Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Pelagia Research Library

Asian Journal of Plant Science and Research, 2013, 3(4):8-12

Asian Journal of Plant Science and Research
Pelagia Research Library

ISSN : 2249-7412
CODEN (USA): AJPSKY

Genotoxic evaluations of *Allium cepa* L. using different concentrations of synthesized butenolide

Rehab Ahmida and Salem Elshatshat*

Department of Botany, Faculty of Sciences, Benghazi University, Benghazi-Libya

---

ABSTRACT

The roles that fire smoke play in the release of dormancy, germination and seedling growth has been previously examined in different studies. More than 100 compounds were identified in smoke and some of those are known to have physiological effects on plants. In this study, we tested genotoxicity of synthesized butenolide using concentrations of 0, 25, 50, 100, 250, 500 and 1000 ppm. Both, low and high concentrations appeared chromosomal aberrations of meristematic cells of Allium cepa L. The bioassay test showed that physiological and clastogenic abnormalities, such as sticky metaphase and anaphase bridges, were found. There were decreases in means of mitotic index at different concentrations.

Key words: Genotoxicity, Fire Smoke, Seed Germination, Mitotic Index, Butenolide.

INTRODUCTION

Fire has the ability to produce flames which send out heat and light as well as smoke, which can be defined as the grey, black or white mixture of gas and carbon that is produced when something burns. One of the many effects of fire is exposing seeds in the soil to the environmental factors (Van Staden *et al.* 2000). Different stages of plant life such as seed germination, seedling establishment, biomass, plant mortality…etc. are affect with the fire. Thus, there is an important positive effect of fire on the conservation and restoration of plant communities (Read *et al.,* 2000; Flematti *et al.,* 2004).

As it mentioned above, fire produces smoke, However, De Lange and Boucher (1990); Brown (1993) and Baldwin and Morse (1994) reported that in the early 1960s, smoke was identified as a vital germination cue in post-fire conditions. Moreover, smoke enhances germination in all seed dormancy classes (Baskin and Baskin 1998) as noticed in laboratory and field conditions. Plants may use smoke as an environmental cue to initiate other adaptive metabolic and growth responses (Calder *et al.* 2010). The influence of smoke on plant emergence ranges from dramatic increases (e.g., 48-fold increases) (Dixon *et al*. 1995, Roche *et al*. 1997) to no effect (Coates 2003). However, excessive accumulation of concentrations can obstruct germination for some species (Dixon *et al.,* 1995; Wills and Read, 2002; Bhalla and Sabharwal 1973, Dixon *et al.* 1995, Pierce *et al*. 1995).

Since 1990, the roles that smoke play in the release of dormancy, germination and seedling growth has been examined. More than 100 compounds were identified in smoke (Radojevic 2003) some of those are known to have physiological effects on plants, including CO_2, SO_2 and NO_2 (Keeley and Fotheringham, 1997). In 2004, germination-active compound, a butenolide, was identified from plant-derived smoke (Van Staden *et al*. 2004) and burned cellulose (Flematti *et al.* 2004). Butenolide (3-methyl- 2*H*-furo [2, 3-*c*]pyran-2-one) is a compound in smoke that induces germination (Flematti *et al*. 2004). It is unknown how the seed perceives the butenolide but there is evidence that it triggers germination by facilitating uptake of water (Jain *et al*. 2008).

Genotoxicity, which refers to the ability of substances to induce a change in the amount or structure of genetic material, was widely studied for different compounds like pesticides (Reddi and Reddi 1985). Root tip systems of various plants have been widely used for determining the harmful effects of mutagens (Khilman 1975; Ma and Grant 1982; Rank and Neilsen 1994), but *Allium* test is a very good bioassay plant for chromosome damage in mitosis by chemicals (Gul *et al*., 2006). A little is known about genotoxicity of synthesized butenolide to higher plants. The question was; is the effect concentration dependent? And if so, how can butenolide affect the plants, especially when seed germination and seedling development inhibited? We thought that destroying the genetic material of cells might behind the effect, in addition, the other factors which reported in the literatures. In this study we tested the genotoxicity effects of different concentrations of butenolide using bioassay test of *Allium cepa* L.

MATERIALS AND METHODS

Chemicals:

Concentrations of 0, 25, 50, 100, 250, 500 and 1000 ppm of synthesized butenolide (ALDRICH, Germany) were prepared and kept in the refrigerator in dark flasks until they used. Chemicals for chromosomal studies were also prepared and used including: 70% ethanol, 1:3 ethanol to glacial acetic acid, 1NHCl, 40% acetic acid and aceto-orcen pigment

Chromosomal study of onion plant:

The plant material used for the genotoxicity test was *Allium cepa* L. (2n=16), the seeds were treated by soaking for 24 hours in different concentration of butenolide: 0, 25, 50, 100, 250, 500, 1000 ppm. After germination process, root tips were fixed in Carnoy for 1 hour and hydrolyzed in 1 N HCl for 11 min using water bath at 60 C^0. This was followed by the preparation of crushed material with aceto orcein for 1 hour dying method. Three slides from each treatment and control were examined. The mitotic index was determined for each treatment and the presence of chromosomes abnormalities were also evaluated. Around 2000, 2653 cells were counted for both evaluations.

Statistical analysis:

The mitotic index and percentage of chromosome aberrations were obtained by the mean of four repetitions of each treatment. The data were submitted to one-way analysis of variance (ANOVA) and comparison between the means of treatments with the means of control was performed using the Tukey test ($p<0.05$).

RESULTS

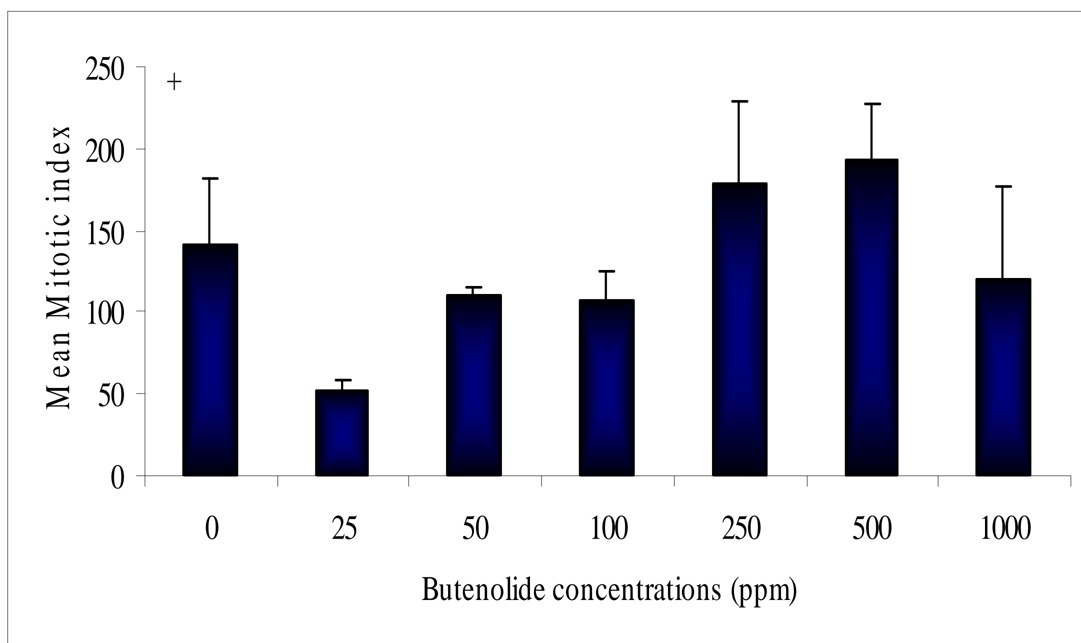
Effect of butenolide on mitotic index (M I):

The results in figure (1) show the effect of different concentrations of butenolide on M I for meristematic cells of *Allium cepa* L. after 24 hours. There were decreases in means of M I at 25, 50, 100 and 1000 ppm of butenolide concentration when they compared to control. However the increase of M I was clearly appeared at 250 and 500 ppm. Statistical analysis showed no significant effect on (MI) between all the treatments.

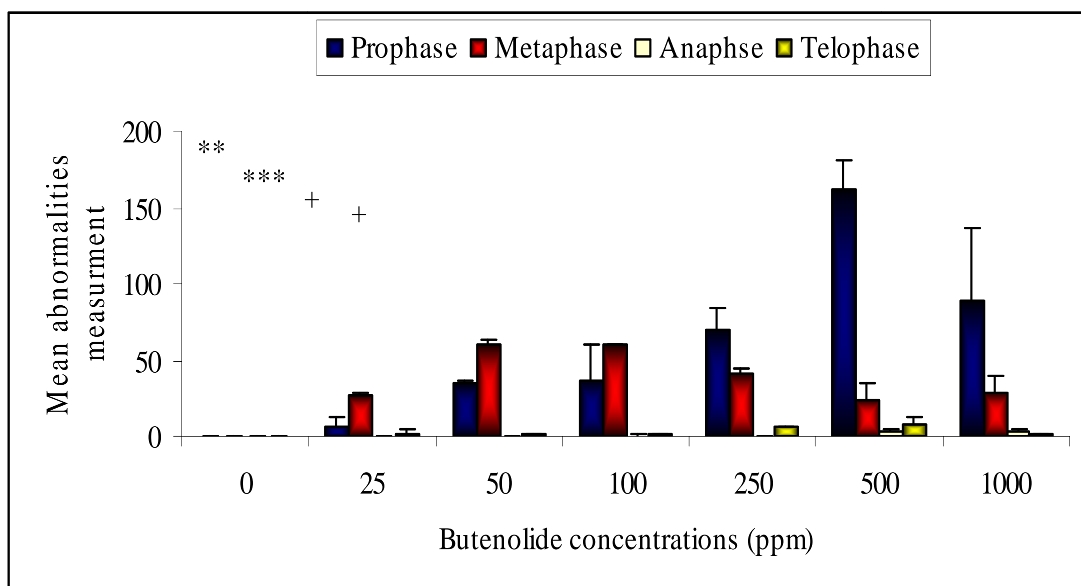
Mutations of *Allium cepa* L. treated seeds:

The results showed that the all concentrations (high or low) of butenolide used in the present study induced important abnormalities of *Allium cepa* L. during mitotic division, when they compared to control condition (Figure1 B). The increasing of aberration is clear at 500 ppm in prophase. At 50 and 100 ppm of butenolide, metaphase appeared the high mean of aberration. The average of aberration in prophase ranged from 0 under control treatment to 161.5 under 500 ppm, and there was significant effect when $p < 0.01$. Metaphase aberration average was varied from 0 under control condition to 60 under 50 and 100 ppm of butenolide concentrations, respectively with high significant when $p < 0.001$. Abnormality cells in anaphase and telophase stage were decreased. The average of anaphase aberrations was ranged between 0 at control condition to 2.5 at 500 and 1000 ppm concentrations. And it was ranged from 0.0 under 0 ppm control condition to 7.6 for 500 ppm in telophase. Statistically, no significant effects were reflected whether in anaphase or telophase.

The most common abnormalities as shown in Plate 1 and 2 were: early condensation in prophase, high condensation in prophase, sticky metaphase, c_ metaphase and lagging chromosome, binucleated cells and multiple nucleated cells, anaphase bridges and telophase bridges.

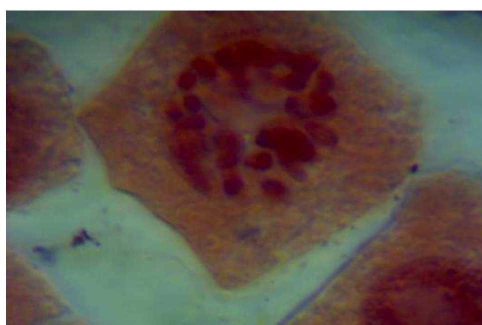


(A)



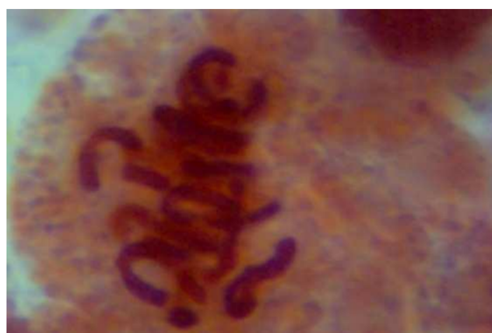
(B)

Figure (1). Effect of different concentrations of butenolide on (A). Mitotic index and (B). abnormalities of prophase, metaphase, anaphase and telophase. (+ = Not significant, **=Significant at $p < 0.01$, * = High Significant at $p < 0.001$, Bars=SE Mean)**

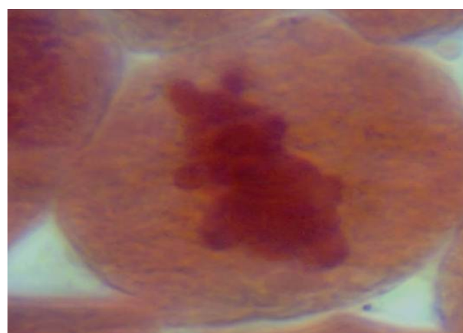


High condensation in prophase

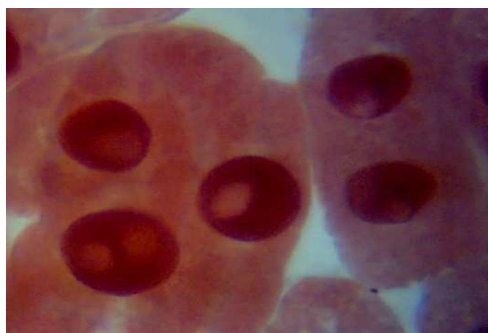
Early condensation in prophase



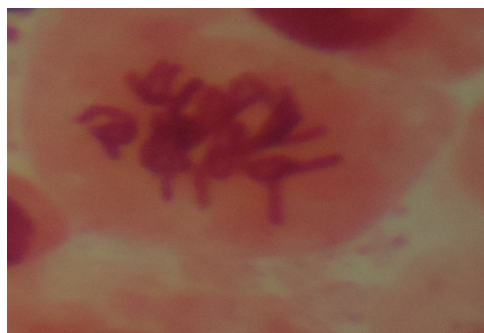
Lagging chromosome



Sticky metaphase

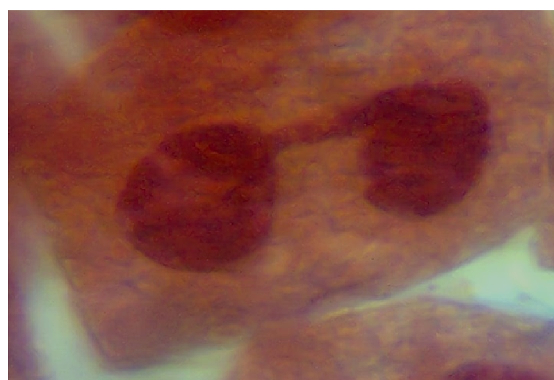


Bi and multiple nucleated cells

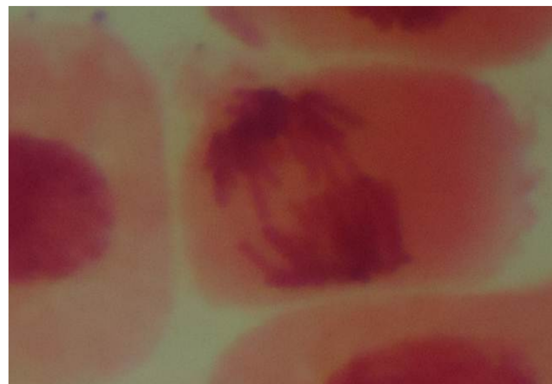


C- Metaphase

Plate1: The physiologica abnormalities of treated meristimic cells of *Allium cepa* L. Most mutations were appeared at all concentrations of butenolide at prophase, which lead to death of cell at the end



Telophase bridges



Anaphase bridges

Plate2: Telophase and anaphase bridges which appeared as clastogenic abnormalities when seeds of *Allium cepa* were treated with different concentrations of 0 to 1000 ppm of butenolide

DISCUSSION

Results showed that the different concentrations of butenolide had an inhibition effect on Mitotic index (MI) at 25, 50,100 and 1000 ppm concentrations but; at 250 and 500 ppm, there were increases in MI (Figure1 A). The reasons behind decreasing MI were stopping the cells in phase G2 and prevent them to enter the stage M of the cell cycle (Steinkilner *et al.*, 1998), or breakdown of DNA and inhibition generate of DNA (EL_Yassiri, 2008). In this research, the mutation effect at different concentrations of butenolide on root tips of *Allium cepa* L. can be shown and mutations can be calculated after soaking seeds in butenolide concentrations for 24 hours. The study revealed that most mutations were in prophase stage (Figure1 B). According to appearing mutations in division meristimic cells of *Allium cepa* L., the effect of different concentrations of butenolide can be classified to;

Physiological abnormalities, which include early condensation in prophase, high condensation in prophase, sticky metaphase, c_ metaphase and lagging chromosome, binucleated cells and multiple nucleated cells. And; Clastogenic abnormalities include anaphase and telophase bridges.

In general, stickiness of chromosome leads to death of cell (Fiskesjo, 1995). Hear, stickiness might become as result of effected chromosomal protein because of butenolide toxicity. Adhesion of chromosomal protein in anaphase cause bridges (Hassan, 2000). This can be noticed clearly in figure (2). The appearance of bridges referring to the ability of butenolide causing broken chromosomes, the emergence of broken chromosomes indicated the direct interaction of butenolide with DNA. Armbruster *et al*., (1991) reported that scattered chromosome result from decreasing ATPs which chromosomes utilize in their movement. The migration of dicentric chromosomes toward opposite spindle poles resulted in telophase stage as bridges (Figure2) due to butenolide effect. Formation of binucleated and multiple nucleated cells is a result of interference between chemicals and cell wall formation (Baeshin *et al*., 1999). We suggest that butenolide has this ability and thus, these cells appeared clearly when the *Allium* treated with this compound. Appearance of early and high condensation in prophase pointed out to the reaction of butenolide with histone protein during mitotic division (Grant, 1978, Topaktas and Rencuzogvullari, 1991). These chromosomes appear short and thickness (Figure 2).

Acknowledgment

We would like to thank Dr. Fawzya AlGarabolli, the genetics Professor, Department of Botany, University of Benghazi, for her assistant during this research.

REFERENCES

[1] Armbruster, B. L., W. T. Molin and M. W. Bugg, *Biochem*., **1991**, Phys. 39: 110-120.
[2] Baeshin, N. A., EL-Seoudy, K. A.A and AI-Nunadi, M.A., *Jam. Pakistan*, **1999**, 113 – 118.
[3] Baldwin, I.T., Morse, L, Up in smoke. II. *J. Chem. Ecol*., **1994**, 20: 2373–2391.
[4] Baskin, C.C., Baskin, J.M., Seeds, Ecology Biogeography and Evolution of Dormancy and Germination, **1998**, Academic Press, San Diego.
[5] Bhalla, P.R., and P.S. Sabharwal, *Environmental Pollution*,**1973**, 4: 237-242.
[6] Brown NAC., *New Phytol*., **1993**, 123: 575–583.
[7] Calder, W. J, Lifferth, G., Moritz, M. A., Clari, S. B. S., Physiological effect of smoke exposure on deciduous and conifer tree species, Forestry Research, **2010**.
[8] Coates, T.D., *Ecological Management and Restoration*, **2003**, 4: 133-139.
[9] Crosti, R., Ladd, P.G., Dixon, K.W., Piotto, B., *Forest, Ecol. Manage* **2006**, 221: 306–312.
[10] De Lange JH, Boucher C., *S Afr J Bot*., **1990**, 56: 700–703.
[11] Dixon KW, Roche S, Pate JS., *Oecologia*, **1995**, 101: 185–192.
[12] El-Yessiri, S.M., Genotoxic Effect of Nickle Sulfate on The Dividing Cells Using Allium cepa and Saccharomyces cerevisiae, M. sc. Thesis, Univ. of Garyonuis Faculty of science, **2008**, Benghazi, Libya.
[13] Fiskesjo G., *Meth. Mol. Biol*., **1995**, 43: 119-127.
[14] Flematti GR, Ghisalberti EL, Dixon KW, Trengove RD. (**2004**). *Science*. 305:977.
[15] Grant, W.F., Chromosome Aberration in plants as monitoring system, Environmental Health Perspectives, **1978**, 27: 37-43.
[16] Gul T, Kaymak F, Muranli F D G, Genotoxic Effects of Avenoxan on Allium cepa L. and Allium sativum L., *Caryologia*, **2006**, (3) : 241-247
[17] Hassan, H.Z., *Conf. Biol. Sci.,* **2000**,7 (8): 448-446.
[18] Jain N, Ascough G D, Van Staden J., *Journal of Plant Physiology*, **2008**, 165:1422—1427.
[19] Keeley, J.E., Fotheringham, C.J., *Ecology*, **1997**, 79: 2320–2336.
[20] Khilman B.A., *Mutation Research*, **1975**, 31: 401-412.
[21] Pierce, S.M., K. Esler, and R.M., *Oecologia*, **1995**, 102: 520-522.
[22] Radojevic, M., *Pure and Applied Geophysics*,**2003**, 160: 157–187.
[23] Rank J. and M.H. Nielsen, *Mutation Research*, **1994**, 312: 17-27.
[24] Read, T.R., S.M. Bellairs, D.R. Mulligan, and D. Lamb, *Austral Ecology*, **2000**, 25: 48-57.
[25] Reddi T.V.V.S. and V.R. Reddi, Cytological effects of chemical mutagens in rice Cytologia, **1985**, 50: 499-405.
[26] Roche S, Koch JM, Dixon KW. , *Restor Ecol.,* **1997**, 5: 191–203.
[27] Steinkellner, H., Mun-Sik, K., Helma, C., Ecker, S., Ma, T.H., Horak, O., Kundi, M and Knasmuller, S., *Environ. Mol. Mutagen*., **1998**, 3: 183-191.
[28] Topaktas, M and Rencuzogvullari, E., *Cytologia*, **1991**, 56 (3): 419-424.
[29] Van Staden, J., N.A.C. Brown, A.K. Jäger, and T.A. Johnson, *Plant Species Biology*, **2000**, 15: 167-178.
[30] Wills, T.J., Read, J., *Australian Journal of Botany*, **2000**, 50: 197-206.