

Influence of Biophysical Priming on Seed Germination and Yield on Two Landraces of Lemon-Balm (*Melissa officinalis* L.)

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Abstract

The aim is to study the effects of physical seed priming on germination and yield of lemon balm. Laboratory and pots experiments were conducted as factorial based on completely randomized design. The treatments are magnetic field with 75 mT for 10 and 15 min durations; laser light with 2 mw.cm⁻² for 20 min; ultrasonic wave with physiotrapy device for 20 min; cs-gamma ray for one hour in lead cell, and control that were applied on two landraces of lemon balm ('Karaj', 'Esfahan'). Then in laboratory with use of special paper and pure water the seeds were cultured in petri-dishes and were putt in germinator with 19°C temperature for 8 days and were counted daily. Some parts of primed seeds were stored in normal condition for 6 months and were cultured in laboratory condition. All the primed seeds were farmed in pots. Three months later the plants were harvested in first blooming level and dry weight and essential oil percentage were measured. Results showed that the influence of various treatments on germination percentage and germination rate were significant. Magnetic field treatment (15 min) showed the highest positive effect on percentage (86%), and rate. The length and weight of seedling in magnetic field treatment were the highest in comparison with other ones. Considering physiological characteristics, the higher Leaf Area Ratio and Leaf Area Index were seen in magnetic field. Effect of different treatments on plant height, biomass dry weight and essential oil concentration was significant.

Keywords: biophysical-priming, essential-oil, germination, lemon-balm, storage

Introduction

Lemon balm (*Melissa officinalis* L.) is a member of *Lamiaceae* family that is native to Europe, central Asia and Iran. Lemony flavor and aroma of lemon balm is due largely to citral and citronellal. Lemon balm contains both vitamin C and vitamin B (Thiamin). The crushed leaves, when rubbed on the skin, are used as a repellent for mosquitos (Shahba *et al.*, 2008). Lemon balm has been shown to improve mood and mental performance. Positive results have been achieved in a small clinical trial involving Alzheimer patients with mild to moderate symptoms (Akhondzadeh *et al.*, 2003). The extract of lemon balm was also found to have exceptionally high antioxidant activity (Dastmalchi *et al.*, 2008). One traditional use of lemon balm tea was in extending age, although this effect has not been proven (Yoon and Kim, 2011). Recent research found a daily dose of lemon balm tea reduced oxidative stress status in radiology staff that was exposed to persistent low-dose radiation during work (Zeraatpishe *et al.*, 2011).

Seed priming is done with various chemical and physical methods. In the priming process, the seed is incited to start cell division and then will be dried. If again the seeds absorb water, it will start emergence from the dried situation (Vasilevski, 2003). The past century was an age of advanced chemical application in agriculture and nega-

tive effects on food products and on the environment is commonly known. Therefore, many scientists believe that this century will be the age of biophysical method application. Using biophysical methods would increase the plant growth, yield and quality. This technique protects plant against diseases and pests and decreases the use of fertilizers and pesticides. So the farmers can reach a crop with more quality and quantity with expensing less time, cost and effort. In this situation the knowledge of the biophysical priming and relevance of plant growth and physical properties appear to be an important factor in the sustainable agriculture (Vasilevski, 2003).

From the present and accessible literature sources, it is possible to select various research results of the application of different biophysical methods on plant production. Sacata *et al.* (2012), Gadyszewska (2011), Wilczek *et al.* (2004) used laser light as a biostimulator. During their investigations they showed effects on the growth and development of plants. Hegazi and Hamideldin (2010), Humarya and Shakil (2012), Abdul *et al.* (2010) explored the effect of gamma ray on germination and yield of plants. In addition, Yaldagard *et al.* (2008) performed the priming technique with ultrasonic wave on barley, and understood that ultrasonic wave could increase germination percentage and reduce germination period. Basiri and Eshagbeigi (2007) increased seeds efficiency by using an electrostatic

separator. Afzal *et al.* (2012), Feghenabi (2007), Hoseini *et al.* (2013); Iqbal *et al.* (2012), Jamil *et al.* (2012), Matwiczuk *et al.* (2012), Socorro and Garcia (2012) applied magnetic field on several plants and seeds with different techniques, and achieved positive results.

The aim of this work was to study the effects of bio-physical seed priming techniques on germination and yield of two landraces of lemon-balm (*Melissa Officinalis* L.) concurrent in laboratory experiments and pot studies in greenhouse condition.

Materials and methods

This study examines the effects of physical seed priming on germination and yield of lemon balm, in the laboratory experiment in conjunction with pot study. Laboratory and pots experiments were conducted as factorial based on completely randomized design (CRD) in three replications (210 seeds per replications). The treatments are magnetic field with 75 mT for 10 and 15 min durations; laser light with 2 mw.cm⁻² for 20 min; ultrasonic wave with physiotrapy device for 20 min; cs-gamma ray for one hour in the lead cell, and a primed treatment in distilled water as control that applied on two landraces of lemon balm ('Karaj', 'Esfahan'). Before the experiment the seeds were disinfected in hypochlorite-Na 5% solution for 3 minutes, then in laboratory by using special paper and distilled water the seeds were cultured in petri dishes and were putt in germinator with 19°C ± 1 temperature for 8 days, and were counted daily. Finally, germination percentage was computed as the cumulative number of germinated seeds with normal radicles. The germination rate (GR) was calculated by using the equation 1 (Maguirw, 1962).

$$GR = \frac{\sum n}{N} \times 100 \quad \text{Eq. (1)}$$

Where: $\sum n$ -total number of germinated seeds at each counting, N -number of total seeds.

Some parts of primed seeds were stored in normal condition for 6 months and were cultured in laboratory condition. All the primed seeds were sown in pots in the greenhouse condition. The soil was sandy-loam with an electrical conductivity of 0.72 dS.m⁻¹ and pH = 7.74. During the growth period, three plants selected randomly, and cut on the floor for measurement the physiological characteristics. Totally, the sampling was done 9 times. LAI (Leaf Area Index) was calculated with putt the plant leaves in a scanner and LAR (Leaf Area Ratio) that view the content of leafy plant was calculated with equation 2 (Sarmadnia and Koocheki, 1999). Three months later the plants were harvested in early blooming stage, and the biomass dry weight was measured, and essential oil concentration was extracted by Clevenger type apparatus.

$$LAR = LAI / PDW \quad \text{Eq. (2)}$$

Where: LAI-Leaf Area Index, PDW-Plant Dry Weight.

The statistical data were analyzed using SAS software. The means of the treatments was compared using the least significant difference test at $p < 0.05$ by LSD method.

Results

There are significant differences between treatments × landraces (AB factor) in germination percentage (GP), germination rate (GR), seedling length (SL), seedling weight (SW). But on storage germination percentage (SGP) there is no significant difference. SGP had only significant effect on B factor (primed treatments). Interaction of landraces × primed treatments was not significant (Tab. 1). Tab. 1 indicates that there are significant differences between lemon balm races and priming effects. The landraces behaved differently by priming methods in all studied characters.

Fig. 1 shows how the priming increased the germination percentage in both of the landraces. Analysis of variance of the laboratory data indicated that, the germination percentage was significantly affected by priming methods. The highest germination percentage was recorded for seeds primed with MAG-15 min (86%). However, germination percentage of unprimed and primed seeds with gamma ray was almost similar (Fig. 1). Tab. 2 shows the effect of different priming techniques on germination rate (GR). All treatments increased GR on both landraces. GR on control had tangible difference on comparison with another. The most GR was shown on MAG-15 min on both landraces. Tab. 2 shows the positive effect of priming on seedling length and weight by almost all treatments. Seed treatments in MAG, laser and ultrasonic wave increased the length and weight of seedling more than other treatments. Maximum seedling length (SL) was depicted in MAG-15 min treatment in Karaj landrace (6.3 mm) and minimum seedling length was noted in control and gamma ray treatments. Also, Maximum seedling weight (SW) was noted in MAG-15 min treatment in Karaj landrace with 0.79 mg (Tab. 2). Analysis of variance revealed that there was significant difference between primed treatments on stored seeds germination percentage (SGP) (Tab. 1). The stored part of seeds showed decreasing in germination per-

Tab. 1. Mean squares of two landraces of lemon balm as affected by physical priming treatment

MS	S.O.V				CV%
	A factor	B factor	AB factor	Error	
GP	0.003 ^{**}	0.03 ^{**}	0.0008 ^{**}	0.0002	0.69
GR	3.98 [*]	137.68 ^{**}	12.03 ^{**}	0.87	3.14
SL	0.03 ^{ns}	13.14 ^{**}	0.84 ^{**}	0.11	8.34
SW	0.01 ^{**}	0.07 ^{**}	0.01 ^{**}	0.0002	2.35
SGP	0.0001 ^{ns}	0.03 ^{**}	0.001 ^{ns}	0.0006	1.63
LAI	0.01 ^{ns}	1.22 ^{**}	0.108 [*]	0.03	5.11
LAR	1.47 ^{**}	0.31 ^{**}	0.43 ^{**}	0.08	5.027
PH	96.69 ^{**}	745.56 ^{**}	43.82 [*]	11.80	6.35
BDW	2256.25 ^{**}	114904.59 ^{**}	13987.9 ^{**}	191.67	1.92
EOP	0.014 ^{**}	0.147 ^{**}	0.0029 [*]	0.0009	7.68
DF	1	5	5	24	-

^{*}, ^{**}, ns: Indicate significant difference at 5%, 1% and not significant

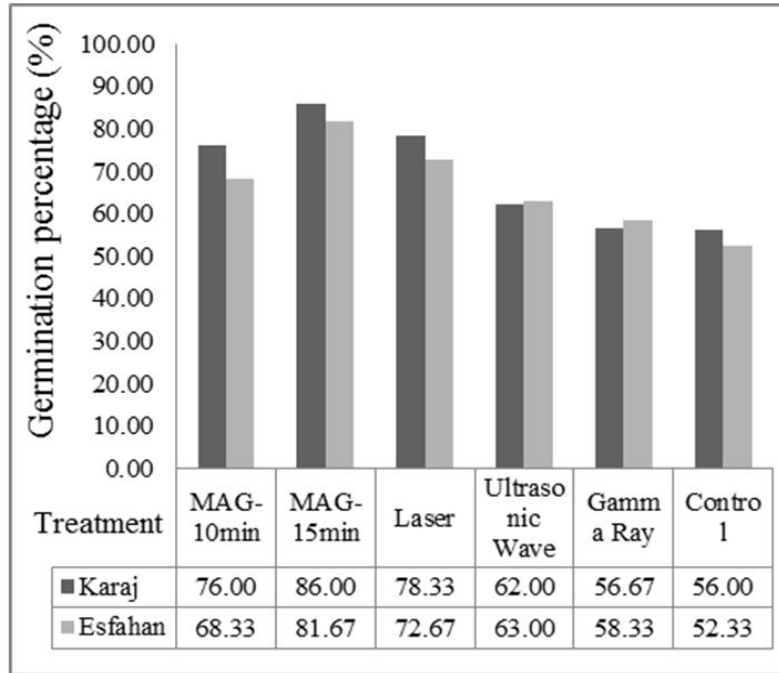


Fig. 1. Germination percentage of two landraces as affected by physical priming treatments

centage. Among treatments, ultrasonic wave showed the most decreasing in germination percentage. Germination percentage in all treatments was less than control (42%) (Tab. 2).

Tab. 1 indicates that there are significance differences within treatments in plant height (PH), biomass dry weight (BDW), Leaf Area Index (LAI), Leaf Area Ratio (LAR), essential oil percentage (EOP). The results of field study have been shown in Tab. 2. Two lemon balm landraces had

different plant height, biomass dry weight and percentage of essential oil. Landraces have affected differently by priming methods in all 3 characteristics (Tab. 2). Results indicated that plant height (PH) influence was significant due to the effect of seed treatments. Priming the seeds in magnetic field for 15 min duration had the greatest effect on plant height at both landraces (Tab. 2). Both duration of MAG and laser were the impressive treatments on biomass dry weight (BDW). MAG-15 min observed the most in-

Tab. 2. Influence of physical seed priming on the yield and quality of two landraces of lemon balm by LSD method ($p < 0.05$)

		Treatment					
		Mag-10 min	Mag-15 min	Laser	Ultrasonic	Gamma Ray	Control
GR	'K'	25.59	33.73	35.01	31.99	29.68	20.29
	'E'	32.34	35.2	30.51	28.93	28.08	21.24
SL (mm)	'K'	5.33	6.3	5.2	3.33	1.91	2.05
	'E'	4.98	5.34	4.87	3.6	2.93	2.8
SW (mg)	'K'	0.72	0.79	0.53	0.59	0.5	0.51
	'E'	0.72	0.76	0.72	0.65	0.51	0.5
SGP	'K'	30	34.33	33	25.67	28	42
	'E'	31	35.33	31.67	24	31	41.67
LAI	'K'	3.73	4.1	3.87	3.2	2.87	2.93
	'E'	4	3.75	3.71	3.44	3.17	2.87
LAR	'K'	5.38	5.6	5.59	4.65	5.48	5.76
	'E'	6.15	5.33	5.89	5.91	5.56	6.05
PH(cm)	'K'	60	75.33	61	51	41	45.67
	'E'	57.33	65	57	55.67	41.33	38
BDW(g/p)	'K'	658.33	1011.67	816.67	640	565	578.3
	'E'	775	855	833.33	705	636.67	560

'K': 'Karaj' 'E': 'Esfahan'

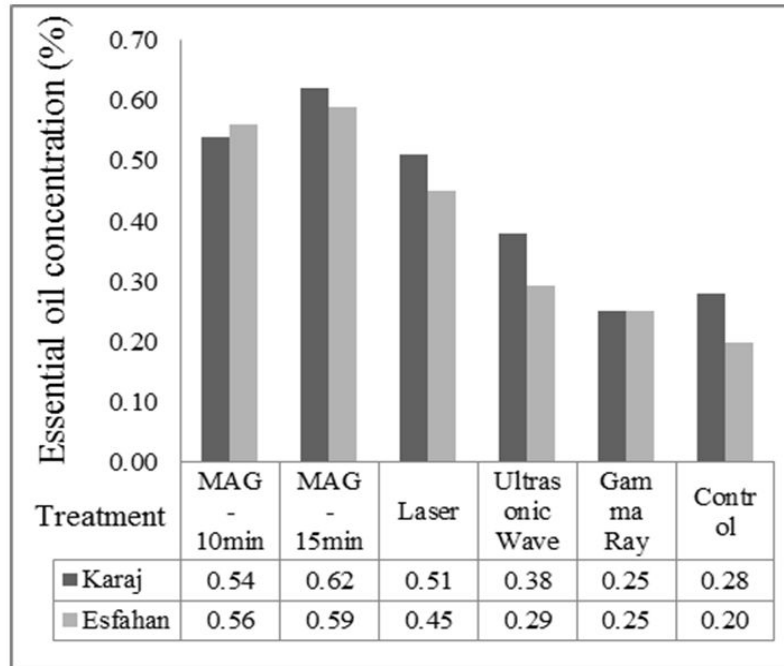


Fig. 2. Essential Oil concentration (%) of two landraces as affected by physical priming treatments

creasing of biomass dry weight (1011.67 g/plant) (Tab. 2). The essential oil percentage (EOP) responded positively and significantly to seed priming methods. Different durations of magnetic field treatment (10 and 15 min) had the most positive effect on essential oil percentage in lemon balm landraces. Essential oil percentage in magnetic field was 2.2 times greater than control. After that other treatments like MAG-10 min and laser affected positively on EOP (Fig. 2). Finally, the physiological characters showed that the priming techniques influenced on Leaf Area Index (LAI) and Leaf Area Ratio (LAR). MAG and laser had the most effect on LAI and LAR (Tab. 2).

Discussion

Fig. 1 shows how the priming had increased the germination percentage in both of the landraces. Treatments with magnetic field for 15 min and laser were effected the seeds more than other ones. The most seed germination percentage was observed in the case of magnetic field 15 min with 86% germination and, the least germination percentage was in relation to gamma ray with 52.33% germination. Germination rate was also influenced by treatments. The most GR resulted from laser and magnetic field treatments (Tab. 2). Also Tab. 2 shows the positive effect of priming on seedling length and weight. The seed treatment in magnetic field in two landraces and laser increased the length and weight of seedling more than other treatments. Various researchers have studied and reported that magnetically treated maize, wheat, sunflower, barley, corn, beans, tomato, fruits and mushrooms etc. showed high performance in terms of germination, seedling es-

tablishment, plant growth, height, yield, mass per spike as well as shoot and root length and assimilation of fresh and dry matter (Jamil *et al.*, 2012). Most of the magnetic field treatments improved significantly seed performance in terms of reduction of time to reach 50% germination and mean germination time with increasing germination energy, germination index, seedling shoot and root lengths compared to the control seeds (Afzal *et al.*, 2012). The germination enhancement with field treatment could be related to enhanced starch metabolism (Afzal *et al.*, 2012). Magnetically treatment may cause an increase in permeability of cell membranes in the seed, acting on calcium ions and inhibiting the growth of harmful microorganisms for the seed germination and plant growth processes (Matwiczuk *et al.*, 2012). Feghenabi (2007) had reported magnetic field priming influence some growth traits and cellular function like mRNA content, protein biosynthesis, enzymes activity and meristem cell metabolism and causes some changes in the tissues. These effects are still waiting for clarification from the theoretical point of view because they involve numerous factors that affect germination and plant development. Modification in chlorophyll content, peroxidase content, increased water adsorption, rate growth increments at later stages of the vegetative cycle and increased yield have been found experimentally (Socorro and Garcia, 2012). Among the various methods of priming it seems that there is little information about physical methods. But in recent years, interest in the use of physical methods of plant growing stimulation has increased (Feghenabi, 2007). As the germination percentage of triploid red clover increased significantly under the influence of laser treatment (Wilczek *et al.*, 2004). The laser

biostimulation method uses the physical phenomenon consisting of the ability to absorb and store light energy by plant cells and tissues. Seeds have the same ability as they absorb light energy, transform it into chemical energy, store it and use it in growth, later. The supply of energy increases the potential power of seeds positively, influencing the intensity of the physiological processes which take place in seeds during germinating (Gadyszewska, 2011). On the other hand, excess of energy absorbed by seeds, may accelerate plant metabolism and finally result in its growth improvement. One of the positive effects of laser biostimulation is the increased concentration of photosynthetic pigments in leaves (Sacata *et al.*, 2012). Investigated the effects of pre-sowing seed treatments with gamma ray on the germination, emergence and seedling establishment of wheat had positive results (Hegazi and Hamideldin, 2010). The gamma irradiation in Mean Germination Time was significantly affected on *Lepidium sativum* (Abdul *et al.*, 2010). The increase in higher germination percentage at higher doses of gamma ray might be due to their stimulating effects on activating RNA synthesis or protein synthesis (Kon *et al.*, 2007). Ultrasonic irradiation of the seeds of wheat, carrots, temperate Cymbidium species, corn, rice, tomatoes, and radishes, as well as sunflowers, in the dry-air state when carried out up to several months before actual sowing, led to the ripening of plants of grain and vegetable crops by 5–10 days sooner than control ones (Aladjajyan, 2002). Using ultrasonic wave increases germination percentage and rate in barley seed. The ultrasonic treatment proved to be efficient in reducing the germination period by 30–45%. The decrease in mean germination time with sonication may be due to initiating metabolic events in primed seeds (Yaldagard *et al.*, 2008).

The stored part of seeds showed decreasing in the germination percentage in laboratory test in all treated seeds compared to control (Tab. 2). It seems that, the storage period decreased seeds vigor and reduced germination viability. In fact, storage period destroyed the embryo of seeds. All the tomato seeds with gamma irradiation decreased in germination percentage during storage period in irradiated and unirradiated seeds (Humarya and Shakil, 2012). Investigations on treated seeds storage, showed different results. The germination test with wheat primed seeds that stored in general showed that 60 days after seed priming was better than other storage duration after seed priming (Shafiei Abnavi and Ghobadi, 2012). Harmful effects of seed treatment while during storage causes activation of DNA-repair due to development in cell cycle. Lipid peroxidation increscent, due to active oxin agent while seed drying and lack of conservation mechanism is effective in reduction of seed preservation (Feghenabi, 2007).

Lemon balm landraces had different plant height, biomass dry weight and essential oil concentration. Landraces had been affected differently by priming methods in all above three characteristics. Magnetic field for 15 min had greatest effect on plant height, biomass dry weight and essential oil concentration at both landraces. After

those other treatments like magnetic field of 10 min duration, laser and ultrasonic wave affected positively all three characteristics (Tab. 2). Results indicated that the plant height was significant due to the effect of seed treatments. The mean plant height achieved from seeds primed with magnetic field and laser that approximately was 1.5 time higher than control (Tab. 2). The highest increase in the biomass dry weight, nearly 1011.67 g/plant, was obtained when seeds were primed with magnetic field, as compared to control (Tab. 2). Ghassemi-Golezani *et al.* (2011) said that, the positive effects on yield and biomass also reported for barley, cucumber, fennel and winter rapeseed. Iqbal *et al.* (2012) reported that the magnetic field treatment at 10 mT for 40 h boosted up the height, mass and crop yield. The main problem for some plants like marigold production is poor plant stand establishment, and unavailability of high quality seeds of marigold is another main reason hindering its production and application of magnetic field treatment can reduce this problem (Afzal *et al.*, 2012).

Different durations of magnetic field treatment (10 and 15 min) had the most positive effect on essential oil concentration in lemon balm landraces. Essential oil concentration in magnetic field was 2.2 times greater than control (Fig. 2). A significant increase in essential oil yield due to seed priming, in comparison to those seeds not primed, was expected because the germination rate, uniformity in seed, seed yield, and oil content in primed seeds were high. Thus, all of these factors contribute to a higher essential oil yield. However, priming may increase yield in directly through its effect on even stand establishment, because uniformity in the stand results in higher yield (Mirshekari, 2012). Feghenabi (2007) understood that, the magnetic field doubled oil percentage in safflower. Scientists found that micro-nutrient treatments increased the essence yield in Cumin from 1.72 kg/ha to 2.56 kg/ha. Also, seed germination with iron and boron enhances germination and yield of Dill (Mirshekari, 2012).

In the present study magnetic field had the most effect on LAI and LAR. After that laser and ultrasonic wave respectively had the most effect on LAI and LAR. Feghenabi (2007) understood that, the magnetic field increased the LAI, LAR and CGR in safflower. In other research conducted by Neamiu and Marariu (2005), magnetic field treatment (120 mT) with duration of 5, 10 min on tomato seeds caused meaningful increase in radical and plumule length, leaf area index, and dry weight.

Conclusions

It has been proven that among treatments of lemon balm seeds the best effective methods are magnetic field in two durations and laser. Magnetic field is the best method of treating seeds to enhance the germination rate and improve the percentage of total germination and essential oil concentration. In the survey between the treatments, the gamma ray and ultrasonic wave had the least influence on lemon balm seeds. The magnetic field with duration of 15

minutes had better result than 10 minutes, so the magnetic field treatment (75 mT for 15 min) is the best method of seed priming in this study. In this situation the knowledge of the biophysical priming and relevance of plant growth and physical properties appear to be an important factor in the sustainable agriculture. The results obtained in this study can be used in the pharmacy, alimentary and sanitary industries.

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