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Phytotoxicity of synthesized butenolide: Response of medicinal plant *Lepidium sativum* L. seed germination and seedling development to different concentrations

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ABSTRACT

Fire is a major factor that affects plants and their development. Different plant life stages like germination, seedling establishment, flowering and seed dispersal can be influenced by fire or one of its derivatives. Fire smoke and its components were described as an important environmental factor to enhance seed germination and to improve seedling vigor in a number of studies. In this study, using different concentrations of 0, 25, 50, 100, 250, 500 and 1000 ppm of synthesized butenolide showed that response of the medicinal plant L. sativum L. seed germination and seedling development is concentration depending. The effect was varying among the concentrations but in general, low concentrations reflect positive effect and, on the other hand, high concentrations caused inhibition due to phytotoxicity of butenolide to the receptor plant cell. While, physiological and clastogenic abnormalities were found.

Key words: Phytotoxicity; Fire Smoke; Synthesized Butenolide; *Lepdium sativum* L.

INTRODUCTION

Fire is interesting phenomena plays a vital role and has great impacts on plants' life. Fire is a major factor in the formation of forests and it seems that it will be a dominant influence in that sense for years to come [12]. It can affect plants and their development i.e. flowering, seed dispersal, germination, seedling establishment, plant mortality, biomass…etc. One of the many effects of fire is exposing seeds in the soil to the environmental factors [22]. In addition to other various effects, fire exposes seeds to smoke [22]. Smoke, which described as a grey, black or white mixture of gas and carbon and it can stay in the air for weeks [16]. Because fire products prefer high seedling establishment they might increase the diversity of species [15, 25]. Fire smoke was identified as a vital germination cue in post-fire conditions [2]. De Lange and Boucher [6] were the first proved that plant derived smoke stimulates seed germination. Smoke treatments also improve post-germinative growth into to large extent (seedling vigor). Smoke is assessed for its characteristic of improving seed germination and growth of plants. In addition, smoke also stimulates somatic embryogenesis [17], flowering [10] and rooting [20].

In 2004 a germination-active compound, a butenolide, was identified from plant-derived smoke [23] and burned cellulose [7].Butenolide (3-methyl- 2H-furo [2,3-c]pyran-2-one) is a compound in smoke that induces germination [7]. It is unknown how the seed perceives the butenolide but there is evidence that it triggers germination by facilitating uptake of water [9].

Lipdium sativum L."Brassicaceae" is a common plant in traditional and folk medicine in different countries and its seed extracts suggested to use as antimicrobial agent [1]. Its oil may be used as row material of soft soap [21], and as food supplement. Due to its importance, sensitivity and and life period, this plant species was suggested to be plant receptor in this study. The aim of this study is to test to what extent that *Lipdium sativum* L. can be response to

synthesized butenolide using different concentrations. And to find out whether there is any toxicity of this compound to the plant receptor or not.

MATERIALS AND METHODS

Plant material:
Plant species *Lipdium sativum* L. was used as plant receptor in this study. Seeds of this plant were purchased from the local market in Benghazi, Libya. and authenticated by the Herbarium of Botany Department, University of Benghazi.

Germination experiment:
Seeds of tested plant species were similar selected in shape and size; these seeds were sterilized by (3%) alkyl dimethyle benzl ammonium sodium hypochlorite for 3 minutes and then washed with distilled water. Seeds were incubated overnight in flasks with different concentrations (25, 50, 100, 250, 500 and 1000 ppm) of synthesized butenolide in a dark place. Distilled water was used as control with concentration of (0.0). subsequently; seeds were placed in Sterilized Petri dishes (diameter 9.0 cm) lined with double layers of Whatmann filter papers. Three Petri dishes were used for each concentration of butenolide and each one contains fifteen seeds of the plant receptor. The filter papers were watered by adding 5 ml of distilled water whenever seeds needed; all replicates were incubated in darkness under $20 \pm 1C^0$ in incubator (BINDER,Tuttlingen, Germany). Germinated seeds were counted daily for the calculations of daily and final germination percentages.

Seedling growth test:
Germinated seeds were allowed to develop into seedlings for another one week under same conditions. Distilled water was added to the Petri dishes whenever they needed. At the end of the growth period in this study; different parameters such as plant shoot and root length, fresh and dry weight were measured. Dry weight was determined by incubating plant parts in oven ((Heraeus, U.K) at 100 C^0 for 48 hours, after that, their dry weight measured using micro balance (Mettler Toledo).

Statistical analysis:
The data were statistically analyzed by one-way test (ANOVA) for testing the differences in means of several groups using a computer program of SPSS version 11, and Dunnet test was used to compare difference between individual's means and control.

RESULTS AND DISCUSSION

Seed germination:
The daily and total germination percentages at different treatments are given in table 1. Results showed that high concentrations (above 250 ppm) decreased seed germination of *L. sativum* L. and germination of seeds was totally inhibited when they treated with 1000 ppm of butenolide (plate 1). In daily and total germination percentages of the seeds, differences between and within treatments ($p < 0.001$) are very clear (Table 1 and plate 1).

Seedling growth:
The fresh and dry seedling growth parameters reflected the response of the treated species to butenolide(Figure 1 and 2). In seedling length, the mean of total elongations of seedlings were approximately the same for most low treatments while concentrations of 500 and 1000 ppm decreased the seedling development. The results showed that the average of length of *L. Sativum* L. was between 7.77 mm for 1000 ppm and 144.44 mm for 25 ppm concentration (Figure 1). And the significant effect between treatments was high when ($p< 0.001$). For fresh and dry weight, increasing concentrations up to 250 ppm was also reduced the mean of these two parameters. The dry weight was averaged between 0.0 mg under 1000 ppm to 1.07 mg for 100 ppm treatment. High significant within and between groups of different treatment ($p < 0.001$) was noted. Seedlings treated with high concentrations revealed decreasing in fresh weight and the means were ranged between 3.0 mg at 1000 ppm to 31.33 mg at control condition (Figure2).

The response of *L. sativum* L. plant speices, which used as receptor, to different concentrations of butenolide has been studied. butenolide was used as a promoter and its effect was measured by calculating seed germination percentages and seedling growth parameters. The role, that butenolide play, on seed germination and seedling growth is well documented for a wild range of plant species, [11, 13, and 24]. Seed germination commences with the uptake of water by dry seed and is completed with emergence of the radical [3]. From the results, it is clear that daily germination percentages of *Lepidium Sativum* L. were reduced when seeds treated with butenolide concentrations of 500 and 1000 ppm, however the other concentrations approximately had the same effect of control

condition (0 ppm). Razanamandranto *et al*., (2005); Sparg *et al*., (2005); Crosti *et al*., (2006) and Daws *et al*., (2007) were reported the ability of smoke treatment to shorten germination time in number of studies. Our results were agreed with those previous studies. Butenolide shortened the germination time of seeds of *L. Sativum* L. at different concentrations (Table1). Butenolide, the chemical compound in smoke that promotes germination, could be involved in early induction of the cell cycle activation and thus accelerate radical emergence in germination seeds [8].

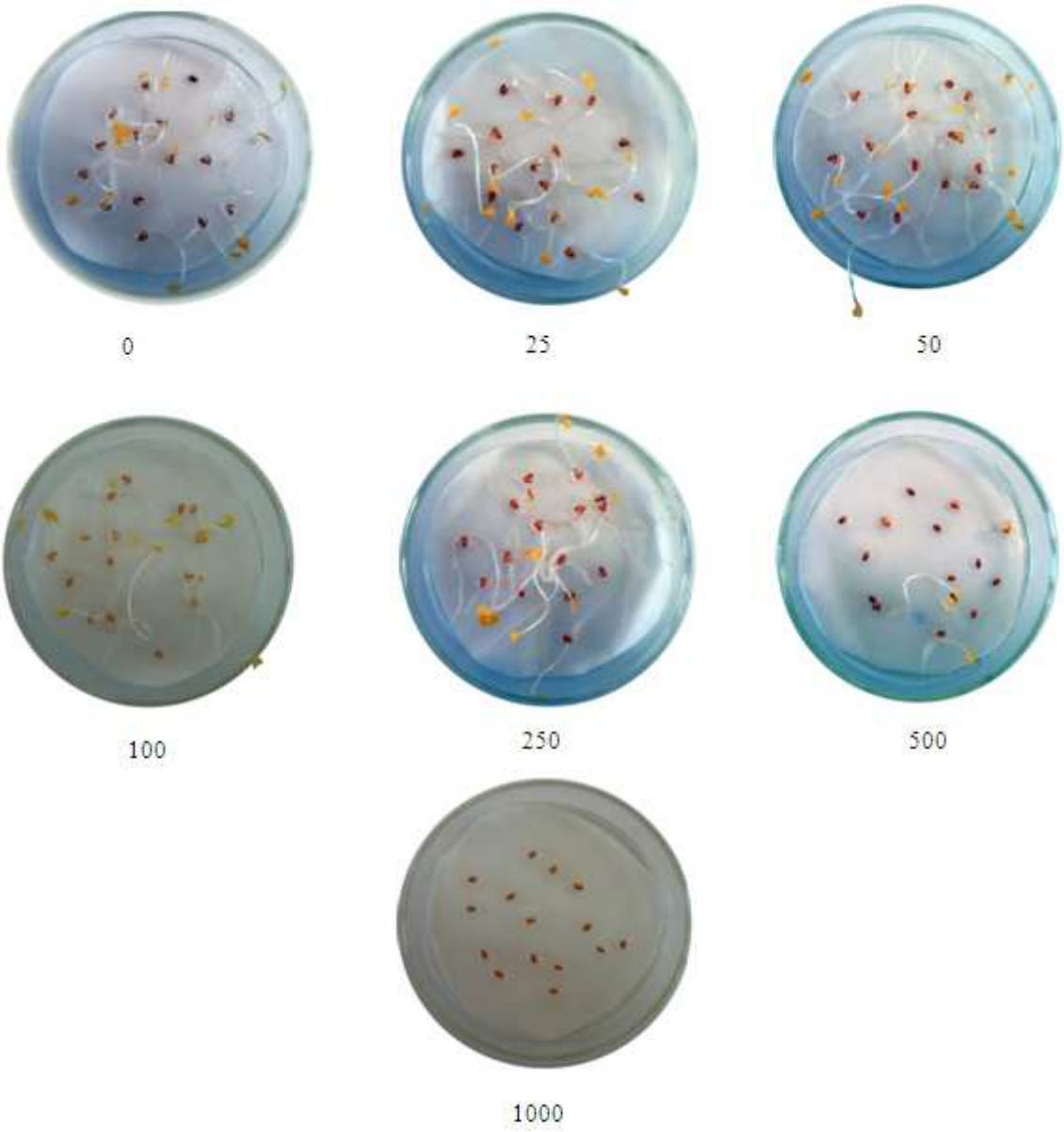

Plate 1. Response of *Lepidium sativum* L. seeds to different concentrations of butenolide(ppm). Concentrations up to 250 ppm decreased and inhibited seed germination. Distilled water was used as control (0 ppm).

Table 1. Daily and total germination of *L. Sativum* L. seeds at different concentrations of butenolide. The total germination percentage was calculated at the end of germination period (7 days).

Days / Conc.ppm	1	2	3	4	5	6	7
0	*** 93.3 ± 6.6	*** 96.6 ± 3.33	*** 96.6 ± 3.33	*** 96.6 ± 3.33	*** 96.6 ± 3.33	*** 96.6 ± 3.33	*** 96.6 ± 3.33
25	76.6 ± 10.0	96.6 ± 3.3	96.6 ± 3.33	96.6 ± 3.33	96.6 ± 3.33	96.6 ± 3.33	96.6 ± 3.33
50	73.3 ± 13.3	86.6 ± 13.3	96.6 ± 3.33	96.6 ± 3.33	96.6 ± 3.33	96.6 ± 3.33	96.6 ± 3.33
100	70.0 ± 3.3	93.3 ± 0.0	93.3 ± 0.0	93.3 ± 0.0	93.3 ± 0.0	93.3 ± 0.0	93.3 ± 0.0
250	50.0 ±3.3	90.0 ± 3.3	93.3 ± 0.0	93.3 ± 0.0	93.3 ± 0.0	93.3 ± 0.0	93.3 ± 0.0
500	0.0 ± 0.0	0.0 ± 0.0	13.3 ± 6.6	13.3 ± 6.6	13.3 ± 6.6	13.3 ± 6.6	13.3 ± 6.6
1000	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

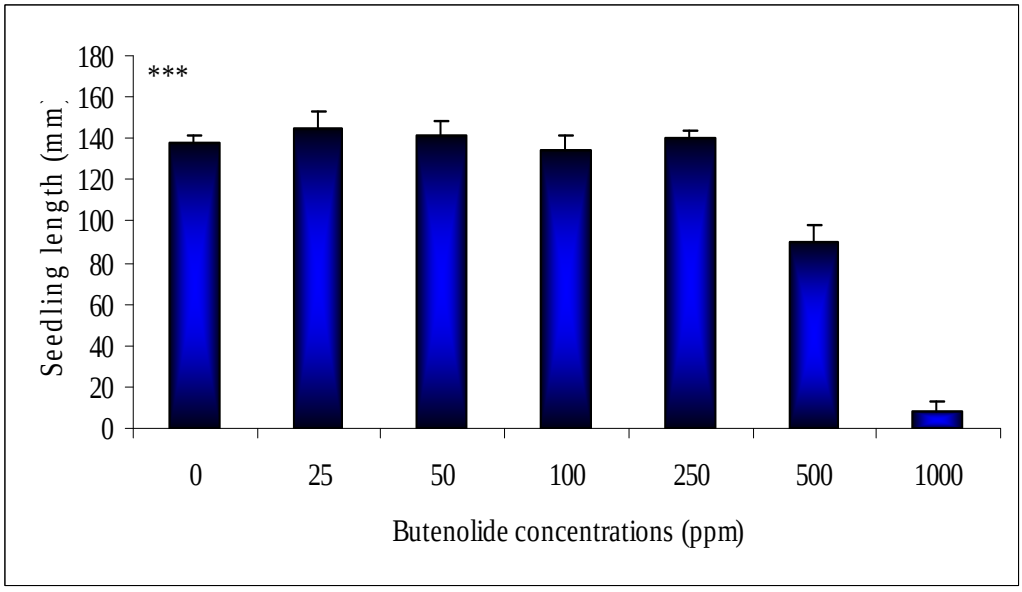

Figure 1. The effect of butenolide on seedling length. The pars are means ± SE. concentration of 1000 ppm was completely inhibited the total length of the receptor plant.

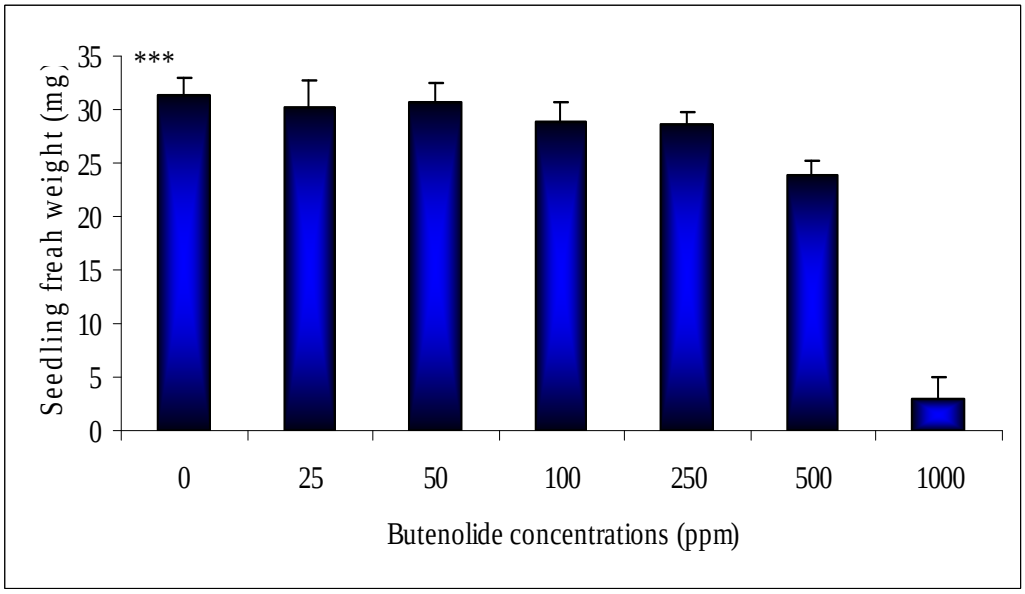

Figure 2. The fresh weight of seedlings of *L. sativum* L. at different treatments. The pars are means ± SE. concentration up to 250 pmm to 1000 ppm was deacresed the fresh weight and from analytical side, Dunnett test indicated high significant between control and 500 and 1000 ppm of butenolide.

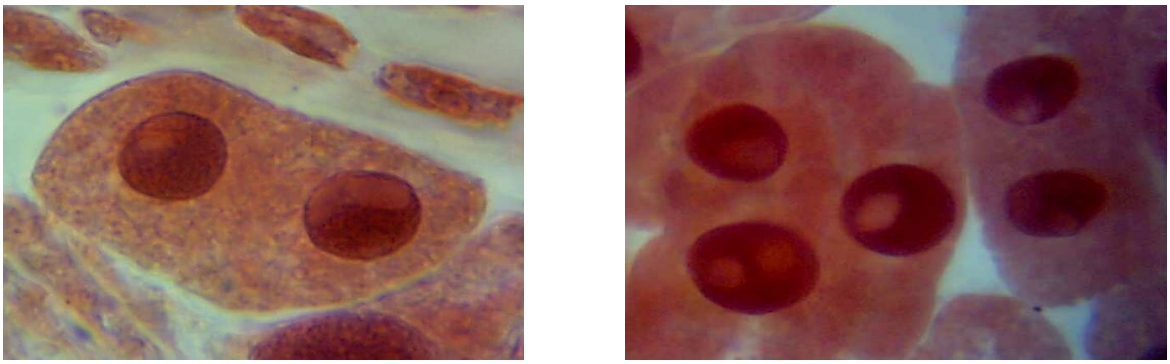

Figure3: The effect of butenolide on merstimic cells of *Allium cepa* L. root tips. The physiological abnormalities appeared clearly when plants treated with high concentrations of the compound. Left; Binucleated cell and right multiple nucleated cell.

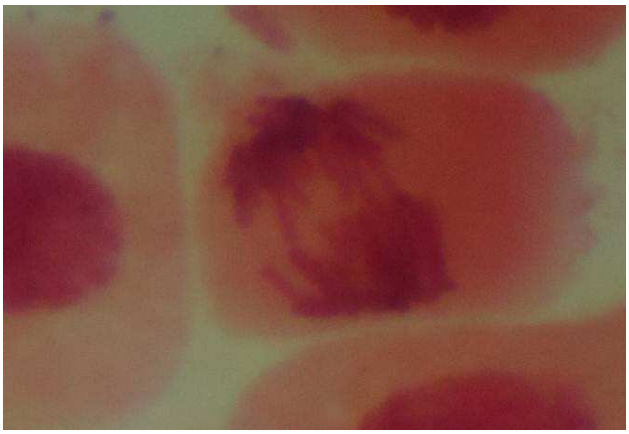

Figure4: clastogenic abnormality (Anaphase bridges) due to the toxicity of butenolide to the merstimic cells of Allium cepa L. rot tips.

Seedling growth was measured, in term fresh and dry parameters of the whole seedling under different concentrations of butenolide. The seedling length of the receptor was high at concentration of 25 ppm, while it decreased when plants treated with 1000 ppm. Dry weight measurements showed that the differences between means of treatments were not significant. Jain *et al*., (2008) reported that butenolide can serve as aquaporin inhibitor. The presence of aquaporin inhibitors reduced seedling water content and subsequently, altered seedling growth and development. Clearly, the high concentrations of butenolide revealed its toxicity to the plant receptor.

Analyzing the genotoxicity of butenolide using Allium test (data are not presented here) showed that the concentrations of (25, 50,100 and 1000 ppm) had an inhibition effect on Mitotic index (MI) but; at (250 and 500 ppm) there were increases in (MI). The mutations appeared in the test were physiological abnormalities like binucleated cells and multiple nucleated cells (Figure3) and clastogenic abnormalities which include anaphase bridges (Figure4). These reasons might behind decreasing seedling development due to breakdown of DNA and inhibition generate of DNA. In addition, stopping the cells in phase G2 [19].

REFERENCES

[1] Adam, S. I., S. A. Salih, Abdalgader W. S., *Asian journal of medicinal science*, **2011**, 3(6): 261-266.
[2] Baldwin, I.T., Morse, L., *J. Chem. Ecol.*, **1994**, 20: 2373–2391.
[3] Bewley JD., *Plant Cell*, **1997**, 9:1055–1066.
[4] Crosti, R., Ladd, P.G., Dixon, K.W., Piotto, B., *Forest Ecol. Manage*, **2006**, 221, 306–312.
[5] Daws MI, Davies J, Pritchard HW, Brown NAC, Van Staden J., *Plant Growth Regulation*. , **2007**, 51: 73–82.
[6] De Lange JH, Boucher C., *S Afr J Bot.*, **1990**, 56: 700–703.
[7] Flematti GR, Ghisalberti EL, Dixon KW, Trengove RD., *Science*, **2004**, 305:977.
[8] Jain, N., Van Staden, J., *Plant Growth Regulation*, **2006**, 50: 139–148.
[9] Jain N, Ascough G D, Van Staden J., *Journal of Plant Physiology*, **2008**, 165:1422—1427.
[10] Keeley, J.E., *S. Afr. J. Bot*., **1993**, 59: 638–639.
[11] Kulkarni, M.G., Sparg, S.G., Light, M.E., Van Staden, J., *J. Agron. Crop Sci*., **2006**, 192: 395–398.
[12] Laughlin, D.C., and P.Z. Fulé., *Canadian Journal of Forest Research*, **2008**, 38: 133-142.
[13] Merritt DJ, Kristiansen M, Flematti GR, Turner SR, Ghisalberti EL, Trengove RD, Dixon KW., *Seed Sci Res*., **2006**, 16: 29–35.
[14] Razanamandranto, S., Tigabu, M., Sawadogo, L., Oden, P.C., *Seed Sci. Technol.*, **2005**, 33: 315–328.
[15] Read, T.R., S.M. Bellairs, D.R. Mulligan, and D. Lamb., *Austral. Ecology*, **2000**, 25: 48-57.
[16] Sandberg D. V., Ottmar R. D., and Peterson J. L., Wild land fire in ecosystems: effects of fire on air," General Technical Report, U.S. Department of Agriculture, Forest Service, **2009**, Ogden, Utah, USA.
[17] Senaratna T, Dixon K, Bunn E, Touchell D., Smoke saturated water promotes somatic embryogenesis in geranium, Plant Growth Regul., 1999, 28: 95–99.
[18] Sparg SG, Kulkarni MG, Light ME, Van Staden J., *Bioresour Technol*., **2005**, 96: 1323–1330.
[19] 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.
[20] Taylor JLS, Van Staden J., *Plant Growth Regul*., **1996**, 18: 165–168.
[21] Van Loon, J., Samenöl von Lipedium sativum L., Z. Lebensm. *Unters.-Forsch*., 1973, 152, 75-78.
[22] Van Staden, J., N.A.C. Brown, A.K. Jäger, and T.A. Johnson, *Plant Species Biology*, **2000**, 15: 167-178.
[23] Van Staden J, Jager AK, Light ME, Burger BV, *S Afr J Bot*., **2004**, 70: 654–659.

[24] Van Staden J, Sparg SG, Kulkarni MG, Light ME., *Field Crops Res*., **2006**, 98: 98–105.
[25] Wills, T.J., Read, J., *Australian Journal of Botany*, **2002**, 50: 197-206.