominent in CP as compared to PZ. In treated cells, fragmentation, stickiness, bridges, multipolar anaphase and diagonal anaphase were observed as aberrations. As compared to control, chromosomal aberrations were higher in CP as compared to PZ. The results of the present study concluded that CP induced chromosomal aberrations were more frequent than PZ; hence it has higher probability to cause genotoxicity in barley.

1. Introduction

Pesticides represent a heterogeneous category of chemicals specifically designed for the control of pests, weeds or plant diseases in modern agricultural practices. Application of pesticides is considered the most effective and accepted means for the protection of plants from pest attack and has significantly contributed to enhance agricultural productivity. Pesticides are potential chemicals and their ingredients possess mutagenic properties leading to chromosomal aberrations or DNA damage in plant cells [1,2]. Biomonitoring of pesticides provides an important tool to estimate the genetic risk derived from application of various chemical ingredients during field experiments. In agricultural fields, plants being direct recipients of agro-toxics may be used for monitoring the ill effects of pesticides [3]. Higher plants provide excellent models for the genotoxicity assessment of environmental chemicals because plant chromosomes are easy to analyze in expression of size, morphology and number, and act in response to treatment with toxins in a similar way to mammals and other eukaryotes [4]. Pesticides have been reported to reduce the germination percentage of seeds, seedling height and mitotic index in barley [5,6].

Genotoxic potential is the primary risk factor for long term effects such as carcinogenic and reproductive toxicology. The majority of pesticides have been tested in a wide variety of mutagenic assays involving gene mutation, chromosome aberration and DNA damage [7,8]. Chlorpyrifos (CP), a crystalline organophosphate insecticide, acts on the nervous system of insects by inhibiting acetylcholinesterase [9]. An important property of organophosphorus compounds is the ability to alkylate DNA [10,11], thus turning them into potential agents for mutagenic and carcinogenic effects. In a study conducted on a fresh water fish (*Channa punctatus*), CP induced high number of micronuclei in the peripheral blood revealing its genotoxic effects on aquatic species [9]. Chlorpyrifos is moderately toxic to humans and its chronic exposure causes neurological effects, developmental disorders and autoimmune disorders. In agriculture, CP remains as one of the most widely used organophosphate insecticides according to the United States Environmental Protection Agency [12]. Propiconazole (PZ) is a triazole fungicide and plant growth regulator. It is also known as a demethylation inhibiting fungicide due to its binding and preventing the 14-α-demethylase enzyme from demethylating a precursor to Ergosterol [13]. Without

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the demethylation step, they are not incorporated into the growing fungal cell membranes, and cellular growth is stopped. According to previous reports, fungicides and insecticides induce the genetic damage and act as mutagens [14–16]. Mutation refers to the change in a DNA sequence which may involve few bases or the large scale chromosomal abnormality. Chromosomal aberrations are considered as reliable indicators of mutagenic activity [17]. Plant chromosomes are excellent material for genotoxic bioassay since they are large in size and sensitive to various environmental conditions [18,19]. Initially, *Allium* test was employed for genotoxic assay of chemicals and hazardous substances [20]. *Hordeum vulgare* is now used extensively for the bioassay experiments [21–23].

In the present study, we have examined the effects of CP and PZ to demonstrate the differential sensitivity of *H. vulgare* L. (variety Karan-16) against the applied pesticides. The present investigation was conducted with the following objectives: (*i*) to find out the efficiency of the selected pesticides on the morphological traits such as germination percentage and seedling height, and (*ii*) to study the comparative effects of selected pesticides on mitotic index, chromosomal aberrations and photosynthetic pigments, if any. To our knowledge, the present investigation is the first study that reports chlorpyrifos and propiconazole induced-genotoxicity in *H. vulgare* L.

2. Materials and methods

2.1. Plant material

The seeds of barley (*H. vulgare* L.) variety Karan-16 were obtained from the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University; Varanasi Barley (*H. vulgare* L., $2n = 2x = 14$) is a diploid and self-fertilizing crop with few but large chromosomes and production of sufficient seeds from a single plant makes it a good experimental material for mutation studies.

2.2. Selection of pesticides, EC_{50} and test concentration determination

In the present study, pesticides were selected on the basis of their frequent use by local farmers in and around Varanasi. Chlorpyrifos (CP) [(O, O-diethyl O-3, 5, 6-trichloro-2-pyridyl-phosphorothioate, EC 20%, CAS number-2921-88-2] is a crystalline organophosphate insecticide known by their trade names Dursban, Lorsban and Rusban. Propiconazole (PZ) [1-{2-(2, 4-dichlorophenyl)-4-propyl-1, 3-dioxolan-2-yl} methyl-1, 2, 4-triazole, EC 25%, CAS number 60207-90-1] is a triazole fungicide. Ethyl methane sulfonate (EMS) was brought from Sigma Aldrich Chemicals, USA. Fixatives/stains used in the present study were obtained from Merck Ltd., Mumbai, India.

For EC_{50} determination, healthy seeds were treated with different concentrations of chlorpyrifos and propiconazole ranging from 0.1 to 1%. As compared to their respective controls, the test concentrations which showed 50% reduction in root length were designated as EC_{50}. EC_{50} values for chlorpyrifos and propiconazole were 0.2 and 0.1%, respectively.

After EC_{50} determination, three different concentrations (0.05%, 0.1% and 0.5%) of CP, PZ and EMS (positive control) were selected as test concentrations. The experimental concentrations were selected on the basis of their doses used by local farmers in agricultural fields [21]. Double distilled water was used as a negative control in the experiment.

Seeds were soaked in double distilled water for complete hydration until 1 h and left for different pre-soaking durations (7 h, 17 h and 27 h) which fall in three different stages of cell cycle, i.e., G_1, S and G_2 phases, respectively [21]. All the pre-soaked seeds were treated with 0.05%, 0.1% and 0.5% of CP, PZ and EMS, respectively for 6 h. Equal number of seeds was also treated with double distilled water to be used as a negative control. The treated seeds were successively washed with double distilled water four times. All the seeds were placed on moist filter paper in petri plates and allowed to germinate at room temperature (22 ± 1 °C) in triplicates. The moistening material was double distilled water. Remaining seeds were sown in the field for analyses of photosynthetic pigments. The field experiment was carried out at the botanical garden of Udai Pratap Autonomous College, Varanasi during winter season between the months of November 2012 to March 2013.

2.3. Cytogenetic assay

The appropriate root tips were randomly collected from each petri plate for cytological studies in triplicate. The collected root tips were washed thoroughly with double distilled water and fixed in Carnoy's solution (ethyl alcohol:chloroform:acetic acid) in the ratio (6:3:1) overnight and preserved in 70% ethyl alcohol at 4 °C. The root tips were hydrolyzed in 1 N HCl and stained with 0.5% hematoxylin. All the observations were made from temporarily prepared slides in triplicates. In each replication, a minimum of 2000–3500 cells were observed. Mitotic index (MI) was calculated as the percentage of the total number of dividing cells/the total number of scored cells. Chromosomal aberrations were counted in the different mitotic stages.

2.4. Extraction of photosynthetic pigments

0.1 g of fresh leaf samples was taken and homogenized in 10 ml 80% acetone. The homogenate was then centrifuged at 6000 rpm for 10 min. The supernatant was taken and optical densities were recorded at 645 and 663 nm, 480 and 510 nm for estimation of chlorophyll pigments and carotenoids. Chlorophyll a (chl a) and chlorophyll b (chl b) were calculated by using the formula described by Maclachlan and Zalik [24]. Carotenoid content was determined by using the formula described by Duxbury and Yentsch [25].

2.5. Statistical analysis

The statistical significance of differences among values of studied parameters (germination percentage, seedling height, mitotic index, chromosomal aberrations and photosynthetic pigments) was analyzed by using New Duncan's multiple test. All the statistical tests were performed using SPSS software (version 16.0, SPSS Inc., USA).

3. Results and discussion

3.1. Morphological parameters

The present study revealed that treatment with the pesticides CP, PZ and mutagenic agent EMS had a detrimental effect on the experimental material. Fig. 1 represents the effect of CP and PZ on germination percentage of seeds. Both the pesticides significantly reduced germination of seeds at 17 h duration (S phase) of cell cycle at all test concentrations with respect to control and the extent was higher in CP. The most pronounced decrease in seed germination was noticed at 17 h pre-soaking duration of 0.5% of treatment. Germination of seeds was significantly decreased by 33.8% and 17.6% under CP and PZ treatment, respectively as compared to their respective controls. Pesticide causes phytotoxic or stimulatory effects on seed germination. Germination of seed is an important stage in plant growth, and is particularly sensitive to contaminants revealing stress tolerance against contaminants [26]. As the concentration of pesticide was increased, reduction in seed germination was

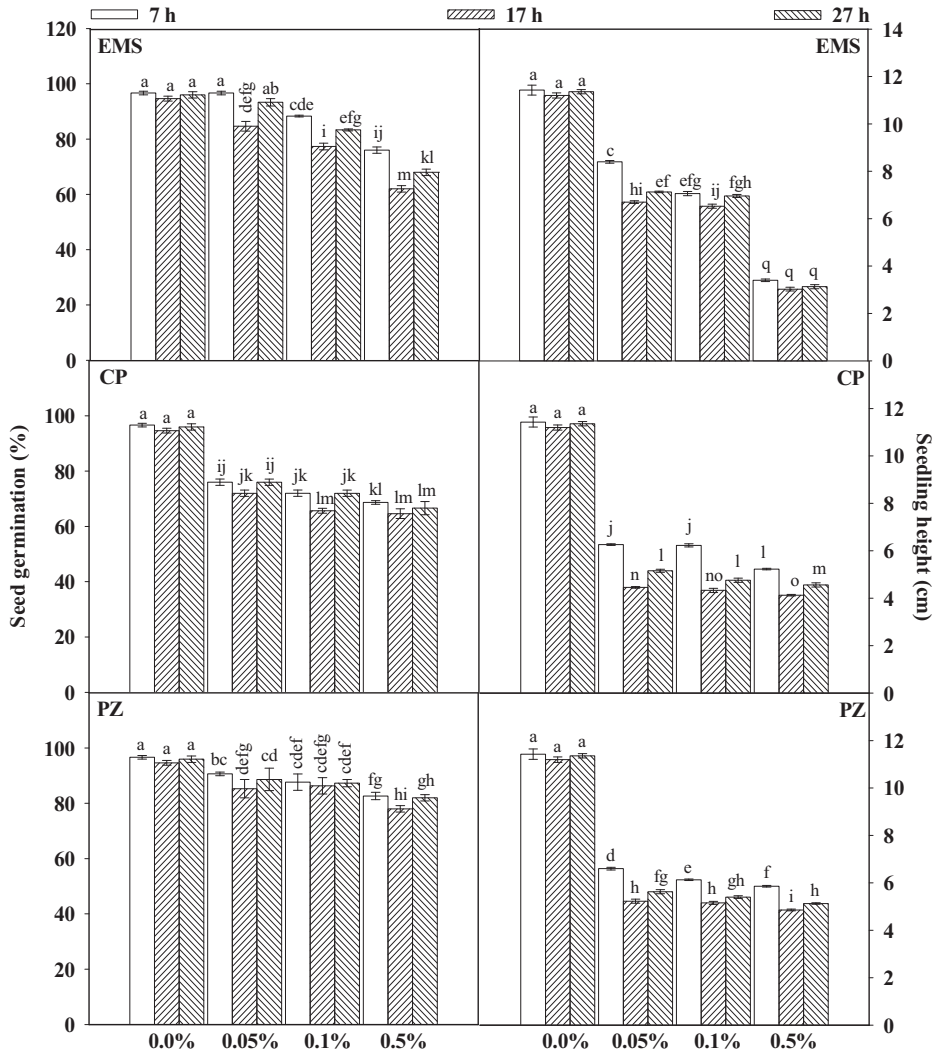

Fig. 1. Germination percentage and seedling height of barley at different pre-soaking durations under various concentrations of EMS, CP and PZ. Bars showing different letters indicate significant differences among treatments according to New Duncan's multiple test at $p < 0.05$.

observed [27]. Treatments with pesticides have been reported to reduce seed germination in barley [5,28]. Pesticides have been reported to pose their toxic effects on the seeds of a range of annual crops [29,30]. Pesticides enter inside the seeds by diffusion and their modes of action have been shown to be internal in the seed, where the synthesis or action of hydrolytic enzymes gets inhibited [20,31]. Inside seeds, vital enzymes such as amylase, ATPase, lipase and protease may be affected [30] which are generally needed for metabolic pathways during germination [32].

The effect of CP and PZ on seedling height is shown in Fig. 1. Higher decrease was noticed in the S phase of the cell cycle at all test concentrations. Significant reductions of 63.1% and 66.4% in seedling height were found at 17 h pre-soaking duration of 0.5% concentration by CP and PZ, respectively as compared to their controls (Fig. 1). Reductions in seedling height may be explained due to inhibition of cell division at the meristematic regions [33,34]. Similar results have been described earlier in *H. vulgare* L. [28,35].

3.2. Cytological parameters

Fig. 2 represents the effects of CP and PZ on MI in the root tip cells of barley exposed to all the three test concentrations i.e., 0.05%, 0.10% and 0.5%. MI is a parameter which estimates the frequency of cellular division [36]. All the experimental concentrations of both CP and PZ affected the MI in an inversely proportional way i.e., as the concentration of pesticides was increased, MI decreased (Fig. 2). The inhibition of MI was most pronounced in the root tip cells exposed to 0.5% concentration of CP at 17 h pre-soaking duration (42.5%), as compared to its respective control. PZ also showed the significant decrease in MI at the same concentration and pre-soaking duration but the extent was less as compared to CP (Fig. 2). In the present study, treatment with pesticides not only brought down the frequency of dividing cells but also resulted in a significant number of abnormalities in the mitotic cells. There was a marked decrease in the MI and a gradual increase in the percentage of chromosomal abnormalities as the concentrations of pesticides were increased. Significant reduction in MI may be ascribed to the mito-depressive action of the chemicals indicating interference in the normal cell cycle resulting in a reduced number of dividing cells [37]. Such type of inverse relation between the MI and concentration, and a direct relation between the concentration and chromosomal aberration have been reported by previous workers [37–40]. Reduction in mitotic index may be either due to the inhibition of DNA in the S-phase or blocking of the G_1 phase suppressing the DNA synthesis or blocking in the G_2 phase of the cell cycle and preventing the cells entering the mitosis [41,42].

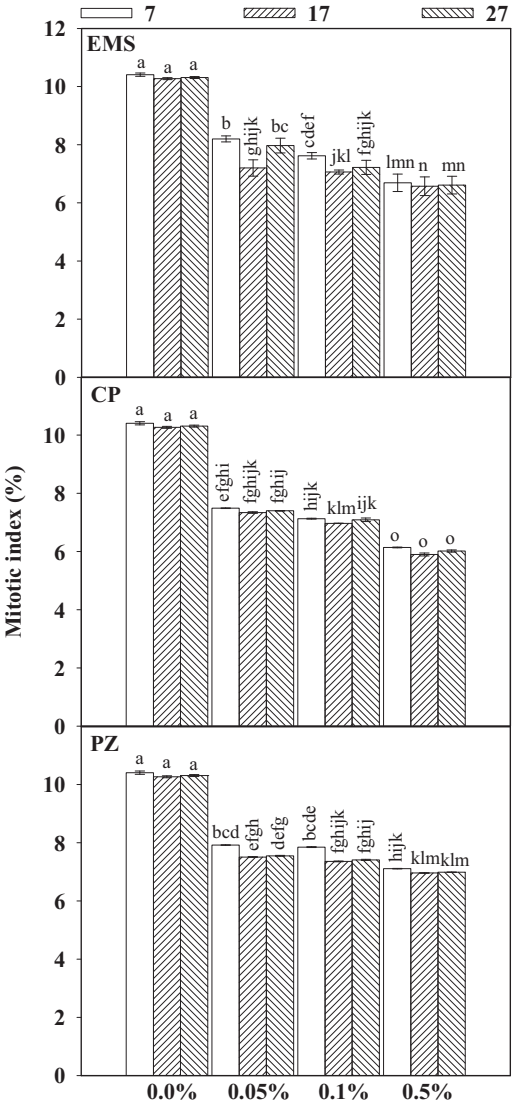

Fig. 2. Mitotic index of barley under different concentrations of EMS, CP and PZ at different pre-soaking durations. Different letters on bars indicate significant differences among treatments at $p < 0.05$ according to New Duncan's multiple test.

Tables 1–3 summarize the total abnormality percentage of chromosomal aberrations in the root tip cells of barley exposed to test concentrations at different pre-soaking durations of EMS, CP and PZ, respectively. The induction of chromosomal aberration was directly proportional to the test concentration. Fragmentation, stickiness, chromosomal bridges, diagonal anaphase and multipolar anaphase were observed as chromosomal aberrations in the treated cells [Fig. 3a–f]. Chromosome fragmentation is a major form of mitotic cell death which is identified through abnormal cytogenetic figures such as multiple breaks. During the process of chromosome fragmentation, the number of chromosomal breaks increases until all chromosomes are completely degraded. Morphologically, chromosome fragmentation can be grouped into at least three groups, (1) early fragmentation where few chromosomes are broken; (2) mid-stage fragmentation where a significant number of the chromosomes have been fragmented; and (3) late stage fragmentation where all or most of the chromosomes have been fragmented, suggesting it is a progressive process [43]. Chemical pesticides possess mutagenic properties and are capable of inducing chromosomal aberrations or DNA damage [1,21]. A high

Table 1
Chromosomal aberrations induced by EMS (positive control) in barley.

Concentration and pre-soaking durations	FRAG	ST	BRI	MA	DA	Total abnormality* (%)
0.0%						
7 h	–	0.17	–	–	–	0.17^{i}
17 h	–	0.55	–	–	–	0.55^{i}
27 h	–	0.34	–	–	–	0.34^{i}
0.05%						
7 h	1.65	2.49	0.00	0.00	0.00	4.14^{h}
17 h	1.95	2.50	1.11	0.00	0.00	5.56^{g,h}
27 h	1.54	1.72	0.93	0.00	0.00	4.20 ^{h}
0.10%						
7 h	2.10	3.15	0.21	0.00	0.00	5.47^{g,h}
17 h	2.22	3.09	0.50	0.00	0.00	5.82^{g,h}
27 h	1.43	2.40	0.62	0.00	0.00	4.46^{h}
0.5%						
7 h	2.94	3.64	0.67	0.23	0.00	7.49^{f,g}
17 h	3.16	4.27	1.13	0.89	0.92	10.39^{d,e}
27 h	1.98	2.83	1.07	0.81	0.81	7.52^{f,g}

* Different letters as superscripts in the column indicate significant differences among treatments at $p < 0.05$ according to new Duncan's multiple test.
FRAG = fragmentation; ST = stickiness; BRI = bridges; MA = multipolar anaphase; DA = diagonal anaphase.

incidence of chromosomal stickiness at all experimental test concentrations was noticed in root tip cells and indicates the toxic effect of CP and PZ. The sticky nature of chromosomes may be due to the delay in chromosome movement due to pesticide treatment. The possible explanation of this trend may be ascribed to non-reaching of chromosomes to the poles which remain scattered in the cytoplasm and appear condensed and sticky [44]. According to McGill et al. [45], chromosome stickiness might be due to intermingling of chromatin fibers which leads to subchromatid connections between chromosomes. The bridge formation might have resulted from the general stickiness of chromosomes at the metaphase stage [46]. The chromosomal bridges noticed in the present investigation may be attributed to the formation of dicentric chromosomes as a result of breaking and reunion of chromosomes [47]. In the present investigation, the non-orientation of chromosomes on the equatorial plate was observed at the anaphase stage of mitosis [Fig. 3f]. These aberrations could be due to

Table 2
Chlorpyrifos induced chromosomal aberrations in barley.

Concentration and pre-soaking durations	FRAG	ST	BRI	MA	DA	Total abnormality* %
0.0%						
7 h	–	0.17	–	–	–	0.17^{i}
17 h	–	0.55	–	–	–	0.55^{i}
27 h	–	0.34	–	–	–	0.34^{i}
0.05%						
7 h	1.94	3.00	1.60	2.28	0.88	9.72^{e,f}
17 h	2.92	3.35	2.46	2.70	1.11	12.55^{c,d}
27 h	2.23	2.69	2.46	2.00	0.90	10.31^{d,e}
0.10%						
7 h	3.05	4.01	1.43	3.52	1.41	13.45^{b,c}
17 h	3.71	3.49	3.25	3.72	1.85	16.04^{a}
27 h	2.43	3.09	2.43	2.43	1.10	11.50^{c,d,e}
0.5%						
7 h	3.38	4.61	2.43	3.15	1.42	15.01^{a,b}
17 h	3.93	4.15	3.46	3.46	2.32	17.33^{a}
27 h	2.20	3.10	2.65	2.88	1.10	11.95^{c,d,e}

* Superscript letters in the column represents significant differences among treatments at $p < 0.05$ according to New Duncan's multiple test.
FRAG = fragmentation; ST = stickiness; BRI = bridges; MA = multipolar anaphase; DA = diagonal anaphase.

Table 3
Chromosomal aberrations observed under propiconazole treatment in barley.

Concentration and pre-soaking durations	FRAG	ST	BRI	MA	DA	Total abnormality* (%)
0.0%						
7 h	–	0.17	–	–	–	0.17^{i}
17 h	–	0.55	–	–	–	0.55^{i}
27 h	–	0.34	–	–	–	0.34^{i}
0.05%						
7 h	1.17	2.68	1.15	1.36	0.00	6.37^{g,h}
17 h	1.83	2.66	1.64	1.65	0.00	7.80^{f,g}
27 h	1.53	1.94	1.35	1.58	1.17	7.59^{f,g}
0.10%						
7 h	1.80	2.35	1.21	1.59	0.00	6.96^{g}
17 h	2.33	2.73	1.92	2.12	1.06	10.19^{d,e}
27 h	1.61	2.00	1.22	1.59	0.79	7.22^{g}
0.5%						
7 h	2.81	3.50	2.11	2.56	1.17	12.17^{c,d,e}
17 h	3.61	4.34	2.89	3.13	1.68	15.66^{a,b}
27 h	2.53	3.48	2.10	2.32	0.93	11.38^{c,d,e}

* Different letters as superscripts in the column indicate significant differences among treatments at $p < 0.05$ according to New Duncan's multiple test.
FRAG = fragmentation; ST = stickiness; BRI = bridges; MA = multipolar anaphase; DA = diagonal anaphase.

the failure of spindle apparatus to organize and function in a normal way rather than inhibition of the spindle fibers and this might lead to irregular orientation of chromosomes [48]. The occurrence of multipolar anaphase indicates that CP and PZ caused inhibition of mitotic spindle formation due to which alteration in chromosome orientation occurred. Such spindle malfunctioning may be due to the interaction of CP and PZ with tubulin-SH groups and may cause inhibition of tubulin polymerization [49]. As compared to PZ, diagonal anaphase was also higher in the root tip cells treated with CP which might have resulted due to non-orientation of two anaphase groups of chromosomes at the same axis in the cell. This observation suggested higher toxic effect of CP on the spindle apparatus in the present study.

3.3. Photosynthetic pigments

Tables 4–6 represent variations in chl a, chl b and carotenoid content, respectively in the leaf samples of barley exposed to test concentrations at different pre-soaking durations. CP increased the chl a content up to 0.1% but a significant decrease was observed at 0.5%. The most pronounced effect of CP was observed at 17 h pre-soaking duration of 0.5% test concentration at which chl a content was maximally reduced by 43.7% as compared to its respective control. A significant reduction by 24.6% in chl a content was observed at 0.5% at all pre-soaking durations in PZ treated leaves. Chl b content increased up to 0.1% under CP and PZ treatment but a significant decrease was observed at 0.5%. Reductions in chlorophyll pigments at higher concentrations of CP may be due to the inhibition of their biosynthesis or breakdown of pigments or their precursor molecules. Another reason for the reduction in chlorophyll pigments may be attributed to the decrease in the leaf area with increasing concentration of pesticides, which is in agreement with the pervious study on the inhibitory action of pesticides on chlorophyll and/or photosynthetic apparatus [50]. Reduction in leaf area under pesticide treatment could be due to obstruction in translocation of photo-assimilates toward leaf expansion [51]. Both pesticides CP and PZ show a slight increase in the carotenoid content at 0.05% and 0.1% and the reason may be ascribed to protection of chlorophyll molecules from photo-oxidative damage. As compared to control, PZ treatment increased the chlorophyll and carotenoid content to a larger extent than CP. Higher level of carotenoid content has been reported in wheat [52] and tomato [53] treated with uniconazole. The increase in pigment concentration under PZ treatment may be attributed to the rise in ABA and cytokinin contents [54].

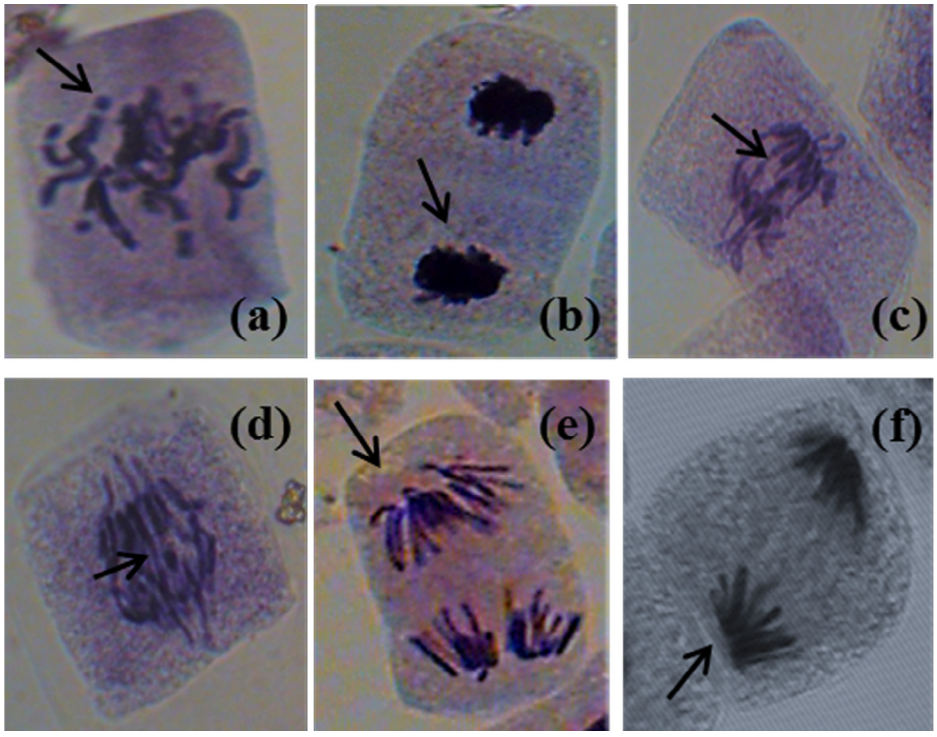

Fig. 3. Chromosomal aberrations in mitotic cells of barley treated with chlorpyrifos and propiconazole; a = fragmentation; b = stickiness; c and d = chromosomal bridges; e = multipolar anaphase; f = diagonal anaphase.

Table 4
Content of chlorophyll a (mg g^{-1} fresh wt.) in different treatments in Barley. Values are mean ± SE.

Concentration and pre-soaking durations	0.05%	0.10%	0.5%
Control (negative control)			
7 h	$0.629 \pm 0.019^{d,e}$		
17 h	0.617 ± 0.015^{e}		
27 h	$0.628 \pm 0.017^{d,e}$		
EMS (positive control)			
7 h	$0.645 \pm 0.036^{c,d,e}$	$0.650 \pm 0.034^{c,d,e}$	$0.530 \pm 0.030^{f,g}$
17 h	0.622 ± 0.028^{e}	$0.628 \pm 0.033^{d,e}$	0.474 ± 0.031^{g}
27 h	$0.627 \pm 0.032^{d,e}$	$0.641 \pm 0.036^{c,d,e}$	$0.528 \pm 0.035^{f,g}$
Chlorpyrifos			
7 h	0.722 ± 0.031^{c}	0.951 ± 0.042^{a}	0.382 ± 0.011^{h}
17 h	$0.715 \pm 0.030^{c,d}$	0.881 ± 0.052^{b}	0.347 ± 0.018^{h}
27 h	0.720 ± 0.033^{c}	$0.947 \pm 0.054^{a,b}$	0.359 ± 0.019^{h}
Propiconazole			
7 h	0.623 ± 0.016^{e}	$0.642 \pm 0.027^{c,d,e}$	$0.528 \pm 0.032^{f,g}$
17 h	$0.602 \pm 0.036^{e,f}$	0.611 ± 0.020^{e}	0.465 ± 0.013^{g}
27 h	0.618 ± 0.019^{e}	0.622 ± 0.017^{e}	0.509 ± 0.016^{g}

Different letters with in a group of a column indicate significant differences among treatments at $p < 0.05$ according to New Duncan's multiple test.

Table 5
Variations in contents of chlorophyll b (mg g^{-1} fresh wt.) in different treatments in Barley. Values are mean ± SE.

Concentration and pre-soaking durations	0.05%	0.10%	0.5%
Control (negative control)			
7 h	$0.260 \pm 0.013^{e,f,g,h}$		
17 h	$0.202 \pm 0.013^{i,j}$		
27 h	$0.217 \pm 0.015^{h,i,j}$		
EMS (positive control)			
7 h	0.365 ± 0.018^{a}	$0.344 \pm 0.023^{a,b}$	$0.152 \pm 0.025^{k,l,m}$
17 h	$0.346 \pm 0.014^{a,b}$	$0.316 \pm 0.013^{b,c,d}$	0.120 ± 0.010^{m}
27 h	$0.353 \pm 0.024^{a,b}$	$0.324 \pm 0.015^{a,b,c}$	$0.131 \pm 0.010^{l,m}$
Chlorpyrifos			
7 h	$0.285 \pm 0.013^{c,d,e,f,g}$	$0.296 \pm 0.018^{c,d,e}$	$0.200 \pm 0.010^{i,j}$
17 h	$0.212 \pm 0.011^{h,i,j}$	$0.239 \pm 0.016^{g,h,i}$	$0.134 \pm 0.011^{l,m}$
27 h	$0.245 \pm 0.014^{f,g,h,i}$	$0.268 \pm 0.012^{d,e,f,g}$	$0.168 \pm 0.013^{j,k,l}$
Propiconazole			
7 h	$0.318 \pm 0.019^{a,b,c}$	$0.351 \pm 0.019^{a,b}$	$0.202 \pm 0.013^{i,j}$
17 h	$0.278 \pm 0.012^{c,d,e,f,g}$	$0.294 \pm 0.014^{c,d,e,f}$	$0.171 \pm 0.012^{j,k,l}$
27 h	$0.287 \pm 0.014^{c,d,e,f,g}$	$0.315 \pm 0.013^{b,c,d}$	$0.195 \pm 0.011^{i,j,k}$

Groups of a column showing different letters indicate significant differences among treatments at $p < 0.05$ according to New Duncan's multiple test.

Table 6
Variations in carotenoid content (mg g^{-1} fresh wt.) induced by chlorpyrifos and propiconazole in barley. Values are mean ± SE.

Concentration and pre-soaking durations	0.05%	0.10%	0.5%
Control (negative control)			
7 h	$0.542 \pm 0.015^{c,d,e}$		
17 h	$0.520 \pm 0.018^{d,e,f}$		
27 h	$0.523 \pm 0.019^{d,e,f}$		
EMS (positive control)			
7 h	$0.561 \pm 0.014^{a,b,c,d,e}$	$0.603 \pm 0.012^{a,b}$	0.423 ± 0.012^{g}
17 h	$0.528 \pm 0.019^{d,e,f}$	$0.569 \pm 0.011^{a,b,c,d}$	$0.381 \pm 0.018^{g,h,i}$
27 h	$0.551 \pm 0.014^{c,d,e}$	$0.580 \pm 0.010^{a,b,c}$	$0.400 \pm 0.007^{g,h}$
Chlorpyrifos			
7 h	$0.560 \pm 0.014^{a,b,c,d,e}$	$0.581 \pm 0.021^{a,b,c}$	$0.366 \pm 0.014^{h,i,j}$
17 h	$0.521 \pm 0.025^{d,e,f}$	$0.539 \pm 0.015^{c,d,e}$	$0.344 \pm 0.014^{i,j,k}$
27 h	$0.537 \pm 0.010^{c,d,e,f}$	$0.550 \pm 0.014^{c,d,e}$	$0.357 \pm 0.012^{h,i,j,k}$
Propiconazole			
7 h	$0.553 \pm 0.011^{b,c,d,e}$	0.608 ± 0.010^{a}	$0.325 \pm 0.011^{j,k,l}$
17 h	0.487 ± 0.020^{f}	$0.554 \pm 0.012^{b,c,d,e}$	0.287 ± 0.010^{l}
27 h	$0.513 \pm 0.017^{e,f}$	$0.572 \pm 0.010^{a,b,c,d}$	$0.313 \pm 0.011^{k,l}$

Different letters with in a group of a column indicate significant differences among treatments at $p < 0.05$ according to New Duncan's multiple test.

The results of present study revealed that higher concentrations of CP and PZ have potential to cause genetic damage to plants by inducing genotoxicity. Since CP induced chromosomal aberrations are more frequently observed than PZ, hence it has higher probability to cause genotoxicity. Therefore, it is essential to test the genotoxic effects of pesticides before considering their applications for agricultural purposes. The study suggests that application of lower concentrations of pesticides in agricultural fields may be beneficial to farmers. The findings of the present study conclude that (*i*) pesticide treatment during the synthesis (S) phase of the cell cycle may result in chromosomal aberrations leading to higher damage to the cells, and (*ii*) the S phase is more sensitive in comparison to other phases of the cell cycle in *H. vulgare* L.

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