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Clethodim induced pollen sterility and meiotic abnormalities in vegetable crop *Pisum sativum* L.

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Abstract. Pesticides are highly noxious materials.Their poisonousness might not be unequivocally precise to the target entities but can unfavorably disturb various procedures in the non-target host plants. In the present study, the effect of application of clethodim on pollen sterility and meiotic anomalies of *Pisum sativum* L are studied. *Pisum sativum* L seeds are treated with different concentrations of clethodim varying from 0.01%, 0.02%, 0.03%, and 0.04% for exposure time of 1 hour and their effect on pollen sterility and chromosomal anomalies were investigated. The outcomes reveal that treatment of clethodim on *Pisum sativum* L seeds induces pollen sterility (PS) and chromosomal anomalies (CA) in a dose-dependent manner. Also in clethodim treated seeds, an elevation in the proportion of abnormal meiotic phases were observed which was time and concentration dependent. Secondary association (SeA), precociuos separation (PS), clumped nuclei (CNu) were reported in metaphase I & II, stickiness (Stc), bridges (Br) and laggards (Lg) in anaphase I & II. The results of the present study reveal that frequently used herbicide clethodim has a substantial cytotoxic effect on meiotic cells of *Pisum sativum* L.

Keywords. Herbicide, Clethodim, Pollen sterility, Meiotic abnormality, *Pisum sativum* L.

INTRODUCTION

The application of herbicides has led to an alteration in the phytosociological properties of weeds and to a choice of biotypes resilient to herbicides. In addition, it also causes effects in the health of humans and environment although they are categorized as an extremely efficient means in the regulation of weeds. According to He *et al*. (2012), all over the world, the most applied chemical materials are the herbicides. In the 90's, the worldwide pesticide sales continued to stay moderately persistent, amongst 270 to 300 billion american dollars, 79% of this amount is related to herbicides. Amongst the three foremost groups of pesticides i.e. herbicides, fungicides and insecticides; herbicides have taken the top slot since 2007 (Zhang *et al*. 2011). In order to upsurge agricultural output, the application of herbicides for regulating weeds is the most widely used practice in worldwide agriculture. Nevertheless, when these chemical substances are applied in an unrestrained

way, they can influence and disturb other organisms, particularly aquatic creatures (Nwani *et al*. 2011; Van Bruggen *et al*. 2018).

Earlier reports have shown that much of the noxious effects of herbicides on plants and animals were inadequately explored (Chevreuil *et al*. 1996; Kim and Feagley 1998; Abdel-Rahman *et al*. 1999). As a result of the absence of evidences about the effect of herbicides in the biotic environments, they can also epitomize a setback to health of human beings (Munger *et al*. 1997; Gorell *et al*. 1998). The influence of pesticide in the environments is determined by its noxiousness, concentration and dispersion manner (Van der Werf 1996). The mutagenic effects of the herbicides can result from several reactions within the organism, as a direct action of the compound on the nuclear DNA; incorporation in the DNA during cell replication; interference in the activity of the mitotic or meiotic division, resulting in incorrect division of the cell (Timbrell 1999). A few herbicides affect directly the elongation, cell differentiation and cell division of plants.

Herbicide clethodim falls into the cyclohexanedione oxime class. From post-emergence, clethodim is active as an herbicide against various species of grass weeds mostly in sunflower, soybean, cotton and other broad-leaved plants (Edwards 2005). Earlier studies have emphasized the adverse effects of the herbicides on plant physiology and cytogenetics. Maleic hydrazide (MH) is one of the few herbicides which prevents the synthesis of proteins and nucleic acids (Siddiqui *et al*. 2008). Mutagenic action of glyphosate, alachor and maleic hydrazide has been reported by Siddiqui *et al*. (2012), diclofop-methyl and lindane has been stated by Anila and Ditika (2013) and anilofos has been described by Arzu *et al*. (2014) on root tip cells of plant.

Pisum sativum L, (Fabaceae) is a plant useful in the manure production, ayurvedic medications and food (Davies *et al*. 1985). It is one of the most used leguminous plant and is a source of protein. There are no studies available in the literature which have assessed the adverse effects of clethodim on *Pisum sativum* L, a potential multipurpose crop. In the current investigation, we have assessed the noxious consequences of clethodim on meiotic cells of *Pisum sativum* L.

MATERIALS AND METHODS

Seeds and chemical

Pisum sativum L seeds, variety ARKIL were obtained from the Indian Council of Agricultural Research - Bbhoj Krishi Vigyan Kendra, Near Village Naktara, P. O. Bankhedi, NH-86 Ext., Raisen Sagar Road, Bhopal, India.

Clethodim herbicide was obtained from the Delta Chemical Company based in Riyadh, Saudi Arabia.

Treatment of Pisum sativum L with clethodim

Healthy and even sized seeds of *Pisum sativum* L were taken and submerged for 6 hours in double distilled water of pH 6.7. In a glass beaker of 250 mL, seeds of *Pisum sativum* L were immersed for 1 hour in 150 mL clethodim solutions of various concentrations (0.01, 0.02, 0.03 and 0.04%). The seeds were shaken frequently for providing ample air to the seeds. For eliminating any remaining amount of clethodim, the treated seeds were properly cleaned with double distilled water. For control, a set of seeds were submerged in distilled water. In order to eradicate any remaining chemical sticking to the seed coat, the seeds were cleaned with tap water. Each set of seeds comprising of 10 seeds per pot were seeded in pots having a height of 24 cm and width of 17 cm. The entire experiment was repeated thrice under similar conditions.

Pollen grain analysis

For experiments related to pollen grains, flower buds of similar age were collected from treated plants and control group. They were fixed in 70% alcohol. For identifying pollen sterility as well as fertility, pollens were stained with 1% propionocarmine. Pollens having uniform size and shape which were stained dark purple in colour and filled with nuclei and cytoplasm were considered as fertile whereas pollen grains which were stained pale yellow colour or colourless, having irregular size and shape and without nuclei and cytoplasm were defined as sterile. Pollen sterility was calculated as the ratio of non viable pollen grains to the total number of pollens and expressed in percentage.

$$\text{No. of sterile pollen grains} = \frac{\text{No. of non viable pollen grains}}{\text{Total no. of pollen grains}} \times 100$$

Meiotic analysis

To assess the effect on meiotic cells, flower buds of similar size were fixed in 1:3 acetic acid saturated with iron and absolute alcohol for 24 hours and than passed to 70% alcohol. The meiotic cell preparations were prepared by traditional acetocarmine squash technique (Man-

Table 1. Percentage of meiotic abnormality in metaphase I & II and anaphase I & II plate of *Pisum sativum* L in PMCS exposed to different concentrations of clethodim for 1 hour.

(%)	Concentration time	Treatment PMC cells	Total		Metaphase I/II (Mean ±S.E)		Anaphase I/II (Mean ±S.E)		
			Sec. asso.	Pre.sep.	Clumped nuclei	Stickiness	Bridges	Laggards	Total anomalies
	1 h								
0.00		110	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	00.0 ± 0.0
0.01		125	1.5 ± 0.34	2.6 ± 0.29	3.5 ± 0.2	1.8 ± 0.3	2.4 ± 0.4	1.4 ± 0.2	13.2 ± 1.73
0.02		115	2.8 ± 1.20	3.8 ± 0.93	4.6 ± 0.9	2.2 ± 0.7	3.1 ± 0.7	2.8 ± 0.6	19.3 ± 5.03
0.03		128	3.2 ± 1.70	4.2 ± 1.04	$6.4^{\$}$ ± 1.3	3.5 ± 1.2	3.8 ± 0.3	3.4 ± 1.2	24.5 ± 7.34
0.04		130	$6.5^{\#}$ ± 2.80	$6.1^{\$}$ ± 2.1	$7.4^{\$}$ ± 2.4	$6.7^{¥\#}$ ± 2.0	$6.5^{\#}$ ± 2.7	$6.2^{\$\#}$ ± 2.1	$39.4^{\$}$ ± 14.1

$p \leq 0.05$; $^{\$}p \leq 0.01$; $^{¥}p \leq 0.001$ compared to control. Data are mean of three replicates ± SE; 0.0 = Control group.

ton 1950). By squashing the anther in an acetocarmine stain, slides were prepared. In normal butnyl alcohol (NBA) series, permanent slides were made and they were mounted in Canada balsam and than dried up at 45°C.

Statistical analysis

Statistical analysis was performed employing one way ANOVA test using GPIS software 1.13 (GRAPHPAD, California, USA) to detect the significance of differences of variables. All values are expressed as mean ± SE.

RESULTS

The occurrence of pollen sterility and meiotic chromosomal abnormalities were listed in (Figs 1, 2 and Table 1). It is clear from the results that clethodim was proficient in inducing pollen sterility and different types of meiotic chromosomal abnormalities such as secondary association (SeA) (Fig. 2, A and B), precociuos separation (PS) (Fig. 2, C and D), and clumped nuclei (CNu) (Fig. 2, E and F) in metaphase I & II and stickiness (Stc) (Fig. 2, G and H), bridges (Br) (Fig. 2, I and J) and laggards (Lg) (Fig. 2, K and L) in anaphase I & II.

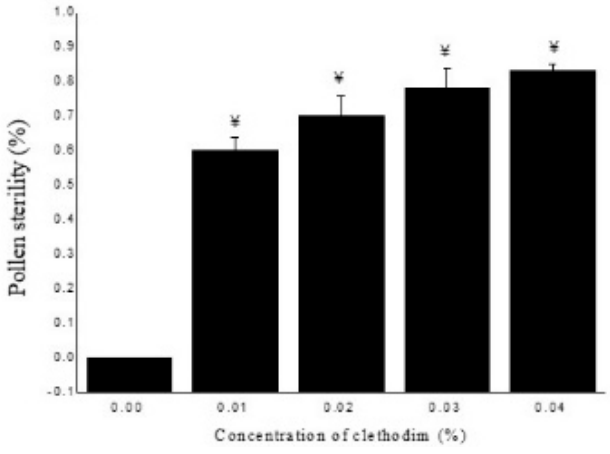

Figure 1. Pollen sterilty of *Pisum sativum* L PMCS exposed to different concentrations of clethodim for 1 hour. $^{¥}p \leq 0.001$ compared to control. Data are mean of three replicates ± SE; 0.0 = Control group.

Effect of clethodim on pollen sterility of Pisum sativum L.

The observation reveals that the percentage of pollen mother cells (PMCS) showing pollen sterility increased with increase in concentrations of clethodim at different exposure times and was null in control (Fig. 1). A lower percentage of pollen sterility were observed at 0.01% concentration which was 60% at one hour and it was highly significant (p>0.001) when compared to control. In addition, at highest concentration high increase in pollen sterility were reported which reached 83% at one hour in clethodim treated PMCS.

Pollen sterility is a sign of disturbance in reproductive process which was observed in clethodim treated pollen mother cells of *Pisum sativum* L at 1 hour (Fig. 1). The increase in the ratio of sterile pollen grains in the clethodim treated groups as the dose increases might be due to their toxic effects on pollen grains.

Effect of clethodim treatment on chromosomal aberration (CA) of Pisum sativum L

Cytological analysis also documented numerous anomalies in the PMCS of clethodim treated plants at one hour, as shown in Table 1. Different types of CA such as SeA, PS, and CNu were noticed in metaphase I & II and Stc, Br and Lg were observed in anaphase I &

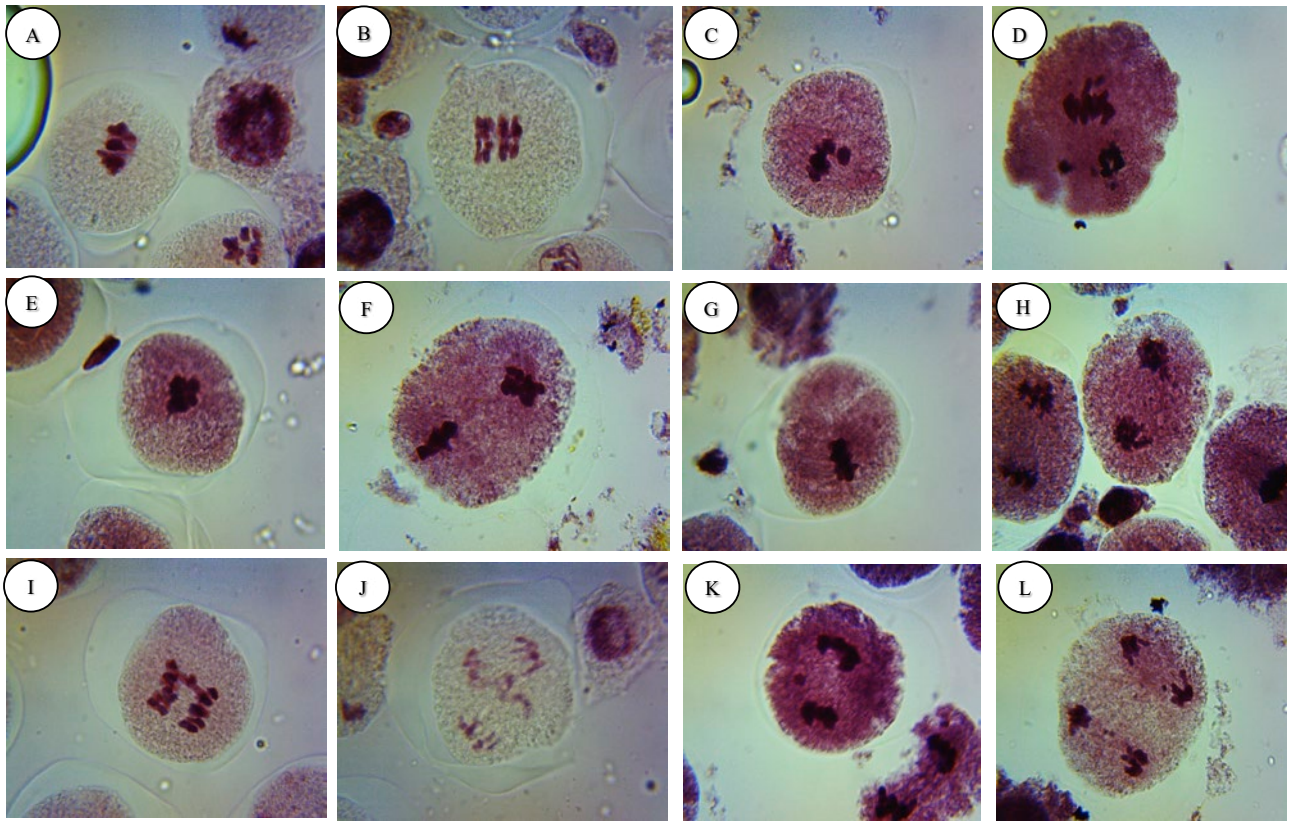

Figure 2. (A-L) Meiotic aberrations induced by clethodim in *Pisum sativum* L PMCS. (A-B) Secondary association; (C-D) Precocious separation; (E-F) Clumped nuclei at Metaphase I & II, (G-H) Sticky chromosome; (I-J) Bridges; (K-L) Laggards at Anaphase I & II in PMCS in *Pisum sativum* L.

II (Table 1 and Fig. 2). Cytological investigations clearly revealed that the level of CAs gradually increased along with increasing concentrations of clethodim at one hour. Studies of different stages of meiotic division shows that almost all the stages of division were affected to a certain extent.

The percentage of formation of SeA, PS, and CNu in metaphase I & II were reported maximum in 0.04% (6.5% - significant $p \leq 0.05$, 6.1% - very significant $p \leq 0.01$ and 7.4% - very significant $p \leq 0.01$) as compared to control, at one hour and reported minimum in 0.01% (1.5%, 2.6% and 3.5%) at one hour of clethodim treated plants.

The percentage of formation of Stc, Br, and Lg in anaphase I & II were reported maximum in 0.04% (6.7% - very significant $p \leq 0.01$ and significant $p \leq 0.05$, 6.5% - significant $p \leq 0.05$ and 6.2% - very significant $p \leq 0.01$) as compared to control at one hour and reported minimum in 0.01% (1.8%, 2.4% and 1.4%) at one hour of clethodim treated plants.

Increased incidence of CAs were in the following order for 1 hour clethodim treatment: CNu > Stc > BR = SeA > Lg > PS.

DISCUSSION

In the present study, the occurrence of laggards and bridges in anaphase I & II were strictly correlated with sterility. Similar finding were reported by Liang *et al.* (1967) on sorghum by Atrazine treatment which affected meiotic stability. Çalli (2008) reported that the Equation Pro (22.5% Famoxadone+30% Cymoxanil) fungicide instigates abnormalities in the meiosis of pollen grains and thus resulting in pollen sterility. Dubey *et al.* (1977) reported a decrease in viability of about 60% in the pollen of eggplants, indicated by the joint effect of a dinitro herbicide and two organophosphate insecticides. Fungicide propiconazole produced damaging effects on pollen tube development and pollen germination of *Tradescantia virginiana* (He *et al.* 1995). Rana and Swaminathan (1964) and Ramanna (1974) found that any deviance in cytokinesis or karyokineses could generate non-viable microspores. Sinha and Godward (1969;1972) found that translocations were liable for reduced pollen fertility. In *H.orientalis*, tube growth and *in vitro* pollen germination was disturbed and affected by the herbicide

Quizalofop-p-ethyl (QPE) treatments. Especially, the maximum QPE concentration instigated alterations in the morphological attributes of *H. orientalis*. Pollen germination is decreased by three times by utmost vigorous QPE application. Within the pollen tube morphological anomalies were also detected (Deveci *et al*. 2017).

Pisum sativum L and *Allium cepa* L are generally used in order to check the genotoxicity and cytotoxicity of several chemicals (Bonciu *et al*. 2018 a and b; Siddiqui 2018). Previous works have revealed that herbicides have mutagenic effect on *Pisum sativum* L and *Allium cepa* L (Siddiqui *et al*. 2008; Rosculet *et al*. 2019). Cytological abnormalities in plants can be a useful monitoring method for the recognition of chemicals present in the environment that might cause a genetic threat. In the current study, we found numerous kinds of chromosomal irregularities such as SeA, PS, CNu in metaphase I & II, Lg, Br, and Stc in anaphase I & II in *Pisum sativum* L after treatments with clethodim of one hour.

The prevalence of secondary association has been found in treated plants. Incidence of secondary association is a regular attribute occurring primarily because of the existence of more than two homologous chromosomes. In the course of secondary pairing, there is a debate on the participation of homologous chromosomes amongst the researchers. Hirayoshi (1957) differed with the postulation that contemplates the participation of homologous chromosome in secondary association and after investigating thorough outcomes in *Zizanieae* and *Oryzeae* offered the inference that secondary association might be an incidence effective under physico and bio-chemical effects and has got no relation to the exact homology of chromosomes.

Secondary pairing within bivalents is well thought to be a sign of polyploid attribute of a species as found in Kidney bean (Girjesh and Nitu, 2014), *Ocimum* (Mukherjee and Datta 2005), *Uraria picta* (Bhattacharya and Datta 2010). As per Stebbins (1950), secondary association may be contemplated to be a phenomenon that illustrates the polyploid attribute of a species but detailed phylogenetic estimations may not be framed from this since secondary pairing within bivalents is substantially altered by further chromosomal alterations.

In the present study, the occurrence of precocious movement also appeared enhanced with rising clethodim concentations. Precocious separations were formed due to unstable spindle process or inactivation of spindle formation and interrupted homology for pairing of chromosomes that might lead to the precocious motion of chromosomes (Umar and Singh 2003). It might also be attributed due to the occurrence of breakage of chromosomes or because of the heavy metals breaching the protein moiety of the nucleoprotein backbone.

In the current study, the prevalence of clumped nuclei also get boosted with rising clethodim concentations. Because of the turbulences at the cytochemical level, clumped nuclei were created. Clumping of the chromosomes can occur with regular arrangement at metaphase and separation may result in the creation of bridges. Some types of gene mutations that cause incorrect coding of some non-histone proteins involved in chromosomal organization can lead to chromosomal clumping. There is a possibility that the mutagen reacts with the available histone proteins and results in an alteration in the surface property of the chromosomes because of incorrect folding of DNA and hence causing the chromosomes to stick or clump (Grant 1978).

Stickiness was also reported with increase in the clethodim concentrations. Stickiness could occur because of incomplete detachment of the nuclear proteins and changes in their design of association or because of incomplete detachment of the nucleoproteins and amendment in their design of association or because of depolymerization of nucleic acids instigated by clethodim treatment. Stickiness might result from disorders in cytochemical balance reaction (Dewitte *et al*. 2010; Rosculet *et al*. 2019). Depolymerisation of nucleic acids due to herbicidal treatment or by incomplete detachment of nucleoproteins (Kaufman *et al*. 1955) or by the limited separation of nucleoprotein variation in their design of organization (Evans 1962) may cause stickness.

Numerous bridges were formed in anaphase I & II in the treated plants. Bridges were probably created by breaking and combination of chromosomes bridges which augmented with the treatment of clethodim. Formation of chromosomal bridges could occur because of the chromosomal stickiness and consequent failure of free anaphase division or because of inversion of chromosome fragments or irregular translocation (Gomurgen 2000). Rosculet *et al*. (2019) reported that the origin of bridges were mainly because of the merger amongst broken chromosomes.

The laggards noticed within the current investigation could be due to the failure of the movement of chromosomes or because of the deferred termination of stickiness of the ends of chromosomes. Lagging of chromosomes might occur because of the interruption of the motion of bivalents towards equatorial plate at metaphase I. The utmost regular occurence was the lagging of single univalent (Zeyad *et al*. 2019). Barthelmess (1957) reported that more than one lagging chromosomes in meiosis might occur because of the interruption of the prometaphase motion of chromosomes supplemented by

union of the centromeric to the adjoining inner surface of plasma. Bridges and laggards might have been formed because of deferred terminalisation of stickiness of the ends of chromosomes (Kaur and Grover 1985). Creation of micronuclei at telophase I is caused by laggards. Generation of micronuclei at telophase II is caused by laggards or acentric fragments and thus it results in the alteration in numeral and size of pollen grains ensuing from mother cells.

As reported in previous findings, our outcomes suggest the possibility of these chemicals to instigate meiotic anomalies (Namrata and Alka 2014; Das *et al*. 2018). The chromosomal abnormalities caused by these herbicides might be because of their impeding effect on spindle proteins and their capability to instigate swap over of sister chromatids (Tartar *et al*. 2006; Siddiqui *et al*. 2009). Previous investigations have revealed that free radicals might be the reason for genomic uncertainty in cells. Damages in DNA, disarray in the cytoskeleton and disproportion in energy metabolism might result in chromosomal anomalies as reactive oxygen species are extremely unstable (Siddiqui *et al*. 2012; Siddiqui 2015).

Due to genetic and physiological disarrays, numerous types of meiotic abnormalities might have been created. It has become clear from the above findings that clethodim used in the current work is probably capable of instigating several types of chromosomal anomalies.

CONCLUSION

Within the experimental settings applied in the current work, clethodim displayed a sturdy genotoxic effect on *Pisum sativum* L. It is obligatory to do additional research work on the attribute of the harvests resulting from seeds and plants subjected to these chemicals in relation to their nutritive worth and their vulnerability to acclimatize with diseases. Additionally, it is significant to note that the pesticide preparations encompassing these active constituents may or may not have a parallel effect on the plant cells. Regarding this, supplementary researches are essential to evaluate their outcome on biological procedures.

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DECLARATION

The authors declare no conflict of interest.

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REFRENCES

Abdel-Rahmam AR, Wauchope RD, Truman CC, Dowler CC. 1999. Runoff and leaching of atrazine and alachlor on a sandy soil as affected by application in sprinkler irrigation. J of Environ Sci and Health-B: Pesti and Food Cont. 34:381–396.

Anila MD, Ditika K. 2013. Cytotoxic and genotoxic potency screening of two pesticides on *Allium cepa* L. Proc Tech. 8:9–26.

Arzu O, Dilek A, Yasin E, Sevim FE. 2014. Potential cytotoxic effect of anilofos by using *Allium cepa* assay. Cytotechnology. 67:783–791.

Barthelmess A. 1957. Chemish induzierte multipalare Mitosen. Protoplasma. 48:546–561.

Bhattacharya A, Datta AK. 2010. Secondary chromosome associations in *Uraria picta* (Jacq.) DC. (Family: Leguminosae). Cytologia. 75:37–40.

Bonciu E, Roşculete E, Olaru AL, Roşculete CA. 2018[a]. Evaluation of the mitodepressive effect, chromosomal aberrations and nuclear abnormalities induced by urea fertilization in the meristematic tissues of *Allium cepa* L. Caryologia. 71(4):350-356.

Bonciu E, Firbas P, Fontanetti CS, Wusheng J, Karaismailoğlu MC, Donghua Liu, Menicucci F, Pesnya DS, Popescu A, Romanovsky AV, Schiff S, Ślusarczyk J, de Souza CP, Srivastava A, Sutan A, Papini A. 2018[b]. An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay. Caryologia. 71(3):191-209.

Çalli İÖ. 2008. Effects of fungicide on meiosis of tomato (*Lycopericon esculentum* Mill.). Bangladesh J Bot. 37(2):121–125.

Chevreuil M, Garmouma M, Teil MJ, Chesterikoff A. 1996. Occurrence of organochlorines (PCBs, pesticides) and herbicides (triazines, phenylureas) in the atmosphere and in the follout from urban and rural stations of Paris area. Science of the Environ. 182:25–37.

Das A, Dutta S, Nag S, Mandal S. 2018. Effect of acephate pesticide on meiosis in onion (*Allium cepa*). Inter J of Agri. 10(6):5456 –5459.

Davies DR, Berry GJ, Heath MC, Dawkins TCK. 1985. Pea (*Pisum sativum* L.). In: Summerfield RJ, Roberts EH (Eds.). Grain legume crops. Williams Collins (London). p. 147–198.

Deveci A, Aksoy O, Al G. 2017. Investigation of the effects of quizalofop-P-ethyl on pollen germination of *Hyacinthus orientalis* L. Caryologia. 70:77–81.

Dewitte A, Eeckhaut T, Van Huylenbroeck J, Van Bockstaele E. 2010. Meiotic aberrations during 2n pollen formation in Begonia. Heredity. 104:215–23.

Dubey PS, Shrivastava A, Shevade A. 1977. Pesticidal toxicity bioassay with pollen damage. Environ. Poll. 13(3):169–171.

Edwards D. 2005. Reregistration Eligibility Decision (RED) for Sethoxydim. United States Environmental Protection Agency. p. 1–27.

Evans HJ. 1962. Chromosome aberrations induced by ionizing radiations. Int Rev Cytol. 13:221–232.

Girjesh K, Nitu C. 2014. Secondary chromosomal association in kidney bean (*Phaseolus vulgaris* L.). Jordan J Biol Sci. 7(1):71–74.

Gomurgen AN. 2000. Cytological effect of the herbicide 2,4-D isooctylester 48% on root mitosis of *Allium cepa*. Cytologia. 65:383–388.

Gorell JM, Jhonson CC, Rybicki, BA, Peterson EL, Richardson RJ. 1998. The Risk of Parkinson's disease with exposure to pesticides, farming, well water, and rural living. Neurology. 50(5):1346 –1350.

Grant WF. 1978. Chromosome aberrations in plant as a monitoring system. Environ Health Per. 27:37–43.

He H, YuJ, Chen G, Li W, He J, Li H. 2012. Acute toxicity of butachlor and atrazine to freshwater green alga *Scenedesmus obliquus* and cladoceran *Daphnia carinata*. Ecotox Environ Saf. 80:91–96.

He Y, Wetzstein HY, Palevitz BA. 1995. The effects of a triazole fungicide, propiconazole, on pollen germination, tube growth and cytoskeletal distribution in *Tradescantia virginiana*. Sex Plant Reprod. 8:210–216.

Hirayoshi I. 1957. The chromosomal relationships in Oryzeae and Zizanieae. Cytologia Supp. Vol Proc Int Genet Symposia. 1956:293–297.

Kaufman BP, McDonald MR, Bernstein MH. 1955. Cytochemical studies of changes induced in cellular materials by ionizing radiations. Ann N Y Acad Sci. 59:553–566.

Kaur P, Grover IS. 1985. Cytological effects of some organophosphorus pesticides. II. Meiotic effects. Cytologia. 50:199–211.

Kim JH, Feagley SE. 1998. Adsorption and leaching of trifluralin, metolachlor, and metribuzin in a commerce soil. Journal of Environmental Science and Health-B: Pest and Food Conta. New York. 33:529–546.

Liang GH, Feltner KC, Liang YYS, Morril JM. 1967. Cytogenetic effects and responses of agronomic characters on grain sorghum following atrazine application. Crop Sci. 7(3):245–248.

Manton I. 1950. Problems of cytology and evolution in the Pteridophyta. Cambridge: Cambridge University Press; p. 316.

Mukherjee M, Datta AK. 2005. Secondary chromosome associations in *Ocimum basilicum* L and *Ocimum tenuiflorum* L. Cytologia. 70:149–152.

Munger R, Isacson P, Hu S, Burns T, Hanson J, Lynch CF, Cherryholmes K, Vandorpe P, Hausler JrWJ. 1997. Intrauterine growth retardation in Iowa communities with herbicides- contaminated drinking water supplies. Environ Health Persp. 105:308–314.

Namrata S, Alka S. 2014. Biomonitoring of genotoxic effect of glyphosate and pendimethalin in *Vigna mungo* populations. Cytologia. 79(2):173–180.

Nwani CD, Nagpure NS, Kumar R, Kushwaha B, Kumar P, Lakra, WS. 2011. Mutagenic and genotoxic assessment of atrazine-based herbicide to freshwater fish *Channa puntatus* (Bloch) using micronucleus test and single cell gel electrophoresis. Envir Toxic and Pharm. 31:314–322.

Ramanna MS. 1974. The origin of unreduced microspores due to aberrant cytokinesis in meiocytes of potato and its genetic significance. Euphytica. 23:20–30.

Rana SR, Swaminathan MS. 1964. Cytological aspects of pollen sterility. Recent Advances in Palynology. p. 276-304. National Botanical garden, Lucknow.

Rosculete CA, Bonciu E, Rosculete E, and Olaru LA. 2019. Article determination of the environmental pollution potential of some herbicides by the assessment of cytotoxic and genotoxic effects on *Allium cepa*. Int J Environ Res Public Health 16: 75.

Siddiqui S, Khan SS, Meghvansi MK, Nazoora SA. 2008. Mutagenic effect of herbicide maleic hydrazide on seed germination and radicle length on *Trigonella foenum-graecum*. Indian J Appl Pure Biol. 23:103–106.

Siddiqui S, Meghvansi MK, Khan SS. 2012. Glyphosate, alachor and maleic hydrazide have genotoxic effect on *Trigonella foenum-graecum* L. Bull Environ Contam Toxicol. 88:659–665.

Siddiqui S, Meghvansi MK, Wani MA, Jabee F. 2009. Evaluating cadmium toxicity to the root meristem of *Pisum sativum* L. Acta Physiol Plant. 31:531–536.

Siddiqui S. 2015. DNA damage in Cicer plant grown on soil polluted with heavy metals. J of King Saud University – Sci. 27:217–223.

Siddiqui S. 2018. Cytotoxicity induced by aluminum sulfate in cells of root meristem of *Pisum sativum* cv. Arikil. Bangladesh J Bot. 47(2):219–226.

Sinha SSN, Godward MBE. 1969. Radiation studies in *Lens culinaris*. Mitosis type of aberration and their elimination in root and shoot apical meristem. J Cytol Genet. 3:80–91.

Sinha SSN, Godward MBE. 1972. Radiation studies in *Lens culinaris*. Meiosis: abnormalities induced due to gamma radiation and its consequences. Cytologia. 37:685–695.

Stebbins GL. 1950. Variation and Evolution in Plants. Columbia Univ Press. New York.

Tartar G, Kaymak F, Muranli FDG. 2006. Genotoxic effects of Avenoxan on *Allium cepa* L. and *Allium sativum* L. Caryologia. 59:241–247.

Timbrell JA. Introduction to Toxicology. 2. Ed. Estados Unidos: Taylor & Francis, 1999. p.167.

Umar G, Singh V. 2003. Comparative analysis of meiotic abnormalities induced by gamma rays and EMS in Barley. J Indian Bot Soc. 82:19–22.

Van Bruggen AHC, He MM, Shin K, Mai V, Jeong KC, Finckh MR, Morris JG Jr. 2018. Environmental and health effects of the herbicide glyphosate. Sci. of the Total Environ. 616–617:255–268.

Van der Werf HMG. 1996. Assessing the impact of pesticides on the environment. Agric Ecosyst Environ. 60:81–96.

Zeyad MT, Kumarb M, Malika A. 2019. Mutagenicity, genotoxicity and oxidative stress induced by pesticide industry wastewater using bacterial and plant bioassays. Biotech Reports. 24: e00389.

Zhang W, Jiang F, Ou J. 2011. Global pesticide consumption and pollution: with Chinas as a focus. Proceedings of the Inter Acad of Ecol and Envir Scies. 1(2):125–144.