Magnetic field effects on seed germination and activities of some enzymes in cumin

Majid Asadi Samani[1]*, Latifeh Pourakbar[2] and Nafiseh Azimi[3]

[1] Medical Plants Research Center, Shahrekord University of Medical Sciences, Sharekord, Iran.
[2] Department of Biology, Faculty of science, Urmia University, Urmia, Iran
[3] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

*** Corresponding author.** Tel.: +983813349509; E-mail address: Biology_2011@yahoo.com, PO Box 88155-137, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Abstract: Failure of germination among Apiaceae family seeds, e.g. cumin (*Cuminum cyminum*), has been reported by many investigators. Until now few works have been done on seed germination and seedling growth of cumin. In this research the effects of the exposure of cumin seeds to magnetic fields on seed germination, early growth, and enzyme activity have been studied under laboratory conditions. Seeds were magnetically exposed to one of three magnetic field strengths, 25, 50 and 75 mT for different periods of time (15, 30, and 60 min). The germination test was performed according to the guidelines issued by the International Seed Testing Association. Enzymes related to the germination process in magnetically exposed and unexposed germinating cumin seeds were assayed after 24 hours of imbibitions in distilled water. Exposure of cumin seeds to different intensities of magnetic fields prior to germination significantly increased germination-related characters. The increase in germination, speed of germination, shoot length, root length, total seedling length, seedling fresh weight, and seedling dry weight was , respectively, 14–17%, 14–57%, 8–27%, 25–62%, 16–39%, 10–29%, and 17–49% compared to untreated control seeds. The calculated vigor indices I and II also increased by 33–73% and 38–72%, respectively. In germinating seeds, enzyme activities of α-amylase, dehydrogenase, and protease were significantly higher in treated seeds in contrast to controls and the maximum value was 50 mT for 60 min exposures. The higher enzyme activity in magnetic-field-treated cumin seeds could trigger fast germination and early vigor of seedlings.

[Majid Asadi Samani, Latifeh Pourakbar and Nafiseh Azimi. **Magnetic field effects on seed germination and activities of some enzymes in cumin.** *Life Sci J* 2013;10(1):323-328]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 53

Keywords: Magnetic Field, Cumin, Germination, Enzyme activity

INTRODUCTION

Plants have important role in earth and studying about the environmental factors which affect plant growth is essential. Electromagnetic fields are a part of environmental factors which affect plant so these effects have attracted considerable attention.

The term "electromagnetic fields" covers all the fields emitted by natural and man-made sources. A distinction is drawn between static fields and alternating fields. In the latter case there is essentially a differentiation between extremely low frequency (ELF) fields, such as domestic electricity, and hyper frequency (HF) fields, which include mobile telephones. Electrical fields are produced by a voltage gradient and are measured in volts/meter whereas magnetic fields are generated by any flow of current and are measured in tesla.

Physical methods for pre-sowing treatment of seeds are not only cost effective; they also significantly improve the yield without adversely affecting the environment. They influence the physiological and biochemical process in the seeds, and thereby contribute to greater vigor and improved crop stand. Therefore, physical pre-sowing seed treatment for enhancing the seed performance, if standardized, can lead to commercial application (Tahir et al., 2010).

Magnetic fields have had many uses in ancient and modern society. Pre-sowing seed treatments with magnetic fields have been reported to enhance the performance of crop plants (Vashisth and Nagarajan, 2008). Exposure of seeds to magnetic fields is one of the safe and affordable potential physical pre-sowing treatments to enhance post-germination plant development and crop stand (Vashisth and Nagarajan, 2010).

The effects of MFs on plant growth were observed on seed germination, early growth, root and shoot growth, seedling growth, seed vigor, fresh weights, dry weights, activities of some enzymes and seed yield (Atak et al., 2007; Balouchi and Sanavy, 2009; Gholami and Sharafi, 2010).

Different plants could be sensitive to different combination of magnetic fields and exposure time (Majd et al., 2009) thus further investigations of the nature of magnetic field stimulation are needed.

Cumin (*Cuminum cyminnum*), from Apiaceae family, is an important medicinal plant which have been planted in Iran and some other countries from ancient times. Although in recent years the production and export of in medicinal plant have been increased in Iran but little work has been done on seed germination and seedling growth of this plant (Khosh-khui and Bonyanpour, 2006).

The aim of this study was to investigate the effects of exposure to static magnetic fields on seed germination and seedling growth as well as enzyme activity of cumin seeds.

MATERIALS AND METHODS

We chose medicinal plant cumin as our research plant. Cumin seeds were exposed to the magnetic field of 25, 50 and 75 mT and exposure times 15, 30 and 60 in a cylindrical shaped sample, made of a nonmagnetic thin transparent plastic sheet. All treatments in the experiments were run simultaneously along with controls under similar conditions.

Seeds have been preliminarily soaked in distilled water for 10 h, presuming that the intra-cell water, due to its magnetic properties, plays a role in the absorption of the energy of magnetic field.

The germination test was performed according to the guidelines issued by the International Seed Testing Association (ISTA Rules, 2007) with slight modifications. After 14 days, germinated seeds were grouped as normal, abnormal seedling, fresh ungerminated and dead seeds. The germination percentage was calculated based on normal seedlings. Ten such seedlings from each replicate were randomly selected to measure root length, shoot length, fresh weights and dried weights.

Seedling vigor was calculated following Vashisth and Nagarajan (2010) as:

Vigor index I = Germination % × Seedling length (Root + Shoot)

Vigor index II = Germination % × Seedling dry weight (Root + Shoot)

Seventy five cumin seeds , divided in to three equal replications , were placed in moistened filter paper in a Petri dish and were incubated at 22 ^{0}C. Seeds were observed daily and considered germinated when the radicle was approximately 2 mm long or more.

The germination speed was calculated according to Eq. (1):

Germination speed = $\sum$ (n/t)

Where n represents the number of newly germinating seeds at the time of t, and t is days of sowing (Vashisth and Nagarajan, 2010).

Enzymes related to the germination process as α-amylases, dehydrogenase and protease in magnetically exposed and unexposed germinating cumin seeds were assayed after 24 hours of imbibition in distilled water at 25 ^{0}C. 1g germinating seeds were taken for enzyme extraction.

The data were analyzed using SPSS software. Two factor analysis of variance (ANOVA) was performed on a split plot randomized complete block design, keeping magnetic field as main plot and duration as sub-plot. The significant levels of difference for all measured traits among magnetic fields, duration of exposure and their interactions were calculated.

RESULTS

Exposure of cumin seeds to different intensities of magnetic fields prior to germination significantly increased germination-related characters , such as germination percentage, speed of germination, shoot and root length, seedling fresh weight, seedling dry weight and seedling vigor indexes. The improvement over untreated control seeds was 14–17% for germination, 14–57% for speed of germination, 8–27% for shoot length, 25–62% for root length, 16–39% for total seedling length, 10–29% for seedling fresh weight and 17–49% for seedling dry weight. The calculated vigor indices I and II also increased by 33–73% and 38–72%, respectively. The percent enhancement of different parameters was not linearly related to magnetic field strength. Among the various magnetic treatments, 75 mT exposure was more effective than others in increasing most of the seedling parameters. Magnetic field exposure time of 15, 30 and 60 min significantly increased germination characteristics. However, exposure for 60 min duration was more effective compared to the others in enhancing seed germination characters.

The interaction of magnetic field and duration of exposure are provided in Fig. 1a–i. Germination percent was significantly higher than control in all treatments and the maximum value was in 50 mT for 60 min and 75 mT for 15 min exposures. Speed of germination was greatest in 50 mT for 30 min and 75 mT for 15 min exposures and the values were significantly higher than the controls in all treatments. Shoot length of seedling and root length of seedling were significantly higher than the controls in all treatments. Seedling fresh weights were significantly higher than the controls in all treatments. Seedling dry weight was greatest in 25mT for 60 min exposure and the values were significantly higher than the controls in all treatments. Seedling vigor I based on seedling length and germination percent showed a trend similar to seedling shoot and root length. Seedling vigor II values based on seedling dry weight and germination percent were highest for 25 mT magnetic fields at 60 min exposure.

Activities of all three enzymes, a-amylase (Fig. 2a), dehydrogenase (Fig. 2b) and protease (Fig. 2c), measured in magnetically exposed seeds maintained significantly higher values than unexposed controls in all stages of germination. Activities of all three enzymes increased significantly until 24 h of imbibition and the maximum value was in 50 mT for 60 min exposures.

DISCUSSION

Exposure of cumin seeds to different magnetic field intensities showed an overall stimulating effect with respect to all germination characteristics. Such enhanced performance of seeds in their germination characteristics have been reported in other plants (Yano, 2001; Aladjadjiyan, 2010).

In this research germination percentage and speed of germination was increased due to magnetic treatment. Similar enhancements in these characteristics due to magnetic field exposure of seeds have been reported in *Festuca arundinacea Schreb*. and *Lolium perenne* L. (Carbonell et al., 2008), and in *Helianthus annuus* (Vashisth and Nagarajan, 2010). Podlesny et al. (2005) confirmed the positive effects of the magnetic treatment on the germination and emergence of both broad bean and pea cultivars. Mo et al. (2011) demonstrated that soybean seeds cultured in an HGMF exhibited increased germination percentage and speed compared to the controls. Seedlings root length and seedling shoot length grown from pre-treated seeds increased in all intensities and duration of exposure. Other researchers (Rajendra et al., 2005; Gholami and Sharafi, 2010) have gained similar results with other seeds. Fresh weight and dry weight of 14-d-old seedlings were higher than control in all treatments. Fischer et al. (2004) reported that sunflower seedlings exposed to magnetic fields showed significant increases in total fresh weight. Also Vashisth and Nagarajan (2010) showed significant increases in dry weight of sunflower seedlings exposed to magnetic fields. Seedling vigor indexes showed significant increases in most intensities and duration of exposure. This result is similar to experiences of Vashisth and Nagarajan (2008) on chickpea seeds.

Change in enzyme activity in seeds treated with magnetic fields reported by Recuciui et al. (2008). It would be desirable to know if magnetic treatment would improve the performance of seed lots with low germination percentages and if it would help counteract the effect of low temperature during germination. In the present study, α-amylase, dehydrogenase and protease activities of the magnetically exposed seeds was greater than that of unexposed seeds. α-amylase is an endohydrolase that cleaves the α-1, 4-linkages between glucosyl residues, liberating smaller glucans. It can use both amylose and amylopectin as substrate, but will not cleave α -1, 6-branch points, nor α-1, 4-linkages in the immediate vicinity of these branch points. Consequently, during α-amylolytic amylopectin degradation, α-limit dextrins are also produced. An increase in germination speed in magnetically treated seeds can be explained as a consequence of increased activity of α-amylase compared with unexposed controls. Bhatnagar and Deb (1978) observed that wheat seeds treated at 150 and 100mT had significantly higher ($P \leq 0.01$) α-amylase activity than the controls. Increase of activity of α-amylase in our study is according to Venkateswar Reddy et al. (2012). They observed that greengram seeds treated for 45 minute had significantly higher α-amylase activity than the controls. Similar to our findings an increase in dehydrogenase activity has been reported in carrot compared with unprimed (Nagarajan et al., 2003). Protease is involved in the degradation of proteins in the germinating seeds and the reduction being initiated by endoproteases, which convert the water in soluble storage protein in to soluble peptides that can be further hydrolyzed to amino acids. In study of Bhardwaj et al. (2012) on cucumber seeds the activities of amylase and protease were greater than the untreated controls. Rajendra et al. (2005), however, reported that, in broad bean protease showed significant decreases in activity on days 2 and 4 of growth at 100 mT, which may be due to the extremely low magnetic fields that were used for exposure in their study compared to the fields used here.

The mechanism of stimulating effect of MF-treatment on seed germination and seedling growth was unknown, but several theories have been proposed, including biochemical changes or altered enzyme activities. Plant processes such as growth, photosynthesis, mineral nutrition, water and transport are quite related to the motion of ca^{2+} ion in cells. Changes in intracellular levels of ca^{2+} and other ionic current density across cellular membrane are important changes which are due to MFs (Florez et al., 2007). The monitoring of the bioluminescence of aequorin in Arabidopsis thaliana showed that combinations of magnetic and electromagnetic fields caused temporary changes in the internal ca^{2+} concentrations and that this phenomenon can be explained according to ion cyclotron resonance (Pazur and Rassadina, 2009). Ions in the cell have the ability to absorb magnetic energy corresponding to specific parameters related to their vibration and rotation energy sublevels. This phenomenon represents a kind of resonance absorption and could explain the stronger effect of applying definite values of magnetic field induction, observed by Martinez et al. (2009). However, most studies have suggested that MFs affect water absorption (Galland and Pazur, 2005; Pang and Deng, 2008). An increase in water uptake rate due to the applied magnetic field has been reported, which may be the explanation for the increase in the germination seed of treated lettuce seeds (Calatayud et al., 2003). Also Mo et al. (2011) suggests that the orientation of growth in a magnetic field may be an important factor in plant germination.

Acknowledgments

This work was supported by Urmia University. We greatly acknowledge Dr H. Sedghi and other colleagues for their support.

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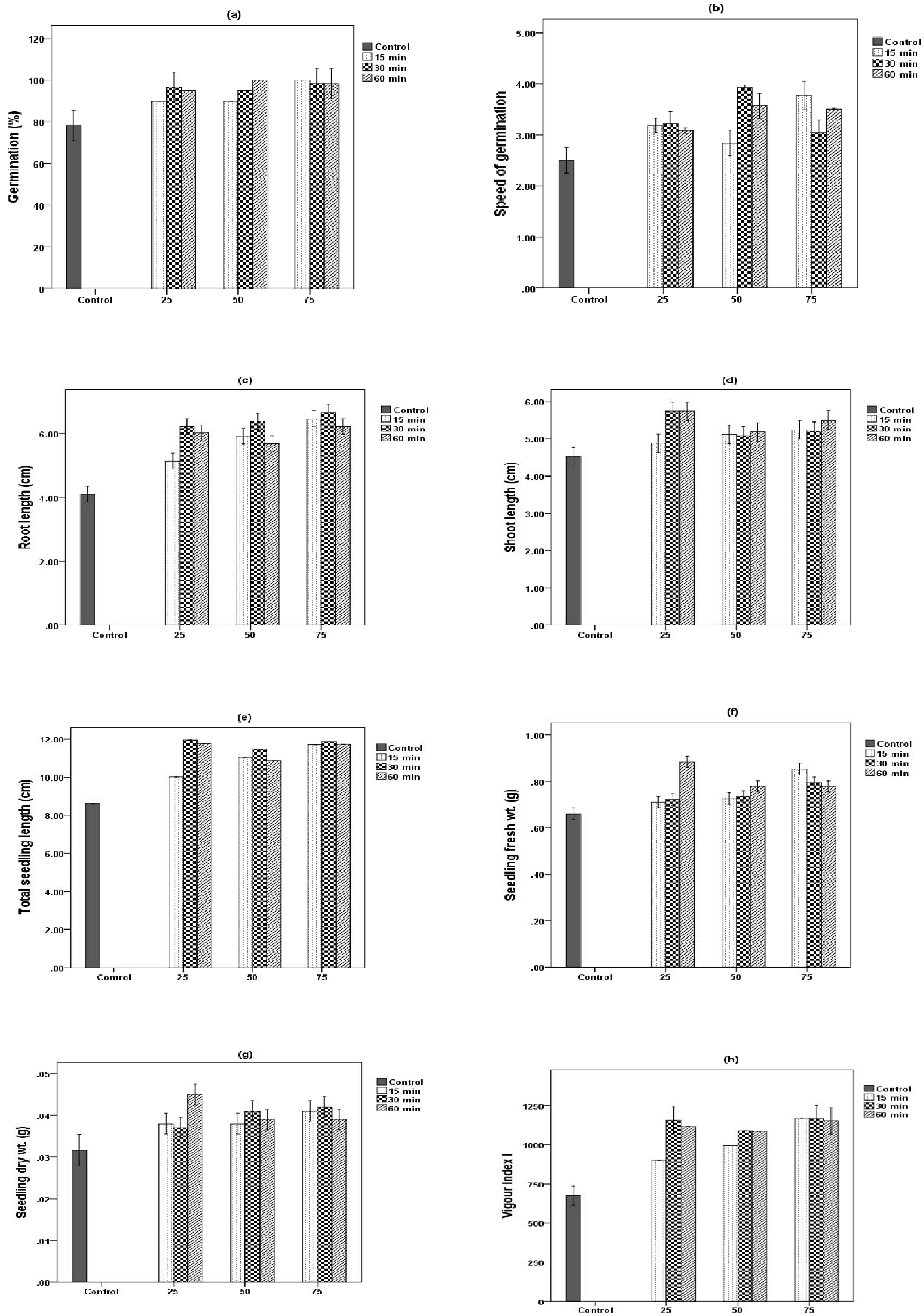
(a)
Germination (%)
Control
15 min
30 min
60 min
Control 25 50 75
(b)
Speed of germination
(c)
Root length (cm)
(d)
Shoot length (cm)
(e)
Total seedling length (cm)
(f)
Seedling fresh wt. (g)
(g)
Seedling dry wt. (g)
(h)
Vigour Index I

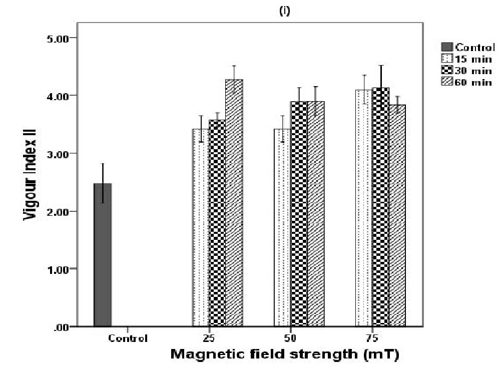

Figure 1. (a–i) Effect of different doses and duration of magnetic field exposure of cumin seeds on (a) percent germination, (b) speed of germination, (c) root length of 14-d-old seedling, (d) shoot length of 14-d-old seedling, (e) total seedling length, (f) seedling fresh weight, (g) seedling dry weight, (h) Vigor index I and (i) Vigor index II.

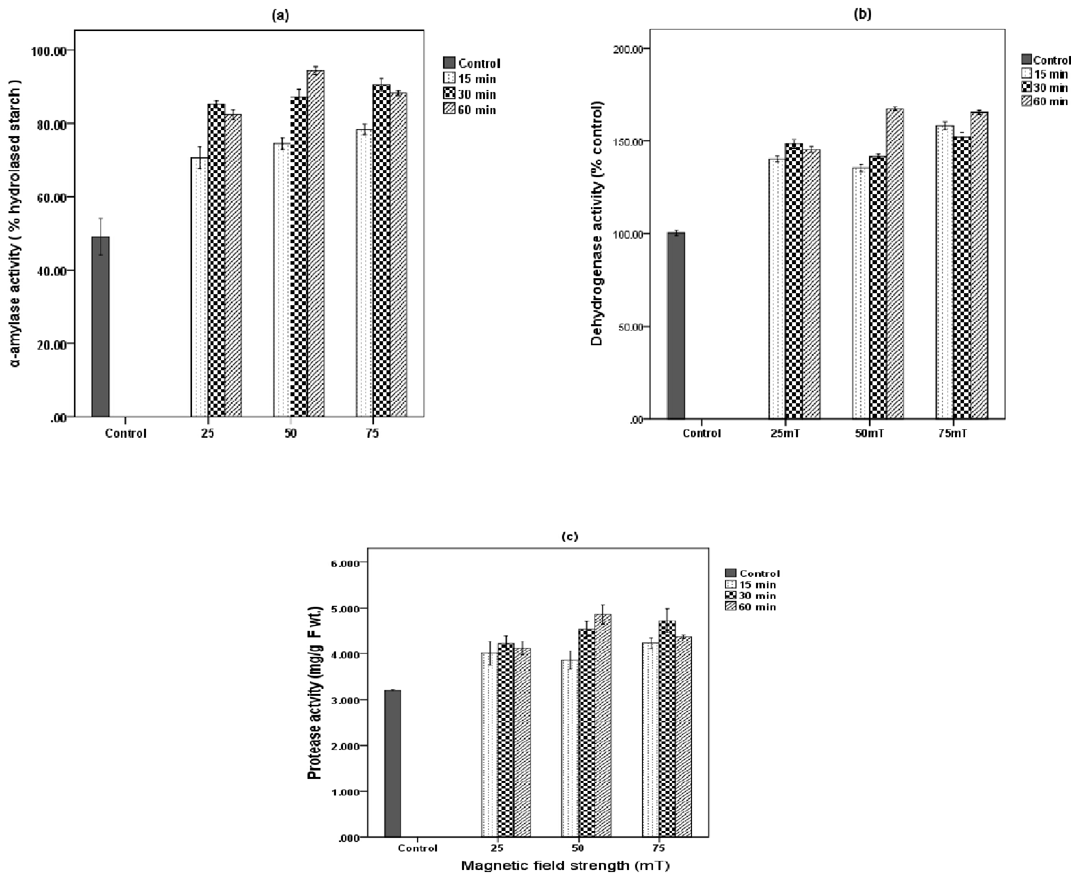

Figure 2. (a–c) Changes in the activities of germination enzymes, (a) a-amylase, (b) dehydrogenase and (c) protease after 24 hours of imbibition in water at 25 C for magnetically exposed and unexposed cumin seeds.