ISSN : 0974 - 7532 Volume 9 Issue 8

Research & Reviews in

BioSciences

Regular Paper

RRBS, 9(8), 2014 [302-310]

Morphological, biochemical and cytogenetic effect of an acaricide fenazaquin on *Hordeum vulgare* L.

K.Babu*, K.M.Umarajan

Genetic Toxicology Lab, Department of Botany, Pachaiyappa's College, Chennai 600030, Tamilnadu, (INDIA)

E-mail: babukplantsci@gmail.com; babu_plantsci@yahoo.co.in

ABSTRACT

The present study has been carried out to investigate the effects of an acaricide Fenazaquin on seed germination, shoot-root growth, total carbohydrate, free amino acid, protein content, mitotic division, chromosomal aberrations and micronuclei in barley (*Hordeum vulgare* L.). Presoaked seeds of barley were treated with various concentrations of fenazaquin *viz*. 25, 50, 100 and 200 ppm for 6, 12 and 24 hr. The treated seeds were washed thoroughly with running tap water and allowed to germinate on moist filter paper in Petri dish at 25±1 C. After 120 hr, germination, growth, biochemical content and cytogenetic damages were analysed. The results showed except total carbohydrate content all the treated samples exhibited significant ($P<0.05$; $P<0.01$) reductions in seed germination, shoot-root growth, total protein, free amino acid content, mitotic index and increases in chromosomal aberrations and micronuclei. These remarkable findings suggest that the acaricide fenazaquin may cause potential toxic effects on the physiomorphology, cell division and genetic materials of barley.

KEYWORDS

Fenazaquin;
Seed germination;
Amino acid;
Protein;
Chromosomal aberration;
Hordeum vulgare.

INTRODUCTION

Pesticides constitute a heterogeneous category of chemicals specifically designed for the control of pests, weeds or plant diseases. Their application is still the most effective and accepted means for the protection of plants from pests, and has contributed significantly to enhanced agricultural productivity and crop yields. A total of about 890 active ingredients are registered as pesticides in USA and currently marketed in some 20,700 pesticide products[1]. Many of these pesticides stay/persist in the environment for several years and cause various toxicity to plants, animals and human beings as well. The majority of pesticides have been tested in a wide variety of mutagenicity assays covering gene mutation, chromosomal alteration and DNA damage[2-6]. Pesticides have been considered potential chemical mutagens and the experimental data revealed that various agrochemical ingredients possess mutagenic properties. Although studies on the biological effects of currently used pesticides have increased in recent years, the complete impacts of these chemicals on biological systems are still largely unknown[7-9].

Fenazaquin (4-[2-[4-(1,1-Dimethylethyl) phenyl]ethoxy]quinazoline), is a white to tan crys-

talline solid, belonging to the quinazoline group of pesticide Figure 1. It is a broad spectrum, non-systemic acaricidal compound, widely used in controlling phytophagus mites infesting a variety of fruits and vegetables crops[10] and tea[11]. It acts as an electron transport inhibitor, acting at Complex I of the mitochondrial respiratory chain. It also reported that this specific acaricide/insecticide has generally no effect on beneficial insects including predaceous mites[12]. Recently European Food Safety Authority (EFSA) published conclusion on the peer review of the pesticide risk assessment of the active substance fenazaquin[13].

To the best of our knowledge, there is little information available so far about the impact of

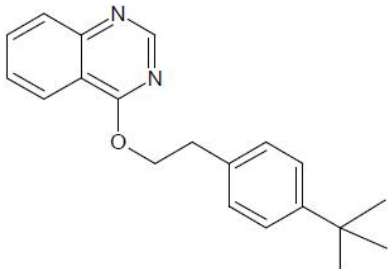


Figure 1 : Structure of fenazaquin

Fenazaquin on higher plants. Therefore, the present study was undertaken to examine the effects of Fenazaquin on seed germination, shoot-root growth, total protein, free amino acid, carbohydrate content, mitotic division and chromosomes of barley, *Hordeum vulgare* L.

MATERIALS AND METHODS

Chemicals

A commercial formulation of Fenazaquin was purchased from local market as Magister (Fenazaquin - 10% EC). Other chemicals were purchased from E. Merck Co. India.

Test system

Seeds of barley, *Hordeum vulgare* L. ($2n = 14$) cv PL172 were used for the present study.

Determination of inhibitory concentration (IC_{50})

Various concentrations (based on the active ingredient) of test solution ranging from 12.5, 25, 50, 100, 200 and 400 ppm were prepared from the stock solution by diluting with tap water. Root elongation test was carried out to determine the IC_{50} according to Environmental Protection Agency (EPA) guidelines[14]. Healthy and uniform sized seeds of barley were selected and surface sterilized with 5 % Tween-20 and 10 % Sodium hypochlorite solution were treated for 10 min and washed thoroughly with distilled water. For each treatment triplicate of 50 seeds were used. From each concentration 7 ml of the freshly prepared test solutions were added to the Petri plates containing filter paper and the seeds were placed on the filter paper with adequate space. The Petri plates were kept in the BOD incubator for 120 hr at 25±1°C temperature in dark to facilitate the linear growth. After 120 hr the root growth was measured.

Root elongations were measured by following formula

$$\text{\% of Relative root elongation} = \frac{\text{Average root elongation in treatment}}{\text{Average root elongation in control}} \times 100$$

Test concentrations

Based on the IC_{50} concentration determined in the preliminary root elongation test, four concentrations were selected *viz*. double the IC_{50} value (200 ppm), the IC_{50} concentration (100 ppm) and two two-fold dilutions of the IC_{50} concentrations (25 and 50 ppm) were selected in order to provide a reasonable range of toxic and non-toxic concentrations.

Measurement of seed germination and shoot-root growth

Seed germination and shoot-root growth assays were carried out similar to root elongation test using above selected four test concentrations. After 120 hr seed germination and shoot-root growth were measured according to EPA[14]. A seed was considered germinated when radicles had attained a length of not less than 5 mm.

$$\text{\% of Relative seed germination} = \frac{\text{No. of seeds germinated in treatment}}{\text{No. of seeds germinated in control}} \times 100$$

Regular Paper

$$\text{\% of Relative shoot root growth} = \frac{\text{Average shoot - root growth in treatment}}{\text{Average shoot - root growth in control}} \times 100$$

Biochemical and cytogenetic assay

Presoaked (12 hr) seeds were treated with above four test concentrations of Fenazaquin for 6, 12 and 24 hrs. After the treatment, the seeds were thoroughly washed with running tap water for 1 hr and allowed to germinate on moist filter paper placed in Petri dishes at 25 ± 1°C in dark. Ethyl methane sulfonate (EMS 10 ppm) and tap water were also maintained simultaneously as positive and negative controls. After germination some of the roots (when the root reached 1-1.5 cm length) were excised and fixed in acetic-ethanol (1:3) for cytogenetic assay and others were left for 120 hr after that shoots and roots were harvested to analyze the total protein[15], carbohydrate and free amino acid content[16]. Cytogenetic assay was performed from the fixed root-tips by haematoxylin squash technique reported as earlier[17]. The frequencies of mitotic index (MI), chromosomal aberrations (CA), such as metaphase and anaphasic abnormalities and interphase cells with micronuclei (MN) were determined as described earlier[17]. For the analysis, a minimum of 5000 cells from 10 root tips were scored for each treatment.

Statistical analysis

All data values are expressed as mean ± SD and the level of significance between the control and treated groups were evaluated by one-way analysis of variance (ANOVA) and multiple comparisons were performed by Tukey's HSD test.

RESULTS

In the preliminary toxicity assay, treatment with 12.5 – 400 ppm fenazaquin produced a concentration-dependent inhibition in *H. vulgare* root growth, accompanied by morphological changes such as stiffness and discoloration of the roots at higher concentrations (i.e., 200 and 400 ppm). The dose response curve for the percent of growth as a function of the log concentration of fenazaquin is presented in Figure 2. The concentration of fenazaquin causing a 50% inhibition of root growth was estimated to be 91.28 ppm (log10 concentration - 1.96). Hence, we used 100 ppm as the IC_{50} of fenazaquin in *H. vulgare* for the subsequent experimentation.

Effects of fenazaquin on seed germination and shoot-root growth

The effects of fenazaquin on seed germination and shoot-root growth are presented Figure 3. Fenazaquin treatments with all concentrations significantly decreased the germination rates. Differences in germination rates between treated and control group were statistically significant ($P<0.05$: $P<0.01$) except 12.5 ppm. The inhibition of germination was observed in dose-dependent manner. The differences of germination rates between all concentrations (50, 100 & 200 ppm) were also statistically significant ($P<0.05$). The lowest germination

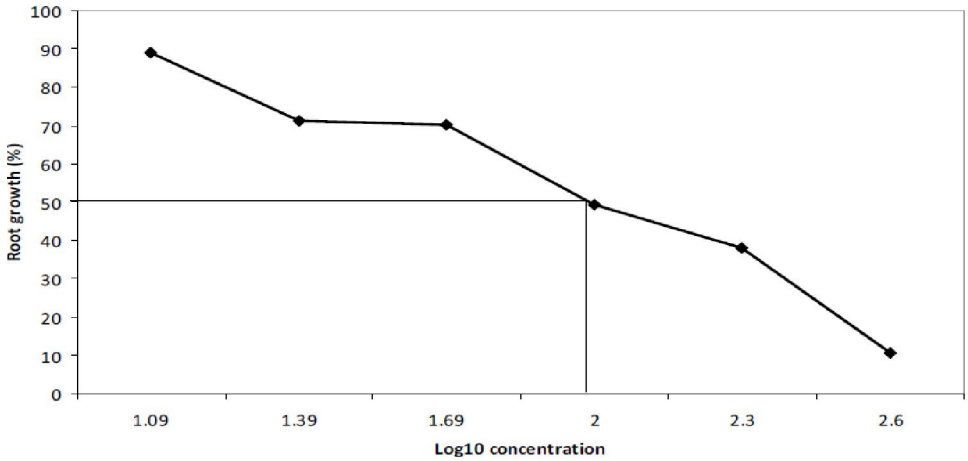


Figure 2 : IC_{50} of fenazaquin on *Hordeum vulgare* roots

Regular Paper

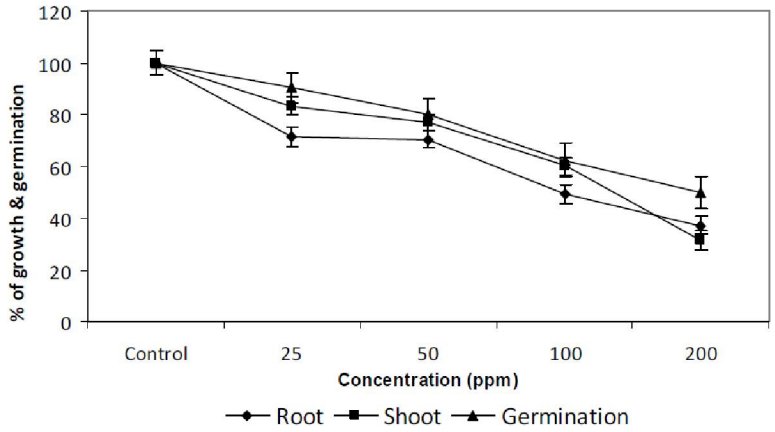


Figure 3 : Effects of fenazaquin on seed germination and shoot-root growth

rate was observed in 200 ppm. Shoot-root growth were affected significantly (P<0.05; P<0.01) in all tested concentrations when compared to control. Among the tested concentration significant differences observed in 100 & 200 ppm and the highest growth retardation was observed in 200 ppm.

Effects on total carbohydrate, free amino acid and protein content

The effects of Fenazaquin on biochemical contents are presented in Figure 4 to 6. It was observed that the total carbohydrate content was significantly (P<0.05) increased in 12 & 24 hrs treatments at all concentrations. Treatment with 12 hr shows more response than the 24 hr. No significant changes observed in 6 hr treatment. The total free amino acid content was significantly (P<0.05) increased in 25 ppm, however, this was immediately decreased in higher concentrations *viz*. 50,100 & 200 ppm. Again, significant (P<0.05; P<0.01) decreases were observed in 12 hr treatment when compared to 6 & 24 hr at all concentrations. The total protein content was significantly (P<0.05) affected in 24 hr treatment at all concentrations of Fenazaquin. No significant changes were observed in 6 & 12 hr treatments. In all these assays, there is no dose-dependent effects were observed. But duration-dependent effects were observed in all cases. Treatment with positive control (EMS 10 ppm) significantly (P<0.05) decreased the carbohydrate, free amino acid and protein content.

Effects on mitotic index (MI), chromosomal aberrations (CA) and micronuclei (MN)

The effects of fenazaquin on the MI, CA and MN of *Hordeum vulgare* root meristems cells are presented in Figure - 7 to 10. The exposure at all concentration of fenazaquin resulted in a dose-depen-

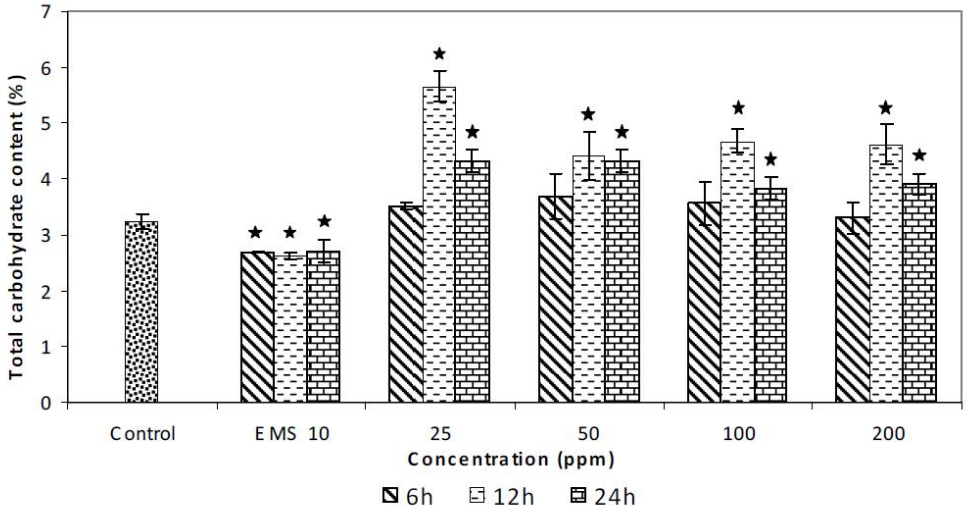
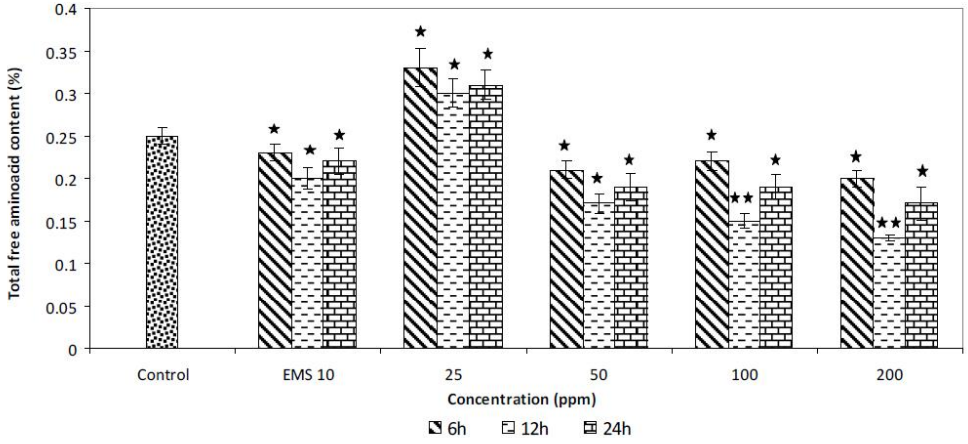


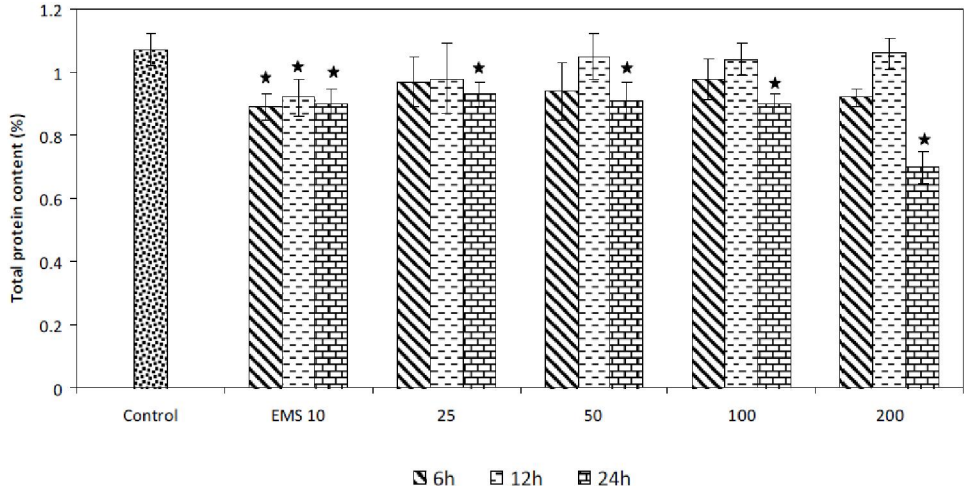
Figure 4 : Effects of fenazaquin on total carbohydrate content

Regular Paper



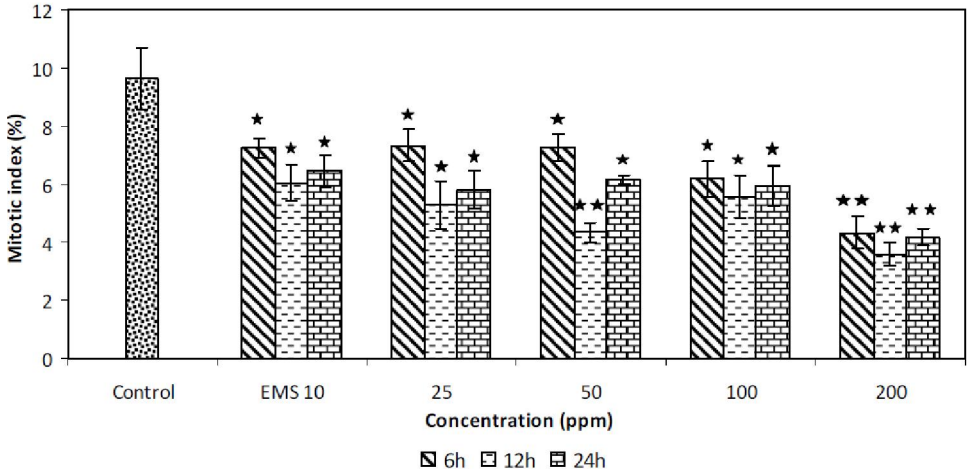
*** - P<0.05; ** - P<0.01**

Figure 5 : Effects of fenazaquin on total free amino acid content



*** - P<0.05**

Figure 6 : Effects of fenazaquin on total protein content

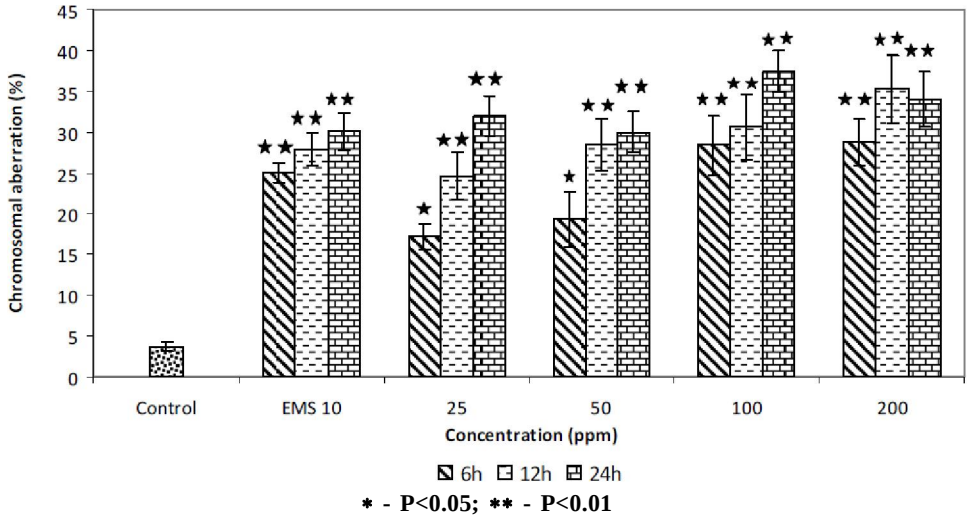


*** - P<0.05; ** - P<0.01**

Figure 7 : Effects of fenazaquin on mitotic index

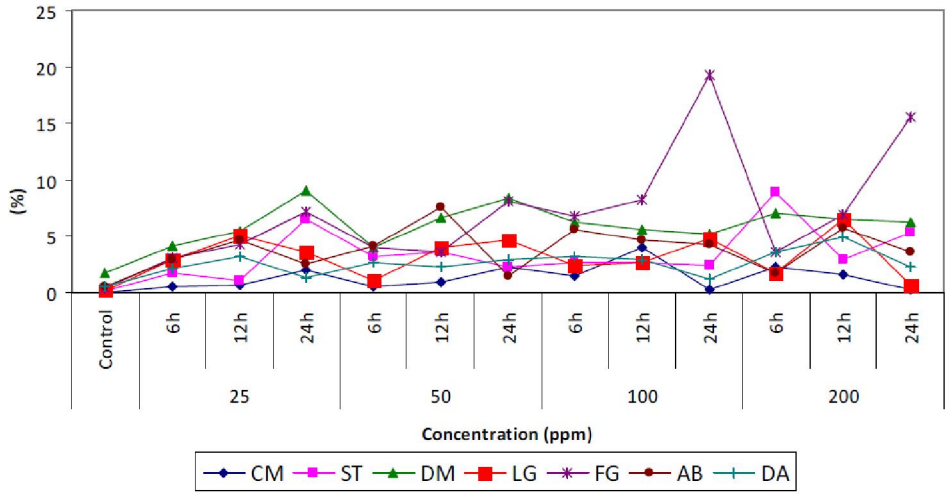
dent inhibition of the MI Figure 7. Though all duration of treatments showed significant inhibition (P<0.05; P<0.01), 12 hr exposure exhibits more reduction in the MI when compared to 6 & 24 h treat-

Regular Paper



*** - P<0.05; ** - P<0.01**

Figure 8 : Effects of Fenazaquin on chromosomal aberration



CM – C-Metaphase, ST – Stickiness, DM – Disturbed metaphase, LG – Laggard, FG – Fragment, AB – Anaphasic Bridge, DA – Disturbed Anaphase

Figure 9 : Varoius types of chromosomal aberrations induced by fenazaquin on root meristem cells of *Hordeum vulgare*

ment at all concentrations. Figure - 8 & 9 shows the frequency and various types of chromosomal and mitotic aberrations. Significant (P<0.05; P<0.01) dose-dependent increases of chromosomal aberration were observed in all concentration of treatments. The highest percent of aberrations were recorded in 200 ppm treatment. The frequencies of C-metaphase, stickiness, disturbed metaphase and anaphasic bridges were found in all concentrations. A gradual increase of fragments was observed when the concentration increased and maximum frequency observed in 24 h treatment at 100 and 200 ppm. The frequency of cells with micronucleus are shown in Figure -10. Statistically significant (P<0.05; P<0.01) increases in MN frequency were detected in root meristems cells of barley after exposure with fenazaquin. The increases of frequency were observed in dose and duration-dependent manner. The highest value was recorded in 200 ppm treatment. Positive control (EMS) exhibits significant (P<0.05) reduction in MI and increases CA and MN.

DISCUSSION

In the present study, the effects of fenazaquin on seed germination, shoot-root growth, total carbohydrate, free amino acid, protein content, MI, CA and MN in barley (*Hordeum vulgare* L.) were investi-

Regular Paper

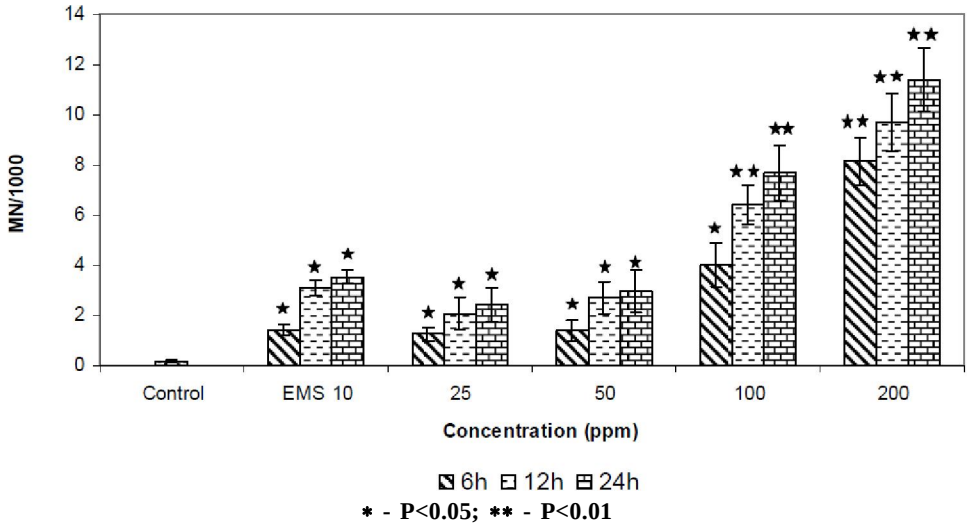


Figure 10 : Effects of fenazaquin on induction of micronuclei

gated. The preliminary toxicity assay with root elongation for the determination of IC_{50} value proved to be useful parameter for selecting the test concentration for the toxicity studies. Treatments with different concentrations of fenazaquin significantly decreased the seed germination and shoot-root growth. The growth inhibition of plants can results from several possible mechanisms such as cell cycle delay, cell death, and photosynthesis damage[18-20]. The reduced germination and shoot-root growth due to inhibitory effect of pesticides and herbicides has been reported earlier[21, 22]. Siddiqui *et al.*,[23] also reported the inhibition of seed germination and seedling growth in *Penesetum americanum* L. due to the application of organophosphate insecticides.

A significant increase of total carbohydrate content was observed in longer duration (12 and 24 hrs) of treatment at all concentrations. This might be due to decrease starch hydrolysis, as reported in bean[24] or enhanced synthesis of cytokinin[25]. Similar observation also reported by Faten[9] in radish treated with cyanophos insecticide and some triazole fungicides *viz*. paclobutrazol, triadimefon and hexaconazole are also known to alter the carbohydrate status in plants[26-27]. In the case of amino acid content, treatment with high concentration significantly reduced the amount. However, in the low concentration (25 ppm) it is significantly increased than the control. Faten[9] also observed treatment with high concentration of cyanophos insecticide reduced the amino acid content in the radish plant.

It has been suggested that the toxicant produced by the application of pesticides inhibits protein synthesis by binding to the larger ribosomal subunits inducing change in the enzyme system[28], ceasing ATP and NADP formation[29]. Application of systemic fungicides Benlate and Calixin also found to decreases the total protein content in *Triticum aestivum*[30]. In this study also we recorded significant decreases of protein content during longer (24 hr) treatments in all concentrations.

The experimental data on the effect of fenazaquin along with positive (EMS) and negative control on MI, CA and MN are presented in Figure 7-10. The results clearly indicate that the acaricide fenazaquin can induce genotoxic effects in plants. Root tip cells of treated seeds of barley with different concentrations of fenazaquin influence the MI and induce the CA and MN. The inhibition of MI and induction of CA and MN in plant cells by several pesticides have been reported earlier by different workers[8, 31-33]. In the present study, we also observed significant reduction of MI and found maximum achieved at 12 hr treatments. The decrease in the MI could be either due to blocking of G_1 suppressing DNA synthesis[34] or inhibition of DNA synthesis at S-phase[34] or blocking in G_2 preventing the cells from entering mitosis[36]. Parul Singh *et al.*,[8, 32] studied the effects of insecticides (Profenophos & Cypermethrin) and fungicides (Mencozeb & Carbendazim) on different stages of cell cycle of barley and found S phase is more sensitive. Our study also akin to the earlier

reports.

Various types of chromosomal abnormalities such as C-mitosis, stickiness, disturbed metaphase, laggard, fragments, bridges, disturbed anaphase and MN were observed after treatment with fenazaquin in barley root tip cells. Fragments, disturbed metaphase, bridges and stickiness were the most predominant abnormalities. High frequency of chromosomal breaks and micronuclei induced by fenazaquin indicates clastogenic potential of the test compound. The induction c-mitosis and disturbed metaphase may be impairment of mitotic spindle function is probably due to the interaction of fenazaquin with tubulin-SH group[37]. The stickiness is presumably due to the intermingling of chromatin fibers which leads to subchromatid connections between chromosomes[38]. The presence of lagging chromosomes may be attributed to the delayed terminalization, stickiness of chromosome ends or failure of chromosome movements[39, 40]. Induction of chromosomal and chromatin bridges may result from stickiness and the separation of daughter chromosomes becomes incomplete even in the presence of spindle fibers and thus remains connected by chromatin bridges[41]. MN, which often results from the acentric fragments or lagging chromosomes that fail to incorporate into either of the daughter nuclei during telophase of the cell cycle, can cause cell death or loss character (s) due to the deletion of primary genes[42]. Similar results also observed by several workers in various pesticides[8, 31-33, 43-45].

European Food Safety Authority (EFSA)[13] was reported there is evidence that fenazaquin is mutagenic *in vitro,* inducing gene mutations, chromosome aberrations and polyploidy, mostly in the presence of metabolic activation and however not genotoxic in *in vivo* studies. But, in our *in vivo* study in barley reveals that fenazaquin may possess potential genotoxic effect.

CONCLUSION

From the present study, it may concluded that the acaricide fenazaquin may possess potential toxic effects on cell division, genetic materials and can bring physio-morphological changes in barley, *Hordeum vulgare* L.

REFERENCES

[1] C.Bolognesi; Mutat.Res., **543**, 251–272 **(2003)**.

[2] N.E.Garrett, H.F.Stack, M.D.Waters; Mutat.Res., **168**, 301–325 **(1986)**.

[3] IARC monographs on the evaluation of carcinogenic risk to humans, Genetic and Related Effects: An Updating of Selected IARC Monographs, **6**, 1–42 **(1987)**.

[4] K.L.Dearfield, H.F.Stack, J.A.Quest, R.J.Whiting, M.D.Waters; Mutat.Res., **297**, 197–233 **(1993)**.

[5] C.Bolognesi, F.Merlo; Biomonitoring of human populations exposed to pesticides, **28**, 673–737 in P.E.Chereminisoff (Eds.), Encyclopedia of Environmental Control Technology, Gulf Publishing Company, Houston, **(1995)**.

[6] K.L.Dearfield, N.E.McCarroll, A.Protzel, H.F.Stack, M.A.Jackson, M.D.Waters; Mutat Res., **443**, 183–221 **(1999)**.

[7] B.Dimitrov, P.G.Gadeva, D.K.Benova, M.V.Bineva; Mutagenesis, **21**, 375–382 **(2006)**.

[8] Parul Singh, A.K.Srivastava, A.K.Singh; Pest.Biochem.Phys., **89**, 216–219 **(2007)**.

[9] Faten A.El-Daly; Res.J.Agri.Biol.Sci., **4**, 210-218 **(2008)**.

[10] M.G.Solomon, J.D.Fitzgerald, M.S.Ridout; Crop Protection, **12**, 255–258 **(1993)**.

[11] A.Shanker, P.Jasrotia, A.Kumar, S.Jaggi, V.Kurnar, C.Sood; Pestology, **25**, 57–60 **(2001)**.

[12] R.M.Hollingworth, K.I.Ahammadsahip, G.G.Gadelhak, J.L.Mclughin; Proc.American.Chem.Soc., **156**, **(1992)**.

[13] [EFSA] European Food Safety Authority, Conclusion on the peer review of the pesticide risk assessment of the active substance fenazaquin, EFSA J., **8**, 1892 **(2010)**.

[14] [EPA] Environmental Protection Agency, Ecological Effects Test Guidelines OPPTS 850, 4200 Seed Germination/Root Elongation Toxicity Test, Prevention, Pesticides and Toxic Substances, **7101**, 712–C–96–154, **(1996)**.

[15] O.H.Lowry, N.J.Rosebrough, A.L.Farr, R.J.Randall; J.Biol.Chem., **193**, 265- 275 **(1951)**.

[16] S.Sadasivam, A.Manickam; Biochemical methods, 2nd Edition, New Age international publishers, New Delhi, **(1996)**.

[17] K.Babu, K.C.Uma Maheswari, K.M.Umarajan, In

Regular Paper

vivo anti-clastogenic effect of tannic and gallic acid on cadmium and lead induced genotoxicity in root meristem cells of Pisum sativum, Nucleus, **51**, 247–258 **(2008)**.

[18] H.X.Wang; Acid rain: Air pollution and preservation, in H.X.Wang (Eds); Pollution Ecology, China Higher Education press and Springer, Heidelberg, Beijing, 171-179 **(2000)**.

[19] H.W.Wang, Y.G.Shen; J.Plant physiol.Mol.Biol., **28**, 247-252 **(2002)**.

[20] H.L.Yi, L.Y.Si, Z.Q.Meng; Bull.Bot.Res., **22**, 305-309 **(2002)**.

[21] S.Siddiqui, M.K.Meghvansi, S.S.Khann, N.S.Aali; Indian J.Appl.Pure Biol., **23**, 103-106 **(2008)**.

[22] F.Jabee, M.Y.K.Ansari, D.Shahab; Turkish J.Bot., **32**, 1-8 **(2008)**.

[23] Z.S.Siddiqui, S.Ahmed, S.S.Shaukat; Pakistan J.Biol.Sci., **2**, 182-184 **(1999)**.

[24] A.Upadhyaya, T.D.Davis, N.Sankhla; Ann.Bot., **57**, 309-315 **(1986)**.

[25] R.A.Fletcher, A.Gill, T.D.Davis, N.Shankla; Hort.Rev., **24**, 55-138 **(2000)**.

[26] J.C.V.Vu, G.Yelenosky; J.Plant Growth Reg., **11**, 85-89 **(1992)**.

[27] M.A.Gomathinayagam, C.A.Jaleel, G.M.Alagu Lakshmanan, R.Panneerselvam; C.R.Biologies, **330**, 644-655 **(2007)**.

[28] C.D.Person, J.Sambroski, F.R.Forysth; Canadian J.Bot., **180**, 1294-1295 **(1957)**.

[29] Z.S.Siddiqui; Appl.Ent.Phytopath., **64**, 17-22 **(1997)**.

[30] Z.S.Siddiqui, S.Ahmed; Turkish J.Bot., **26**, 127-130 **(2002)**.

[31] P.N.Saxena, L.K.S.Chauhan, S.K.Gupta; Toxicology, **216**, 244-252 **(2005)**.

[32] Parul Singh, A.K.Srivastava, A.K.Singh; Bull.Environ.Cont.Toxicol., **81**, 258-261 **(2008)**.

[33] P.Gadeva, B.Dimitrov; Mutat.Res., **652**, 191–197 **(2008)**.

[34] M.H.Schneidermann, W.C.Dewey, D.P.Highfield; Exper.Cell Res., **67**, 147-155 **(1971)**.

[35] R.Sudhakar, K.N.Ninge Gowda, G.Venu; Cytologia, **66**, 235-239 **(2001)**.

[36] A.A.El-Ghamery, A.I.El-Nahas, M.M.Mansour; Cytologia, **65**, 277-287 **(2000)**.

[37] R.Kuriyama, H.Sakai; J.Biochem., **76**, 651-654 **(1974)**.

[38] I.Klasterska, A.T.Natarajan C.Ramel; Hereditas, **83**, 153-162 **(1976)**.

[39] K.Permjit, I.S.Grover; Cytologia, **50**, 199-211 **(1985)**.

[40] B.C.Patil, G.L.Bhat; Cytologia, **57**, 295-264 **(1992)**.

[41] A.Kabarity, A.El-Bayoumi, A.Habib; Biol.Plant., **16**, 275-282 **(1974)**.

[42] H.Yi, J.Liu, K.Zheng; Ecotox.Environ.Safety, **62**, 421-426 **(2005)**.

[43] M.A.Hamoud, A.Badr; Cytotoxic effects of the insecticide Birlane in root meristems of *Vicia faba* and *Zea mays*, Proc.6th Arab Pesticide Conference, Tana University, **1**, 435-444 **(1985)**.

[44] I.S.Magda, T.H.Ghada; Biotechnology, **3**, 140-154 **(2004)**.

[45] T.C.Askin; Pakistan J.Biol.Sci., **9**, 2508-2511 **(2006)**.