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# Cytomorphological Effects of Two Insecticides on Mitotic and Meiotic Cells of Barley (*Hordeum vulgare* L.)

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**Abstract**

Many insecticides are being used now-a-days to control insects which may alter the morphological and cytological constitution of plants. Present investigation was undertaken to study the effects of two most commonly used insecticides namely Morter-$48_{EC}$ and Safathrin-$10_{EC}$ on the morphological and cytological characters of barley **(***Hordeum vulgare* L.). Data on morphological characters such as germination percentage, coleoptile length, root length, plant height, ear length, seeds/ear, grain weight/ear and 100-grain weight indicated that seeds treated with Safathrin-$10_{EC}$ affected these characters with the increase of its doses whereas no detrimental effect was found in control. In case of shoot length, Morter-$48_{EC}$ showed significant effect than Safathrin-$10_{EC}$ and ear length and seed/ear were little affected by the different doses of Morter-$48_{EC}$ compared to that of control. For cytological parameters *viz.*, interphase chromosome volume, percent (%) of pollen sterility, mitotic and meiotic abnormalities were found to be proportional with increasing concentration of both insecticides except mitotic index. It can be concluded that these insecticides have adverse impacts on morphological and cytological characters of barley which may lead to reduce yield.

**Keywords:** *Hordeum vulgare*, insecticide, morphological effect, cytological effect.

## INTRODUCTION

Barley is the fourth most important cereal crop in the world which play vital role in enhancing the food security because of its high yield per unit area and also for its multidimensional usages. But, insect-attacks are the key factors for decreasing crop yield. In order to protect the crop plants from the insect attacks, chemical control is being considered as the most accepted measure at present time. In this regards, the use of organophosphate and pyrethroid insecticides in current agricultural practices is steadily increasing (Haux *et al*., 2000 and Aurbek *et al*., 2006). On the other hand, in Bangladesh, majority of the farmers are not educated and that is why they apply insecticides without knowing the population of insect pests, crop condition and specific recommended doses. For this reason, these insecticides cause the morphological and cytological abnormalities of various crop species (Shehata *et al*., 2000). Halil (2011) reported that mitotic index was decreased with the increasing concentration of insecticide "Dichlorvos" in barley seedlings which indicated its genotoxic and mutagenic effects. Moreover, in Bangladesh the cytological effects of insecticides on barley plant have been studied by Biswas (1979) who observed chromosomal aberration both in root tips and pollen mother cells as well as some abnormalities in some of the phenotypic traits and pollen sterility. But, there is not much information on the cytomorphological changes in plants induced by the insecticides Morter-$48_{EC}$ and Safathrin-$10_{EC}$.

The objective of the present investigation was to make a keen and comparative study regarding the effects of the two insecticides on morphological and cytological characters of barley.

## MATERIALS AND METHODS

This experiment was conducted in Professor S. Alam Cytogenetics Laboratory, Department of Botany, University of Rajshahi, Bangladesh during the year 2011-2012. Seeds of barley variety named BARI barley-5 [(*H. vulgare* L., 2n=14)] were used as plant material. Two insecticides namely, Morter-$48_{EC}$ ($C_9H_{11}Cl_3NO_3PS$) and Safathrin-$10_{EC}$ ($C_{22}H_{19}Cl_2NO_3$) with three different concentrations *viz.*, 1.0%, 2.0% and 4.0% were used for treatment while distilled water for control. Approximately, 450 fresh dry barley seeds (according to the suggestions of International Seed Testing Association) were soaked in three different doses (aqueous solution) of both the insecticides in separate beakers for 6 hrs and the treated seeds were washed thoroughly with tap water. The seeds for control were also presoaked in distilled water for the same period of time. Morphological study was made from both the plants grown in laboratory and earthen pot. In laboratory, part of the seeds was allowed to germinate in the petri dishes at room temperature (25°-30°C). For mitotic study, germinating roots of 1.0-1.5 cm in length were fixed in 1 acetic acid: 3 alcohol for 48 hrs and were stored in 70% ethanol. Chromosomes were stained with 0.5% haematoxylin following the method of Haque *et al.* (1976). Data were recorded on interphase chromosome volume (ICV), mitotic index (MI) and various mitotic abnormalities from different stages in case of somatic cells. The nuclear volume (NV) was calculated by using the formula for a sphere, $NV = 4/3\ \pi r^3$ (Nayar *et al.*, 1970). The mean nuclear volume divided by somatic chromosome number gave the ICV.

For meiotic study, young inflorescences from pot plants of various treatments along with control were collected and immediately fixed in Carnoy's fixative (6 ethanol: 3 chloroform: 1 acetic acid). After 48 hrs, these inflorescences were transferred to 70% ethanol and stored in a refrigerator. To collect data on meiotic abnormalities and pollen sterility, temporary slides were prepared from suitable anthers by acetocarmine smear technique. All the cytological parameters were observed at 10X magnification by a research microscope and results obtained in this study were analyzed statistically following MSTAT-C package program. The mean values recorded for all the treatments were compared by Duncun's Multiple Range Test (DMRT) along with the estimation of LSD values following Gomez and Gomez (1984).

## RESULTS

### Morphological study

In this investigation, various morphological characters as mentioned earlier were considered for morphological study. For all the characters, control always showed good performance over the treatments except shoot length, in that case Morter-$48_{EC}$ gave maximum length (Table 1). Moreover, Safathrin-$10_{EC}$ showed more adverse effect on the morphological characters than Morter-$48_{EC}$ in almost all cases except germination percentage, with the increase of doses. Yield and yield components were also reduced when higher doses (2% and 4%) of both the insecticides were used, which was evident from the data collected on the grain weight/plant and 100-grain weight (Table 1).

**Table 1.** Effect of the two insecticides on morphological characters of *H. vulgare*.

| Treatments | | Characters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemical | Doses (%) | Germination (%) | | Coleoptile length (cm) | Root length (cm) | Shoot length (cm) | Plant height (cm) | Ear length (cm) | Seed no./ear | Grain weight/ plant (g) | 100 grain weight (g) |
| | | Petri dish | Pot | | | | | | | | |
| Water | - | 92.00a | 96.00a | 3.50a | 9.12a | 12.81a | 80.00a | 8.80a | 38.97a | 1.28a | 4.97a |
| Morter-$48_{EC}$ | 1.0 ($D_1$) | 80.00cd | 88.00b | 3.19b | 8.47ab | 11.27abc | 78.00a | 8.00abc | 36.00b | 1.63b | 4.78a |
| | 2.0 ($D_2$) | 76.00de | 80.00d | 3.12bc | 7.63abc | 9.95cd | 77.50a | 7.50cd | 32.37c | 1.34cd | 4.40b |
| | 4.0 ($D_3$) | 68.00f | 72.00e | 3.09bc | 6.25cd | 8.51de | 75.00b | 7.20cd | 30.00d | 1.17e | 3.97c |
| Safathrin-$10_{EC}$ | 1.0 ($D_1$) | 84.00bc | 92.00ab | 3.17b | 8.00abc | 10.47bcd | 76.00b | 7.60bcd | 32.00c | 1.47c | 4.80a |
| | 2.0 ($D_2$) | 80.00cd | 84.00c | 3.10bc | 6.89bcd | 8.91de | 71.00c | 7.16cd | 29.00d | 1.20de | 4.38b |
| | 4.0 ($D_3$) | 72.00ef | 80.00cd | 3.00c | 5.11d | 7.45e | 69.00d | 6.70d | 24.00e | 0.92f | 3.72d |
| CV % | | 3.29 | 2.65 | 2.44 | 10.23 | 9.48 | 5.69 | 7.03 | 9.46 | 8.12 | 2.26 |

**Cytological study**

Some important cytological parameters such as ICV, MI, pollen sterility, and mitotic and meiotic abnormalities were examined in this study. Barley seeds treated with 4% Safathrin-$10_{EC}$ gave maximum ICV value (1.86µ³) while control gave minimum (0.76µ³). Between the two insecticides along with control, Safathrin-$10_{EC}$ caused more effect than Morter-$48_{EC}$ for ICV (Fig. 1). In case of doses for both insecticides, the maximum ICV was found in 4% dose while the minimum was found in 1% dose (Fig. 2).

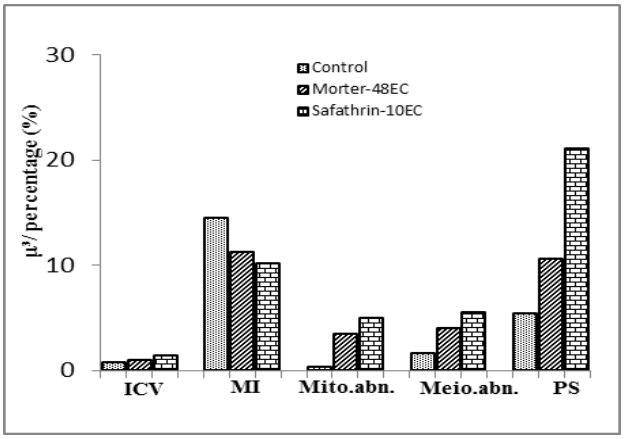


Fig. 1. Effect of insecticides along with control on cytological characters of *H. vulgare*.

Mitotic index was found to be the highest (14.92%) in case of control while the lowest value (8.80%) was observed for 4% Safathrin-$10_{EC}$ which was similar to 4% dose of Morter-$48_{EC}$ (Table 2). Mitotic index value alteration was obtained in root tip cells due to the use of Morter-$48_{EC}$ and Safathrin-$10_{EC}$ as presented in Fig. 1 and the lowest and highest values were recorded for 1% and 4% doses of both the insecticides, respectively (Fig. 2).

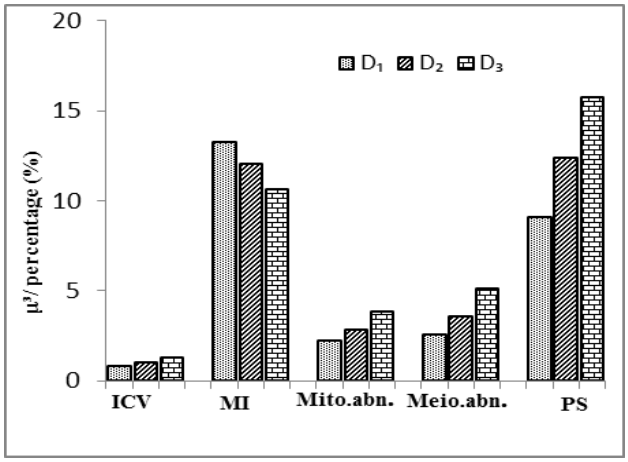


Fig. 2. Effect of doses of insecticides on cytological characters of *H. vulgare*.

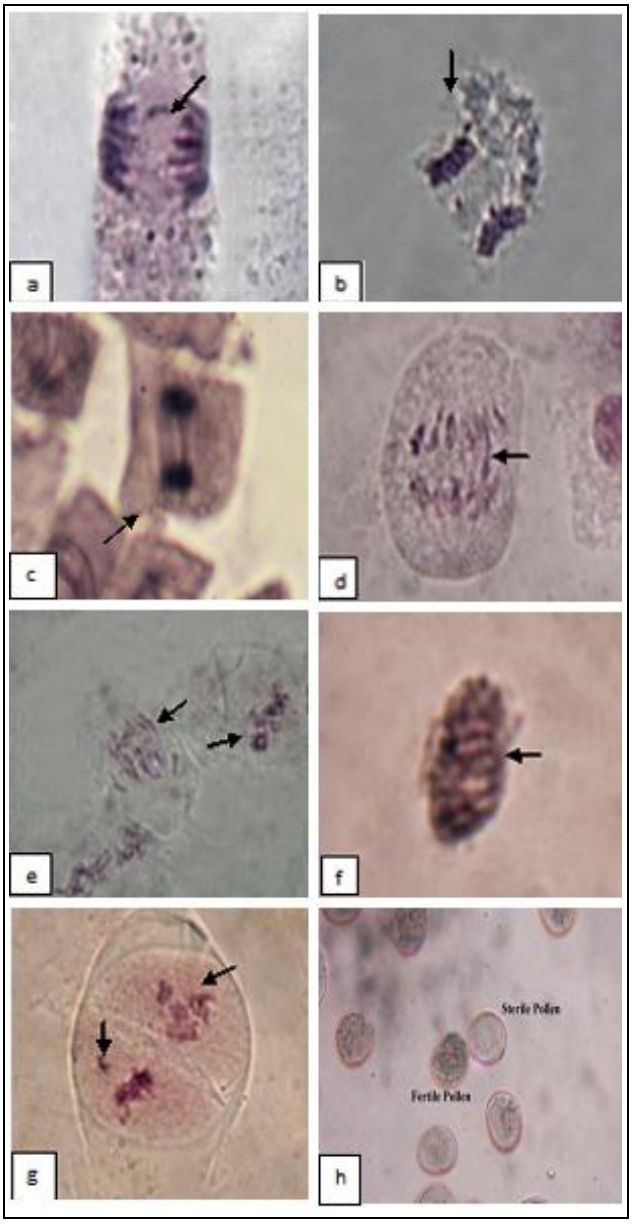


Fig. 3. Different types of aberrations induced by the two insecticides in mitotic and meiotic cells of *H. vulgare*. a. Anaphase with laggard chromosome, b. Anaphase (late) with chromosome fragment, c. Telophase with chromosome bridge, d. Anaphase I with chromosome bridge, e. Anaphase I with chromosome bridge & metaphase I with ring chromosome, f. Sticky prophase chromosome, g. Metaphase II with double chromosome fragment, h. Photograph of sterile and fertile pollen observation.

Mitotic study in the root tip cells of *H. vulgare* emerged from the seeds treated with different doses of the two insecticides along with control showed various types of

mitotic abnormalities such as bridge, fragment, laggard, etc. (Fig. 3). The highest percentage of mitotic abnormality (6.80%) was observed due to 4% Safathrin-$10_{EC}$ treatment and the lowest (0.33%) was found in control (Table 2). Overall, Safathrin-$10_{EC}$ induced more abnormalities than Morter-$48_{EC}$ (Fig. 1) and in case of concentrations 4% dose caused highest percentage of mitotic abnormality and 1% dose showed lowest percentage of mitotic abnormality (Fig. 2).

Meiotic study of the pollen mother cells (PMCs) of treated *H. vulgare* seeds with three different doses of the two insecticides showed various types of chromosomal aberrations such as fragment, bridge, laggard, stickiness, etc. (Fig. 3). These types of aberrations were found more or less in both Meiosis I and Meiosis II. DMRT estimation indicated that the highest percentage of meiotic abnormality (7.75%) was observed in 4% Safathrin-$10_{EC}$ whereas, the lowest value (1.79%) was found in control (Table 2). Safathrin-$10_{EC}$ produced maximum abnormalities than Morter-$48_{EC}$ (Fig. 1). On the other hand, the highest percentage of meiotic abnormalities was obtained due to higher dose (4%) and lowest due to lowest dose (1%) (Fig. 2). Moreover, in both cases the percentages of different types of chromosomal abnormalities were increased with the increase of insecticidal concentrations (Table 2).

**Table 2.** Effect of the two insecticides on cytological characters of *H. vulgare*.

| Treatments | | Characters | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemical | Doses (%) | ICV ($\mu^3$) | MI | % of pollen sterility | Mitotic abnormalities | | | | Meiotic abnormalities | | | | |
| | | | | | Mean (%) | Laggard | Fragment | Bridge | Mean (%) | Laggard | Fragment | Bridge | Stickiness |
| Water | - | 0.76f | 14.92a | 5.50f | 0.33d | 0.33 | - | - | 1.79e | 0.26 | - | 0.25 | 1.28 |
| Morter-$48_{EC}$ | 1.0 ($D_1$) | 0.84e | 13.54b | 8.33e | 2.39c | 1.59 | 0.80 | - | 2.82d | 0.47 | - | 0.94 | 1.41 |
| | 2.0 ($D_2$) | 0.86de | 11.34cd | 11.00d | 3.60b | 2.06 | 1.54 | - | 3.57c | 0.51 | 0.25 | 1.03 | 1.78 |
| | 4.0 ($D_3$) | 1.30c | 8.97e | 12.66c | 4.48b | 1.92 | 1.28 | 1.28 | 5.81b | 0.69 | 0.24 | 2.09 | 2.79 |
| Safathrin-$10_{EC}$ | 1.0 ($D_1$) | 0.91d | 12.05c | 13.66c | 3.82b | 2.65 | 0.88 | 0.29 | 3.29cd | 0.52 | - | 0.75 | 2.02 |
| | 2.0 ($D_2$) | 1.41b | 10.28d | 20.66b | 4.50b | 1.90 | 1.34 | 1.26 | 5.62b | 0.51 | 0.26 | 2.04 | 2.81 |
| | 4.0 ($D_3$) | 1.86a | 8.80e | 29.00a | 6.80a | 2.56 | 2.97 | 1.27 | 7.75a | 1.25 | 0.75 | 2.25 | 3.50 |
| CV % | | 2.49 | 7.87 | 10.71 | 9.55 | - | - | - | 10.96 | - | - | - | - |

Plants which were raised from seeds treated with 4% of Safathrin-$10_{EC}$ showed the highest percentage of pollen sterility (29.00) while the lowest (5.50) percentage was found in control (Table 2). Between the two insecticides, Safathrin-$10_{EC}$ caused maximum pollen sterility and control showed minimum values for it (Fig. 1). Among the treatments, 4% dose showed highest pollen sterility and 1% dose showed lowest value for this character (Fig. 2).

In case of both the morphological and cytological studies, analysis of variance (ANOVA) revealed that all characters were highly significant, whereas, coleoptile length, root length, shoot length, plant height and ear length were non-significant in case of chemical × dose interaction.

## DISCUSSION

In the present experiment, data on morphological characters except germination percentage indicated that Safathrin-$10_{EC}$ decreased the values with an increase of its doses compared to seeds treated with Morter-$48_{EC}$ and distilled water. But in case of shoot length, Morter-$48_{EC}$ showed increased result over Safathrin-$10_{EC}$ and control. Similar results were also obtained by Jayakumar and Selvaraj (2003), Dhakshanamoorthy *et al.* (2009) and Aruna *et al.* (2010). According to Schjoerring *et al.* (1993), Hansen (2000) and Nazarko *et al.* (2003) excessive use of insecticide can reduce the grain size and grain production, which are in agreement with our findings on yield and yield components.

The relationship of ICV with different concentrations of insecticides indicated that with an increase of the concentration of insecticides there was an increase in ICV than that of control. Similarly, Kabir (1981) also observed increased ICV with increased doses of two insecticides on barley. This increase of ICV, in general, might be due to alteration of cell membrane configurations, modification of chromosomal protein and changes in sensitivity of chemicals.

The mitotic index (MI) is a cytogenetic test which is a reliable predictor of cell proliferation in the tissue that is used to characterize proliferating cells and to identify the compounds that inhibit mitotic progression resulting in a decrease in the MI of that population. In the present study, cytological observation revealed that the insecticides reduced mitotic index in root tip cells of barley which is in agreement with the result of Ilbas *et al*. (2005).

Cytological analysis with respect to either mitotic or meiotic behavior is considered as one of the most dependable indexes to estimate the potency of mutagen. In the present study, the treated seeds of barley with Morter-$48_{EC}$ and Safathrin-$10_{EC}$ showed varying degrees of chromosomal abnormalities like bridges, fragments, laggards, etc. at different stages of root tip mitosis. The chromosomal abnormalities were increased generally with increasing concentrations of both these insecticides. This result showed resemblance with Zaman and Saleh (2005) who obtained increased frequency of chromosomal changes like chromatid bridges, chromosome fragments, laggards, etc. of wheat with the increased doses of chemical mutagen ethylene glycol.

The number of abnormal pollen mother cells (PMCs) found in the flower buds of barley plants was obvious with all the concentrations of insecticides and also increased with the increasing concentrations of the two insecticides. The most frequent types of abnormalities observed were stickiness, laggards, bridges, fragments etc. at meiosis-I and meiosis-II stages. The results obtained by Haiba *et al.* (2011) showed that all the concentrations and different durations of treatment with the pesticides Telliton and Dithane M-45 on *Vicia faba* induced a number of chromosomal aberrations in PMCs *viz.*, stickiness, bridges, laggards and micronuclei which is in good agreement with result of the present study.

In the present investigation, the percentage of pollen sterility in different treatments was higher than the control and gradually increased with an increasing concentrations of insecticides in barley. The present observation is in accordance with Bhat *et al.* (2007) who reported that the pollen sterility was increased as the concentration of chemical mutagen EMS increased in case of *Vicia faba* L.

## CONCLUSION

From the foregoing results and discussion, it can be inferred that the insecticides had negative effects on the morphological parameters including yield and yield traits and cytological parameters specifically on chromosomes of barley in producing various kinds of abnormalities and they were found to be in proportion with the increase of concentrations of the insecticides used. So, the farmers should be aware about the indiscriminate use of these insecticides and should keep the use of insecticides at optimum level to prevent yield loss. Moreover, they should practise the alternative methods of ecofriendly biological control of insects which have no such adverse effects on crops.

## REFERENCES


Aruna, J., M. Prokash and B. S. Kumar. 2010. Studies on effect of physical and chemical mutagens on seedling characters in brinjal (*Solanum melongena* L.). International Journal of Current Research, 3: 38-41.

Aurbek, N., H. Thiermann, L. Szinicz, P. Eyer and F. Worek. 2006. Analysis of inhibition, reactivation and aging kinetics of highly toxic organophosphorus compounds with human and pig acetylcholine-esterase. Toxicology, 5: 224-228.

Bhat, T. A., S. Parveen and A. H. Khan. 2007. Meiotic studies in two varieties of *Vicia faba* L. after EMS treatment. Asian Journal of Plant Science, 6(1): 51-55.

Biswas, A. K. 1979. Cytological effects of insecticides, chemical mutagens and gamma radiation on barley (*Hordeum vulgare* L.). M.Sc. Thesis, Dept. of Botany, Rajshahi University.

Dhakshanamoorthy, D., R. Selearaj and A. Chidambaram. 2009. Physical and chemical mutagenesis in *Jatropha curcas* L. to induce variability in seed germination, growth and yield traits. Romanian Journal of Plant Biology, 55(2): 113-125.

Gomez, K. A. and Gomez, A. A. 1984. Statistical Procedures for Agricultural Research. An international rice research institute book. A Wiley-Interscience Publication, New York.

Haiba, A. A., N. R. A. El-Hamid, E. A. A. A. El-Hady and E. R. M. F. Al-Ansary. 2011. Cytogenetic effect of insecticide telliton and fungicide dithane M-45 on meiotic

cells and seed storage proteins of *Vicia faba*. Journal of American Science, 7: 19-25.

Halil, E. E. 2011. The cytogenetic effects of organophosphorus insecticide Dichlorvos in barley (*Hordeum vulgare* L.) seedlings. Pakistan Journal of Botany, 43(5): 2441-2443.

Hansen, L. M. 2000. Establishing control threshold for bird cherry-oat aphid (*Rhopalosiphum padi* L.) in spring barley (*Hordeum vulgare* L.) by aphid-days. Crop Protection, 19(3): 191-194.

Haque, A., M. A. Ali, M. Wazuddin and M. A. Khan. 1976. Squash method for the mitotic chromosomes of grasses. Current Science, 45(10): 382-383.

Haux, J. E., G. B. Quistad and J. E. Casida. 2000. Phosphobutyrylcholinesterase: Phosphorylation of the ester site of butyrylcholinesterase by ethephon [(2-chloroethyl) Phosphonic acid] dianion. Chemical Research in Toxicology, 13: 646.

Ilbas, A. I., Y. Eroglu and H. E. Eroglu. 2005. Effects of the application of different concentrations of $NaN_3$ for different times on the morphological and cytogenetic characteristics of barley (*Hordeum vulgare* L.) seedlings. Journal of Integrative Plant Biology, 47: 1101-1106.

Jayakumar, S. and R. Selvaraj. 2003. Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in sunflower. Madras Agriculture Journal, 90(7-9): 574-576.

Kabir, G. 1981. Cytological effects of insecticides (Carbicron-$100_{EC}$ and Vapona-$50_{EC}$) on barley (*Hordeum vulgare* L.). M. Sc. Thesis, Dept. of Botany, Rajshahi University.

Nayar, G. G., K. P. Geroge and A. R. Gopal. 1970. The relation between cytological abnormalities and interphase chromosome volume (ICV) in plants growing in a high radiation area. Radiation Botany, 2: 175-178.

Nazarko, O. M., R. C.Van Acker, M. H. Entz, A. Schoofs and G. Martens. 2003. Pesticide free production of field crops. Agronomy Journal, 95(5): 1262-1273.

Schjoerring, J. K., A. Kyllingsbaek, J. V. Mortensen and S. BYSKOV–NIELSEN. 1993. Field investigations of ammonia exchange between barley plants and the atmosphere. I. Concentration profiles and flux densities of ammonia. Plant, Cell & Environment, 16(2): 161-167.

Shehata, M. M., A. Habib, N. S. Khalifa and M. S. Salama. 2000. Cytological and biochemical effects of 5-florouracil and colchine on *Vicia faba* plants. Egyptian Journal of Biotechnology, 7(4): 218-233.

Zaman, S. and M. A. Saleh. 2005. Mutagenic effect of ethylene glycol on somatic cells of wheat (*Triticum aestivum* L.). Journal of Life and Earth Science, 1(1): 43-49.