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Effect of Magnetic Field on Peroxidase Activity and Growth of *Panicum miliaceum* L. Seeds

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ABSTRACT

In latest years in agricultural sector, physical techniques based on magnetic field are being designed. The present work studied the impact of magnetic field on the germination of fresh and partially-aged *Panicum miliaceum* L. (PM) seeds. The germinated PM seeds were categorized into 6 groups. Groups 1,2 and 3 were fresh seeds, and Groups 4, 5 and 6 (LV30%) were left for 6 days to lose about 30% of their viabilityLV30% seeds. Groups 1 and 4 were normal unexposed seeds. Groups 2 and 5 were seeds exposed to magnetic field intensity of 100mT, while those of 3 and 6 to 200mT for 60 minutes. Germination was monitored on days 2, 5, 7, 10, 12, 14 and 16. The effect of magnetic field on the % germination, growth characteristics of radical and shoot, protein concentration and peroxidase activity in fresh and LV30%were studied. Both the exposed and unexposed seeds lost their viability by 16 days of ageing in fresh and 14 days in (LV30%). Exposed seeds to magnetic fields 100 and 200 mT revealed significant increase in the length of root and shoot compared to normal unexposed seeds. The magnetically exposed aged seeds had significantly higher protein than in the unexposed aged seeds. The peroxidase activity decreased with ageing and the magnetically exposed seeds showed higher activity than the corresponding aged unexposed normal seeds. The present study suggests the magnetic field could accelerate the germination of PM seeds, accelerate their growth characteristics and increase soluble protein content. In addition the peroxidase activity significantly improved.

Introduction

Physical and chemical regimen used during seed storage play an important role in maintaining seed quality. The quality of stored seeds declines dramatically with temperature and relative humidity in combination. Today's sustainable agricultural systems focus on finding physical and biological technology that is friendly environmental and increase crop yield and seedling vigor. Magnetic field was used as physical treatment to accelerate germination of seeds and it was found that its one of the safe methods in the system of crop production (1).

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The effects of magnetic field have been studied in many researches on plants as physical treatment that can improve the quality of seeds. In addition, the growth and yield of seeds have been improved by pre-sowing the seeds with both magnetic fields (MF) and electromagnetic fields (EMF) (2). Studies reported that stationary magnetic field stimulated the growth of seeds *via* its invasive external germination. Magnetic field of 0.08 T was found to have positive results in stimulating the growth of bean, pea, corn and soy seeds (3).Vashisth and Nagarajan, 2007 have reported earlier that subjecting *Zea mays* seeds to MF of 100mT for two hours and 200 mT for one hour have significantly increased seed germination and improved theirvigor

characteristics (4). In another study, Vashisth and Nagarajan, 2009 showed that magnetically-exposed seeds during ageing revealed decreased leachate conductivity, and increased activity of antioxidant enzymes (peroxidase, catalase and superoxide dismutase) and soluble protein level in comparison with the unexposed seeds. In addition, MF reversed the deteriorated status of the aged seeds regarding seedling length and dry weight compared to normal control aged seeds(5).

Though the mechanism of the effect of static magnetic field on the plant physiology is not clear, yet the responses of the plant depends on the duration of exposure to the magnetic field and its intensity, as well as the plant species (6). Aladjadjiyan, 2010 reported that the paramagnetic properties of some atoms contained in the plant pigments and cells may be responsible for the positive response of plants to magnetic fields. He added that the different magnetic properties of plant molecules convert the magnetic energy to which they expose to different form of energy consumed by specific organelles in the plant cells and activating them (7).

The present research was conducted to study the effect of the magnetic field on the germination characteristic and the activity of peroxidase enzyme as well as soluble proteins in *Panicum miliaceum* L. (PM) seeds.

Materials and Methods

Plant materials and crude extract preparation

PM seeds were purchased from the local markets and classified at Department of Life Scinces,Faculty of Education, University of Anbar, Iraq (Reference number GC4665). The fresh seeds were ground using mortar and pestle and the ground powder was homogenized in 20 mM Tris-HCl buffer, pH 7.2 usingOmni Tissue Homogenizer (TH115,USA). The homogenate was then centrifuged at 10,000 xg at 4^{o}C for 20 minutes, and then the supernatant was collected as PA crude extract (8).

Magnetic treatment of partially-aged seeds

PM seeds were placed over sieve in desiccators containing water at its bottom to accelerate the ageing of MP seeds at 40^{o}C and 95-100% humidity. Seeds (LV30%) were left for 6 days to lose about 30% of their viability. The seeds were then subjected to two different intensities of magnetic field, 100mT for 120 minutes and 200mT for 60 minutes (5).

Generating magnetic fields and experimental pattern

The magnetic field was generated following the protocol conducted by Florezet et al. Using ring magnets with magnetic induction values 100mT and 200mT, the magnetic field was produced. Following the instructions issued by the International Seed Testing Association (9), the germination tests were performed. Seeds were placed between two layers of moist filter papers in 4 replications, 25 seeds each. To reduce surface evaporation, the seeds were then wrapped in wax paper and placed in an upright position with 25 cm distance apart. The seeds were kept under adjusted conditions of 12 h light/12 h dark artificial light cycle, while the day temperature was 23 ± 3^{o}C and night temperature 9 ± 2^{o}C.The germinated PM seeds were categorized into 6 groups. Groups 1,2 and 3 were fresh seeds, and Groups 4, 5 and 6 LV30% seeds. Groups 1 and 4 were normal unexposed seeds. Groups 2 and 5 were seeds exposed to magnetic field intensity of 100mT, while those of 3 and 6 to 200mT. Germination was monitored on days 2, 5, 7, 10, 12, 14 and 16 and the seeds were counted as germinated when their radical measured at least 2 mm length. The percentage of germination of all the seeds was calculated with respect to control unexposed seedlings according to the following formula:

%Germination =**(**Number of seeds that germinated / Number of seeds planted)*100

Protein concentration measurement

The protein content of the PA crude extract was assessed following Bradford method and using bovine serum albumin as a standard (10).

Proxidase activity assessment

Following the protocol of Miranda et al, 1995(11), the experiment of the present study was conducted. Briefly, a quantity of the enzyme (50µL)was mixed with the mixture solution, 20 mM H2O2, 50 mM guaiacol, 0.1M sodium acetate buffer, pH 5.5 to a final volume of 1 mL. The change in absorbance due to guaiacol oxidation was recorded at 470 nm with 30 second interval.The relationship between the absorbance and

time was plotted until 3minutes and the activity of peroxidase was measured following the formula below:
Enzyme activity (U/mL) =(3.05/(0.05 * 1* 6.4)) * slope
Where: 3.05 = the total volume of reaction solution (mL)
0.05= enzyme solution volume (mL)
1 = the light path (cm)
6.4=the absorbance molarity (ε) of oxidizing guaicol molecule ($mM^{-1}cm^{-1}$)

Statistical analysis

All results were recorded as mean ± SD. The statistical analysis of the differences between groups was performed based on Tukey analysis and the one way ANOVA test (SPSS ver.14). A *p*-value less than 0.05 were recorded as being significant.

Results and discussion

Germination

Change in % germination of exposed and unexposed seeds of PM to magnetic field kept at accelerating ageing condition are shown in Figures**1 a**,**b**and **2**. After germination, the seedling growth was observed as compared to control. Both the exposed and unexposed seeds lost their viability by 16 days of ageing in fresh and 14 days in (LV30%).Initial germination percentage was marginally higher for the exposed seeds as compared to the unexposed control and remained higher only up to 7 days of ageing. After 10 days the germination percentage reduced sharply similar to control. Therefore the longevity of the seed viability does not change by the application of magnetic treatment.

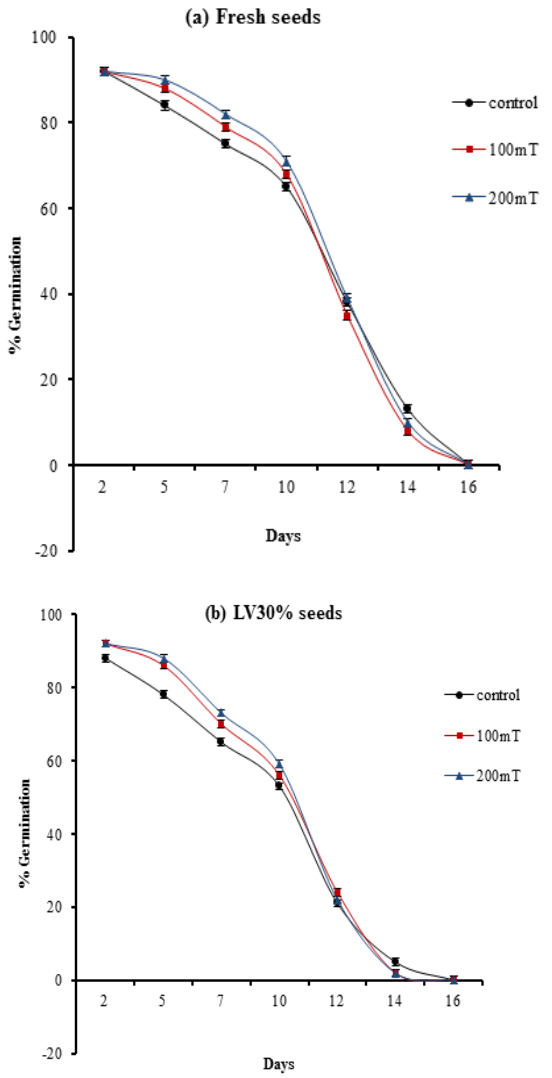
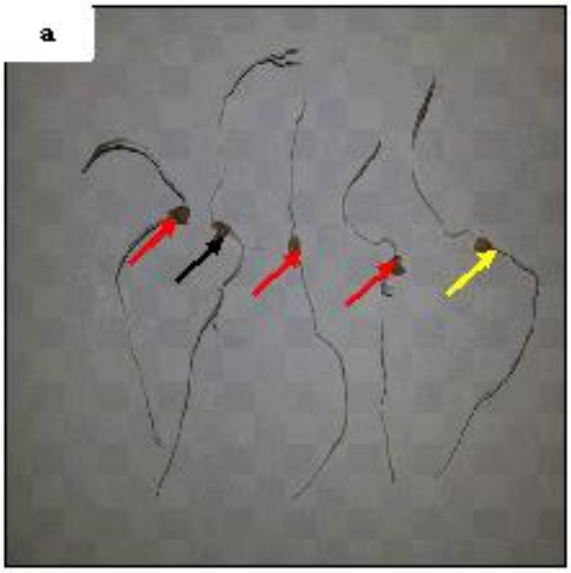


Figure 1: Effect of magnetic field intensity on PM seeds % germination.



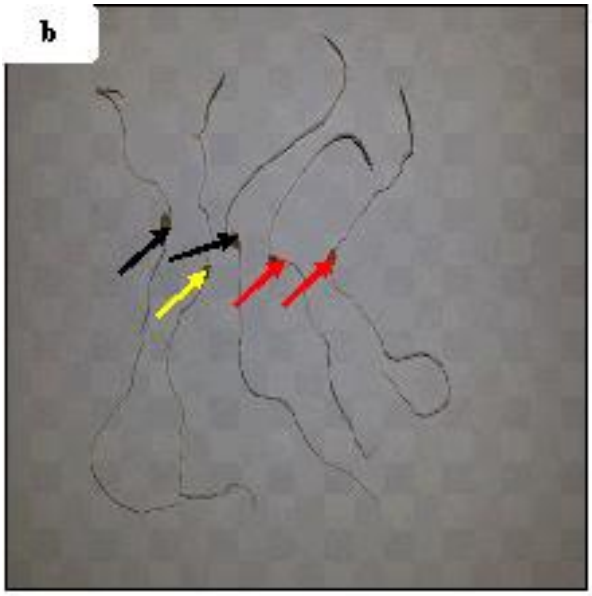


Figure 2: Effect of magnetic field on the germination of PM seeds. (a) Fresh seeds of PA. (b) LV30% seeds of PA which lost 30% of their viability. Control seeds (red arrow), seeds exposed to 100mT (yellow arrow) and seeds exposed to 200 mT (black arrow).

Growth characteristics of PM seeds

Pre-sowing magnetic treatment significantly enhanced the growth characteristics such as shoot and root length as shown in Table **1**. Results showed that the exposed seeds to magnetic fields 100 and 200 mT revealed significant increase in the length of root and shoot compared to normal unexposed seeds. The stimulatory effect of the application of different magnetic doses on the germination is in agreement with that obtained by other researchers. Florez et al, observed an increase in the initial growth stages and an early sprouting of rice and maize seeds exposed to 125 and 250 mT stationary magnetic fields (12). Martinez et al. (3) observedsimilar effects on pea and lentil seeds magnetically treated. Alexander and Doijode (13) reported that pre-germination treatment improved the germination and seedling vigor of low viability rice and onion seeds. Similarly, Fischer *et al.* (14) observed higher germination and growth of sunflower as compared to untreated seed samples.

Soluble protein concentration

The effect of magnetic field on the soluble protein content measured from the crude extract of fresh and LV30%PM seeds on 5,7,10 days are illustrated on Figure **2a** and **b**. Soluble protein was significantly decreased during artificial ageing. The magnetically exposed aged seeds had significantly higher protein than in the unexposed aged seeds. Magnetic field application could induce the protein synthesis in plant and it might be the reason of more accumulation of protein (15). Results of other studies showed that protein content decreased in the treated plants in comparison with the control group in *Helianthus annuus* L (16). Weak magnetic field causes intensification of protein synthesis and disintegration in plant roots(17).

Peroxidase activity

The results of the magnetic field effect on the activities of peroxidase in the present study are illustrated on Figure **3a** and **b**. The data showed that the peroxidase activity decreased with ageing. The magnetically exposed seeds showed higher activity than the corresponding aged unexposed normal seeds. Loss of seed viability during accelerated ageing at 40°C and 100% RH (LV30%) was associated with a decrease in the activity of peroxidase in both magnetically treated and untreated control seeds compared with fresh seeds. Similar decrease in the activities of anti-oxidant enzymes have been reported in peanut seeds (18) and soybean (19). Effects of magnetic field have been related to uncoupling of free radical processes in membranes and enhanced redox status generation. It has been experimentally proven that magnetic field can change activities of some scavenging enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione transferase (GT), peroxidase (POD), ascobtate peroxidase (APX), and polyphenoloxidase (POP) (20, 21). In addition, it was reported that the exposure to increased magnetic field causes accumulation of reactive oxygen species and alteration of enzyme activities. Moreover, it was suggested that apoplastic constituents may work as potentially important redox regulators sensing and signaling magnetic field changes and weak magnetic is involved in antioxidant-mediated reactions in the

apoplast, resulting in overcoming a possible redox imbalance (22).

Conclusion

The present study suggests the magnetic field could accelerate the germination of PM seeds, accelerate their growth characteristics and increase soluble protein content. In addition the peroxidase activity significantly improved.

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Conflicts of Interest

No conflicts of interest declared.

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Table **1**: Effect of magnetic field on the root and shoot length of PM seeds during germination.

Parameter	Root length (cm)				Shoot length (cm)			
	LV30% seeds		Fresh seeds		LV30% seeds		Fresh seeds	
Treatment	Exposed (200 mT)	Exposed (100 mT)	Normal uesposed	Normal unexposed	Exposed (200 mT)	Exposed (100 mT)	Normal uesposed	Exposed (200 mT)	Exposed (100 mT)	Normal unexposed
Day 2	2.10 ± 0.24*#	1.78 ± 0.23*	1.48 ± 0.18	1.20 ± 0.12	1.10 ± 0.12*#	0.80 ± 0.15*	0.60 ± 0.09	0.90 ± 0.12*	0.80 ± 0.13*	0.49 ± 0.16
Day 5	5.59 ± 0.21*#	5.03 ± 0.25*	3.78 ± 0.32	4.08 ± 0.15	2.90 ± 0.16*	2.70 ± 0.23*	2.40 ± 0.17	3.50 ± 0.16*	3.30 ± 0.18*	2.10 ± 0.26
Day 7	6.69 ± 0.21*#	6.18 ± 0.37*	4.70 ± 0.27	5.19 ± 0.17	3.70 ± 0.18*	3.60 ± 0.27*	3.30 ± 0.22	4.70 ± 0.23*	4.50 ± 0.19*	3.60 ± 0.30
Day 10	8.24 ± 0.25*#	7.50 ± 0.37*	5.80 ± 0.31	6.41 ± 0.18	4.90 ± 0.19*	4.80 ± 0.26*	4.10 ± 0.28	5.80 ± 0.24*#	5.40 ± 0.25*	4.90 ± 0.32
Day 12	9.33 ± 0.35*#	8.45 ± 0.30*	6.50 ± 0.31	7.08 ± 0.18	5.80 ± 0.31*	5.60 ± 0.28*	4.60 ± 0.27	6.40 ± 0.26*#	6.10 ± 0.29*	5.40 ± 0.25
Day 14	9.83 ± 0.36*#	9.16 ± 0.52*	7.10 ± 0.43	7.79 ± 0.23	6.60 ± 0.27*#	6.20 ± 0.31*	5.10 ± 0.32	6.70 ± 0.32*	6.50 ± 0.34*	5.70 ± 0.31
Day 16				8.03 ± 0.28				7.10 ± 0.28*	6.80 ± 0.32*	5.90 ± 0.37

Data are represented as Mean ± S.D (n=10), where P<0.05 is considered significant. *P<0.05 compared to the normal unexposed seeds. #P<0.05 compared to seeds exposed to 100 mT.

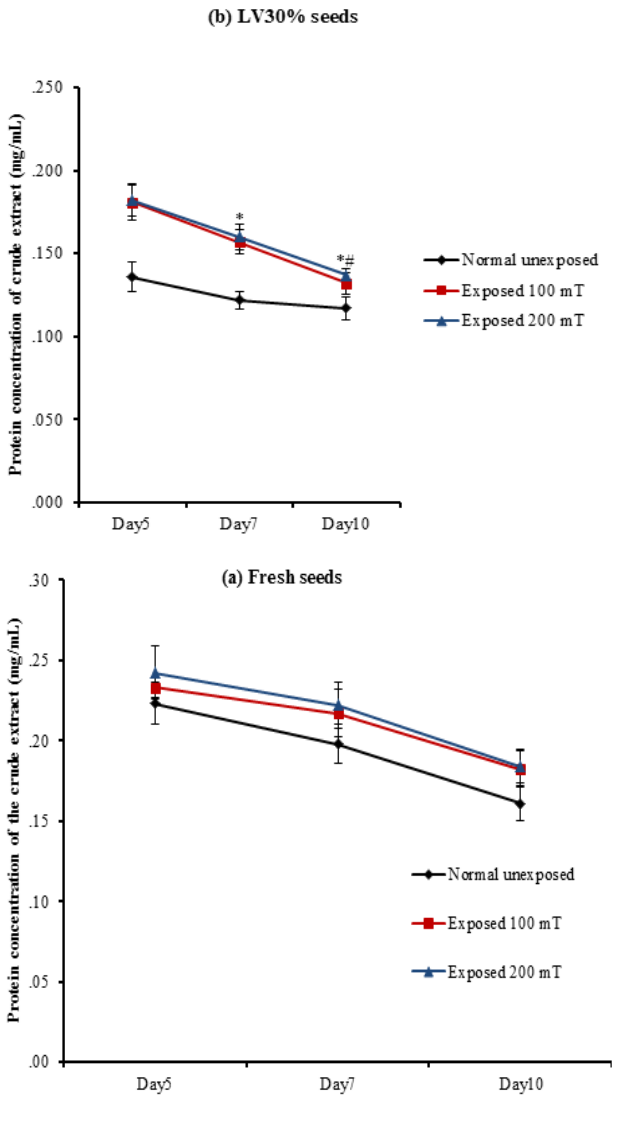

Figure **2**: Effect of magnetic field intensity on the soluble protein concentration of the crude extract of PM seeds. (a) Fresh seeds of PM. (b)LV30% seeds of PM which lost 30% of their viability.Data are presented as Mean ± S.D (n = 10). **P*<0.05 compared to the normal unexposed seeds on day 5, 7 and 10.$^{\#}$*P*<0.05 compared to seeds exposed to 100 mT on day 5, 7 and 10.

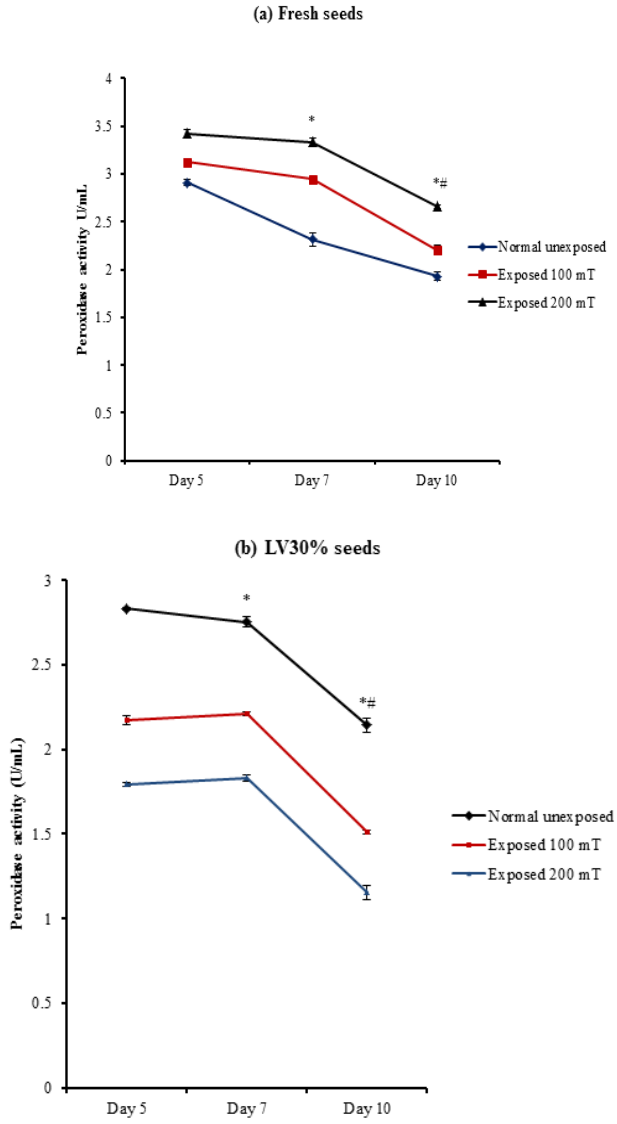

Figure 3: Effect of magnetic field intensity on the peroxidase activity of PM seeds.Data are presented as Mean ± S.D (n = 10). *P<0.05 compared to the normal unexposed seeds on day 5, 7 and 10. #P<0.05 compared to seeds exposed to 100 mT on day 5, 7 and 10.