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IN-*VITRO* AND IN THE FIELD COMPARISON STUDY OF *LEPIDIUM SATIVUM* CHEMICAL COMPOSITION.

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Abstract:

The aim of this experiment is to study the effect of different concentrations of BAP plant growth regulator on the chemical composition of Lepidium *sativum* plantlets in the case of micropropagation in *vitro* and chemical analysis of them, and to compare these results with the results of the chemical analysis in the case of cultivation of L. *sativum* in the field. In *vitro*, MS media was used supplemented with different concentrations of cytokinin BAP at (0.0, 1, 2, 3, 4, 5 mg/L BAP) to produce the plantlets from the seeds. By comparing the chemical analysis of the chemical components of the plantlets produced in *vitro* and the chemical analysis of the plants those were grown in the field, the comparison revealed a higher value of chemical compounds in the field plants compared to the chemical compositions resulting from micropropagation of the plantlets in *vitro*.

From our study we obtained the following, the maximum values of shoots number, plantlet length, plantlet weight, plantlet wide, was recorded with 3.0 mg/L BAP and the maximum value of reading spad, chlorophyll a, b and chlorophyll a+b were recorded with 5.0 mg/L BAP, on the other hand the maximum values of carotenoids, total phenolics, flavonoids contents and antioxidants activity in plantlets were recorded with 4 mg/L BAP. While cultivation of *Lepidium sativum* in the field gave values in chemical compounds greater than those obtained when cultivation in the laboratory.

Keywords: *Lepidium sativum*, in *vitro*, total Phenolics, Flavonoids, Antioxidant activity, Ch.a, Ch.b, BAP.
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Introduction

Garden cress (GC), scientific name: *Lepidium sativum L.* (L.s.), it is an erect annual herb, belonging to family Brassicaceae (Sharma and Agarwal, 2011). GC is native to Egypt and south west Asia and was referred to over many centuries ago in Western Europe. *L.S.* is an important medicinal crop, seeds, leaves and roots are economically important, however the crop is mainly cultivated for seeds, in some regions garden cress is known as garden pepper cress, pepper grass, cress, curly cress (Doke and Guha, 2014). Common name in arabic: habb al-rashad, rashad, thufa (Al sanafi, 2019**b**). Tuncay et al. (2011) showed that *L. sativum* seeds were sown in the winter season, the optimal month of sowing are the cool months of November, January, and February in the Mediterranean climate. *L. sativum* an annual herb, 10-60 cm in height, the fruiting stage of *L. sativum* L. occurred when all inflorescence was developed and turned into fruits with mature seeds at the age of 16 weeks, after two weeks green fruits turned into yellow fruits at harvest time 18 weeks (Farag and Shaaban, 2021). Abd El-Salam et al. (2019) found that nineteen flavonoid compounds were quantitatively identified in GC seeds extract, in addition, it contained remarkable levels of sodium and calcium. GC seeds are free from bromine, selenium, cadmium and nickel.

The pharmacological investigation revealed that *Lepidium sativum* possessed antimicrobial, antidiabetic, antioxidant, anticancer, reproductive, gastrointestinal, respiratory, anti-inflammatory, analgesic, antipyretic, cardiovascular, hypolipidemic, diuretic, central nervous, fracture healing and protective effects (Al-Snafi, 2019$_b$).

Doke (2014) and Al-Sheddi et al. (2016) and Raish et al. (2016) reported that Several studies have revealed that the extract of *L. sativum* possesses antioxidant, antidiarrheal, antispasmodic, antimicrobial, anti-inflammatory and hepatoprotective effects against oxidative damage.

Parts used medicinally, Seeds, oils and leaves (Al-Yahya et al., 1994 and Sharma and Agarwal, 2011and Adam et al., 2011).The preliminary phytochemical analysis of *Lepidium sativum* showed that it contained cardiac glycoside, alkaloids, phenolic, flavonoids,

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cardiotonic glycosides, coumarins, glucosinolates, carbohydrates, proteins and amino-acids, mucilage, resins, saponins, sterols, tannins, volatile oils, triterpene, sinapic acid and uric acid (Ahmad et al., 2015and Ghante et al., 2011and Ghalem and Ali, 2017). The active constituents of GC are Sulfur-containing compounds that in this plant called Diallyl Disulfide (Allicin) and glucosinolates (GLS) that are Cancer prevention materials (Mazloom et al., 2013). Moser. et al. (2009) reported that *L. sativum* seeds contain 24% oil. Phenolic compounds are very important plant constituents because of their scavenging ability due to their hydroxyl groups, twenty one phenolic compounds have been quantified in GC seed extract (Oktay et al., 2003).

Sosnowski et al. (2023) showed that phytohormones are regulators of plant growth and development. Despite the fact that they are synthesized by plants in small quantities, they are highly active physiologically. According to their action, phytohormones can be divided into two categories, as either activators of plant growth and development or as inhibitors, auxins and cytokinins belonging to the activators group. cytokinins, are formed in root tips and in unripe fruits and seeds, These hormones are responsible for stimulating the growth of lateral shoots, they also stimulate cytokinesis and, consequently, cell division. the most important role of cytokinins affect cell division, which in turn affects plant growth, but they also stimulate lateral buds and inhibit the aging of organs and plant tissues (Ortiz and Campos, 2020 and Holalu et al. 2021). The success of the micropropagation method depends on several factors like genotype, media, plant growth regulators and type of explants, (Lin et al., 2000 and Pati et al., 2006). Most important of these parameters are the plant growth regulators included in the culture media (Gomes and Canhoto, 2003). Plant growth regulators act like signals to stimulate, inhibit or regulate growth in the media of plant cultivation by micropropagation (Mercier et al.,1997).

Materials and Methods

The following experiment was performed at the Tissue Culture and Plant micropropagation Laboratory of Horticulture Department, Faculty of agriculture, Damanhour University since September 2020 to June 2023.

2.1 *Lepidium sativum* seeds

Lepidium sativum seeds were brought from commercial store in Cairo which were imported from India.

2.2 Sterilization

All seeds were soaked in 20% sodium hypochlorite for 10 minutes then washing thoroughly with sterile distilled water for five minutes, the sterilized seeds were dried with sterilized filter paper

2.3 Treatments

2.3.1 Media Preparation and Micropropagation of *Lepidium sativum* seeds

Media preparation: (Murashige and Skoog. 1962) medium was used in the present study, 4.4 g/L of MS media supplied with sucrose 30 mg/L (3%), and supplemented with different concentrations of BAP plant growth regulator at (0.0, 1.0, 2.0, 3.0, 4.0, 5.0 mg/L). and pH of the medium was adjusted to 5.7 ± 0.1 using HCL or NaOH then solidifying with 7 g/L (0.7%) agar. Then poured 50 ml of media in each glass vessels airtight, the media was autoclaved at 121 C° and 15 lbs.(15 psi) pressure for 20 minutes. In the micropropagation room(culture room), the seed inoculation was done with help of sterile forceps. After inoculation glass culture bottles were wrapped and incubation was done in growth room, maintained at 24 C° ± 1 under 16 hours photoperiod and 8 hours darkness provided by cool –white fluorescent lamps emitting a photosynthetic photon flux density of 40 µmol m^{-2} s^{-1}, Data were reported after four weeks.

2.3.2 Direct propagation of *Lepidium sativum* seeds in *vitro*

The effect of different concentration of BAP (0, 1, 2, 3, 4 and 5 mg/L) were used in reproductive culture of *Lepidium sativum* seeds. then two subcultures via explants from plantlet, the first subculture, from plantlet resulting from the seeds. thereafter, the second subculture from new plantlets resulting from the first subculture, (Photo 1) is represented of the subculture 1, (Photo 2) is represented of th subculture 2.

2.3.3 Experiment layout:

The experiment was conducted in completely randomized design (CRD) with three replications and one factor.

2.4 Parameters

2.4.1 Plantlets length (cm)

The length of plantlets was measured by a ruler and the average was calculated

2.4.2 Plantlet weight (g)

Each plantlet was taken and cleaned then weighted and average was calculated.

2.4.3 Plantlet wide (cm)

Was measured by a ruler and average was calculated.

2.4.4 Number of shoots

Was numbered and average was calculated.

2.4.5 Measurement of spad

Was measured by spad device and average was calculated.

2.4.6 Chlorophyll a, b and carotenoids content (mg/L)

Chlorophyll A, B and beta carotene were determined according to Wintermans and Mats (1965) as follows: half gram of fresh leaves was extracted by about 15 ml. of 85 % acetone with 0.5 g calcium carbonate, the mixture filtrated through a glass funnel and the residue was washed with a small volume of acetone and completed to 25 ml. the optical density of constant volume of filtrated was measured at a wave length of 662 nm. for chlorophyll A, 644 nm. for chlorophyll B and 440 nm. for carotene using spectrophotometer device.

The following equations were used to calculate the chorophyl a and b, as well as total carotenoids conyents in mg/L:

Chl.A = 9.784 E.662- 0.99 E 644 =mg/ L

Chl. B = 21.426 E 644 – 4.65 E. 662 = mg / L

Carotene = 4.695 E.440 – 0.268 (Chl. A + Chl. B) =mg/ L

Where E = optical density at the wave length indicated.

2.5 Chemical analysis of *Lepidium sativum* plantlets (Determination of total phenolics content, flavonoids, and antioxidant activity):

2.5.1 Preparation of extract:

Half gram of the dry plantlets was mixed 8 ml of 70 % methanol and stored at room temperature, after 48 h. the infusions were filtered through Whatman No. 1 filter paper.

2.5.1.1 Determination of total phenolic content (mg of gallic/g of extract)

Total phenolics content (mg / g) of each treatment were determined in the representative dry herb samples according Singleton et al., (1999) as follows:

The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared,

containing 0.5 ml methanol, 2.5 ml of 7.5% $NaHCO_3$. The samples were thereafter incubated at room temperature for 45 min. The absorbance was determined using spectrophotometer at λmax =765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

2.5.1.2 Determination of flavonoids content (mg of rutin/g of plantlet)

Flavonoids content (mg/g) of each treatment were determined in the representative dry plantlet samples according Quettir et all., (2000) as follows:

The reaction mixture was prepared by mixing 1 ml of methanolic solution of extract and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λmax =415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for standard solution of rutin and calibration line was construed. Based on the measured absorbance. The concentration of flavonoids was read (mg/ml) on the calibration line; then the content of flavonoids in the extracts was expressed in terms of rutin equivalent (mg of RU/g of herb).

2.5.1.3 Antioxidant activity of plantlet extract

the ability of the plantlets extract scavenge DPPH free radicals was assessed by the standard method (Tekao et al.,1994). Adopted with suitable modifications (Kumarasamy et al., 2007) as follows:

DPPH was prepared by dissolving 10 mg in100 ml 70% methanol. A Know volume (60 µl) of methanolic extract of the dry plant, callus and seeds was dissolved in 440 µl of 70% methanol then mixed with DPPH (0.5 ml). The mixtures were well shaken and then placed in darkness at room temperature for 30 min. The absorbance was recorded at 517 nm. The control samples contained all the reagents except the extract. Methanol was used to zero the spectrophotometer. The inhibition

percentage of the DPPH radical was calculated according to the formula: 1% = [(AB- AS)/AB] × 100
Where 1 = DPPH inhibition %, AB = absorbance of control sample and AS = absorbance of a tested sample at the end of the reaction. Each assay was carried out in triplicate.

2.6 Parameters of direct micropropagation of *Lepidium sativum* seeds in *vitro*

Measurement parameters of weight, number of shoots, height, spad of *Lepidium sativum* plantlets.

2.7 Chemical analysis of *Lepidium sativum* plantlets

All of total phenolics content (mg gallic /g), flavonoids content (mg rutin /g) and antioxidant activity of dry plantlets extract were determined as mentioned in micropropagation experiment in this study, and determinations**,** chlorophyll a, b and carotenoids from fresh garden cress.

2.8 statistical analysis

Analysts' variance with Costat software (Costat statutical software. California: CoHort software, 1989) was carried out on the test treatments data. Treatments means were compared using LSD test at 5% level of probability.

3. Results and discussion

3.1. Micropropagation of *Lepidium sativum* seeds

3.1.1. Plantlets fresh weight per explant

The maximum average plantlets weight (4.43 g) of *Lepidium sativum* was observed with using BAP at level of 3 mg /L compared to other concentration, followed by the treatments 5, 2, 1, 4, 0.0 mg/ L BAP respectively as shown in Figure (1) and Photo (1to 6).

3.1.2. Plantlets number of shoots

The maximum average number of plantlets shoots (24 shoots) of *Lepidium sativum* was observed with using BAP at level of 3 mg /L compared to other concentration, followed by the treatments 4, 5, 2, 1, 0.0 mg /L BAP respectively, as shown in Figure (2).

3.1.3. Plantlets height (cm)

The maximum average of plantlets height (5.8 cm) of *Lepidium sativum* was observed with using BAP at level of 3 mg /L compared to other concentration, followed by treatments 5, 2, 4, 1, 0.0 mg/ L BAP respectively as shown in Figure (3).

3.1.4 Plantlets wide (cm)

The maximum average of plantlets wide (5.8 cm) of *Lepidium sativum* was observed with using BAP at level of 3 mg /L compared to other concentration, followed by the treatments 5, 4, 2, 1, 0.0 mg/ L BAP respectively as shown in Fig. (4).

3.1.5. Spad measurement of *Lepidium sativum* plantlet

The maximum spad of plantlets (10.8 spad) of *Lepidium sativum* was observed with using BAP at level of 5 mg /L compared to other concentration, followed by the treatments 4, 2, 3, 1, 0.0 mg/ L BAP respectively as shown in Figure (5).

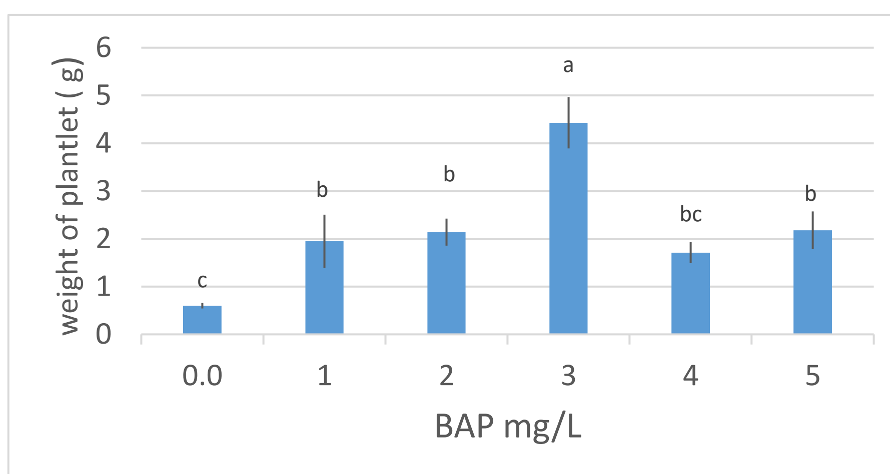

Fig. (1) Effect of different concentrations of BAP supplemented to MS media on the weight of *L. sativum* plantlet. Data are the mean values ± SE. Bars with the same letters are not significantly different at P ≤ 0.05.

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Photo (1)

Effect of 0.0 mg/L on *L. sativum* weight

Photo (2)

Effect of 1 mg/L on *L. sativum* weight

Photo (3)

Effect of 2.0 mg/L on *L. sativum* weight

Photo (4)

Effect of 3.0 mg/L on *L. sativum* weight

Photo (5)

Effect of 4.0 mg/L on *L. sativum* weight

Photo (6)

Effect of 5.0 mg/L on *L. sativum* weight

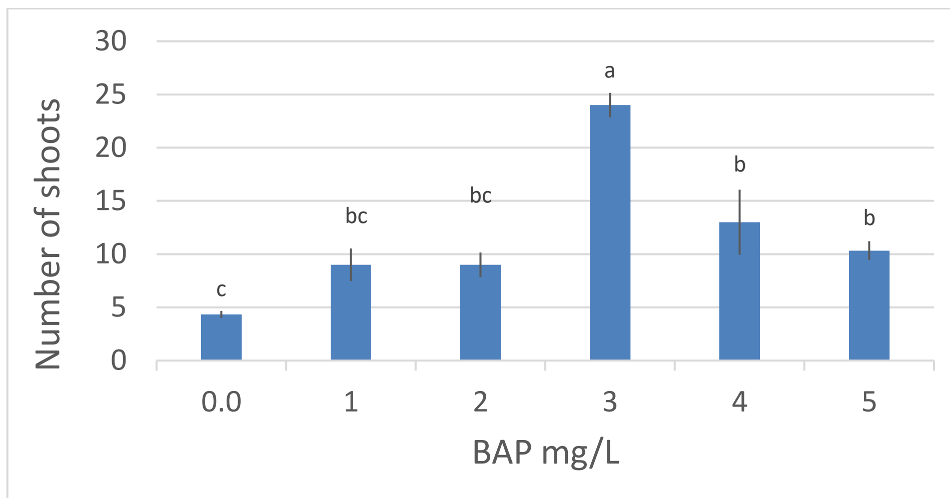

Fig. (2) Effect of different concentrations of BAP supplemented to MS media on **number of shoots** of *L. sativum* plantlet. Data are the mean values ± SE. Bars with the same letters are not significantly different at $P \leq 0.05$.

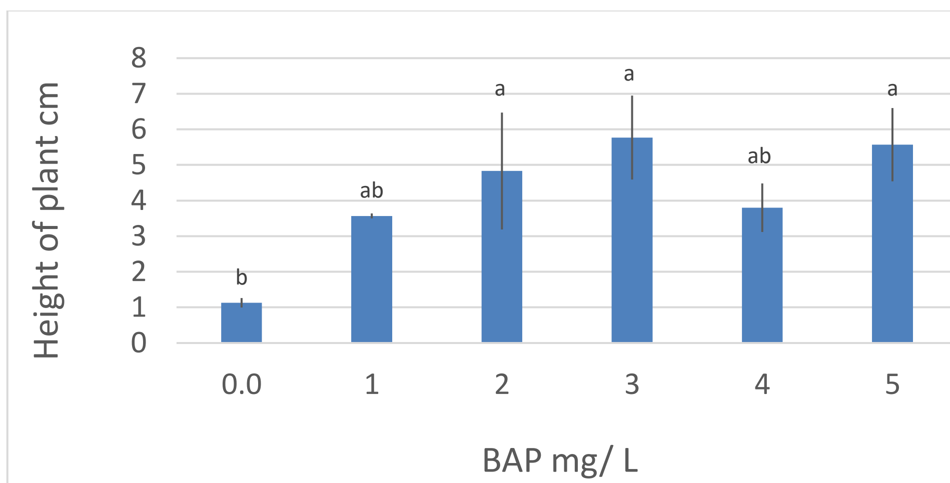

Fig. (3) Effect of different concentrations of BAP supplemented to MS media on **height** of *L. sativum* plantlet. Data are the mean values ± SE. Bars with the same letters are not significantly different at $P \leq 0.05$.

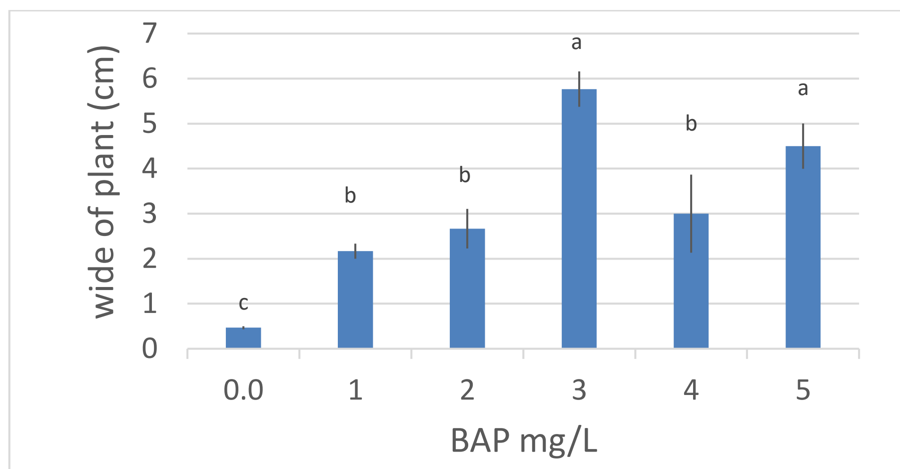

Fig. (4) Effect of different concentrations of BAP supplemented to MS media on **wide** of *L. sativum* plantlet. Data are the mean values ± SE. Bars with the same letters are not significantly different at P ≤ 0.05.

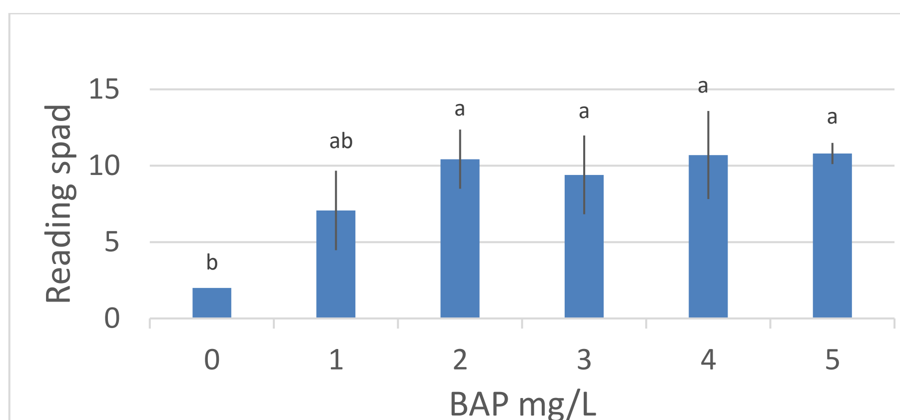

Fig. (5) Effect of different concentrations of BAP supplemented to MS media on spad of *L. sativum* plantlet. Data are the mean values ± SE. Bars with the same letters are not significantly different at P ≤ 0.05.

From the previous results obtained, it was found that, application of BAP to the MS media increased weight, height, wide and number of plantlet shoots. Increasing BAP concentration increased parameters up to 3 mg/L, then decreased with increasing BAP either to 4 or 5 mg/L BAP. Application of BAP to the MS media increased the parameters comparing to control treatments. Our results are consistent and agree with previous studies.

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Of the previously mentioned of the results it was found that the number of *Lepidium sativum* shoots increased with an increase of BAP concentration, these results agree with Danial et al. (2019) who reported that the role of cytokinins as a plant growth regulator causes shoots induction by stimulating cells division, decreasing apical dominance in addition of the role of cytokinins via breaking the apical dominance in buds and induce subsidiary meristem grown into shoots. El Kinany et al. (2019) who reported that cytokinins play an important role in counteraction or elimination the apical dominance and stimulating the release of axillary buds from apical dominance and lead to increment in number of branches per plant. Rabani et al. (2001) found that when BAP was used in moderate concentration, it can significant improvement in *vitro* multiple shoot induction.

Kishor and Devi (2009) showed that the application of cytokinins is very important for the induction of multiple shoots in many plants. Danci (2007) reported that the micropropagation of plants depends on the genotype, the nutrients in the culture media and the hormonal balance and plant growth hormones supplemented in the nutrient medium are one of the most significant factors affecting the efficiency of micropropagation.

The cytokinins are derived from adenine (aminopurine) and play an important role in the in *vitro* manipulation of plant cells and tissues (Torres et al., 2001). Cytokinins stimulate plant cells to divide, and they were shown to effect of many other physiological and developmental process. These effects include the delay of senescence in detached organs, the mobilization of nutrients, chloroplast maturation, and the control of morphogenesis (Taiz and Zeiger, 1991).

Hagar et al. (1991) mentioned that the initial response of explants to shoot formation due to addition of cytokinin by an increase in the cytosolic calcium concentration which is promoted by cytokinin high uptake from the media. Kishor and Devi (2009) showed that the application of cytokinins is very important for the induction of multiple shoots in manycrops. Mohamed et al. (2007) mentioned that Phytohormones play an important role to influence the growth of explant in *vitro* regeneration. Zhang et al. (2005) and Madhavam et al. (2009) reported that BAP is a cytokinin synthesized in the laboratory that is utilized in tissue culture to stimulate cell division, bud development, and stem branch expansion its agree with the results

obtained in this study as the number of shoots, length, wide, and weight of GC plant had lower results in the case of that the media was free of the Plant growth regulator.

On the other hand, in our study is noted that the concentraion more than 3 mg/L BAP gave less measurement of plant weight, height, wide and number of shoots of *Lepidium sativum*, these results agree with Wang et al. (2018) who found that high concentrations of cytokinins have negative effects on shoot regeneration.

and agree with Hoque (2010) who noticed that, in higher concentration of both the hormones, the number of shoot regeneration was decreased, the negative effect of higher concentration of PGR_s was observed on shoot multiplication.

Each explant responds differently in the presence of phytohormones, cytokinins play a vital role in regulating growth and morphogenesis in plants. Its main function is to induce shoots (Leopold, 1987).

Ganapathi and Nataraja (1993) found that involvement of cytokinins especially in shoot-bud formation has been reported in many plant species.

3.1.6. Plantlets chlorophyll A

The maximum average chlorophyll A of plantlets (2.81 mg /L) of Lepidium sativum was observed with using BAP at level of 5 mg /L compared to other concentration, followed by 4, 2, 3, 1, 0.0 mg/L BAP respectively and compare the results with the field plant where it was recorded (4.36 mg/L), as shown in Fig (6)

3.1.7. Plantlets chlorophyll B

The maximum average chlorophyll B of plantlets (2.33 mg / L) of *Lepidium sativum* was observed with using BAP at level of 5 mg /L compared to other concentration, followed by 3, 2, 4, 1, 0.0 mg/ L BAP respectively and compare the results with the field plant where it was recorded (3.12 mg/L), as shown in Figure (7).

3.1.8. Plantlets chlorophyll A+ B

The maximum average chlorophyll A, B of plantlets (5.15 mg /L) of *Lepidium sativum* was observed with using BAP at level of 5 mg /L compared to other concentration. Followed by 4, 2, 3, 1, 0.0 mg/ L BAP respectively and compare the results with the field plants where it was recorded (7.48 mg/L), as shown in Figure (8).

3.1.9. Plantlets total carotenoids

The maximum average total carotenoids of plantlets (0.69 mg /L) of *Lepidium sativum* was observed with using BAP at level of 4 mg /L compared to other concentration, followed by 5, 3, 2, 1, 0.0 mg/L BAP respectively and compare the results with the field plant where it was recorded (1.69 mg/L), as shown in Figure (9).

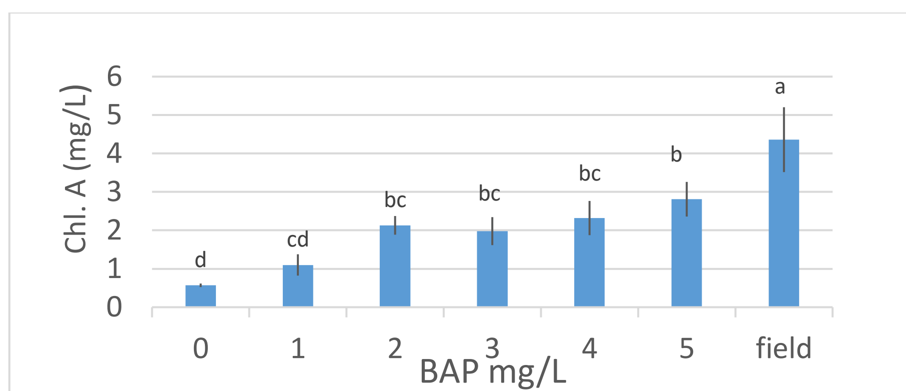

Fig. (6) Effect of different concentrations of BAP supplemented to MS media on **Chl. A** of *L. sativum* plantlet and its comparison with cultivation in the field. Data are the mean values ± SE. Bars with the same letters are not significantly different at P ≤ 0.05.

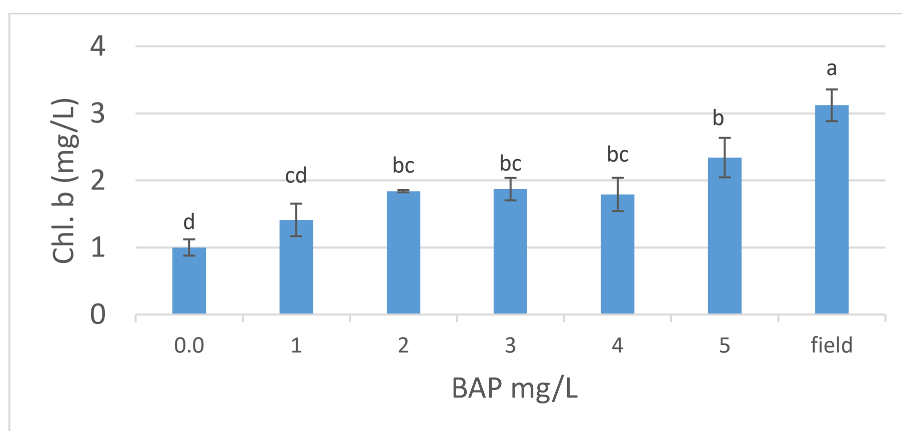

Fig. (7) Effect of different concentrations of BAP supplemented to MS media on **Chl.b** of *L. sativum* plantlet and its comparison with cultivation in the field. Data are the mean values ± SE. Bars with the same letters are not significantly different at P ≤ 0.05.

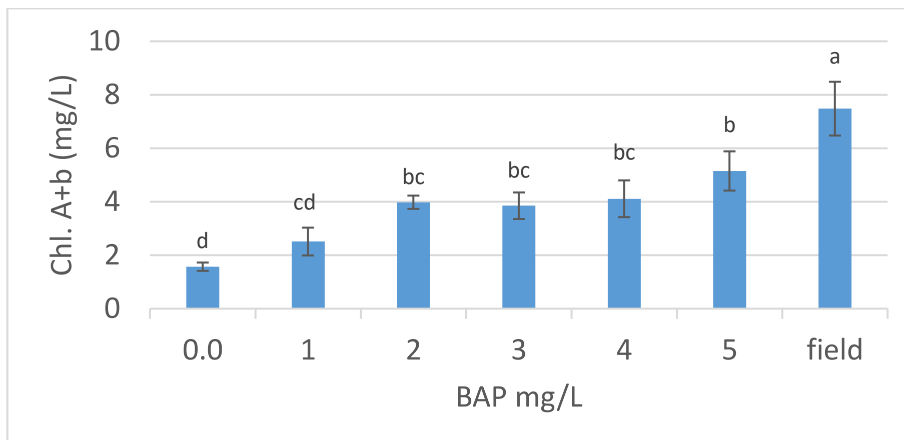

Figure (8) Effect of different concentrations of BAP supplemented to MS media on **Chl.a+b** of *L. sativum* plantlet and its comparison with cultivation in the field. Data are the mean values ± SE. Bars with the same letters are not significantly different at $P \leq 0.05$.

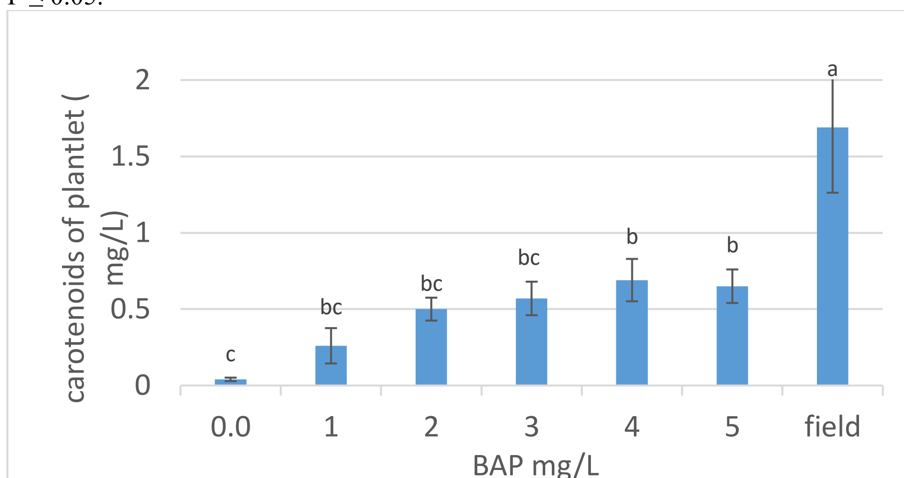

Fig. (9) Effect of different concentrations of BAP supplemented to MS media on **carotenoids** of *L. sativum* plantlet and its comparison with cultivation in the field. Data are the mean values ± SE. Bars with the same letters are not significantly different at $P \leq 0.05$.

From the previous results obtained, it was found that, application of BAP to the MS media increased the chlorophyll a, b and carotenoids contents comparing to control treatments. Increasing BAP concentration gradually increased both chlorophyll a, b up to 5 mg/L BAP. While carotenoids increased up to 4 mg/L BAP. The chlorophyll

a, b and carotenoids contents of plants resulted from cultivation was more than of those plantlets resulted from in *vitro*. This results agree with (Halim and phang, 2017 and Atteya and El Gendy, 2018_a ; Atteya and Elgendy 2018_b) who found that the plant growth regulators play an important role in the growth, synthesis of chlorophyll, carotene, and secondary metabolites in the different plants.. Abd Elaleem et al. (2009) found that significant differences inside each cultivar and among the different concentrations of cytokinins.

3.1.10. Total phenolics content (mg gallic /g) of dry *Lepidium sativum* plantlets.

The highest mean value of total phenolics (2.63 mg / g) of L S were recorded with the level of 4 mg/L BAP compared to other concentration, followed by 5, 3, 2, 1, 0.0 mg/L BAP respectively and compare the results with the field plants where it was recorded (5.27 mg/g), as shown in Figure (10).

3.1.11. Total flavonoids content (mg rutin /g)

The highest mean value of total flavonoids (0.92 mg / g) were recorded with the level of 4 mg/L BAP compared to other concentration, followed by 1, 5, 3, 2, 0.0 BAP respectively and compare the results with the field plant where it was recorded (1.38 mg/g), as shown in Figure (11).

3.1.12 Antioxidant activity of *Lepidium sativum*

The highest mean value of antioxidants activity (42.6%) was recorded with the level of 4 mg/L BAP compared with the other concentration, followed by 1, 5, 3, 2, 0.0, mg/L BAP respectively and compare the results with the field plant where it was recorded (72.53 mg/g), as shown in Figure (12).

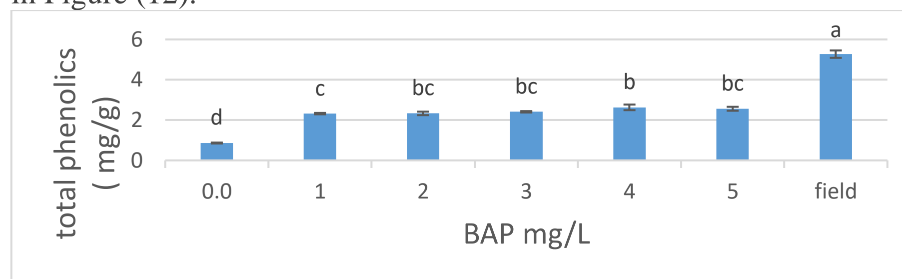

Fig. (10) Effect of different concentrations of BAP supplemented to MS media on **total phenolics** of *L. sativum* plantlet and its comparison with cultivation in the field. Data are the mean values ± SE. Bars with the same letters are not significantly different at $P \leq 0.05$.

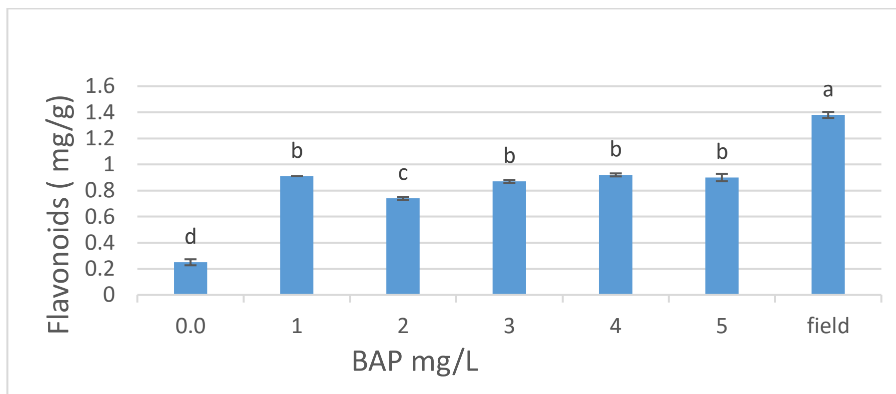

Fig. (11) Effect of different concentrations of BAP supplemented to MS media on **flavonoids** of *L. sativum* plantlet and its comparison with cultivation in the field. Data are the mean values ± SE. Bars with the same letters are not significantly different at $P \leq 0.05$.

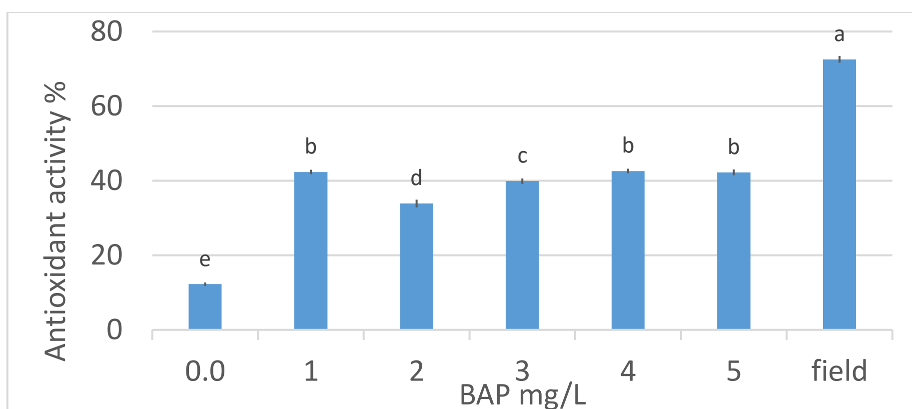

Fig. (12) Effect of different concentrations of BAP supplemented to MS media on **antioxidant activity** of *Lepidium sativum* plantlet and its comparison with cultivation in the field. Data are the mean values ± SE. Bars with the same letters are not significantly different at $P \leq 0.05$.

From the previous results obtained, it was found that, application of BAP to the MS media increased the total phenolics, flavonoids, antioxidants activity contents comparing to control treatments. Increasing the BAP concentration did not show significant increase in phenolics contents up to 5 mg/L BAP. The total phenolics, flavonoids, antioxidants activity contents of plants resulted from cultivation was more than of those plantlets resulted from in *vitro*.

From the obtained results of this study, it is clear that, the concentrations under study of BAP were able to improve total phenolic, flavonoids content and antioxidant activity of *Lepidium sativum* plantlet. it agrees with Elkeltawi and Croteau (1987) who showed that BAP effect on the plant metabolites composition as a secondary product of plant.

Sayd et al. (2010) who found that antioxidant activity, total phenolics and flavonoids content were higher in plantlet cultured in all concentration of cytokinins compaired to medium free PGR_s, this agree with the results obtained in this study,where application of BAP to MS media increased the chl a,b, carotenoids, total phenolics, flavonoids and antioxidant activity in *L. sativum* plantlet compared to control treatments.

Khalil et al. (2016) reported that higher phenolic and flavonoid compounds in *vitro* shoots of stevia (*Stevia rebaudiana*,Compositae family) plant treated with growth regulators compared to the control non-treated one. Zifkin et al. (2012) and Sakakibara et al. (2006) found that the stimulatory role of micropropagation in increasing phenolic content might be because of plant growth regulators used in media on biosynthesis of phenolic compounds through influencing the expression or up-regulation of genes involved in the biosynthetic pathway of secondary metabolites.

Selek et al. (2018) found that the antioxidants content and activity of the methanol extract of *Lepidium sativum* was investigated in *vitro*,the extract contained high amounts of phenolic and flavonoid compounds and showed significant antioxidant activity.

3.1.13. Cultivation of *Lepidium sativum* in the field:

Garden cress plant is a winter plant and is grown in Egypt in the period from mid-September to mid- December, as it gaves the best group at that time. And its cultivation in Egypt is limited. In this experiment garden cress was cultivated in November, and the chemical compositions of the plant were analyzed.

From our study was found that, chlorophyll a and b, total phenolics, flavonoids and antioxidant activity of plant resulted from cultivation was more than those plantlets resulted from *in vitro*, and this is attributed to the exposure plant in the field to biotic and abiotic factors that stimulate the plant to produce more secondary metabolites such as flavonoids, phenols and antioxidants, and this agree with Abdelaleem et

al. (2019) in their experiment showed that the abiotic factors such as adding sugar or salt to the MS media in *vitro*, at certain concentrations stimulate the production of total phenols and antioxidant activity in *Lepidium sativum*. As well Cheynier et al. (2013) who explained that Polyphenols synthesis and accumulation in plants is generally stimulated in response of abiotic or biotic stress. This was found and agree with our study, where secondary metabolites such as, total phenols, flavonoids and antioxidant activity resulted from the field plants are higher compared to secondary metabolites resulted from *vitro* plantlets.

4- Conclusion

Chemical compositions produced from the field plants are higher compared to the chemical compositions produced from *vitro* plantlets. Micropropagation of *Lepidium sativum* in *vitro* has the advantage of being able to propagate at any time of the year under sterile and controlled conditions to obtain effective chemical compounds used in pharmaceutical manufacturer. Cultivation of garden cress in Egypt is limited; its cultivation must be expanded due to its many medicinal benefits.

Abbreviations

L.S= *L.sativum* = *Lepidium sativum*.
GC= Garden cress= Cress
L.S.S = *Lepidium sativum* seeds
BAP = 6-Benzyl amino or N6-benzylaminopurine= BA: Benzyl adenine.
KIN= Kinetin
MS medium = Murashige and Skoog medium.
Chl.a = Chlorophyll a.
Chl.b = Chlorophyll b.
SE = Standard Error
PGR_S = Plant growth regulators
ML= Milliliter.
L= Litter.
gm (g) = Gram.
Mg = Milligram.
mg/L= Milligram per litter.
mg/g = Milligram/gram.
DPPH = 1.1-diphenyl-2-Picrylhydrazyl. Hydrate.

Cm = Centimeter.
pH = potential of hydrogen ion.
LSO = *Lepidium sativum* oil.

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Significance statement

The study represented comparison study of *Lepidium sativum* chemical compositions in *vitro* and in the field

Authors' contributions All authors contributed to the design and performance of the experiment and also to the data analysis, writing of the manscript, and following up the publication with the journal (correspondence).

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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دراسة مقارنه للمكونات الكيميائية لنبات حب الرشاد في حاله الزراعة في المعمل والحقل

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الملخص العربي:

تم اجراء هذه الدراسة في معمل زراعة الانسجة الاكثار الدقيق للنبات قسم البساتين / كلية الزراعة / جامعة دمنهور من شهر سبتمبر2020 الي يونيو 2023 وتم التحليل الكيميائي لكلوروفيل ا، ب والكاروتينويدات والفينولات والفلافونويدات ومضادات الأكسدة للنبات في حاله الاكثار الدقيق في المعمل وفى حاله الزراعة في الحقل.

- وكان الهدف من الدراسة مقارنه التركيب الكيميائي لنبات حب الرشاد فى حاله الزراعة في المعمل والحقل.

وفي المعمل تم استخدام تركيزات مختلفة من منظم النمو النباتي بنزيل أمينو بيورين (0.0، 1.0، 2.0، 3.0، 4.0، 5.0ملجم/لتر) لإنتاج النبات من البذرة وإعادة زراعة النبات مرتين من جزء نباتي صغير من الفرع النباتي.

وسجلت نتائج التحليل الكيميائي ارتفاع محتوى التركيب الكيميائي في نبات الحقل عنه في نبات المعمل في جميع المعاملات المختلفة من منظم النمو النباتي بنزيل أمينو بيورين التي تمت في المعمل.

وفى المعمل سجلت أعلى قيمه من قيمه من كلوروفيل ا، ب عند المعاملة 5 مجم/لتر بينما سجلت أعلى قيمه من الكاروتينويدات والفينولات والفلافونويدات ومضادات الأكسدة النشطة عند المعاملة 4 مجم/لتر بنزيل أمينو بيورين.

يمتاز الاكثار الدقيق لنبات حب الرشاد في المعمل بأنه يمكن زراعته طول العام تحت ظروف معقمه ومتحكم فيها للحصول على مركبات كيميائية فعاله تستخدم في التصنيع الدوائي.
الكلمات المفتاحية: Lepidium sativum، في المختبر، إجمالي الفينول، الفلافونويدات، النشاط المضاد للأكسدة، Ch.a، Ch.b، BAP.