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RESPONSE OF DIFFERENT PLANT RECEPTORS TO FURANONE.

BY

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قوله تعالى:

وما أوتيتم من العلم إلاّ قليلا

سورة الإسراء: (85)

TO MY PARENTS

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Great thanks to Almighty Allah who granted me health, patience, capability and knowledge to execute this study.

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I express my thanks to my:

1- Mother Sikna Khris, Father Ibrahim El. Tajori.

2- My brothers and my sisters.

Finally, I dedicate this work to my beloved (mother and father).

Abstract

Smoke act as a promoter of seed germination, that the active compound in it called butenolide 3-methyl-2H-furo[2,3-c]pyran-2-one and this last effect on post germination and germination on different plant species. The present study reports on the effect of butenolide on seed germination and seedling growth on four plant species that are: *Solanum lycopersicum L, Lens culinaris L, Lepidium Sativum L and Hordeum vulgare L.* Study chromosomal abnormalities on *Allium cepa L.* tip roots. Results of seed germination and seedling growth showed significality different concentrations of butenolide had no effect on, *Lepidium Sativum L.* and *Hordeum vulgare L*., but; in the case of *Solanum lycopersicum L.* had a perfictal effect on seed germination and seedling growth. However *Lens culinaris L.* in both seed germination and seedling growth gave good results under low concentrations of butenolide. Tomato seeds soaking in different of butenolide solutions for 24 hours prior to planting, significantly improved root and fresh weight after 60 days, the number of leaves survival were also greater in some of butenolide treated plants. The effect on *Allium cepa L.*tip roots gave inhibition in Mitotic index (MI) at low concentrations of butenolide and appeared many different abnormalities chromosomes when the seeds soaking in different concentrations of butenolide such as: sticky metaphase, lagging chromosome, binucleated cell, Anaphase and Telophase bridge.

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Introduction

Nature, which is a collective name for all facts, has many phenomena that surrounding us some of them can be considered ambiguous or, in other words, partly of them are known to us and partly unknown. However, one of these interesting phenomena plays a vital role in our daily life. It, actually, has great impacts on human, animals as well as plants' life, which we called fire's phenomenon.

Fire's phenomenon is something amazing. It 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 very small pieces of carbon that is produced when something burns. However, 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 (Laughlin and Fulé 2008). It is anticipated that fire activities will increase in southwestern forests as a result of warmer temperatures and the melting of snow in climate change scenarios (Westerling *et al.* 2006). To understand the effects of fire in forests due to the fact that there is an increase in wildfires as well as the use of fire (Collins and Stephens 2007).

One of the many effects of fire is exposing seeds in the soil to the environmental factors and the plants too. (Van Staden *et al.* 2000). That

affects both plants and development i.e. flowering, seed dispersal, germination, seedling establishment, plant mortality, biomass…etc. So, fire is an essential element in the seed bank dynamics as about 40% of species have enhanced germination following fire. There is an important positive effect of fire on the conservation and restoration of plant communities, (Read *et al.,* 2000; Flematti *et al.,* 2004); because fire products prefer high seedling establishment they might increase the diversity of species (Read *et al.,* 2000; Wills and Read, 2002; Enright and Kintrup, 2001). There can be a reduction in the biotic stress pressure that plants are subjected to due changes of environmental conditions that take place following fires (Calder, 2010). (Marschner, 1995), that plants may use smoke as an environmental cue to initiate other adaptive metabolic and growth responses. However it is crucial to understand the influence of fire forests, due to the increase in wildfires and the use of fire (Collins and Stephens 2007). In addition to other various effects, fire exposes seeds to smoke (Van Staden *et al*. 2000).

Germination is triggered in fire-prone areas by high temperature, plant-derived smoke, ash and charred wood which are fire products (Keeley and Fotheringham, 2000; Van Standen *et al.,* 2000). Smoke that results from fires varies in fuel loads, intensity as well as duration of burning as it can stay in the air for weeks (Sandberg *et al.,* 2009). In addition smoke

that results from wildfires is a crucial chemical stimuli for the germination of fire-adapted species (Todorovic *et al.*, 2005).

As it mentioned early, fire produces smoke, which has been described as a grey, black or white mixture of gas in addition carbon. However, De Lange and Boucher, 1990; Brown, 1993; Baldwin and Morse, 1994 reported that in the early 1960s, smoke was identified as a vital germination cue in post-fire conditions. De Lange and Boucher (1990) were the first proved that plant derived smoke stimulates seed germination. Smoke may be used a chemical cue to increase permeability of seed coat or stimulate metabolic activity (Baldwin *et al*. 1994, Keeley and Fotheringham 1998). Moreover, smoke enhances germination in all seed dormancy classes (Baskin and Baskin 1998) as noticed in laboratory and field conditions. Therefore; the action of smoke is not influenced by life form, phylogenetic relationship, geography and seed type (Chiwocha *et al.*, 2009). At three different scales: individual seeds, soil seed bank samples, and in field plots, smoke is assessed either as germination or emergence cue in laboratory and field settings (Abella, 2009). Products of smoke were demonstrated to enhance germination from natural soil seed banks (Lloyd *et al.* 2000) and in post-mining rehabilitation operations (Roche *et al.* 1997). 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).

Affirms that smoke does not influence germination of all species, it instigates the process of germination of a different number of species in both frequent- and infrequent-fire ecosystems. Since 1990, the role that smokes plays in the release of dormancy, germination and seedling growth has been examined and only 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). Calder *et al*. (2010) say that plants can utilize smoke for the beginning of other adaptive metabolic growth responses. There are various compounds in smoke, and the one that is responsible for enhancing germination puzzled researchers because the promotive effect was found to be well documented (Baldwin *et al.* 1994,. Brown and Van Staden 1997). From the Physical side, the production of smoke may lead to high-vapor pressure deficits that can instigate stomata closure (Guehl and Aussenac 1987) and from the chemical side of things more than 100 compounds were identified in smoke, (Radojevic 2003) some of those are known to have physiological effects on plants, including NO2 (Keeley and Fotheringham, 1997), CO2, SO2 , and O3 (Robinson *et al*. 1998).

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[A]). . One of the essential climatic factors is temperature which plays an important role in systemizing the process of seed germination (Jain *et al.*, 2006) However, in butenolide treated seeds the ratio of cells with replicated DNA was increased (Jain and Van Staden, 2006). Flematti *et al*. (2004, 2005) pointed out that butenolide provides the potential to transfer smoke technology into field benefits. In addition to enhancing percentage of germination, Butenolide is also capable of widening the environmental window over which germination can occur as a complex process, seed germination is controlled by different internal and external factors. A number of weed species have witnessed positive effect as a result smoke solutions and farmers may potentially utilize smoke-water to promote sake of eradication before planting the new crop a thing that decreases the burden of the weed on the crop (Light and Van Staden, 2004) .

By means of enhancing seedling vigor, smoke and butenolide have proved to have a post-germination positive influence (Sparg *et al.*, 2005; Jain and Van Staden, 2006; van Staden *et al.*, 2006; Daws *et al.*, 2007). Seedling vigor as well as survival rates were improved as a result of applying butenolide in some South African indigenous medicinal plants

(Sparg *et al.,* 2005), a commercial maize cultivar (Sparg *et al.,* 2006), rice (Kulkarni *et al.,* 2006), vegetables such as tomatoes, okra and beans (Jain and Van Staden, 2006; Van Staden *et al.,* 2006), grasses (Baxter and Van Staden, 1994; Blank and Young, 1998) and woody Acacia species (Kulkarni *et al.,* 2007[A]). Various numbers of short-term studies are based on *in vitro* and *in vivo* tests, which utilized for the discovery of and monitoring of many types of environmental chemicals with mutagenic and carcinogenic potential (Ashby *et al.,*1985; 1988).

Chromosomal aberrations can be accepted as indicators of genetic damage induced by 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).

Aims of the study:-

The main aim of this study is to test to what extent that synthesized butenolide can affect seed germination, seedling development and plant establishment using different plant species as receptors, and either the effect is species and concentration dependent or not, and to study the effect of different concentrations of butenolide on cell division and chromosomal abnormalities of onion root tips.

1.1**Literature review:**

(Moritz and Odion, 2005) reported that fire may obstruct pathogen activity by way of increasing the availability of Ca, which is vital for plant resistance to disease. Jain *et al*., (2006) attempted to elucidate the role of the butenolide in overcoming detrimental effects of low and high temperatures on tomato seed germination and seedling growth. they reported that the germination percentage followed a parabolic curve for temperatures ranging from 10 to 40 C^o, with 25 C^o being the optimum for all treatments. Control seeds showed radical emergence at two extreme temperatures (10 and 40C^o) and seedlings failed to develop further, even upon prolonged incubation. Furthermore, seedlings developed in the presence of the butenolide had about a 1:1 correspondence between root and shoot length. Jain *et al*., (2008[b]) reported that butenolide can serve as aquaporin inhibitor. This suggests enhanced activity of aquaporins. Seedlings raised in the presence of butenolide had higher moisture content (93%) compared to those imbibed in water only (85%). Jain *et al*., (2008[A]) reported that, The effects of butenolide, known aquaporin inhibitors (HgCl2 and ZnCl2), along with several chemical agents known to reverse the inhibitory effects of mercuric chloride on the activity of aquaporins were tested. The presence of aquaporin inhibitors (HgCl2 and ZnCl2) reduced seedling water content and altered root development. The presence of HgCl2 (10, 20 or 30 mM) reduced the percentage imbibition

of seeds by 11–12%. Consequently, Daws *et al*., (2008) tested whether butenolide also functions as germination stimulant for parasitic weeds. Butenolide stimulated germination of both *Orobanche minor* and *Striga hermonthica.*These results suggest that the germination stimulatory activity of butenolide may result from analogy with strigolactones. (Zhou *et al*., 2011) Smoke treatments also improve post-germinative growth into to large extent (seedling vigor); Smoke is assessed for its characteristic of improving germination of seeds and growth of plants. In addition, smoke also stimulates somatic embryogenesis (Senaratna *et al*., 1999), flowering (Keeley, 1993) and rooting (Taylor and van Staden, 1996). In a preliminary experiment on *Watsonia borbonica* (spring-flowering hybrid), a treatment of 1:500 (v/v) smoke water increased flowering from 20% to 90% (Light et al., 2007). Smoke is capable of reducing photosynthesis by way of physical and/or chemical processes (Calder el al., 2010).

(Jain et al., 2008[B]) found the changes induced by the butenolide at the level of macromolecules (DNA, RNA and proteins) during seed germination. Total number of bands recorded for 25 primers in control and butenolide treated seedlings were not significantly different from each other according to the non-parametrical Kruskal– Wallis test. Smoke also remarkably better post germinative growth (seedling vigour) in seeds of the Amaryllidaceae, regardless of the fact that in these species no

influence on germination was noticed (Brown *et al*. 2003; Sparg *et al.* 2005). Demir *et al*., (2001) have studied the effect of butenolide priming treatments on seedling emergence and growth of Pepper (*Capsicum annuum* L.) and salvia (*Salvia sp*.) seeds they were found Butenolide-primed seeds emerged faster and produced larger seedlings as indicated by fresh and dry weight compared to the water controls for both species. Butenolide-primed seeds had higher catalase activity than that of the controls suggesting that the enhancement obtained from priming may be due to changes in enzymatic activity. Kulkarni *et al*., (2010) have showed effects on a number of agricultural and horticultural crops. In (onion) *Allium cepa L*. plants were treated with smoke-water solution or a butenolide solution under greenhouse conditions. Onion plants supplied with smoke-water and butenolide solution exhibited a significantly greater number of leaves, increased leaf length, and a higher fresh and dry leaf weight than untreated plants In addition, smoke-water and butenolide-treated onion plants exhibited a significantly higher bulb diameter and bulb weight than untreated plants.

The species of Acacia investigated were *A. hebeclada* (deciduous shrub), *A. mearnsii* (invasive tree, native to Australia) and *A. robusta* (deciduous tree). Seeds of *A. hebeclada* germinated under different light conditions with smoke-derived butenolide solution, exhibited a significantly greater germination percentage than untreated seeds. Whereas *A. mearnsii* seeds

exposed to constant dark conditions showed a significantly better germination percentage than the control. However, there was a non-significant improvement for *A. robusta* seeds. All three species responded positively to the butenolide treatment after incubating for 10 days under constant dark conditions, achieving a higher vigour index and seedling mass in comparison to the controls (Kulkarni *et al*., 2007[A]). Van Staden *et al*., (2006) investigated the post-germination effect of smoke-water on tomato (*Lycopersicon esculentum*), okra (*Abelmoschus esculentus*) and bean (*Phaseolus vulgaris*) under laboratory conditions. Tomato seedlings that were treated with solution had 10-times greater root length than the water control, whereas in okra and bean, root length was 3-times more. There was also a significant increase in shoot length of all three crop seedlings. Furthermore, smoke-water (1:500, v/v) significantly improved the weight of the tomato and okra seedlings.

A study conducted by Kulkarni *et al*. (2006) indicated that smoke can be a useful treatment for improving the vigor of rice (*Oryza sativa*) Results showed that smoke-water significantly promoted shoot length and a low concentration achieved maximum root length and seedling mass. seedlings produced a greater number of lateral roots than untreated ones. In addition, this study was undertaken to gain insight into the physiological events involved in seed germination and seedling development and which are affected by butenolide using tomato

(*Lycopersicon esculentum* Mill.) cultivar seeds. No stimulatory role on the seed germination of tomato was recorded following the use of the butenolide, however, post-germinative growth of tomato seedlings was significantly improved over the control. The emergence of the radicle and elongation of the hypocotyls and radicles were accelerated in seeds imbibed with butenolide.

Flow cytometry studies showed that in butenolide treated seeds the ratio of cells with replicated DNA was increased. Seedling vigour and weight were significantly increased by the butenolide (Jain *et al*., 2006). Kandari *et al.* (2011) has been suggested that, seed germination of *Solanum viarum* was markedly stimulated by different concentrations of smoke-water solutions. which resulted in greater vigor indices than the control seedlings. The effects of foliar application of smoke-water and a butenolide on seedling growth of okra [*Abelmoschus esculentus (L.) Moench*] and tomato (*Lycopersicon esculentum Mill*.). Treating okra seedlings with smoke-water showed a significant increase in shoot/root length; shoot fresh/dry weight, number of leaves, total leaf area, and stem thickness compared with the control treatment. Treatment of okra seedlings with smoke-water significantly increased the absolute growth rate (AGR) per week. However, the seedling vigor index (SVI) did not improve as a result of no change in root fresh weight. On the other hand, foliar application of smoke-water and butenolide showed a pronounced

effect on the seedling growth of tomato. Most of the growth parameters examined for both the treatments were significantly increased, resulting in a significantly higher SVI and AGR than the control (Kulkarni *et al*., 2007[B]).

Jain and Van Staden, (2007) found that, the potential of the butenolide as a priming agent of tomato (*Solanum esculentum* Mill.) seeds. Flow cytometry data revealed that butenolide-primed seeds had a higher percentage of nuclei at the 4C stage than water-primed seeds. Emergence of the radicle was much faster in the primed seeds. After 36 h of imbibition, a higher percentage of the butenolide-primed seeds (22%) exhibited radicle emergence compared to the water-primed seeds (12%). While butenolide-primed seeds initially germinated more rapidly than either water-primed or unprimed seeds in a 48-h period, water-imbibed seeds reached a similar germination level as the butenolide-primed seeds by 60 h of incubation. The butenolide-primed seeds produced significantly more vigorous seedlings than water-primed seeds or seeds in the continuous presence of either water or butenolide. A gradual decrease in the seedling vigour index was recorded for both water and butenolide-primed seeds with increased seed storage at room temperature. Nevertheless, the vigour index was significantly greater in the butenolide-primed seeds than the water-primed seeds. Vigour indices were significantly higher for the butenolide-primed seeds under various stress

conditions (salinity, osmoticum or temperature) compared to control or water-primed seeds. (Kulkarni *et al*., 2006), have studied the effects of butenolide on shoot and root elongation of a local rice variety. Butenolide (10^{-8} M) treatments significantly increased shoot length. A low concentration of butenolide (10^{-10} M) promoted maximum root length and seedling weight, which were significantly different from the control. Butenolide-treated (10^{-8} M) seedlings had a significantly greater number of lateral roots than untreated seedlings. The vigour index of butenolide-treated (10^{-8} M) rice seeds was significantly higher than that of untreated seeds. Roche *et al*. (1997) suggested that smoke provides protection for seeds and seedlings against microbial attack. Examined the influence of smoke on seed lots of rye, barley, wheat and oats, which is well established old method used for drying grains (Paasonen *et al*., 2003). During their study it was found that germination was better and the grains experienced a reduction in microbial contamination by endophytic species. Nautiyal *et al*. (2007) proved that the smoke generated by 'combusting wood and a mixture of odoriferous and medicinal herbs' eradicated some of the bacteria that are harmful to both agricultural and horticultural plants.

Sparg *et al.*, (2005), Jain and Van Staden (2006) and Daws *et al.* (2007) reported that seeds which were treated by the use butenolide can germinate quicker and increased vigor and fresh weight. Smoke can also

stimulate the germination of species from non-fire prone environments such as a number of temperate arable weeds (Adkins and Peters 2001), *lettuce Lactuca sativa L.,* (Drewes *et al.* 1995), celery *Apium graveolens L.*, (Thomas and Van Staden 1995) and red rice *Oryza sativa*, (Doherty and Cohn 2000), indigenous maize (Modi, 2002, 2004), carrot, parsley and leek (Merritt *et al.,* 2005), commercial bean (Van Staden *et al.*, 2006), bush tomato (Ahmed *et al.*, 2006), okra (Kulkarni *et al.*, 2007[B]), commercial tomato (Kulkarni *et al.,* 2008) and tef (Ghebrehiwot *et al.*, 2008).

Chapter2

Materials and method

2.1. Plant material:

Different plant species related to different groups were used as plant receptors in this study. Seeds of these plants were certified and purchased from the local market in Benghazi. The seeds used in this study are shown in table 2.1.

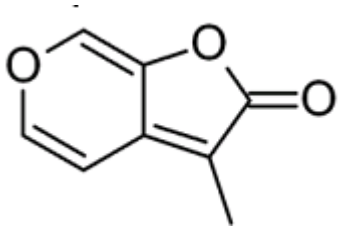
Table 2.1. Scientific name, common name, plant family and plant group of plant species those used in this study:

Common name	Scientific name	Family	Plant group
Tomato	*Solanum lycopersicum* L.	Solanaceae	Dicot
Lentil	*Lens culinaris L.*	Fabaceae	Dicot
Cresson	*Lepidium Sativum* L.	Brassicaceae	Dicot
Onion	*Allium cepa L.*	Alliaceae	Dicot
Barly	*Hordeum vulgare L.*	Poaceae	Monocot

All tested seeds were used to examine the effect of butenolide concentrations on seed germination and seed development with exception of onion plant which used for chromosomal study. Tomato seedlings were used as an example for established plant test.

2.2. Chemicals:

5 ml of synthesized butenolide:



3-methyl-2H-furo[2,3-c]pyran-2-one (ALDRICH, Germany) was obtained from Bonn university, department of plant ecophysiolog, and concentrations of (0, 25, 50, 100, 250, 500 and 1000 ppm) were prepared and kept in the refrigerator in dark flasks until they used. (3%) alkyl dimethyle benzl ammonium sodium hypochlorite (Clorox) was used to prevent microbial growth on seeds after planting. Chemicals for chromosomal studies including: 70% ethanol, 1:3 ethanol to glacial acetic acid, 1NHCl, 40% acetic acid and aceto- orcen pigment were also prepared and used.

2.3. Seed germination test:

Seeds of tested plant species were similar selected in shape and size, these seeds were sterilized by (3%) alkyl dimethyle benzl ammonium sodium hypochlorite (Clorox) for 3 minutes and then washed with distilled water. Seeds were incubated in flasks with different concentrations of butenolide and soaking for 24 hours in a dark place. After that, seeds were placed in petridishes (diameter 9.0 cm) lined with double layers of whatmann filter papers. The petri dishes were used for each concentrations of butenolide contains fifteen seeds of all different

tested species. Five ml of distilled water were added to every replicate. Distilled water was added whenever seeds needed; all replicates were incubated in darkness under 20 ± 1C^0 in incubator (GALLENKAMP, U.K). Germinated seeds were counted daily for the calculations of daily and final germination percentages for tested plant species under the effect of different concentrations of butenolide.

2.4. Seedling growth test:

Germinated seed of different species 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 of plants used in this study; different parameters were measured as following:-

A. Length of plants shoots and roots (mm) by using a ruler.

B. Fresh weight of plants, shoots and roots (mg) by using balance (Mettler Toledo).

C. Dry weight that roots and shoots were covered with aluminum foil and then placed in an oven (Heraeus) at 100 C^0 for 48 hours, after that, their dry weight determined (mg). Root / shoot ratio was calculated.

2.5. 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. 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 (Darlington and La Cour 1976). 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 evaluation.

2.6. Established plant test:

Tomato species were selected in this study for established plant, that thirty seeds of similar shape and size for each concentrations of butenolide. The following concentrations of butenolide: 0.0, 25, 50, 100, 250, 500 and 1000 (ppm) soaking for 24 hours in a dark place and then potted in pots (size 10 cm in diameter) filled thoroughly clean sandy soil, each single seedling was placed in a pot and were potted in a plastic container and then placed in greenhouse. Each container of tomato plant was re-watered with distilled water whenever plants needed. After two months, fresh and dry parameters were measured include:

Length of shoots and roots (mm) by ruler, fresh weight of shoot and root system (mg) using (Mettler Toledo balance), and number of leaves per plant.

Dry weight of roots and shoots (mg). Here, plant parts were covered with aluminum foil and placed in oven at 100 C^{o} for 72 hours.

Root/ shoot ratio (mg/mg) was calculated for each treatment as following:

$$\text{Root / Shoot ratio (R / S)} = \frac{\text{Dry weight of root}}{\text{Dry weight of shoot}}$$

2.7. Statistical analysis:

A. For different measurements of plant species:

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.

B. For chromosomal study:

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 ($p<0.05$).

Chapter3

Results

3.1. Response of *Hordeum vulgar L. (Barley)* to different concentrations of butenolide :

3.1.1. Seed germination:

The effect of different concentrations of butenolide on daily germination percentages of *Hordeum vulgar* L. (Barly) is shown in table (3.1). Results showed that, high concentrations (up to 250) of the butenolide solution decrease the growth of seeds and approximately caused inhibition at (1000 ppm) concentration (plate 3.1, A and B).

3.1.1.2. Seedling growth:

(Table 3.2) shows the influence of different concentrations on root length, fresh and dry parameters of (Barley). The best mean was obtained at (50 ppm) in length, but; above (500 ppm) the results revealed decreasing in length. The range of elongation was between 66.63 mm under (1000 ppm) to 326.06 mm under (50 ppm) of butenolide concentration. There are highly significant between and within groups ($p < 0.001$). Fresh weight gives the best result at (25 ppm). However fresh weight average was ranged from 51.66 mg under (1000 ppm) to 238.9 mg under (25 ppm), and in dry weight the parameters similarly the same with exception at (1000 ppm). The dry weight ranged from 3.13 mg under (1000 ppm) to 11.66 mg under (100 ppm).

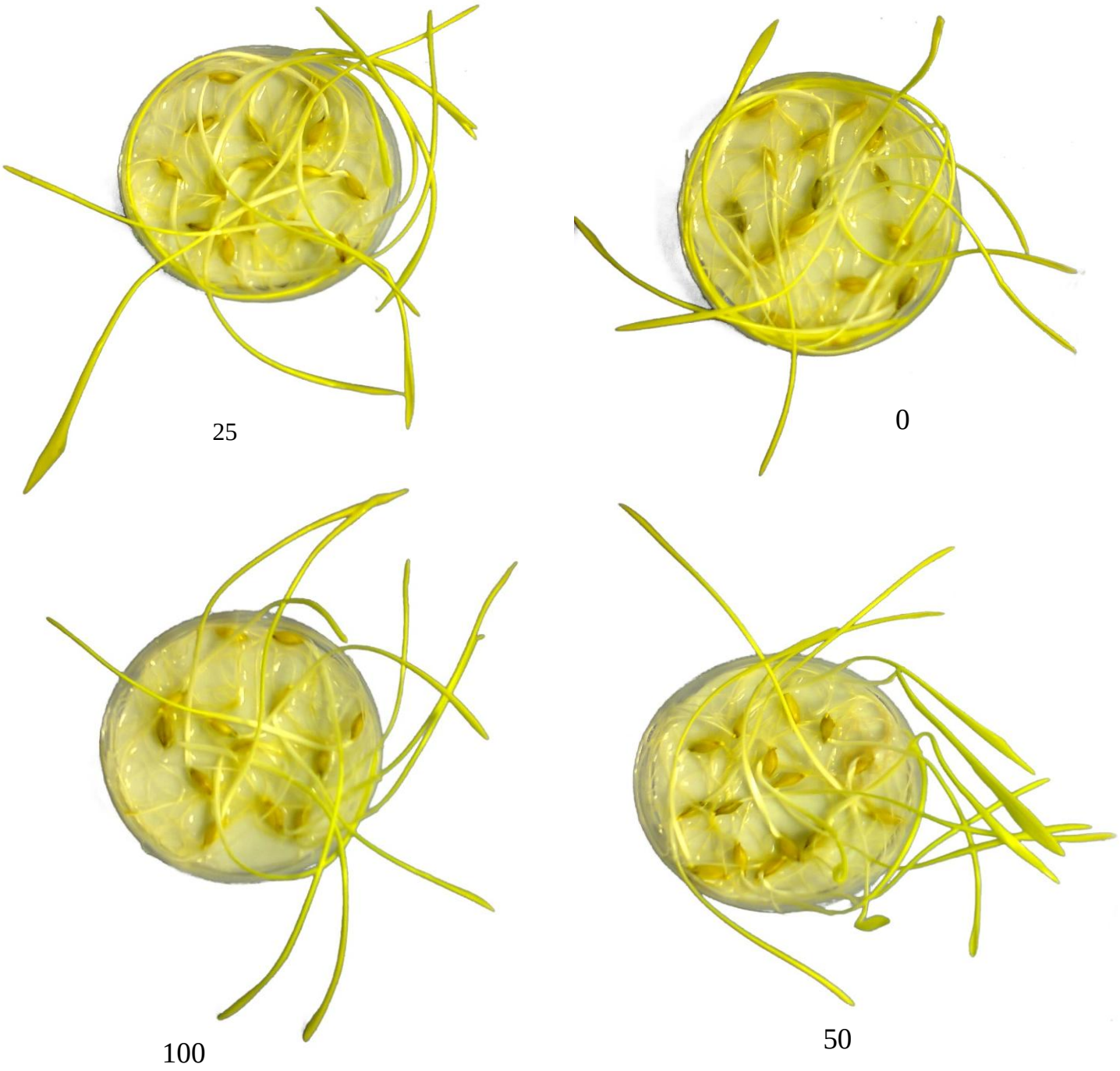


Plate 3. 1, A. Response of *Hordeum Vulgar L.* (Barley) to different concentrations of butenolide.

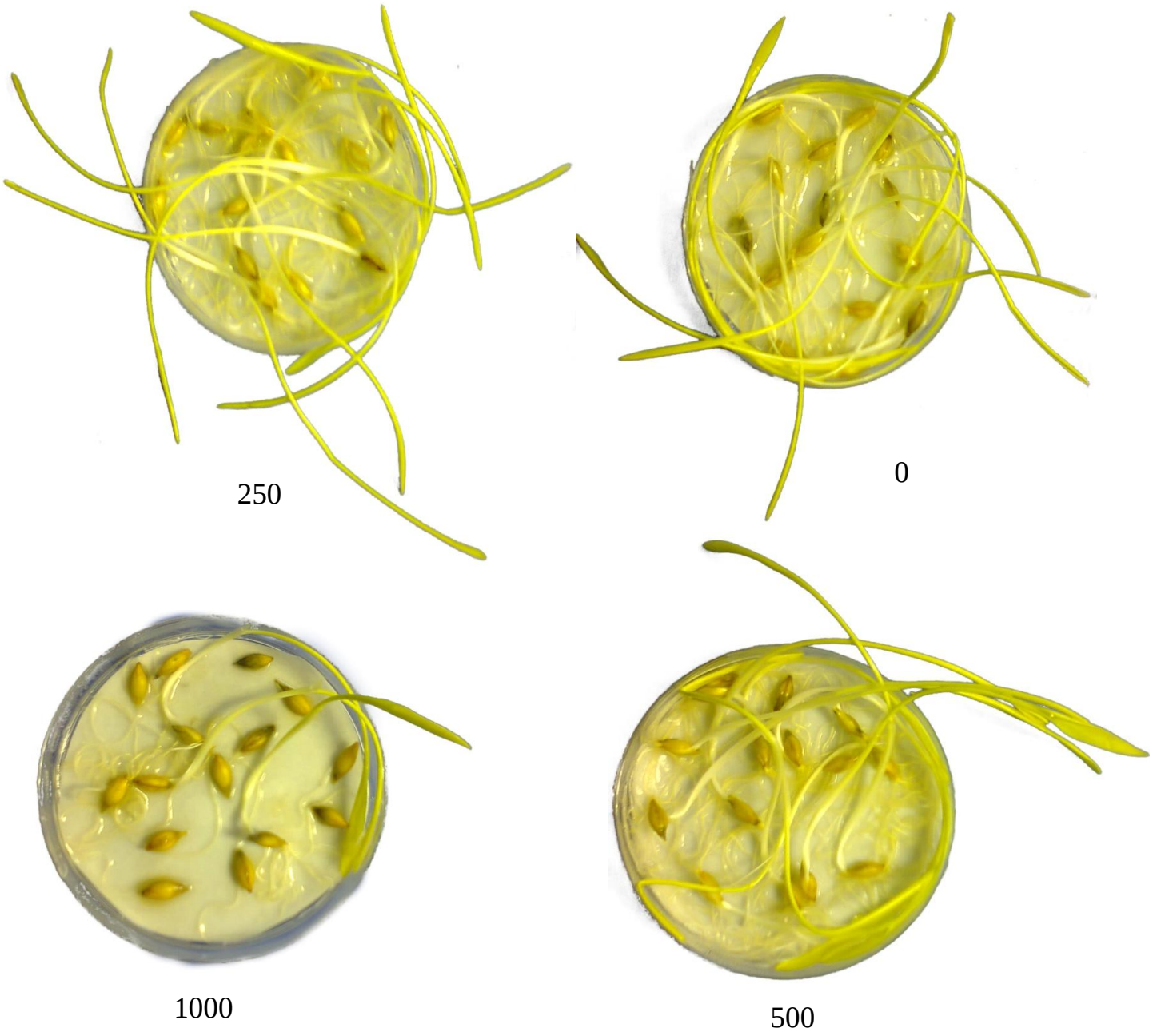


Plate 3. 1, B. Response of *Hordeum Vulgar (Barley)* seeds to different concentrations of butenolide.

Table 3. 1. Response of *Hordeum vulgar L. (Barley)* seeds to different concentrations of butenolide and their effects on daily germination percentages. (* = Significant at P < 0.05, *** = High Significant at P < 0.001, ± = SE Mean).

Concentration (ppm)	Day1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0	* 16.6 ± 3.33	*** 70.0 ± 3.3	*** 86.6 ± 0.0	*** 86.6 ± 0.0	*** 86.6 ± 0.0	*** 86.6 ± 0.0	*** 86.6 ± 0.0
25	50.0 ± 3.33	76.6 ± 10.0	86.6 ± 6.6	86.6 ± 6.6	86.6 ± 6.6	86.6 ± 6.6	86.6 ± 6.6
50	33.3 ± 13.3	73.3 ± 13.3	96.6 ± 3.3	96.6 ± 3.3	96.6 ± 3.3	96.6 ± 3.3	96.6 ± 3.3
100	26.6 ± 0.0	83.3 ± 3.3	90.0 ± 3.3	90.0 ± 3.3	90.0 ± 3.3	90.0 ± 3.3	90.0 ± 3.3
250	23.3 ± 4.7	86.6 ± 0.0	93.3 ± 6.6	93.3 ± 6.6	93.3 ± 6.6	93.3 ± 6.6	93.3 ± 6.6
500	20.0 ± 18.8	86.6 ± 6.6	86.6 ± 6.6	86.6 ± 6.6	86.6 ± 6.6	86.6 ± 6.6	86.6 ± 6.6
1000	0.0± 0.0	10.0 ± 3.3	20.0 ± 0.0	20.0 ± 0.0	20.0 ± 0.0	20.0 ± 0.0	20.0 ± 0.0

Table 3. 2. Response of *Hordeum vulgar* L (Barley) to different concentrations of butenolide. (*** = High Significant at p < 0.001, ± = SE Mean).

Concentration (ppm)	Length (mm)	Fresh weight (mg)	Dry weight (mg)
0	*** 269.13 ± 21.51	*** 188.00 ± 23.01	*** 10.43 ± 1.47
25	279.80 ± 21.6	238.93 ± 21.79	11.43 ± 1.59
50	326.06 ± 14.22	228.13 ± 11.55	11.46 ± 1.59
100	280.43 ± 20.00	200.00 ± 17.51	11.66 ± 1.63
250	285.30 ± 16.87	213.66 ± 15.82	10.86 ± 1.52
500	256.36 ± 28.02	180.33 ± 20.71	10.80 ± 1.50
1000	66.63 ± 24.88	51.66 ± 19.35	3.13 ± 1.19

In fresh and dry weight there are high significant in both ($p < 0.001$). That:

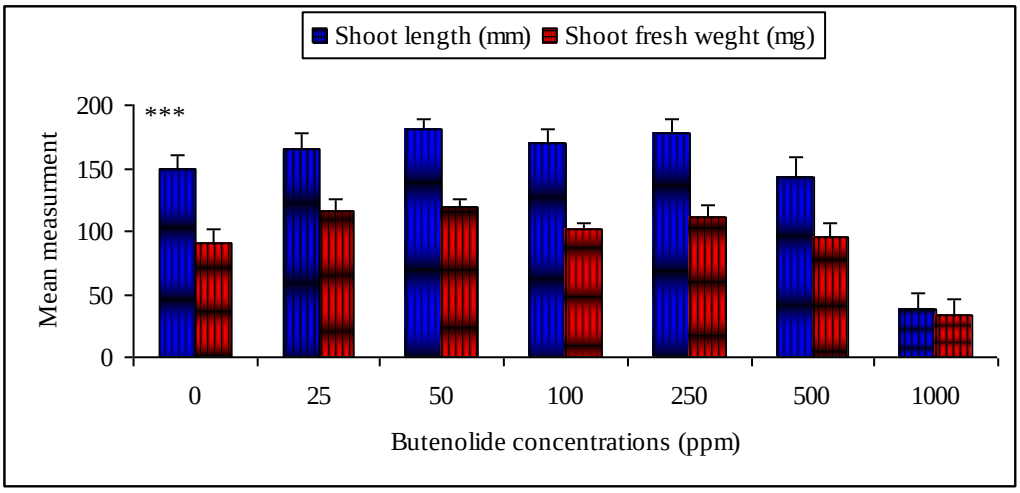
a. Fresh parameters:

[Figure 3.1] shows the effect of different concentrations of butenolide on fresh parameters of (Barley) seedlings. There are no difference in shoot length and fresh weight of shoot when seeds treated with different concentrations, but; these two parameters were decreased at concentration (1000 ppm). The average length of shoots was ranged from 37.46 mm under (1000 ppm) to 181.56 mm under (50 ppm) concentration, fresh weight of shoot was varied between 33.3 mg under (1000 ppm) to 118.33 mg under (50 ppm) of butenolide solution and there are highly significant in both shoot fresh weight and shoot length. In root length, the graph shown the high value in length at (50 ppm) and also the root weight value at (25 ppm), but; at (1000 ppm), the decrease happen in both length and weight of (Barley). Root length was ranged from 29.23 mm under (1000 ppm) to 144.50 mm under (50 ppm) and root fresh weight was 18.33 mg under (1000 ppm) to 123.66 mg under (25 ppm) of butenolide concentration, and the results showed that, there are highly significant in root length and fresh weight of length.

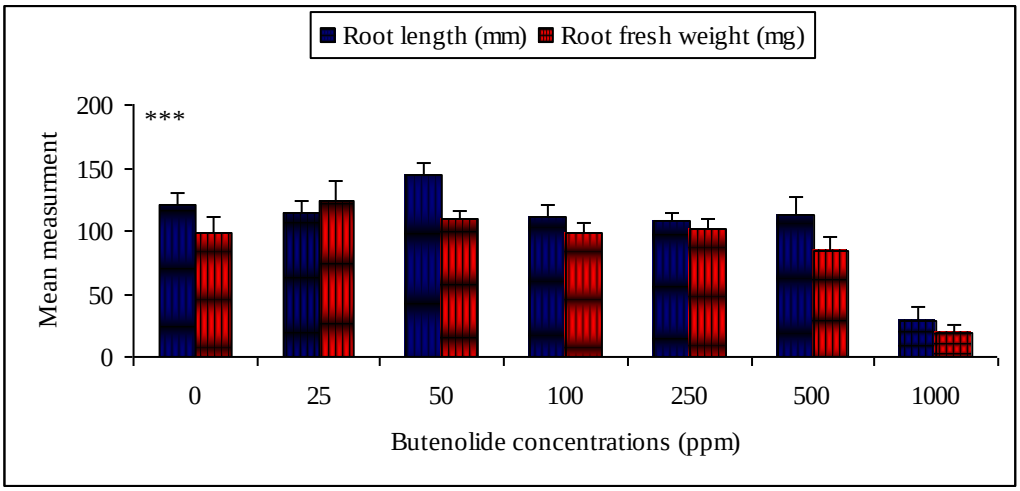
From statistical point of view, it is found by Dunnett test highly significant between control condition and (1000 ppm) of butenolide concentration in all fresh parameters.

b. Dry parameters:

[Figure 3.2. (A)] shows the shoot and root dry measurement of (Barley) seedlings under different concentrations of butenolide solution. In shoot dry weight, the best result was at concentration of (100 ppm) and in root dry weight approximately similar results were obtained in dry weight except at (1000 ppm), there are decrease of dry weight on shoot and root of barley. The shoot dry weight was ranged from 1.06 mg under (1000 ppm) to 7.060 mg under (100 ppm) concentrations, root average was varied from 2.06 mg under (1000 ppm) to 4.80 mg under (50 pm) of butenolide concentration. There is no significant in root dry weight; but in shoot dry weight there is high significant result can be noticed ($p < 0.001$). By Dunnett test there are highly significant values between control and (1000 ppm m) concentration in shoot dry weight and the significant in root dry weight between control and (1000 ppm) of butenolide concentration. Root / Shoot ratio [Figure 3.2 (B)], the results showed no high difference between the means and they were approximately the same.

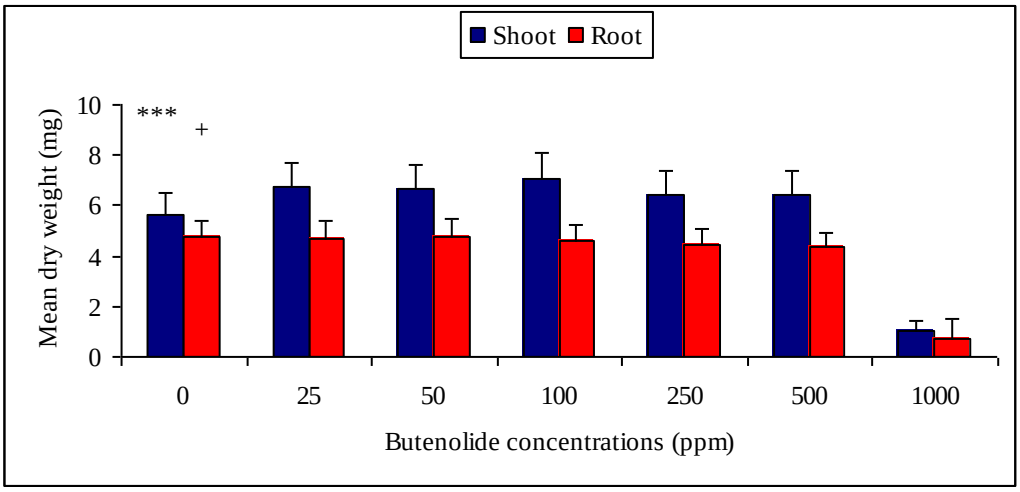


(A)

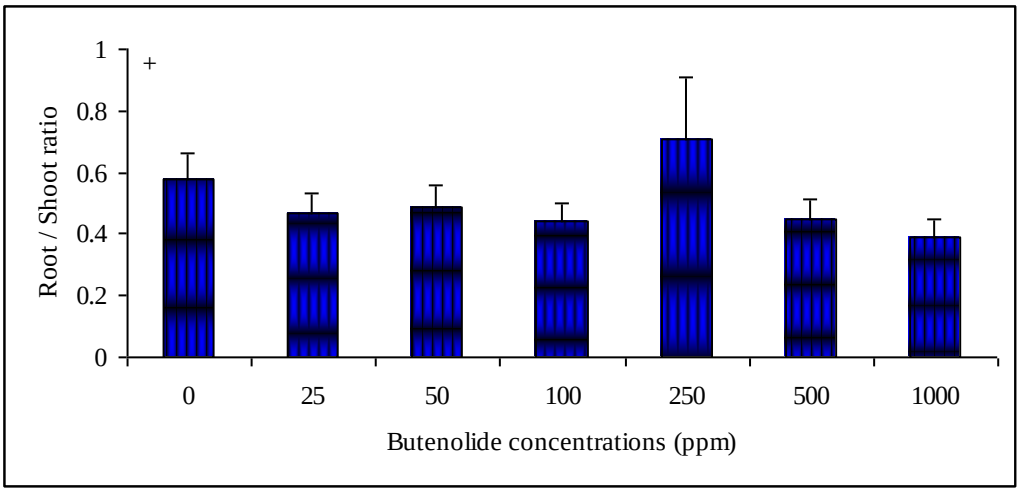


(B)

Figure 3. 1. Response of *Hordeum vulgar L.* (Barley) to different concentrations of butenolide. (A): shoot length and shoot fresh weight. (B): root length and root fresh weight. (*** = High Significant at $P< 0.001$. Bars = SE Mean.)



(A)



(B)

Figure 3. 2. Response of *Hordeum vulgar L. (Barley)* to different concentrations of butenolide, (A): dry weight of shoot and root. (B): root / soot ratio. (+ = Not significant, *** = High Significant at $P < 0.001$, Bars = SE Mean).

3.2. Response of *Lens culinaris* (Lentil) seeds to different concentrations of butenolide:

3.2.1. Seed germination:

Daily germination percentages of (*Lentil*) are shown in (Table 3.3), that the seeds were grown in all concentrations [plate 3.2 (A,B)].

3.2.2. Seedling growth:

As shown in (Table 3.4) of (*Lentil*) seedlings , The different of length, fresh and dry weight were clear, that in length showed that the concentrations above (25 ppm) is better than that at control condition(0.0 ppm). The range of length is from 133.77mm under (0.0 ppm) control condition to 206.60mm under (250 ppm) of butenolide concentration. In fresh and dry weight, the control was the lowest mean in all concentrations while the other concentrations were better than this mean. The average of fresh weight was ranged between 115.77 mg under (0.0 ppm) to 206.33 mg under (250 ppm) and dry weight was 6.6 mg at (0.0 ppm) to 10.8 mg at (250 ppm) of butenolide concentration. There are highly significant between groups in length, fresh and dry weight of (Lentil) ($p < 0.001$), that:

a. Fresh parameters:

The length and fresh weight of (Lentil) shoot and root as shown in [Figure 3.3 (A, B)], as in the graph, the shoot length is better at (250 ppm) and there is decrease in length at (50 and 100 ppm) butenolide.

25

0

100

50

Butenolide concentrations (ppm)

Plate 3. 2, A. Response of *Lens culinaris L.* (Lentil) seeds to different concentrations of butenolide.

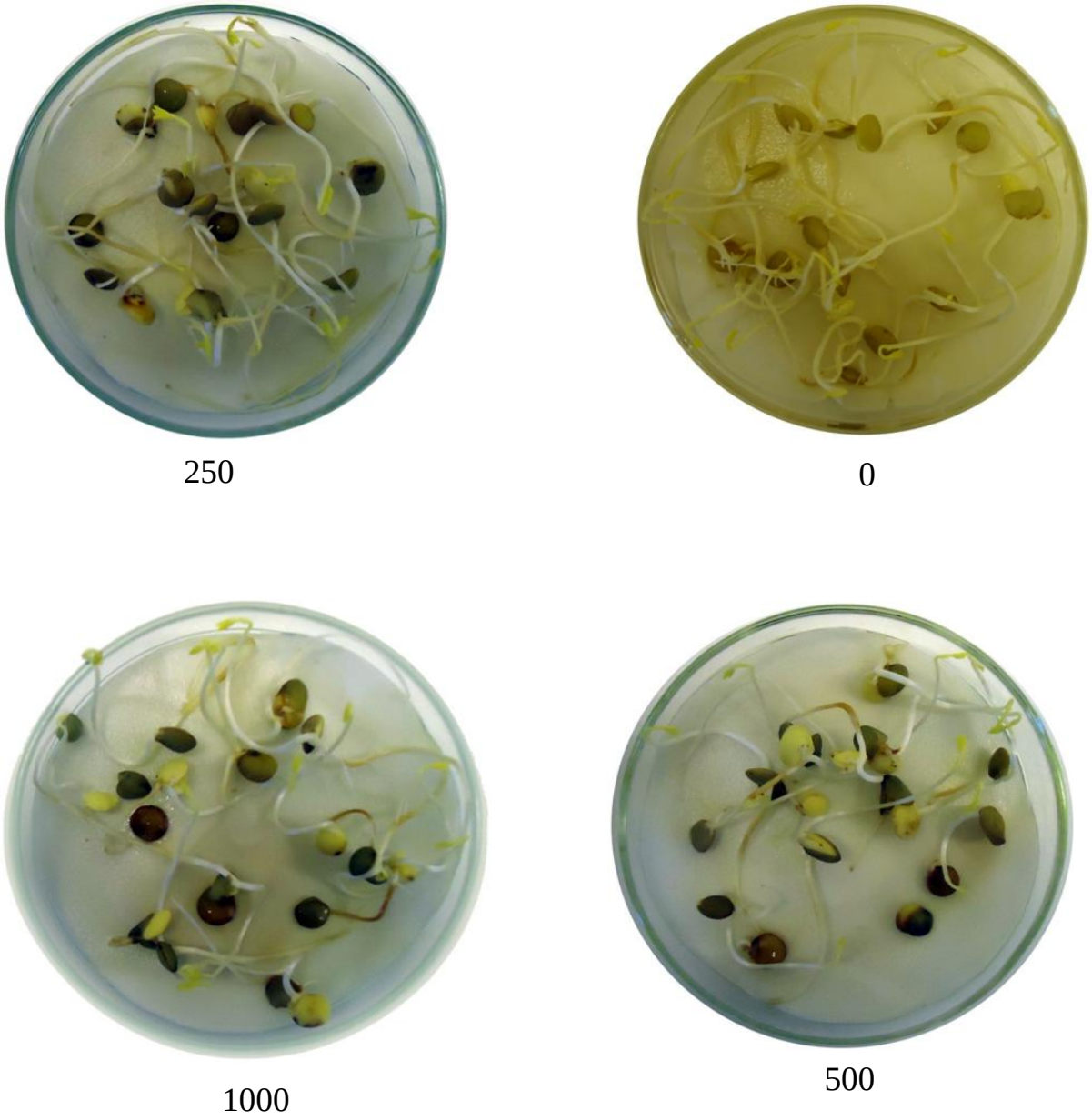


Plate 3. 2, B. Response of *Lens culinaris L.* (Lentil) seeds to different concentrations of butenolide.

Table 3. 3. Response of *Lens Culinaris.L (Lentil)* seeds to different concentrations of butenolide and their effect on daily germination percentages. (+ = Not significant, * = Significant at P < 0.05, ** = Significant at P < 0.01, ± = SE Mean)

Concentrations (ppm)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0	** 56.6 ± 23.3	+ 100.0 ± 0.0	* 100.0 ± 0.0	* 100.0 ± 0.0	* 100.0 ± 0.0	* 100.0 ± 0.0	* 100.0 ± 0.0
25	96.6 ± 3.3	96.6 ± 3.3	96.6 ± 3.3	96.6 ± 3.3	96.6 ± 3.3	96.6 ± 3.3	96.6 ± 3.3
50	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
100	93.3 ± 6.6	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
250	80.0 ± 6.6	90.0 ± 10.0	93.3 ± 6.6	93.3 ± 6.6	93.3 ± 6.6	93.3 ± 6.6	93.3 ± 6.6
500	50.0 ± 3.3	80.0 ± 0.0	83.3 ± 3.3	83.3 ± 3.3	83.3 ± 3.3	83.3 ± 3.3	83.3 ± 3.3
1000	43.3 ± 3.3	76.6 ± 10.0	90.0 ± 3.3	90.0 ± 3.3	90.0 ± 3.3	90.0 ± 3.3	90.0 ± 3.3

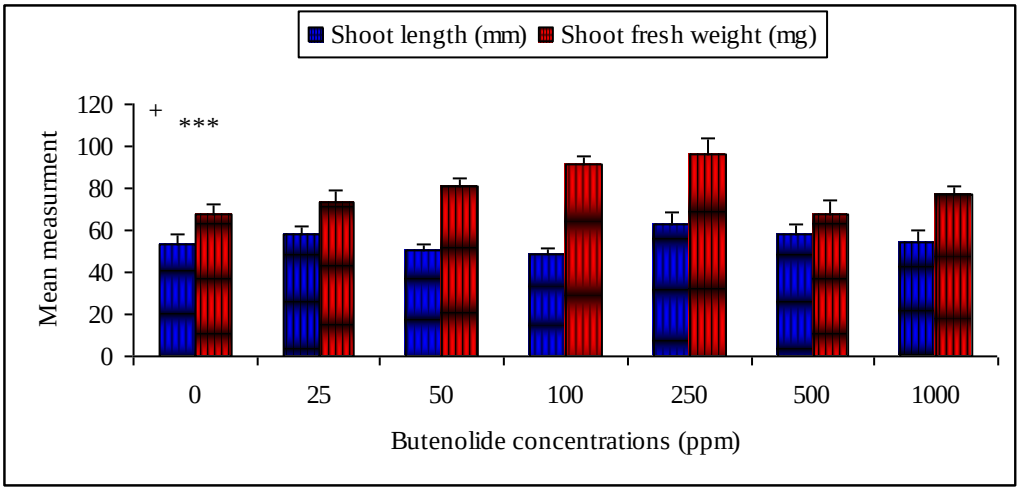
Table 3. 4. Response of *Lens culinaris L. (Lentil)* to different concentrations of butenolide. (*** = High Significant at $p < 0.001$, ± = Mean SE)

Concentration (ppm)	Length (mm)	Fresh weight (mg)	Dry weight (mg)
0	*** 133.7 ± 7.3	*** 115.7 ± 9.4	*** 6.6 ± 0.6
25	134.8 ± 17.7	151.7 ± 10.5	10.4 ± 0.2
50	188.3 ± 11.9	199.7 ± 18.2	10.3 ± 0.7
100	157.2 ± 11.1	198.0 ± 10.6	10.0 ± 0.7
250	206.6 ± 15.3	206.3 ± 16.3	10.8 ± 1.2
500	156.8 ± 8.2	148.0 ± 8.5	9.4 ± 0.5
1000	172.6 ± 10.8	168.4 ± 11.3	9.4 ± 0.8

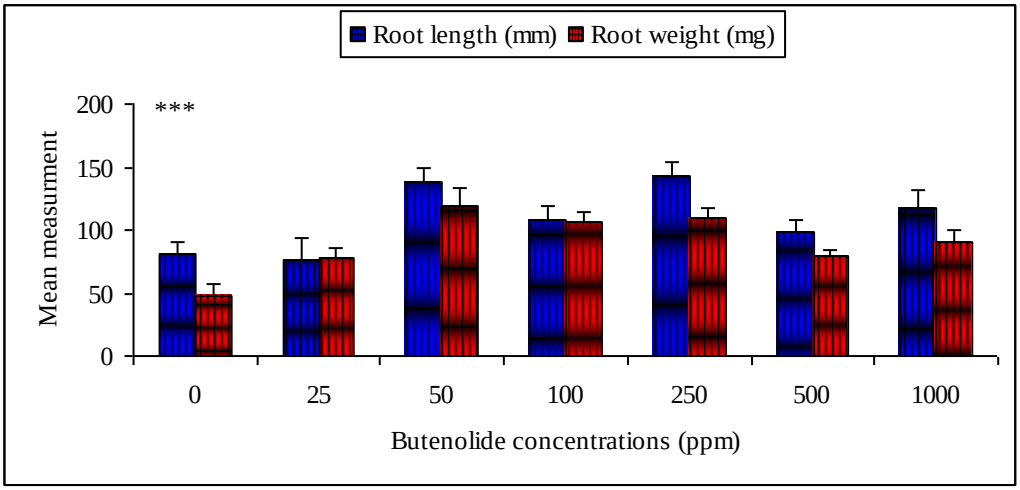
However there is no effect at (500 ppm) in shoot fresh weight. Average of these parameters was ranged between 48.88 mm under (100 ppm) to 63.11 mm under (250 ppm) of butenolide concentration, and shoot fresh weight was varied from 67.55 mg under (0 ppm) control condition to 96.66 mg under (250 ppm) concentration. There is no significant in shoot length; but in shoot fresh weight a high significant ($p < 0.001$) was observed. By Dunnett test the significant between control condition (0 ppm) and (100 and 250 ppm) of butenolide concentrations. In root length there are high in the mean of length of all concentrations of butenolide except at (25 ppm) there is no effect. However the butenolide solution effect on root fresh weight in all concentrations (25, 50, 100, 250, 500 and 1000 ppm). Where there are highly significant in length and fresh weight in root ($p < 0.001$), that the range of root length between 76.44 mm under (25 ppm) to 143.55mm under (250 ppm) concentration, root fresh weight is range from 48.22 mg at (0 ppm) control condition to 118.88 mg at (50 ppm) of butenolide concentration. By Dunnett analysis the data found that there were highly significant in both root length and root fresh weight, the root length between control (0 ppm) and (50 and 250 ppm) of butenolide concentrations. But; the root fresh weight between control condition (0 ppm) and (50, 100, 250 and 1000 ppm) of butenolide concentrations.

b. Dry parameters:

[Figure 3.4] show the effect of butenolide concentrations on shoot and root dry weight of (Lentil). The shoot and root dry weight values were high in all concentrations of butenolide compare to control condition (0 ppm). In shoot dry weight, the means ranged from 3.44 mg under (0 ppm) to 5.55 mg under (250 ppm) of butenolide concentration and there are significant at ($p < 0.05$). Using Dunnett test showed that the significant is between control and 25, 250 and 1000 ppm) of butenolide concentrations. In root dry weight, the mean of control is the lowest, all the concentrations are better than it. Root average was varied from 3.22 mg under (0 ppm) control condition to 5.44 mg under (50 ppm) of butenolide concentration. From the analytical side of Dunnett, it is found that there are significant between control and (50 and 250 ppm) of butenolide concentrations. Root/shoot ratio appeared fluctuations in means of effect of butenolide concentrations (Figure 3.6), on the other hand, There is no significant differences in shoot / root ratio of lentil plants.

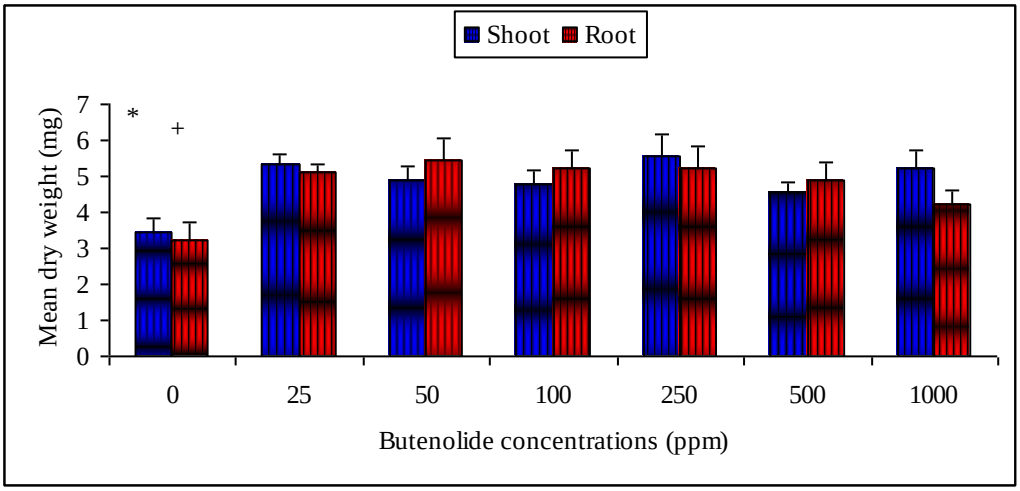


(A)

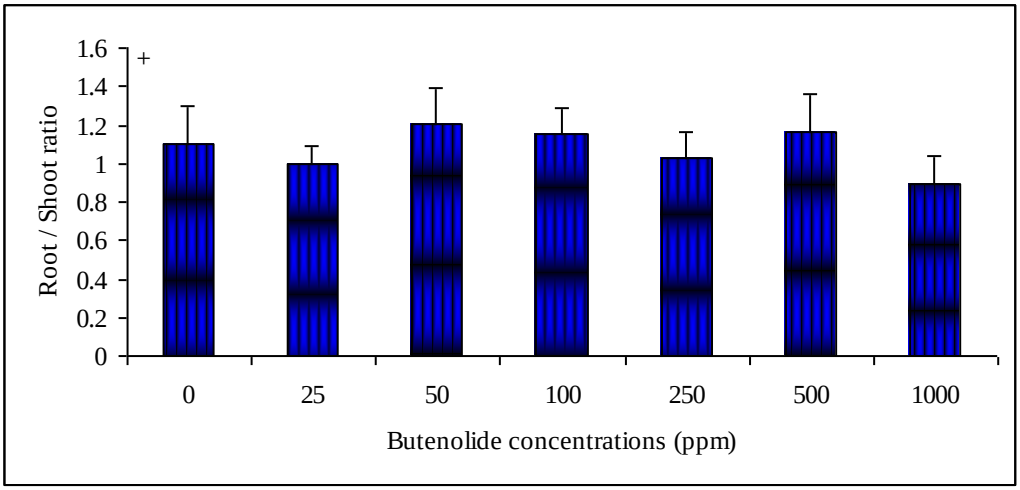


(B)

Figure 3. 3. Effect of different concentrations of butenolide on *Lens culinaris* (Lentil) : (A) length and fresh weight of shoots, (B) length and fresh weight of roots. (+ = Not significant, *** = High significant $p < 0.001$, Bars = SE Mean).



(A)



(B)

Figure 3. 4. Response of *Lens culinaris (Lentil)* to different concentrations of butenolide, (A): dry weight of shoot and root. (B): Root / Shoot ratio. (+ = Not significant, * = Significant at $P < 0.05$, Bars = SE Mean).

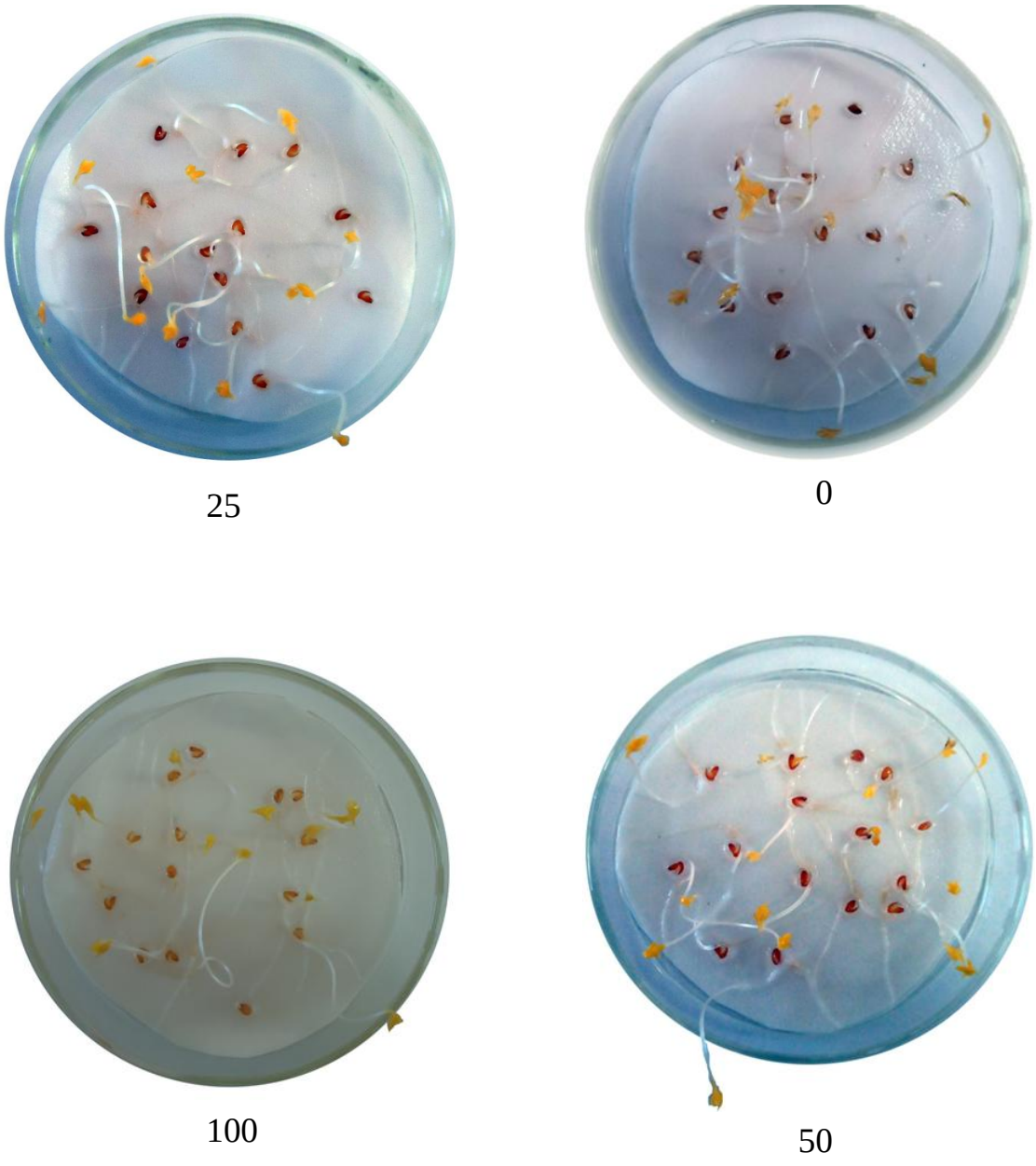
3.3. Response of *Lepidium Sativum L.* (Cresson) seeds to different concentrations of butenolide:

3.3.1. Seed germination:

Daily germination percentages of *Lepidium Sativum* (Cresson) seeds under different concentrations of butenolide are calculated (Table 3.5). The results showed that, high concentration above (250 ppm) occur decrease in growth and inhibition at (1000 ppm). In daily germination percentages of (Cresson) grain [(Plate 3.3 (A,B)] differences between and within groups ($p < 0.001$) are very clear.

3.3.2. Seedling growth:

Fresh and dry parameters of *Lepidium Sativum L.* seedling under different concentrations of butenolide solution were measured (Figure 3.5 (A,B)] for fresh parameters, [Figure 3.6] for dry weight. Increase concentrations of butenolide up to (250 ppm) reduced these parameters and caused inhibition of development of *Lepidium Sativum L*. In elongation of total plant the means of length are approximately the same except at (500 and 1000 ppm) of butenolide concentrations the mean was decrease. However, the average length of *Lepidium Sativum* L. between 7.77 mm under (1000 ppm) to 144.44 under (25 ppm) of butenolide concentration, that the length of *Lepidium Sativum L.* is high significant ($p< 0.001$). In fresh weight of *Lepidium Sativum L.* there are not a big differences in means at (0, 25, 50, 100 and 250 ppm), however at (500



Butenolide concentrations (ppm)

Plate 3. 3, A. Response of *Lepidium sativum L.* (Cresson) seeds to different concentrations of butenolide.

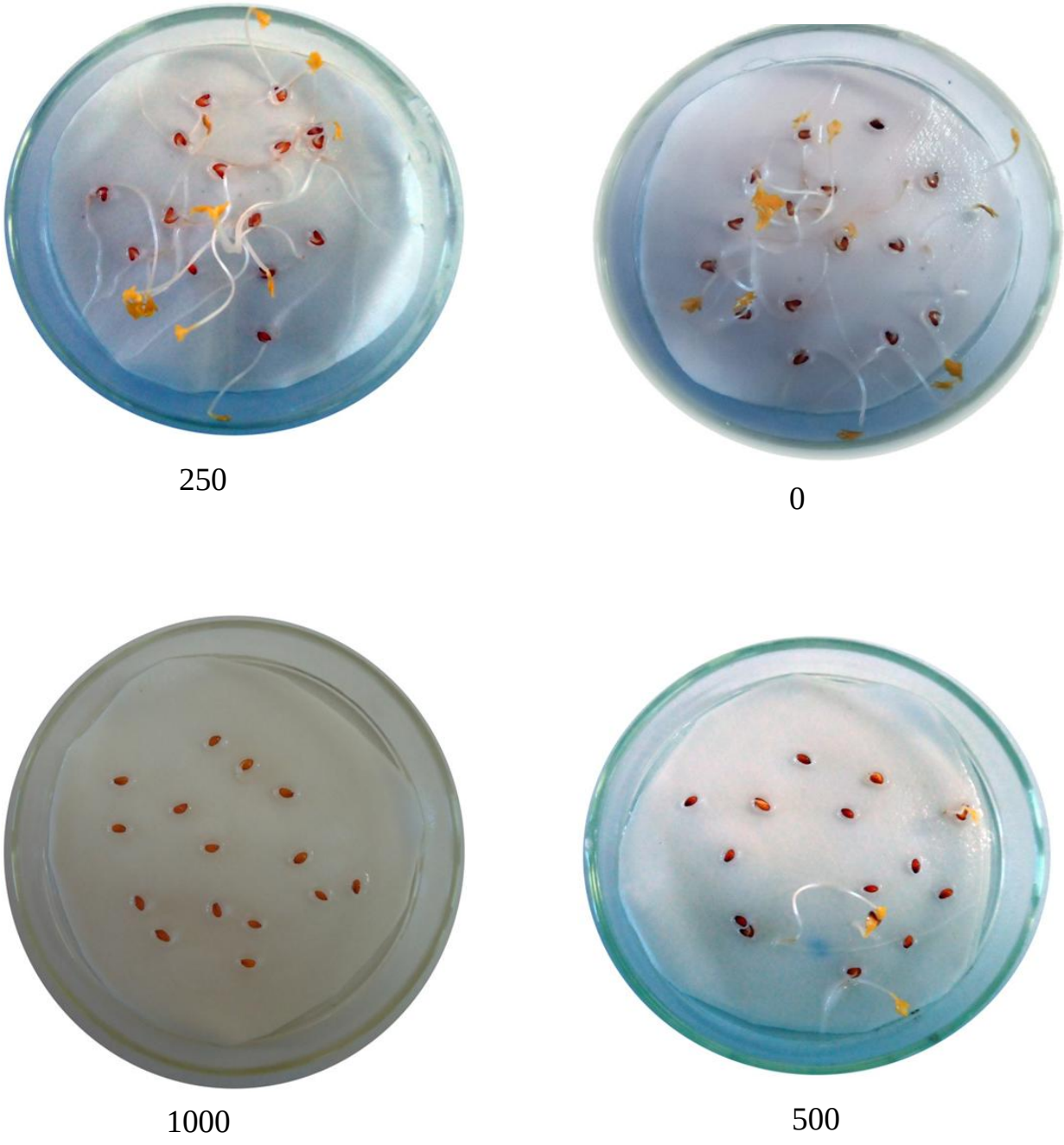
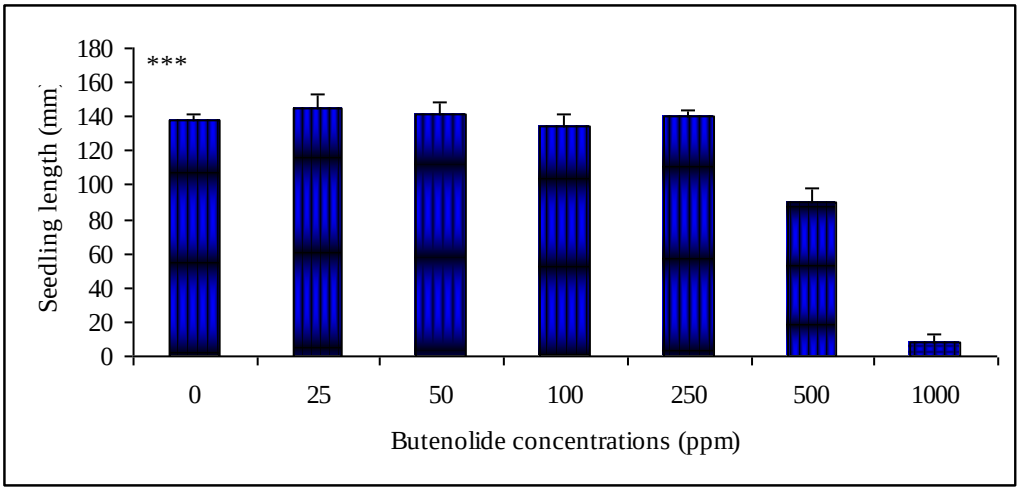


Plate 3. 3, B. Response of *Lepidium sativum L.* (Cresson) seeds to different concentrations of butenolide.

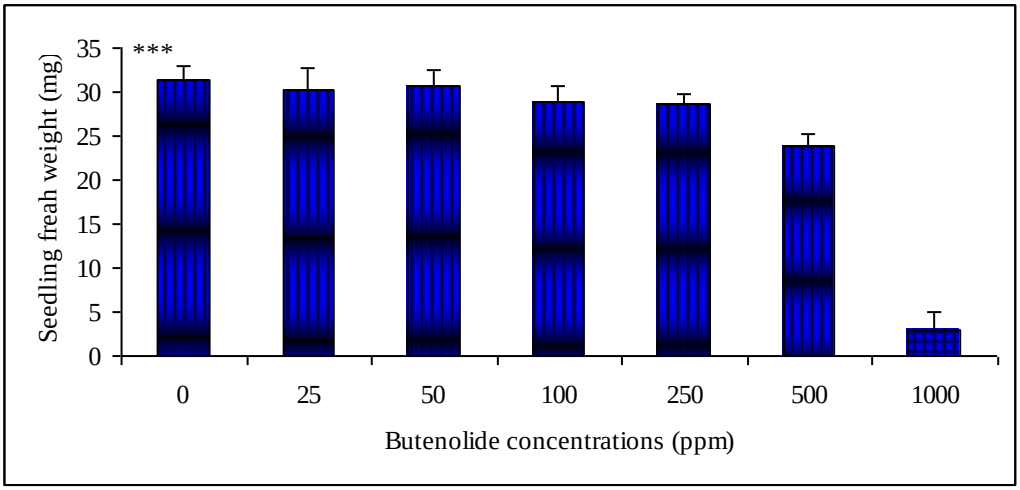
Table 3. 5. Response of *Lepidium Sativum L.* (Cresson) seeds to different concentrations of butenolide and their effects on daily germination percentages. (*** = High Significant at P < 0.001, ± = SE Mean)

Concentration (ppm)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 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

and 1000 ppm) the effect of butenolide concentration showed that the solution make decrease in fresh weight, and the range was from 3.0 mg at (1000 ppm) to 31.33 mg at (0 ppm) control condition. In dry weight of Cresson there are not big differences in means of different concentrations of butenolide, at (1000 ppm) the mean was disappeared. The average of dry weight was between 0.0 mg under (1000 ppm) to 1.07 mg under (100 ppm) of butenolide concentration, High significant within and between groups of different treatment ($p < 0.001$) was noted. From the analytical side, Dunnett test indicated high significant between control and (500 and 1000 ppm) of butenolide concentrations in elongation of fresh weight. However in dry weight the significant is between (0 ppm) and all concentrations of butenolide solution.



(A)



(B)

Figure 3. 5. Response of *Lepidium Sativum L. (Cresson)* to different concentrations of butenolide, (A): Length, (B): Fresh weight. (*** = High significant $p < 0.001$, Bars = SE Mean).

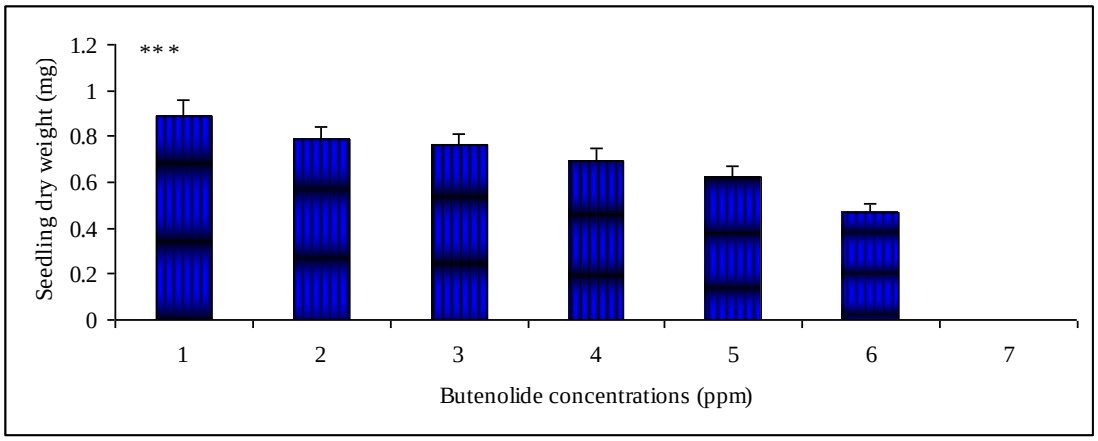


Figure 3. 6. Dry weight of *Lepidium Sativum L. (Cresson)* at different concentrations of butenolide. (*** = High significant $p < 0.001$, Bars = SE Mean).

3.4. Response of *Lycopersion esculentum* L. (Tomato) seeds to different concentrations of butenolide:

3.4.1. Seed germination:

The seeds of *Lycopersion esculentum L.* (Tomato) were germinated under all concentrations of butenolide [Plate 3.4]. However there is a delay in growth at (0 ppm) control condition (Table 3.6), where treated seeds started their germination from the fourth day under (25, 50, 100 and 250 ppm) but; in control condition (0 ppm) they started after seven days to germinate.

3.4.2. Seedling growth:

[Figure 3.7], show the influence of different concentrations of butenolide on fresh and dry parameters are shown at (Figure 3.8). These parameters were increased at all concentrations, and were better than control condition (0 ppm). In length of (Tomato), the means of concentrations are clearly appear, they are higher than control. The best mean in length and fresh weight of tomato was at (100 ppm), there are a significant of all of them ($p < 0.01$). The average length was ranged from 153.88 mm under (0 ppm) to 196.66 mm under (100 ppm) of butenolide concentration. In mean of fresh weight of tomato, it was varied between 31.22 mg under control condition (0 ppm) to 42.66 mg under (100 ppm) of butenolide concentration and there was significant at ($p < 0.01$). Dry weight at (100 ppm) was gave the best mean effect of butenolide

concentration on tomato. But; there are no significant statistically. By Dunnett test there is a highly significant between control and (100 ppm) concentration in length and the significant in fresh weight between (0 ppm) at control condition and (100 and 1000 ppm) of butenolide concentrations.

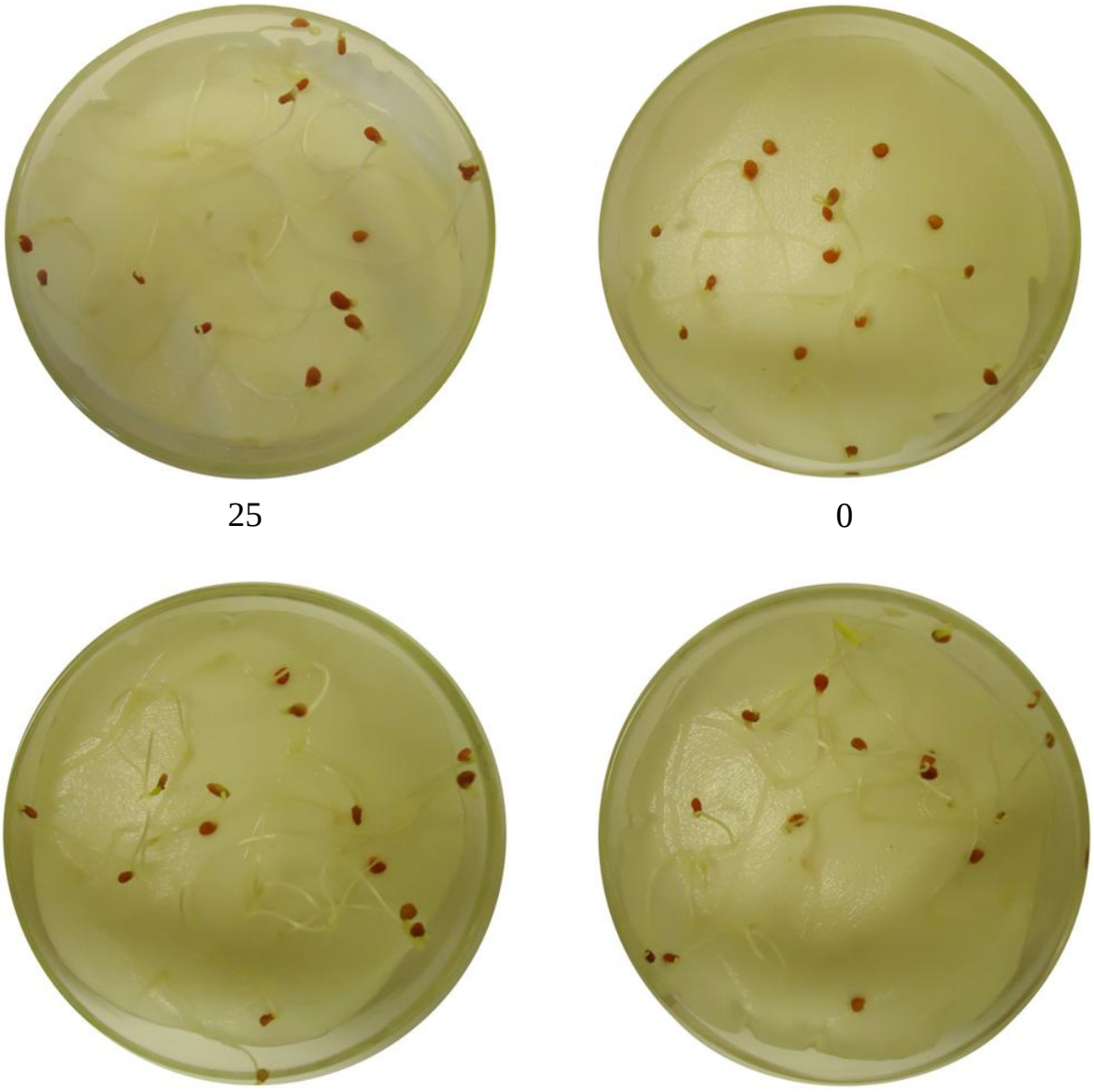
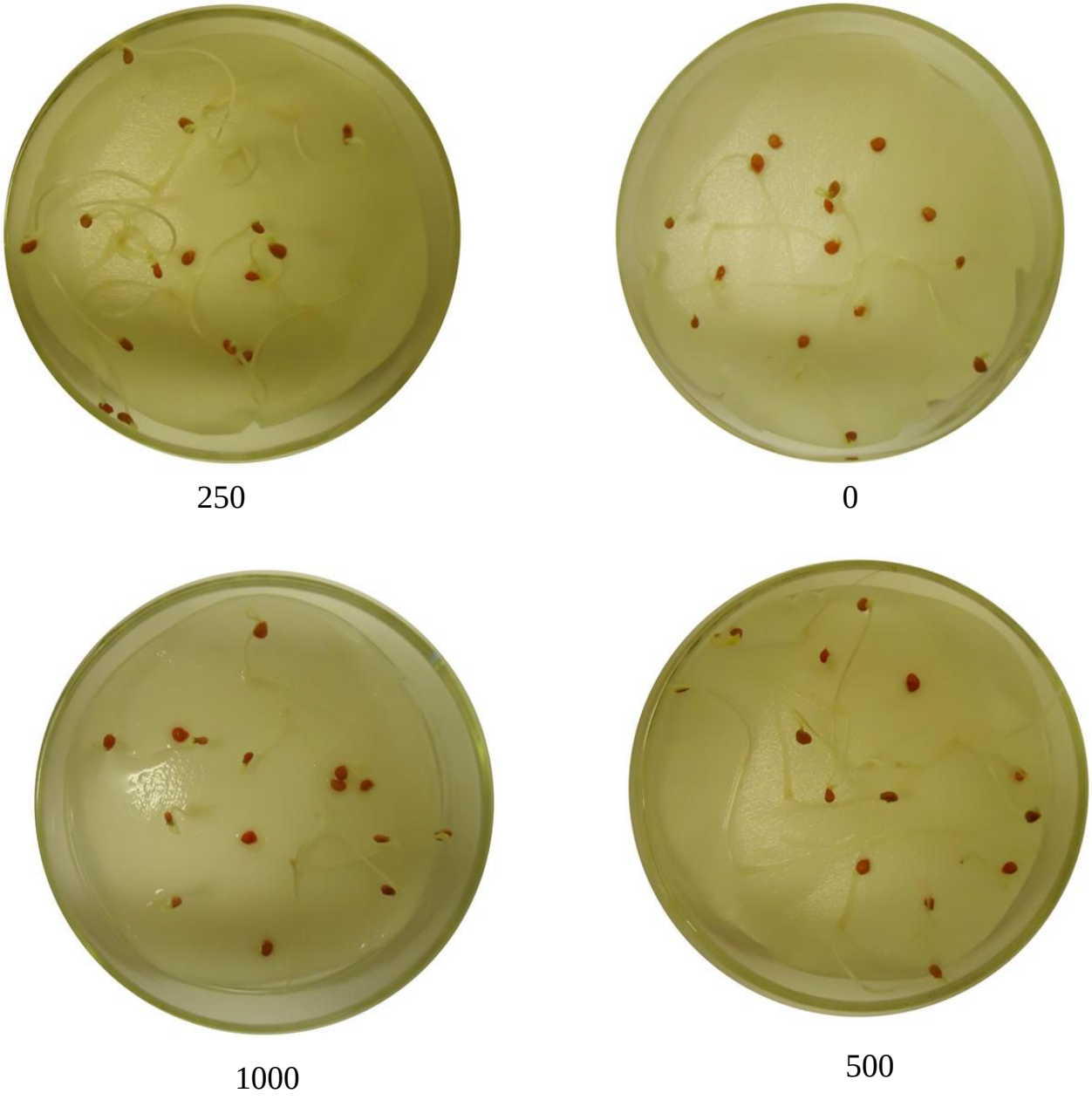


Plate 3. 4, A. Response of *Lycopersion esculentum L.* (Tomato) seeds to different concentrations of butenolide.

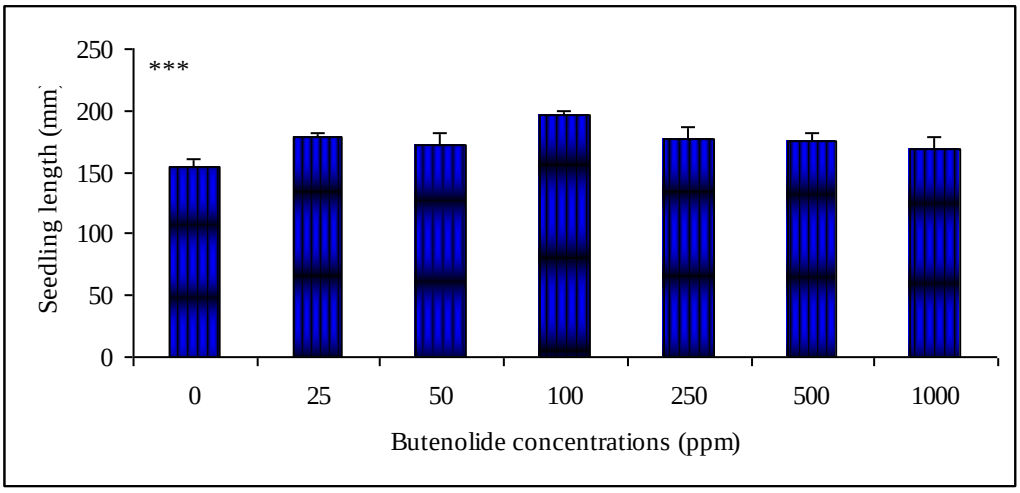


(Butenolide concentrations (ppm)

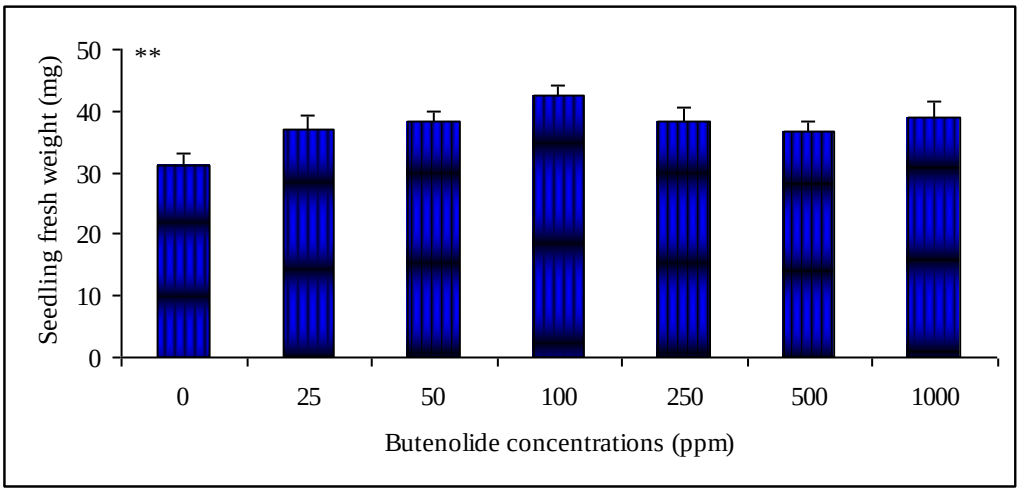
Plate 3. 4, B. Response of *Lycopersion esculentum L. (Tomato)* seeds to different concentrations of butenolide.

Table 3. 6. Response of *Lycopersion esculentum L.* (Tomato) seeds to different concentrations of butenolide and their effects on daily germination percentages. (*** = High Significant at P < 0.001, ± = SE Mean).

Concentrations (ppm)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	30.0± 10.0	43.3 ± 3.3	50.0 ± 10.0
25	30.0 ± 3.3	30.0 ± 3.3	76.6 ± 3.3	93.3 ± 0.0	96.6 ± 3.3	96.6 ± 3.3	96.6 ± 3.3
50	23.3 ± 3.3	23.3 ± 3.3	56.6 ± 10.0	73.3 ± 0.0	76.6 ± 3.3	83.3 ± 3.3	90.0 ± 3.3
100	26.6 ± 0.0	26.6 ± 0.0	50.0 ± 3.3	86.6 ± 0.0	90.0 ± 3.3	96.6 ± 3.3	96.6 ± 3.3
250	10.0 ± 3.3	43.3 ± 10.0	76.6 ± 10.0	76.6 ± 10.0	80.0 ± 6.6	96.6 ± 3.3	96.6 ± 3.3
500	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 3.3	26.6 ± 13.3	53.3± 0.0	86.6± 6.6	86.6 ± 6.6
1000	0.0 ± 0.0	0.0± 0.0	0.0± 0.0	3.3 ± 3.3	13.3 ± 0.0	43.3 ± 3.3	43.3 ± 3.3



(A)



(B)

Figure 3. 7. Fresh parameters of *Lycopersion esculentum L.* (Tomato) plant (A), length and (B), fresh weight at different concentrations of butenolide. (** = Significant at $p < 0.01$, *** = High significant $p < 0.001$, Bars = SE Mean).

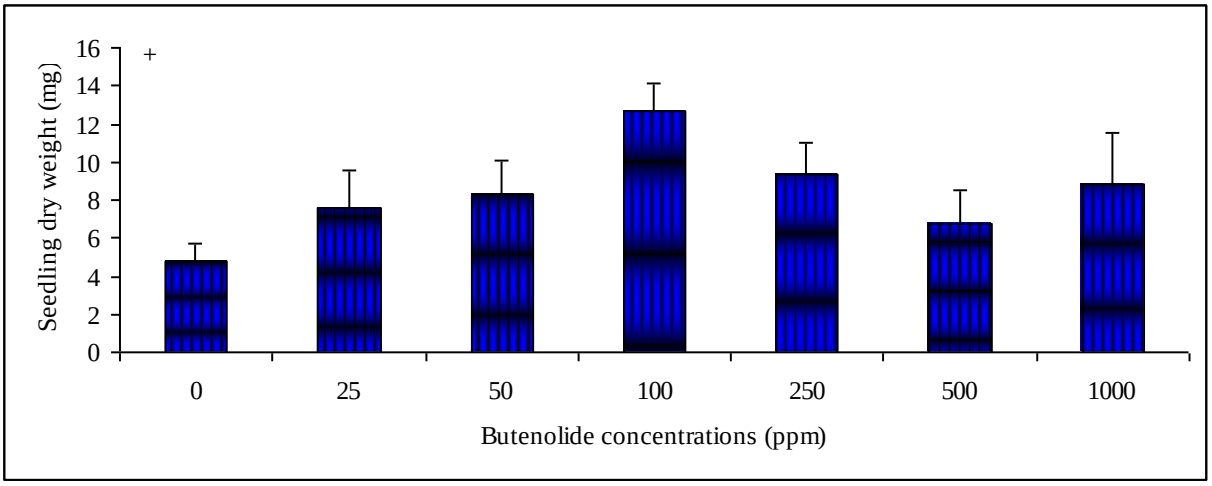


Figure 3. 8. Dry weight of *Lycopersion esculentum L.* (Tomato) seeds to different concentrations of butenolide. (+ = Not significant, Bars = SE Mean).

3. 5. Response of *Lycopersion esculentum L.* (Tomato) plant growth to different concentrations of butenolide:

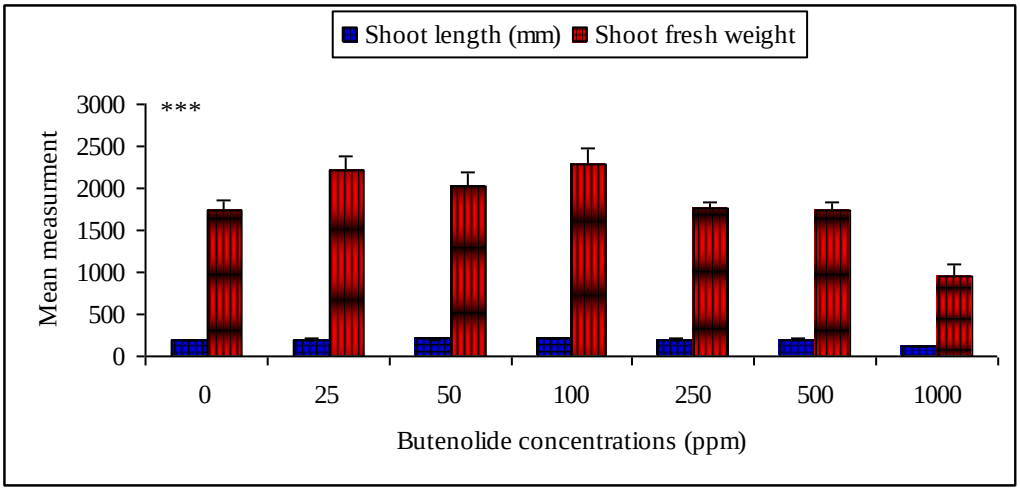
Fresh parameters:

(Plate 3.5) show the effect of different concentrations of butenolide on fresh parameters of (Tomato) plants. Fresh parameters are better than control effect at all concentrations except at (1000 ppm) concentration where the decrease of parameters is appearing clearly. The best elongation was at (250 ppm) and best fresh weight was at (100 ppm) of butenolide concentration (Table 3.7). The best mean of Number of leaves (Figure 3. 9) was given at (25 and 100 ppm) concentrations. There are highly significant in all fresh parameters. The shoot length and shoot fresh weight as given in [Figure 3.10 (A)], showed The best mean in shoot length was at (100 ppm) of butenolide concentration and it ranged from 118.63 mm under (100 ppm) to 210.90 mm under (100 ppm) concentration. In shoot fresh weight the mean was ranged from 941.36 mg under (1000 ppm) to 2291.54 mg under (100 ppm). There are high significant in both shoot length and shoot fresh weight ($p < 0.001$). By Dunnett test, there is highly significant between control and (1000 ppm) in shoot length but; in shoot fresh weight between control and (100, 1000 ppm) of butenolide concentrations. In root length and fresh weight of root as shown in [Figure 3.10 (B)], the high length clearly appeared at (250 ppm) concentration.

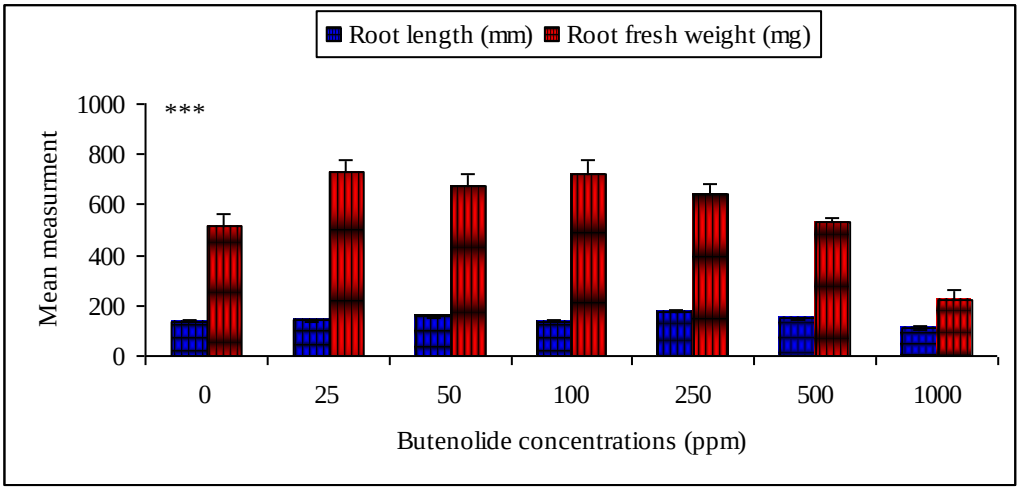
Plate 3. 5. Response of *Lycopersion esculentum L.* (Tomato) plant growth to different concentrations of butenolid.

Table 3.7. Response of *Lycopersion esculentum L.* plant to different concentrations of butenolide. (*** = High Significant at P < 0.001, ± = SE Mean)

Concentration (ppm)	Length (mm)	Fresh weight (mg)	Dry weight (mg)
0	*** 325.6± 7.8	*** 2250.0 ± 154.5	*** 242.0 ± 19.9
25	336.6 ±7.2	2951.6 ± 215.7	266.0 ± 24.2
50	365.5 ±10.2	2709.0 ± 182.3	263.36 ± 21.7
100	349.0 ± 6.4	3012.4 ± 230.1	311.27 ± 25.3
250	379.0 ± 6.7	2397.9 ± 107.7	244.54 ± 13.0
500	343.4 ± 8.9	2266.7 ± 112.4	230.3 ± 14.3
1000	229.7 ± 6.9	1164.8 ± 191.0	66.4 ± 14.0



(A)



(B)

Figure 3. 9. Response of *Lycopersion esculentum L.* (Tomato) plant to different concentrations of butenolide, (A): Shoot length and fresh weight, (B): root length and fresh weight. (*** = High significant $p < 0.001$, Bars = SE Mean).

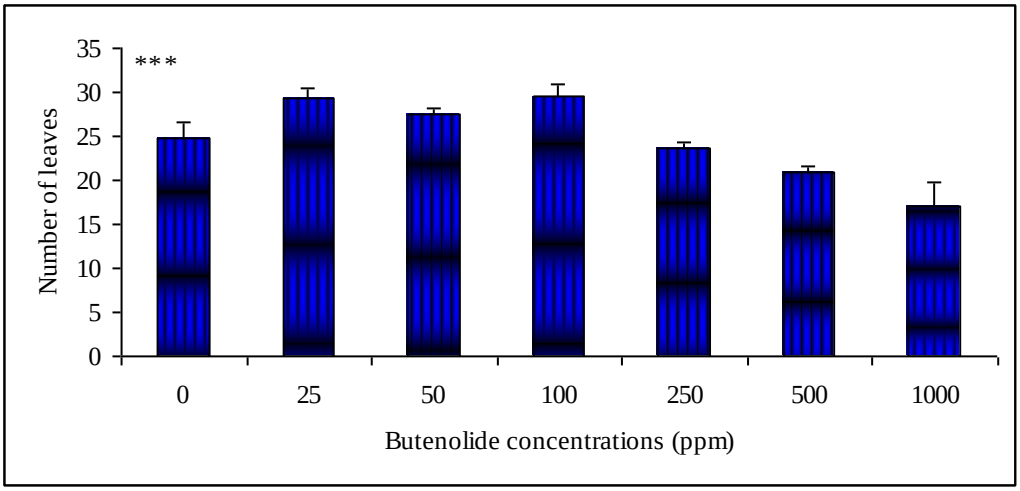
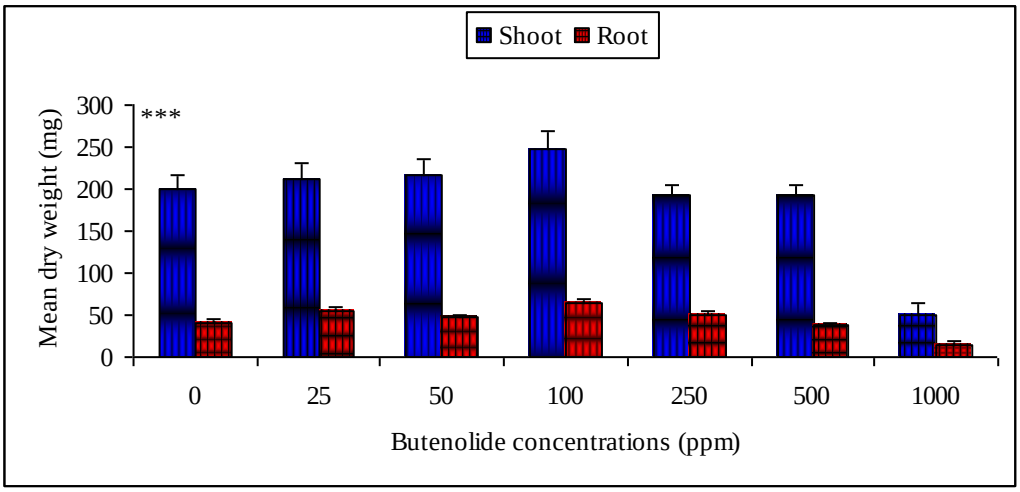


Figure 3. 10. The number of leaves of *Lycopersion esculentum L.* (Tomato) plant at different concentrations of butenolide. (*** = High significant $p < 0.001$, Bars = SE Mean).

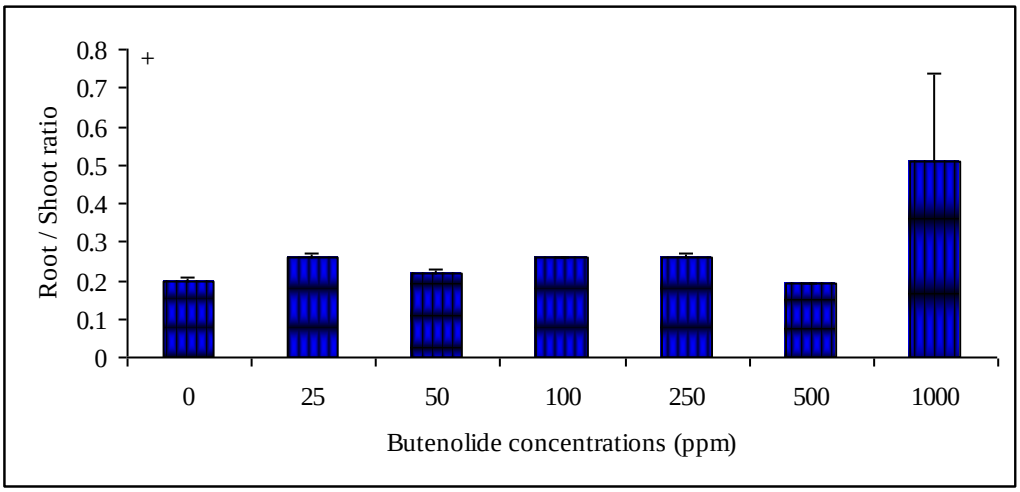
And its range was varied from 111.09 mm under (1000 ppm) to 178.45 mm under (250 ppm) of butenolide concentration. And there is high significant ($p < 0.001$). But; in root fresh weight the best mean was at (25 ppm) of butenolide concentration. The range of root fresh weight was from 223.45 mg under (1000 ppm) to 728.72 mg under (25 ppm) of butenolide concentration. There is high significant in root fresh weight ($p < 0.001$). From the analytical side by Dunnett test, we found that there is highly significant in root fresh weight between control condition (0 ppm) and (50, 250 and 1000 ppm), But; In root fresh weight between control and (25, 50, 100 and 1000 ppm) of butenolide concentration. In number of leaves parameters, the best result was obtained at (100 ppm) and results revealed high significant at this concentration when $p < 0.001$. By Dunnett analysis, the significant was between control and (1000 ppm) of butenolide concentration.

Dry parameters:

Dry parameters of *Lycopersion esculentum L.* (Tomato) under different concentrations were measured [figure 3. 11. (A and B)]. The best result of dry weight of shoots and roots measurement was appeared at (100 ppm) concentration (figure 3. 11, A). The dry weight was decreased at concentration (1000 ppm). Root / shoot ratio as shown in (figure 3. 11, B), a very small increase at (1000 ppm) of butenolide concentration, but there is not significant effects.



(A)



(B)

Figure 3. 11. Response of *Lycopersion esculentum* L. (Tomato) plant to different concentrations of butenolide, (A): shoot and root dry weight, (B): root / shoot ratio. (+ = Not significant, *** = High significant $p < 0.001$, Bars = SE Mean).

3.6. Effect of butenolide on mitotic index (M I):

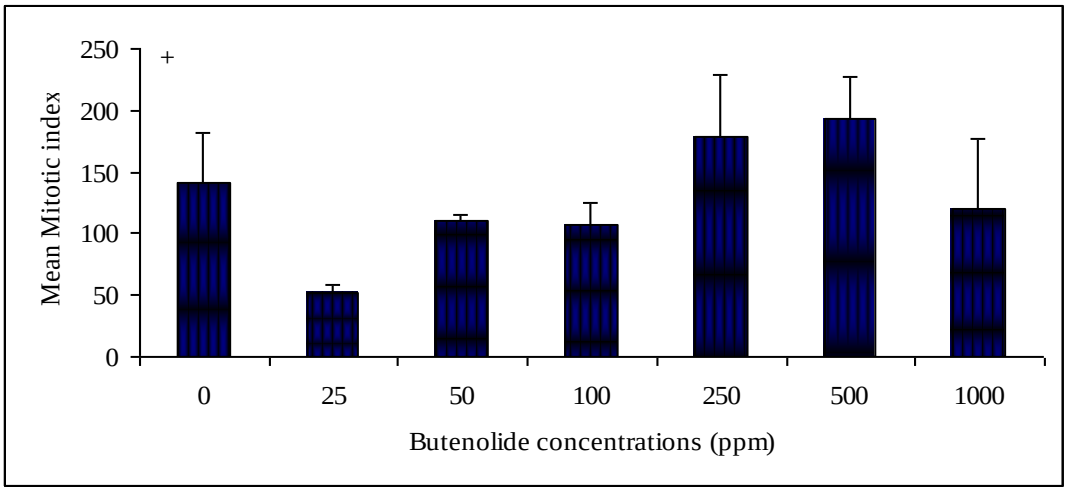
The results in figure (3. 12, A) show the effect of different concentrations of butenolide on (M I) for meristematic cells of *Allium cepa L.* after 24 hours. There are decreases in means of (M I) at (25, 50, 100 and 1000 ppm) of butenolide concentration when they were compared with control. However the increase of (M I) was clearly appeared at (250 and 500 ppm). By statistical analysis, the results showed no significant effect of all treatment on (M I).

3.6.1. Effect of different concentrations of butenolide on mutation of *Allium cepa L.* :

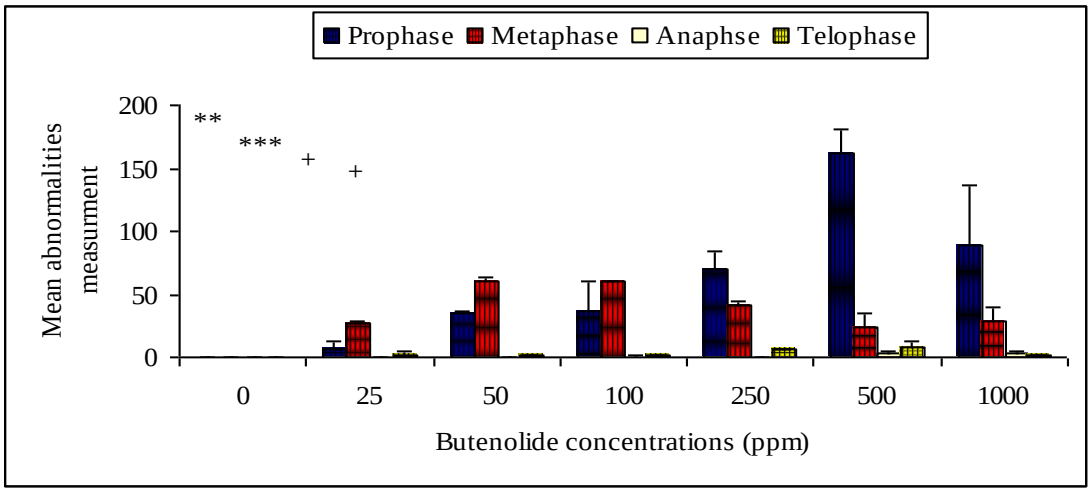
The results showed that all the concentrations of butenolide used in the present study induced important abnormalities during mitotic division when they compared to control condition (0 ppm) in *Allium cepa* L. (Figure 3.12, B) shown the effect of different concentrations of butenolide on aberration of meristematic cells of *Allium cepa* L. through 24 hours. The increasing of aberration is clear at (500 ppm) in prophase. At (50 and 100 ppm) concentration in metaphase had the best mean in high of aberration. The average of aberration in prophase ranged from 0 under (0 ppm) concentration condition to 161.5 under (500 ppm), and there was significant when ($p < 0.01$). Metaphase aberration average was varied from 0 under control condition (0 ppm) to 60 under (50 and 100

ppm) of butenolide concentrations. There was highly significant ($p < 0.001$). There are decrease in abnormality cells in anaphase and telophase. The average of anaphase aberrations was ranged between 0 at control condition to 2.5 at (500 and 1000 ppm) concentrations. In aberrations of telophase, it was ranged from 0.0 under (0 ppm) control condition to 7.6 under (500 ppm) of butenolide concentration, and there were no significant effects in both anaphase and telophase.

The most common abnormalities as shows in [Plate 3.6 (A, B and C)] 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.

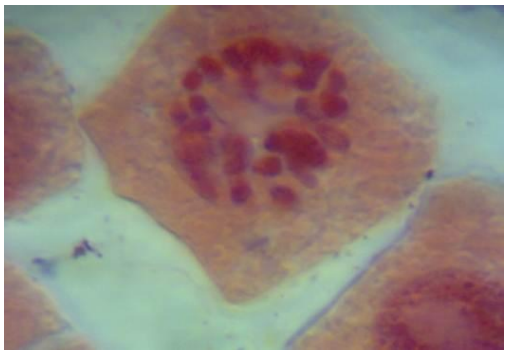


(A)

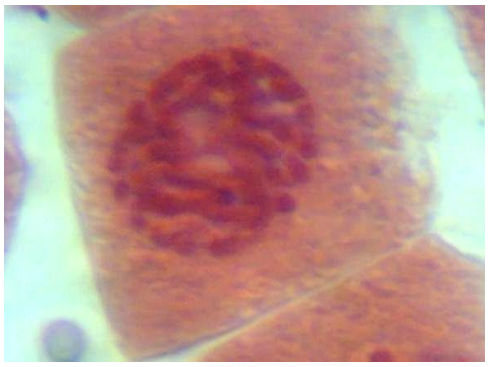


(B)

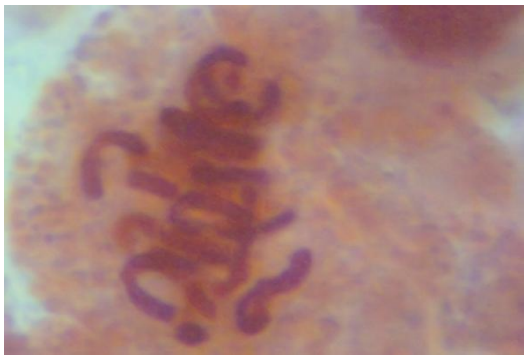
Figure (3. 12). Effect of different concentrations of butenolide on (A). Mitotic index and (B). abnormalities of prophase, metaphase, anaphase and telophase. (+ = Not significant, **=Significant at $p < 0.01$, *** = High Significant at $p < 0.001$, Bars=SE Mean)



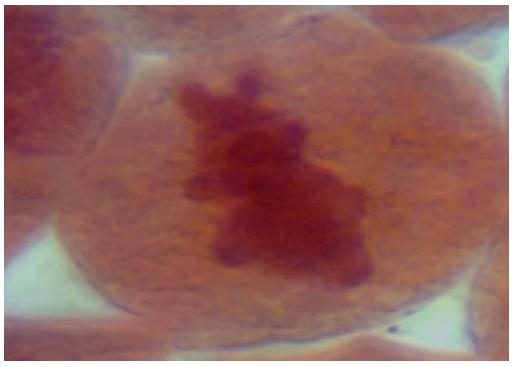
High condensation in prophase



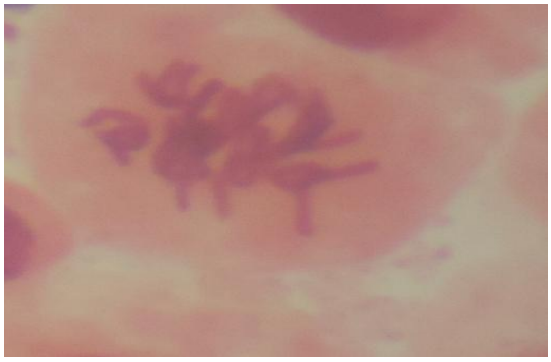
Early condensation in prophase



Lagging chromosome

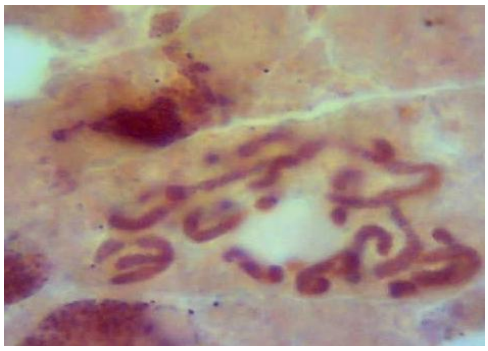


Sticky metaphase

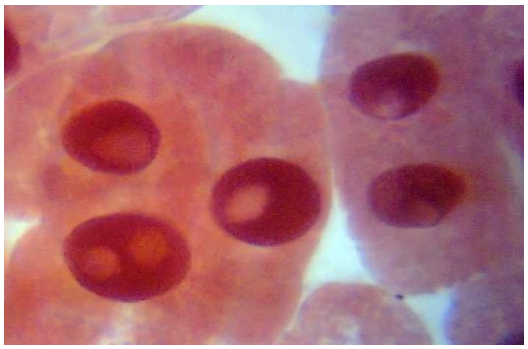


Multipolar Metaphase

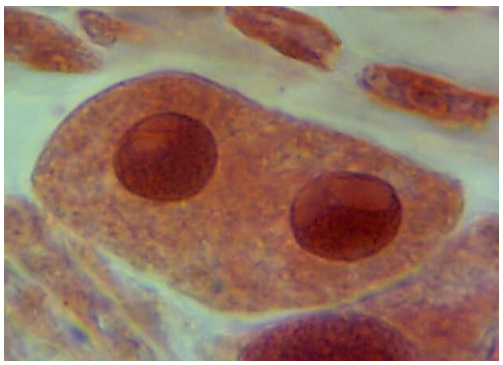
Plate 3. 6. A. Effect of different concentrations of butenolide on apical merastematic cells during cell division of *Allium cepa L.* root tips.



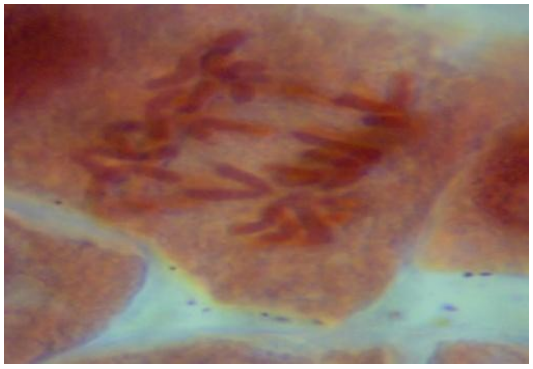
C- Metaphase



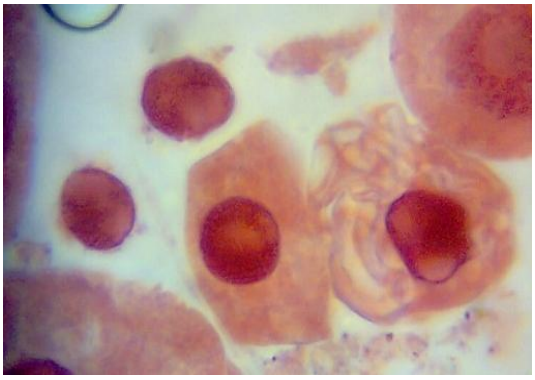
Multiblnucleated cell



Binucleated cell

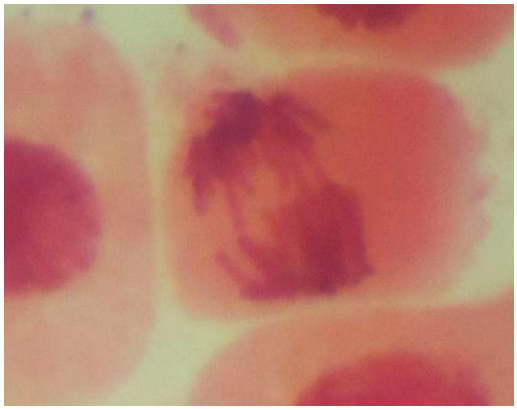


Disturbed Anaphase

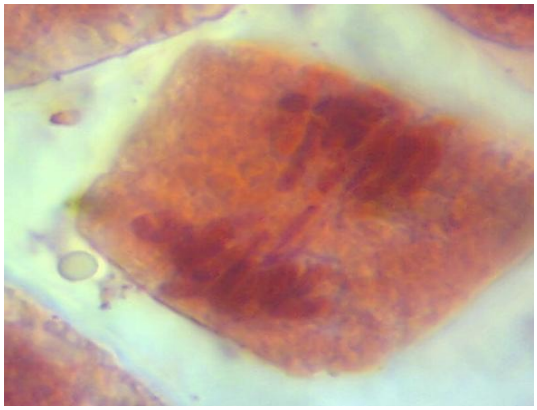


Nucleic outside the cells

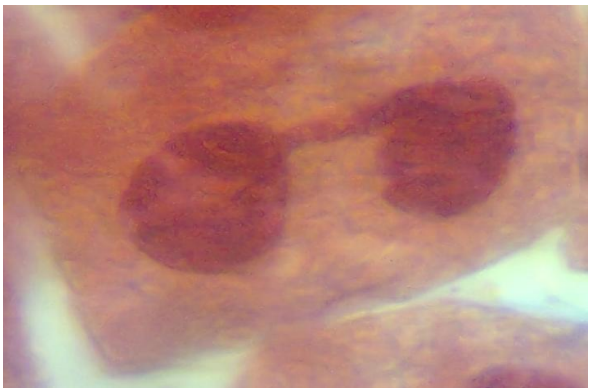
Plate 3. 6. B. Effect of different concentrations of butenolide on apical merastematic cells during cell division of *Allium cepa L*. root tips.



Anaphase bridges



Anaphase bridges



Telophase bridges

Plate 3. 6. C. Effect of different concentrations of butenolide on apical merastematic cells during cell division of *Allium cepa L*. root tips.

Chapter 4

Discussion

In this present study the response of some seeds of plants to different concentrations of butenolide has been studied. Five plant species were exposed to this compound and they are *Hordeum vulgar L.* (Barley), *Lens culinaris* (Lentil), *Lepidium Sativum L.* (Cresson), *Lycopersion esculentum L.* (Tomato) and *Allium cepa L.* (Onion) which used for chromosomal study. The promotive role of butenolide on seed germination and seedling growth is well documented for a wild range of plant species, irrespective of fire sensitivity (Kulkarin *et al.*, 2006; Merritt *et al.*, 2006; Van Staden *et al.*, 2006). The study employed the use of the butenolide as a promoter; the effect was measured by calculating seed germination percentages, seedling growth parameters, seedling establishment of tomato plant and chromosomal study on *Allium cepa* L.

Seed germination commences with the uptake of water by dry seed and is completed with emergence of the radical (Bewley 1997), the seed germination process of different plant species under different concentrations of butenolide solution was influenced in some of them. From the results; the response of *Hordeum vulgar* L. (Barley) to different concentrations of butenolide, daily germination percentages were decrease under (1000ppm) concentration (Table 3.1). Where *Lens culinaris* (Lentil) germination percentage is approximately gave the same

effect except at the concentration of (500ppm), there was reduction in it (Plate 2. 2, B). In *Lepidium Sativum L.* daily germination percentages were reduced when seeds treated with butenolide concentrations at (500 and 1000 ppm), however the other concentrations approximately had the same effect of control condition (0 ppm) showed at (Table. *Lycopersion esculentum* L. (Tomato) seen to be more sensitive than Cresson seeds in term daily germination percentages while all the concentrations were better than the control except at (1000 ppm), there was a reduction in daily germination percentages (Table 3.6).

The ability of smoke treatment to shorten germination time was reported in previous studies (Razanamandranto *et al*., 2005; Sparg *et al*., 2005; Crosti *et al*., 2006; Daws *et al*., 2007) and our results were agreed with those previous studies. Butenolide shortened the germination time of seeds of *Hordeum vulgar* L. *(Barley), Lens culinaris* (Lentil), *Lepidium Sativum* L. (Cresson) and *Lycopersion esculentum* L. (Tomato). Butenolide (the chemical compound in smoke that promotes germination could be involved in early induction of the cell cycle activation and thus accelerate radicle emergence in germination seeds (Jain and Van Staden, 2006).

Seedling growth of different plant species that mentioned in above was measured, in term fresh and dry parameters of the whole seedling under different concentrations of butenolide. Parameters which include seedling

length of barley gave the best increase in length at (50ppm), Lentil and Cresson at (25 ppm) and Tomato at (100 ppm) of butenolide concentration. However the length were reduction at (1000 ppm) in Barley and Cresson but in Lentil and Tomato all concentrations of butenolide were gave better effect than control (0 ppm).

Shoot and root parameters were different from plant species to another and this lead to say that the effect is not species dependent. The results showed as example that there was increasing in shoot length at (50, 250, ppm) of butenolide concentrations for Barley, Lentil gradually (Figures 3.1, A and 3.4, A). However in root length the increasing was at (50 and 100 ppm) concentrations for lentil and tomato.

In fresh weight parameters, it was clearly appear that the increasing in parameters of lentil in (table 3.4) and tomato at (Figure 3.9, B) was better in all treatment than control. In barley there was decrease in fresh weight at high concentration (1000 ppm) of butenolide solution in (table 3.2). However, there were no effects on Cresson but it was decreased at (1000 ppm) of butenolide solution (Figure 3.7, B).

In dry weight parameters, measurements were gave better results in different concentrations of butenolide than control condition (0 ppm) except in Cresson, that there were not a big differences between means (Figures 3.8).

The results in this study showed that using different concentrations of butenolide with different receptors gave a number of facts agreed with number of previous studies. And the effects of this compound must take in account in any further work related to seed germination and seedling development.

The temperature which used here (22 C^o) is very close to that which used by Jain *et al.*, (2006) and ranged from 10 to 40 C^o, with 25 C^o being the optimum for all treatments. They reported that the germination percentage followed a parabolic curve for these temperatures. Kulkarni *et al.*, (2010) reported that *A. mearnsii* seeds exposed to constant dark conditions showed a significantly better germination percentage than the control, and this agree with our results while we used dark conditions at different concentrations of butenolide, but; the results were varying depending on species its self.

The post-germination of plants were also differ from plant to other, especially in root length and this result is strongly dealed with Van Staden *et al.*, (2006) who investigated the post-germination effect of smoke-water on tomato (*Lycopersicon esculentum*), okra (*Abelmoschus esculentus*) and bean (*Phaseolus vulgaris*) under laboratory conditions. Tomato seedlings that were treated with solution had 10-times greater root length than the water control, whereas in okra and bean, root length was 3-times more. There was also a significant increase in shoot length

of all three crop seedlings. Jain *et al*., (2008[A]) reported that butenolide can serve as aquaporin inhibitor. This suggests enhanced activity of aquaporins. The presence of aquaporin inhibitors reduced seedling water content and altered root development.

For chromosomal study, 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). The reasons of decrease 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 study the influence of 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. Most mutation in prophase stage (Figure 3.15, A).

The effect of different concentrations of butenolide due to appear of mutations in division meristimic cells of *Allium cepa* L., which are classified to;

A_ (physiological abnormality) which include early condensation in prophase, high condensation in prophase, sticky metaphase, c_ metaphase and lagging chromosome, binucleated cells and multiple nucleated cells.

B_ (clastogenic abnormality) which include anaphase bridges and telophase bridges.

In general, stickiness of chromosome leads to death of cell (Fiskesjo, 1995). Stickiness maybe the result of affected chromosomal protein due to butenolide toxicity.

Presence of bridges (Figure 3.15, C) may result from the adhesion of chromosomes in anaphase (Hassan, 2000). The appearance of bridges referring to the ability of butenolide in causing broken chromosomes, the emergence of broken chromosomes indicated the direct interaction of butenolide with DNA. Scattered chromosome result from decrease of ATP for movement of chromosomes (Armbruster *et al*., 1991). And telophase bridge result from the migration of dicentric chromosomes toward opposite spindle poles. binucleated cells and multiple nucleated cells can be caused from the ability of butenolide and its interference with cell wall formation (Baeshin *et al*., 1999). Appearance of early and high condensation in prophase pointed out the apply of butenolide to reaction with histone protein during mitotic division that chromosomes appear short and thickness (Grant, 1978, Topaktas and Rencuzogvullari, 1991).

Summary

Fire is one of the important factors that affect the plant life. The fires produce a number of different compounds when it occurs. The fire smoke, which described as one of the important productions of fire, consists of a group of chemicals appear as gases or dusts. Butenolide, which play a major role in plant growth and seed germination, had recently studied. In our work, the effect of different concentrations and the role of different plants as receptors were studied. Concentrations of (0, 25, 50, 100, 250, 500 and 1000 ppm) of Butenolide were used. Five different plant species *Hordeum vulgare (*Barley) *Solanum lycopersicum* L. (Tomato), *Lens culinaris* (Lentil), *Lepidium sativum* L. (Cresson) and *Allium cepa L.* (Onion) were used as receptors to test the effect of different concentrations of butenolide. Even though some concentrations revealed significant effect, the results showed that the effect is not species dependent. In general, concentrations of (100 ppm) approximately was an optimum for all species in seed germination, but; at (1000 ppm) of Butenolide concentration the seed germination percentages was decreased with exception of lentil and tomato plant.

Dry and fresh weight of seedlings appeared no significant effect in both roots and shoots at all concentrations with exception at (1000 ppm) concentration, there was high significant by decreasing the parameters with barley plant. On the other hand, tomato plants showed response at

(100 ppm) of Butenolide concentration in root length. In both concentrations of (50 and 250 ppm) the root length was increased and fresh weight of stem was at high level at (100 and 250 ppm), when lentil was used as plant receptor. For the same species, the root fresh weight was revealed the high amount at (50, 100, 250 and 1000 ppm) of Butenolide concentration. The results of fresh weight of Cresson also appeared significant values when the concentrations increased up to (250 ppm) of Butenolide concentration.

The mitotic index (MI) of the chromosomal study was gave very important results when *Allium cepa* L. (Onion) plant treated with the concentrations at (25, 50, 100 and 250 ppm), the (MI) decreased but; when (500 and 1000 ppm) used it increased under the light microscope when it was investigated. Different chromosomal disorder like sticky metaphase, nucleic outside the cells and others explained some results related to these abnormalities.

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جامعة بنغازى

كلية العلوم

قسم النبات

استجابة مستقبلات نباتية مختلفة للفيورانون

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قدمت هذه الرسالة استكمالا لمتطلبات درجة الإجازة العليا (الماجستير)

بقسم علم النبات ، كلية العلوم ، جامعة بنغازى ، ليبيا.

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الملخص العربي

هذه الدراسة كانت عبارة عن دراسة أو معرفة تأثير مادة البيوتينوليد (butenolide) على مجموعة من بذور المحاصيل وتشتمل هذه البذور: العدس *Lens culinaris L.* ويتبع العائلة (Fabaceae)، حب الرشاد *Lepidium Sativum* L. ويتبع العائلة (Brassicaceae)، الطماطم *Solanum lycopersicum* L. ويتبع العائلة (Solanaceae)، البصل *Allium cepa L.*ويتبع العائلة (Alliaceae) وتعتبر جميع تلك المحاصيل من ذوات الفلقتين وآخر نوع من البذورالمستخدمة فى هذه الدراسة هى بذور الشعير *Hordeum vulgare L.* التى تتبع العائلة (Poaceae) وهى تعتبر من ذوات الفلقة الواحدة.

أجريت التجارب المعملية بقسم علم النبات التابع لكلية العلوم – جامعة بنغازى, وتم الحصول على البذور من محل خاص لبيع البذور فى بنغازى. وقد تم تحضير تراكيز مختلفة من محلول butenolide لغرض دراسة تأثيره على البذور ومشاهدة مدى تأثر النبات به, حيث عوملت البذور للنباتات المختلفه بنقعها لمدة 24 ساعة فى التراكيز المختلفة من محلول butenolide والتي كانت على النحو التالى (0، 25، 50، 100، 250، 500، 1000 ppm) حيث أن التركيز (0) عبارة عن ماء مقطر. ثم زرعت البذور في أطباق بتري بشكل متساوٍ حيث كل طبق زرع به 15 بذرة. ورويت ب 5 ملليتر بالماء المقطر ثم وضعت فى الحاضنة عند ظروف ملائمة لمدة أسبوعان، وطول تلك الفترة يتم سقاية البذور حسب احتياجها من الماء. وتم قياس النسبة المئوية للإنبات اليومى والنهائى لمدة أسبوع. أما على مستوى البادرات فأخذ المتوسط الإحصائي لكل من أطوال الريشة والجذير و أوزانها الطرية والجافة مع معدل الجذير / الريشة. أيضا تم دراسة تأثير التراكيز المختلفة من محلول butenolide على المستوى الخلوى حيث تم حساب معدل انقسام الخلايا المرستيمية ونسبة الطفرات فى جميع الأطوار وتم استخدام برنامج spss للتحليل الإحصائى.

بينت الدراسة أن:

نسبة الإنبات تتفاوت من نبات لآخر حيث أن:- فى الشعير كانت نسبة الإنبات مرتفعة عند التركيز(50 ppm) حيث وصلت نسبته إلى 96% وحدوث تثبيط عند تركيز (1000 ppm) . أما العدس فكانت نسبة الإنبات جيدة فى كل التراكيز إلا أن هناك انخفاض ضئيل جدا عند التركيز (500 ppm) فكانت نسبة الإنبات 83%. بالنسبة لنبات الطماطم كانت نسبة الإنبات فى التراكيز المختلفة على التوالى (25، 50، 100، 250، 500) ppmأفضل من الكنترول. أما نبات حب الرشاد فلم يكن هنالك أى تغير ملحوظ فى نسبة الإنبات فكانت ذات نتائج متقاربة سواء عند الكنترول او عند (25، 50، 100 و 250) ppm أما عند (500) ppm فحدث تثبيط قوى وعند التركيز (1000) ppm لم يحدث أى إنبات .

أما نتائج تأثير المركب ومدى إستجابة النباتات المستقبلة له وجدت اختلافات ظاهرية لنبات الشعير ولكن عند التحليل الإحصائى لم توجد هنالك أى تأثيرات من قبل التراكيز المختلفة للمحلول على نبات الشعير بإستثناء الإنخفاض الملحوظ عند التركيز (1000) ppm سواء فى الإستطالة أو فى أوزانها الطرية و الجافة.

أما العدس فتأثره بمحلول butenolide كان مختلفا تماما عن تأثر نبات الشعير به، حيث وجد أن الإستطالة قد زادت عن الكنترول عند التركيزى (50 و 250) ppm وكانت هذه الإستطالة فى الجذر وليست فى الساق. أما بالنسبة للأوزان الطرية فوجدت زيادة فى وزن الساق عند (100 و 250) ppm والجذر كانت الزيادة عند (50، 100، 250 و 1000)ppm . والأوزان الجافة بالنسبة للساق عند (25، 250 و 1000) ppm ، والجذر كانت نسبة الزيادة عند (25، 50، 100 و 250)ppm .

أما بذور حب الرشاد فكان تأثره بالتراكيز المختلفة لمحلول butenolide مختلفاً تماما عن نباتى الشعير والعدس فلم يلحظ أى تأثير محفز للمحلول بإستثناء كان هنالك تأثير مثبط للساق عند التركيز (1000)ppm والجذر كان التثبيط واضحاً عند تركيزى (500 و 1000)ppm . كما

أن نتائج الطماطم بُينت الزيادة فى طول الجذر عند (100) ppm والوزن الرطب عند (100 و 1000) ppm ، أما فى الوزن الجاف فكانت الزيادة فقط عند التركيز (100)ppm .

عند زراعة نبات الطماطم فى التربة لوحظ أن بعض التراكيز المختلفة من محلول butenolide لهل تأثير إيجابى على بذور الطماطم حيث أن للمحلول تأثير تحفيزى واضح على الجذور إلا عند التركيز (1000)ppm فكان التأثير تثبيطى ولوحظ التحفيز عند التراكيز (50 و 250) ppm، أما بالنسبة للوزن الطرى فكانت الزيادة ملحوظة فى الوزن عند التركيز (100) ppmبالنسبة للساق. أما بالنسبة لوزن الجذر فكانت الزيادة عند التراكيز (25، 50 و 10) ppmوانخفاض الوزن كان عند التركيز (1000) ppm فى كل من الجذر والساق.

فى عدد الأوراق كان الإختلاف ملحوظاً فقط بالعين المجردة ولكن بالتحاليل الإحصائية أظهرت النتائج عدم وجود أى فروقات معنوية. بالنسبة للوزن الجاف كان هنالك انخفاض عند التركيز (ppm100) فى الوزن الجاف للجذر.

أظهرت الدراسة الكروموسومية لخلايا البصل انخفاض نسبة الخلايا المرستيمية فى بعض التراكيز مع وجود ارتفاع النسبة المئوية للطفرات فى كل من الطورى الإستوائى والتمهيدى.