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Effectiveness of magnetic field in stimulation of biochemical and enzymes activities in seedling and callus of *Nigella sativa*

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Abstract---This current study investigated the influence of Magnetic Field from Winding coil magnet about (400 mT) for different exposure periods (0, 30, 60, 90, 120, 150 min) on seed germination, callus initiation, and callus fresh weight, protein, carbohydrate and proline content and vitality , in addition to the efficacy of some antioxidant enzymes . The results indicated that the highest seed germination percentage was when exposed for an(90 Minutes). Also found that the best exposure period for acceleration of callus initiation was (30 minutes). Fresh weight, protein, proline, carbohydrates content and callus vitality got a better result under (90 minutes) of magnetic field exposure . On the other hand, the antioxidant enzymes activity significantly increased with 150 minutes M.F treatment.

Keywords---magnetic field, callus, *nigella sativa*, antioxidant.

Introduction

Nigella sativa L. (Ranunculaceae), a highly valued medicinal herb. Seeds known as (Black cumin) in English, In Arabic, they are known as (Habba Al-Sauda), (Habba Al-Bar-akah) or (Kalonji) (Sultan *et al.*, 2012). *N. sativa* is native to South west Asia and Africa. and grown in Mediterranean countries, Turkey, Syria, India, Srilanka, Iran , Albania , Saudi Arabia and other (Naz, 2011 ; Chaudhry *et al.*, 2020) . *Nigella sativa* seed is one of the most traditional medicinal plants used for diseases treatment, such as asthma, headache, inflammation, eczema, bronchitis, rheumatism, hypertension, anorexia, amenorrhea, paralysis and mental debility. *N. sativa* is known to display diverse pharmacological actions, antioxidant (Erol *et*

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al., 2017), anti-inflammatory (Abd-Elbaset *et al.*, 2017), fungicidal (Almshawit and Macreadie, 2017), anticancer (Almoyad, 2018), nephroprotective (Kotb *et al.*, 2018), good antimicrobial activity against both the Gram-negative and Gram-positive bacteria (Ecrastian *et al.*, 2021) and prevention diabetes mellitus disease type 2 (Tigi *et al.*, 2021), recently *Nigella sativa* seeds extract used as reducing agent for the silver nanoparticles (AgNP) production (Anwar *et al.*, 2021)

Plant tissue culture branches of biotechnology depends on the cell totipotency, which is the ability of any single cell to produce all the cells differentiate into organs, and regenerate into a whole plant (Trigiano and Gray, 2016). In sterile and specific environmental conditions without depending on a geographical region, season or climate for cultivation (Chandran *et al.*, 2020).

Magnetic field a directional quantity, can be represented by imaginary lines called lines of magnetic flux. the number of magnetic lines perpendicular to a unit area is called magnetic flux density. (MF) act as a kind of abiotic stresses which can affect growth, metabolic and many physiological processes and compounds of medicinal plants, (Verpoorte *et al.*, 2002) (Hassapour *et al.*, 2014) as well as M.F effect on photosynthesis, redoxstatus, lipid, carbohydrate, and protein composition. (M.F) considered as a biophysical treatment change the growth and development parameters. (Golbaz & Kaviani, 2019).

Materials and Methods

Seed sterilization and Exposing to magnetic field

The seeds were cleaned and sterilized according to (Albaker, 2002) by submerge seeds in 96% ethanol for (2) minutes with constant stirring, then under sterile condition, seed were transferred to v/v sodium hypochlorite diluted with distilled water (1:2) respectively, after (4) minutes washed them with sterile distilled water 5-7 times, the seeds placed on sterile filter paper. ten seeds were planted in each of the glass tubes containing MS medium (Murashige and Skoog, 1960). The seeds were immediately exposed to the magnetic field of (400 mT) generated by a winding coil magnet at 22 temperature conditions for different periods of time (0, 30, 60, 90, 120, 150) minutes, Then the seeds were incubated at $22 \pm 2^\circ\text{C}$ with photoperiod at 16h light / 8h dark / day. The percentage of seed germination was calculated after 7

days, the average length of the shoot and root system was recorded after three weeks. A winding coil magnetic field device from the Physics department/ Sciences college, is shown in the figure (1).



Figure (1): Magnetic field device used for exposure Seed, Explant and Callus cultures

Exposing Explants to the magnetic field

Seedlings were taken at the age of 21 days and cut using sterile scissors. Different segments were cultured on solid MS medium supplemented with (10^{-6}) M of 2,4-D (Albaker, 2002). Then it was exposed directly to the winding coil magnet (400 mT) of MF intensity, for successive periods of time (0, 30, 60, 90, 120, 150) minutes, at 22°C temperature room conditions. The glass tubes containing explants were incubated at 22°C with photoperiod at 16h light / 8h dark / day. The response of callus induction from explants was recorded after 7 and 14 days.

Exposing the callus of *N. sativa* to the magnetic field

One gram of callus was subcultured in each glass tube on MS media containing (10^{-6}) M of 2,4-D and exposed directly to a magnetic field in the same way as before.

Callus initiation and measurement of fresh weight

The response of callus initiation from exposing explants to M.F was recorded after 20 days, also fresh weight of the callus was recorded for after 30 days.

Callus vitality

The vitality of the callus exposed to the magnetic field for all treatments and control sample was measured based on the ability of the live callus cells to reduce triphenyltetrazolium chloride to red formazan according to (Towill and Mazur, 1975) method.

Measurement of protein in callus cultures

The total protein content of the callus exposed to the magnetic field was measured for all parameters and control according to (Laurie *et al.*, 1951), based on the standard curve of bovine serum (albumin).

measurement of Total soluble sugars

Total soluble sugar was determined following method given by (Herbert *et al.*, 1971) to extract and quantify the amount of sugars dissolved in Callus for all treatments exposed to magnetic field and control at 30 days of age and by using the standard curve for glucose.

Determination of proline content

The amount of proline was estimated in 30-day-old callus samples exposed to magnetic field and control according to the method followed by (Bates *et al.*, 1973), according to absorbents at 520 nm using spectrophotometer.

Measurement of Catalase activity

The activity of CAT enzyme was measured relay on (Goth, 1991) method based on the intensity of light absorption by the yellow complex formed by the reaction of hydrogen peroxide with ammonium molybdate at 405 nm.

Measurement of Peroxidase activity

The activity of POD enzyme was measured according to (Kim and Yoo, 1996), a colorimetric method based on the formation of a colored product whose optical absorbance can be measured at 470 nm.

Experimental design and statistical analysis

The data analyze according simple and factorial experiments system, using Complete Randomize Design. The treatments are took same the letter did not differ between them significantly under 1% level probability.

Result and Discussion

Effects of magnetic field on seed germination and growth parameter

Magnetic field effect on plant in various way according to many factors as intensity, duration and application method. (Mroczek-Zdyrska *et al.*, 2021) also the plant genetic structure of the species, age and severity and environmental conditions such as humidity and temperature can effects on plant metabolism (Cakmak *et al.*, 2010). The results obtained from exposing the seeds to magnetic field (400 mT) at different times showed a significant effect that reduced the mean germination time and the percentage of germination began to increase with the increase in the exposure time up to 90 minutes which gave maximum value (96%), then the percentage began to decrease when the exposure time increased

to 120 and 150 minutes , the last one exhausted maximum time to germinate and gave minimum value of germination percentage (82 %), figure (2).

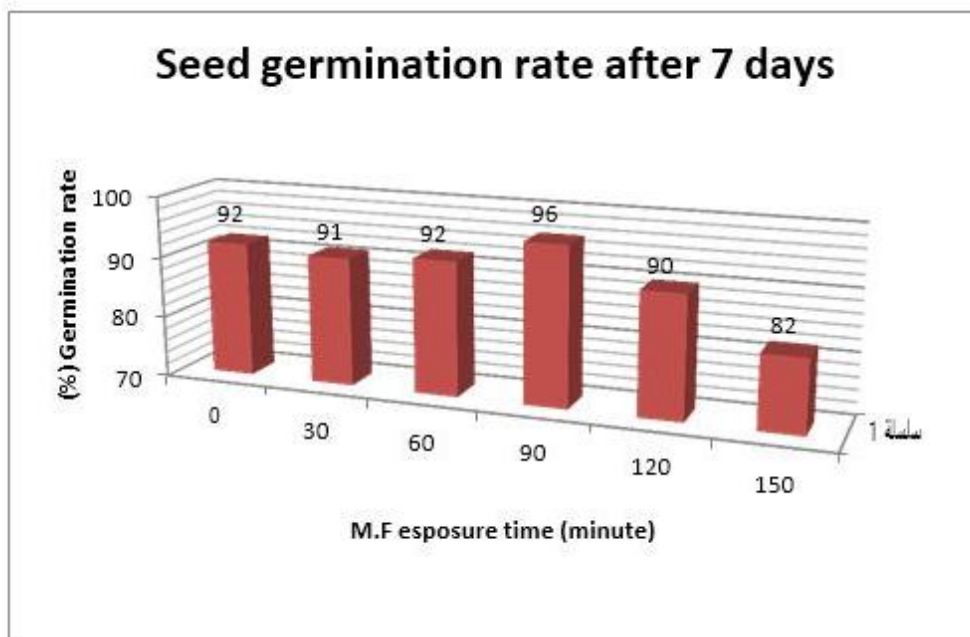


Figure (2): Effects of magnetic field on seed germination

Also the results in Table (1) demonstrated that best exposure time to the magnetic field that stimulate seedling growth was (90) minute after 21 days from seed culture, the average lengths of roots and shoots of seedlings reached (7.5, 9.5)cm respectively, while (150)minute exposure period had a negative effect on the seedlings growth and gives the lowest average lengths of roots and shoots (4.5, 5.5)cm which was less than the control treatment, figure(3). This significant stimulatory effect of Magnetic field on the seed germination and seedling growth are in contrast with some other researches . (Kargarshooraki and Majd, 2016) reported that magnetic field application enhanced *Nigella sativa* seed performance, germination rate, root length, shoot length, , stomatal density. According to (Pourakbar *et al*, 2012) This is duoto the increase of α -amylase, dehydrogenase and protease enzymes in *Nigella sativa* M.F treated seeds

M.F exposure time(minute)	Mean root length(cm)	Mean shoot length(cm)
0	5.5 c	7.5
30	7.5 a	8
60	6 b	8.5
90	7.5 a	9.5
120	5 c	7.3
150	4.5 c	5.5

Table (1) : Effect of M.F on root and shoot length of *N. sativa* seedling after 21 days from culture

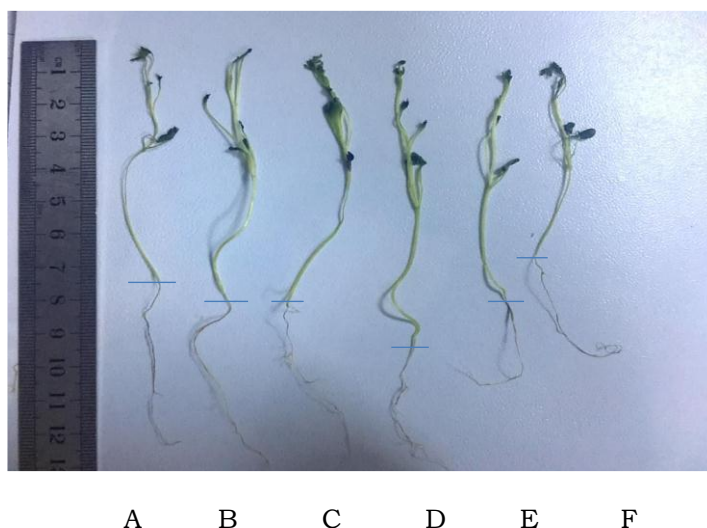


Figure (3): Effect of M.F on seedling growth :

(A) 0 minute M.F

(B) 30 minute M.F

(C) 60 minute M.F

(D) 90 minute M.F

(E) 120 minute M.F

(f) 150 minute M.F .

The positive effect of M.F on seedling growth maybe a reflection of nitrogen metabolism and photosynthesis enhancement, according to (Javed *et al.*, 2011; Anand *et al.*, 2012) study on maize seedlings . Another study showed that the Chlorophyll content was increased in leaves of M.F treated soybean plants. plants grown from M.F- treated seeds had higher reducing power with higher efficiency of electron transport and more active reaction centers, additionally recorded increase of ATP supply through the enhancement in (Baghel *et al.*, 2018).

Effect of magnetic field in callus induction from explant

The explants of *Nigella sativa* seedlings which exposed to the magnetic field for different periods of time showed a clear and positive variation superior to the control sample. Data in table (2) showed that best treatment stimulate callus initiation was observed in exposure to M.F for (30) minute with (99%) callus initiation after (20) days compare with stander treatment which un exposure to M.F (0)minute induced callus initiation with (92.7 %). MF stimulates many processes as increasing mitotic activity in meristematic cells of the common bean (Mroczek-Zdyrska *et al.* , 2021)

M.F exposure time(minute)	Callus initiation(%)
0	92.7
30	99.0
60	97.0
90	92.0
120	88.3
150	86.0

Table (2) : Effect of M.F in stimulation of callus induction from *Nigella sativa* segments after 10 days

also observed other treatment of M.F exposure time , higher or less than (30) minute, induced callus initiation but with less percentage figure (4) , it is worthy to mention, these results certified the great role of M.F and exposure times in stimulate induction and growth of callus , this callus was friable and bright green.

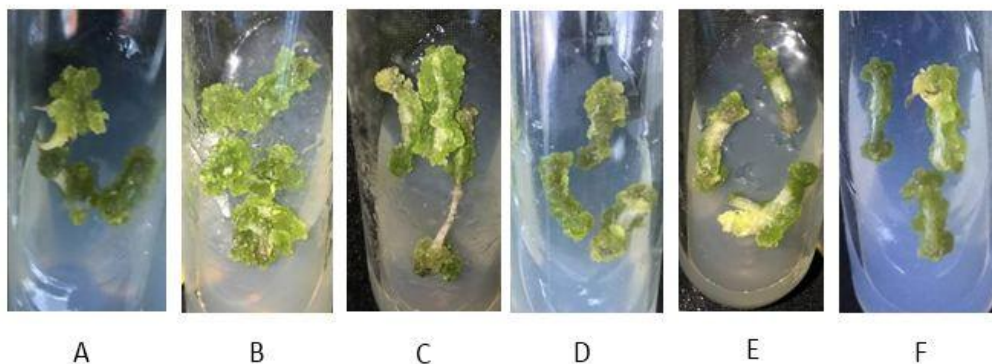


Fig (4): : Effect of different times of M.F on callus induction from *Nigella sativa* segments :

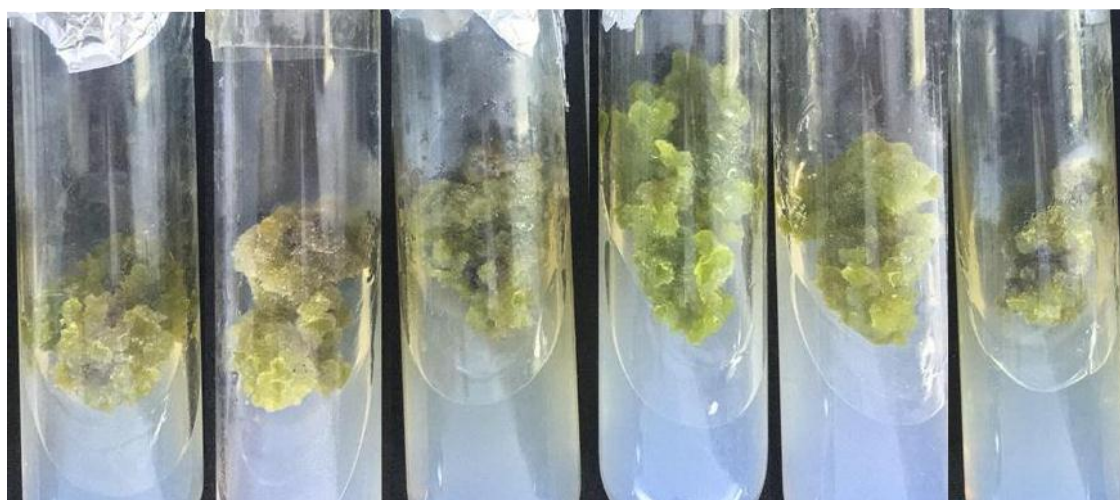
(A) 0 minute M.F (B) 30 minute M.F (C) 60 minute M.F
(D) 90 minute M.F (E) 120 minute M.F (f) 150 minute M.F .

Determiration of callus fresh weight

After exposing the callus to M.F noticed a significant increase in fresh weight of callus ,results in the table (3) showed a successful growth of callus culture specially at (90) minute under (400 mT) M.F intensity which produced 99.25% with 5 g after 30 days as well as result in table (3) demonstrated that increase and decrease at the time of exposure to M.F about 90 minute cause a increase in callus growth and fresh weight. It is worthy to mention, that the exposure of callus cultures to M.F enhanced callus growth and fresh weight, compare with stander sample (which was not exposed to M.F . this positive result perhaps due to the role of magnetic field in stimulating the growth process by reducing the resistance of the cell walls to the elongation of cells (MsQueen and Cosgrove, 1994) .Callus texture become more soft and greenish in color figure (4) , these results refer to the great role of M.F in stimulate induction and growth of callus. (obid Altimimi , 2012) found that the influence of (200 mT) M.F on *Nigella sativa* callus gave the highest fresh weight .

MS + 10 ⁻⁶ M 2,4-D	M.F exposure time (minute)	Callus growthafter 20 days (%)	fresh weight after30 days (g)
	o	92.0	1.9 c
	30	93.0	2.0 c
	60	94.2	2.3 c
	90	99.2	5.3 a
	120	98.6	4.7 a
	150	86.3	3.5 b

Table (3) : Effect of magnetic field on callus growth and fresh ewight



A B C D E F

Figure (5) : Effect of M.F on callus growth :
 (A) 0 minute M.F (B) 30 minute M.F (C) 60 minute M.F
 (D) 90 minute M.F (E) 120 minute M.F (f) 150 minute M.F .

Effect of M.F in callus vitality, protein and dissolved sugars

According to the aforementioned chemical methods and based on the standard curves, the measurement of the protein and carbohydrate concentration in the callus was calculated after 25 days from exposure to the magnetic field for different periods of time and compared to the control sample . Results in figure (6) showed that the highest value for callus vitality, protein and carbohydrates concentration was in callus cultures treated with (400 mT) M.F for (90) minutes. Where the value of the vitality ranged between (56.0 - 95%) for all cultures exposed to M.F, and it outperformed the callus cultures that were not exposed to the magnetic field, in which the vitality reached (48.7) % . As for the

estimation of the amount of total protein in the callus culture , it ranged between (28-95) mg/ml, where the callus cultures exposed to the magnetic field for 90 minutes outperformed the rest of the cultures exposed to different periods, especially the culture that were not exposed to the magnetic field, in which the amount of total protein reached 28 mg /ml . This indicates that exposing the callus cultures to the magnetic field caused an increase in protein synthesis. When estimating the amount of sugars in the callus cultures exposed to the magnetic field for several times, the results showed a clear discrepancy in the treatments, which ranged between (34_122) mg/g , as the lowest amount was when the callus cultures were exposed to the magnetic field for 30 minute , and the highest estimate of the dissolved sugars reached when exposed for a period of time. 90 minutes, This refers to the role of M.F treatments in stimulation the biochemical activity that corresponds with results of (Asghar *et al.*, 2016) when soybean seeds exposed to a magnetic field of different intensity, noted a clear effect of

M.F on the content of protein, sugars, protein and antioxidants system, as well as M.F facilitating the movement of water inside the cells in contact with the food medium, by improving the permeability of the cell membrane and increasing the ion exchange through it resulting from the change in osmotic pressure inside and outside the cell (Reina and Faschal, 2001). It can also be associated with the effect of magnetism on ion and water absorption, hormonal balance, cytoplasmic flow, plant cell growth processes related to intracellular mass and the regulation of charge transfer. (Belyvaskaya, 2001), this lead to metabolites accumulation and antioxidant in plant under the abiotic effect of M.F (Hassanpour *et al.*, 2019).

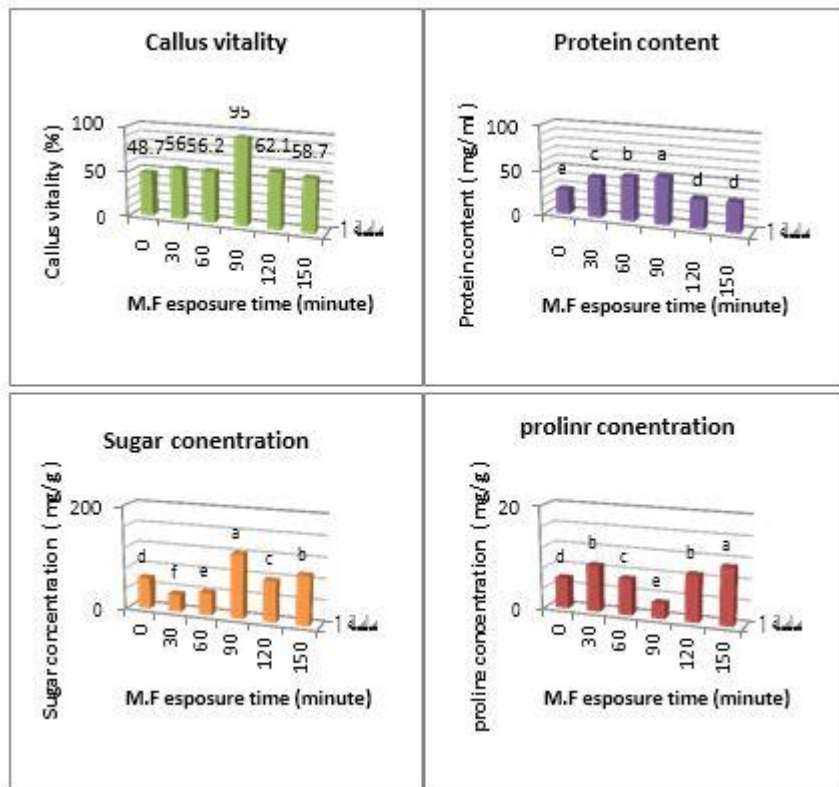


Figure (6): Effect of M.F in biochemical indicators of *N. Sativa* callus culture after 25 days from exposure

Antioxidant activities (non-enzymatic and enzymatic)

The positive influence of magnetic effect on biomass and development was related with the stimulation of the -ROS scavenger by enhancement of enzymatic and non-enzymatic antioxidant system (Latef *et al.*, 2020).

non- enzymatic antioxidant activities (Proline content)

Proline, one of the most important endogenous antioxidant, suggested to protect the structural and functional properties of cell membrane systems under stress, by preventing oxidative damage that caused by free radical (Baxter *et al.*, 2014) (kaur and asthir, 2015), and Modulation of calcium signaling.

(Radhakrishnan,2019). Data in table (4) demonstrated the significant increase in proline content when callus exposed to M.F comparing with control . this result corresponding with (Abdullahi *et al.*,2019) study.

M.F exposure time(minute)	Proline concentration(μ /g)
0	6 b
30	9 d
60	7 c
90	3 e
120	9 b
150	11 a

Table (4): Proline level in *Nigella Sativa* callus responding to M.F effect

Catalase (CAT) protects the cell from toxicity by converting H₂O₂ -which produced by SOD- to water and oxygen molecule, dismutation reaction (Sahebjamel *et al.* , 2007). Peroxidase (POD) utilizes pyrogallol and guaiacol as a substrates for H₂O₂ detoxification. Statistical analysis results in Table (5) generally indicate that exposing the callus of the *Nigella sativa* to different time periods of magnetic field led to a significant increase in the catalase and peroxidase enzymes activity with increasing vperiods of exposure to the M.F compared to the control treatment, and the obtained

Results confirmed that the exposure time increased to 150 Minute cause a significant increase in the catalase and peroxidase enzymes as the enzymes activity was (0.95, 0.739) for CAT and POD respectively after 25 days. Present result indicate the role of M.Fas protective system depending on exposure time and intensity , similar improvement of antioxidant enzymes in plant cells by M.F indicated in lettuce (Latef *et al.*, 2020) and lemon balm (Ulgen *etal.*, 2021).

M.F exposure time (minute)	CAT (U/ml)		POD (U/ml)	
	After 5 days	After 25days	After 5 days	After 25 days
0	0.673 e	0.672 e	0.426 f	0.427 f
30	0.690 e	0.750 d	0.489 ef	0.620 bc
60	0.733 d	0.811 c	0.519 de	0.649 bc
90	0.708 de	0.838 bc	0.532 de	0.680 ab
120	0.745 d	0.880 b	0.528 de	0.690 ab
150	0.749 d	0.950 a	0.590 cd	0.739 a

Table (5): The effects of SMF treatments on the activity of antioxidant enzymes (Catalase and Peroxidase) of *Nigella sativa* callus

Conclusion

The data presented in current report showed that the magnetic field is highly efficient in stimulation the biochemical processes of *Nigella sativa* seedling and callus , it is worthy to mention that the exposure time of magnetic field has been significant role in stimulating these biological activities, seed germination ,

seedling growth, callus initiation and primary metabolite content, and promote plant tolerance against stress (antioxidant capacity). This indicates the importance of magnet technology in tissue culture and vegetative propagation of medicinal and economic plants, as the most important of which is the *Nigella sativa*. Through this research, we encourage the expansion of understanding and exploitation of magnet mechanics on plant cell.

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