Seed Research, Vol. 44(2): 120-126, 2016

Seed Quality Enhancement Using Pulsed Electromagnetic Field in Mungbean (*Vigna radiata* L. Wilczek)

SIMARJEET KAUR, RUPINDER KAUR* AND GEETA BASSI

Seed Technology Centre, Punjab Agricultural University Ludhiana 141 004
*rupinder261@gmail.com

ABSTRACT: Considering the importance of pulsed electromagnetic field (PEMF) in seed quality improvement, present study was conducted using fresh and revalidated mungbean seed after exposing to different PEMFs (1 Hz, 10 Hz, 50 Hz and 100 Hz). The effect was registered in terms of seed germination per cent, total soluble sugars (TSS), enzymatic activity and free amino acid content. Overall, all the frequencies improved the quality parameters under study but 100 and 50 Hz were found to be superior and at par with each other in their effect in both seed lots. In fresh seed lot there was increase in germination per cent (98% and 97%) over control (78%) and increase in TSS (552.3 and 540.9 mg/g respectively) over control (522.7 mg/g). The amylase activity also enhanced to 4.6moles $min^{-1}g^{-1}$ and 4.5moles $min^{-1}g^{-1}$ over control (3.3moles $min^{-1}g^{-1}$). Likewise, free amino acid content also augmented to 56.85 mg/g and 55.65 mg/g respectively over 53.14 mg/g in control. Similarly in revalidated seed lot, higher germination per cent (92.8% and 92%) over control (75%) and higher TSS (528.2mg/g and 520.6mg/g) over control (500.9mg/g) were registered. The amylase activity also elevated to 3.9 moles $min^{-1}g^{-1}$ and 3.8 moles $min^{-1}g^{-1}$, over the control (2.9moles $min^{-1}g^{-1}$). Likewise free amino acid content also increased to 53.50 mg/g and 53.31mg/g, respectively over the control (52.26mg/g). Hence present findings indicated that the mungbean seed may be given an exposure of 50 Hz or 100 Hz with PEMF before sowing, to improve its quality.

Key words: Mung bean, Pulsed electromagnetic fields, Seed quality

Mungbean (*Vigna radiata* L. Wilczek) is an important pulse crop of Asia, Africa and Latin America. India is one of the leading mungbean cultivator, covering up to 55% of the total world acreage and 45% of total production [1]. Mungbean serves as imperative source of vegetable protein (22-25%), mineral (0.18-0.21%), carbohydrates (59-61%), amino acids (65%) and other essential elements [2].

Seed quality is a complex trait that is determined by interactions between multiple genetic factors and environmental conditions. Seed quality enhancement comprises a broad spectrum of techniques that are being performed on seeds before sowing and after harvesting, to improve their performance. Pre-sowing seed quality enhancement treatments include mechanical, physiological and physical techniques or their combinations. Physical techniques like magnetic fields, microwaves, ionizing radiations, ultraviolet radiations, ultrasonic radiation etc. are being used to improve germination and elevate protein content, amino acids, carbohydrates and vitamins. Application of magnetic field of low frequencies positively affects seed germination, shoot development, plant length, fresh weight, fruit production and mean fruit weight [3-5]. Similarly Audus and Pittman [6, 7] studied the effect of magneto-tropism on root development in cress, corn, oat and sunflower. Thus, PEMFs have been found with significant outcomes at cellular level. This technique for seed invigoration is more economic and eco-friendly and has no side effects on quality of seed. It is less time consuming as it has shorter exposure time. The exposure of seeds to PEMFs before planting

Received : June 2016 Revised : July 2016 Accepted : August 2016

stimulate a series of biochemical effects in seeds. Magnetic treatments are used to enhance seed vigor by triggering the biochemical process though stimulating enzyme activities and proteins. However, its physiological mechanism is still not clearly understood [8]. Therefore, the present investigation was carried out to assess the seed quality enhancement of mungbean using pulse electromagnetic field of different frequencies and constant intensity.

MATERIAL AND METHODS

Two different lots of mungbean (*Vigna radiata* L. Wilezek) *cv.* SML668 (fresh and revalidated) having seed moisture content of 8.5 percent, were used for present studies. All laboratory experiments were conducted at Seed Technology Centre of Punjab Agricultural University, Ludhiana during 2012, 2013 and 2014.

Pulsed electromagnetic field exposure

After testing the initial germination per cent, the two lots were sent to the Madras Institute of Magnetobiology (MIM) Chennai, Tamil Nadu, India for PEMF exposure. Fifty grams of dry green gram seeds were exposed to magnetic field of varying frequencies *i.e.* 1Hz,10 Hz, 50 Hz and 100 Hz and constant intensity of 1500 nT (nano Tesla) using Sine wave form, for 5 hours per day for a period of 15 days. Seeds without exposure to PEMF served as control. The exposure details are given in Table 1.

Exposure setup

The pulsed magnetic fields used in the present investigations were generated in a specially fabricated controlled magnetic field (CMF) enclosure. This coil system of the CMF enclosure, designed after the primary equations of Fansleau and Braunbeck was made up of 2 sets of circular coils, the inner two of large diameter and the outer two of smaller diameter, all the four being mounted co-planar and co-axial. The 4 coils were wound with the same number of turns of enameled copper wire, all the coils being electrically connected in "series-aiding" configuration.

Table 1. Exposure details of the treatments

Treatment	Frequency (Hz)	Intensity (nT)	Waveform
Control	-	-	-
TEST 1	1	1500	Sine wave
TEST 2	10	1500	Sine wave
TEST 3	50	1500	Sine wave
TEST 4	100	1500	Sine wave

The ratio of the diameters of the 2 sets of coils and also the separation (or spacing) in between them were so adjusted that the entire disc-shaped volume between the inner (larger) coils offers the most uniform *i.e.* homogeneous magnetic field. This configuration gave an estimated degree of homogeneity of about 1 part of 5000. The Fansleau-Braunbeck coil system is a refined version of the classical Helmholtz-2-coil system offering the most practical advantage of large volume of highly uniform magnetic field of the order 20 to 30 times than that offered by a Helmholtz coil of identical physical dimensions. The coil system was energized by pulsed electric current from a function generator in which the strength, frequency and wave-form of output current can be controlled to any desired values, thus offering along the axis of the coil system a highly uniform or homogeneous pulsating magnetic field. To ensure that the alternating current power supply to the function generator (domestic electric power) is not interrupted during the PMF exposure by possible power failure, an uninterrupted power supply (UPS) was built into the supply circuit, which delivered uninterrupted power regardless of any power failure. As regards the geometry of presentation of PMF, the controlled magnetic field (CMF) coil assemblies were kept with their axis vertical so that the axis of PMF passes through the test objects vertically.

Germination test of treated seeds

After exposing to pulsed electromagnetic fields of different frequencies, seeds were subjected to seed germination test. The germination was tested with "Between the Paper" method as per Rules

of International Seed Testing Association [9] in quadruplicate of 100 seeds each at 25±1°C and 95 % RH in a germinator. According to the method, the mungbean seeds were sown between two layers of moistened germination paper which were then wrapped in wax paper to retain its moisture and placed inside transparent polyethylene bags. After final count day *i.e.* eighth day, germination per cent was computed as the percentage of normal seedlings.

Biochemical analysis

Total soluble sugar content was estimated using method of Dubois *et al.* [10]. Similarly the activity of α-amylase, was estimated using the method recommended by Jayaram [11], while free amino acids were determined by following the method suggested by Lee and Takahashi [12].

Statistical analysis

Statistically, the correlation of biochemical parameters with germination was determined using sigma plot software, where significance level of 0.05 was used for all statistical interpretation.

RESULTS AND DISSUSION

Germination per cent

Electromagnetic exposure of all the frequencies enhanced seed germination in both seed lots. The results on germination as influenced by different electromagnetic frequencies in both lots are presented in Table 2 and Fig. 4. In fresh seed lot, germination per cent was increased (98%) with 100Hz frequency, following 97% with 50Hz treatment over the control (78%). Similarly at 1Hz and 10 Hz frequencies, seeds exhibited 82% and 86% germination respectively as compared to control. Revalidated seed lot, when exposed to 50Hz and 100Hz, also showed significantly high germination *i.e.* 92% and 92.8% respectively over the control (75%). EMF exposure of even 1Hz and 10 Hz also showed its positive impact on germination per cent in revalidated seed lot too. It was 80% with 1Hz and 10Hz frequencies, while untreated seed exhibited 75% germination. In other studies on soybean by Radhakrishnan and Kumari [13], 10 Hz magnetic field treatment and as per Badiger *et al.* [8] 50 Hz and 100Hz, enhanced the germination and seedling growth of soybean.

Total Soluble Sugars (TSS)

In the present study, the TSS improved with increased PEMFs frequency rates. The readings of the TSS in both lots are presented in Table 2. In fresh lot, 50Hz and 100Hz elevated TSS from 522.7 (mg/g) (control) to 540.9 (mg/g) and 552.3(mg/g) respectively. There was 3.48% and 5.66% increase in total soluble sugars over control, respectively. Using 1 Hz and 10 Hz, small increase in TSS was observed *i.e.* 522.7mg/g

Table 2. Seed quality as influenced by PGMFs in mungbean (Pooled Data of three years)

Treatments	Total soluble		α-amylase enzyme sugar (mg/g)		Free amino acids moles $min^{-1}g^{-1}$		Germination rate (mg/g)	
	F	R	F	R	F	R	F	R
Control	522.7	500.9	3.3	2.9	53.14	52.26	78	75
1 Hz	525.8	508.8	3.5	3	53.27	52.53	82	78
10 Hz	532.9	512.9	3.8	3	53.58	52.41	86	80
50 Hz	540.9	520.6	4.5	3.8	55.65	53.3	97	92
100 Hz	552.3	528.2	4.6	3.9	56.85	53.5	98	92.8
CD5%	14.88	13.09	0.73	0.6	2.06	0.7	9.75	10.24

*F-Fresh seed lot; *R- Revalidated seed lot

(control) to 525.8mg/g and 532.9mg/g respectively, while in revalidated seed lot the sugar content increased from 500.9 mg/g (control) to 528.2 mg/g and 520.6 mg/g at 50Hz and 100Hz respectively. Higher frequencies *i.e.* 50 Hz and 100 Hz, increased TSS by 3.93% and 5.45% respectively. The per cent values of TSS in both seed lots are presented in Fig. 1. Lower frequencies of 1 Hz and 10 Hz also indicated higher TSS content *i.e.* 500.9 mg/g (control) to 508.8 mg/g and 512.9mg/g, in there validated seeds correspondingly.

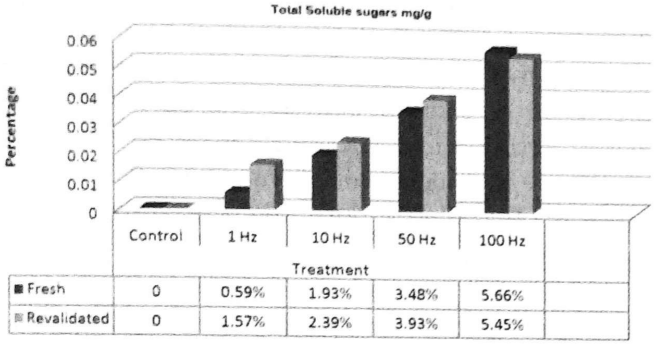

Fig. 1. Soluble sugar percentage as affected by different frequencies of PEMFs in mungbean

α-amylase activity

Enzymatic activity is key component of seed during its germination. Activity of amylase enzyme is involved in the germination process, therefore α-amylase activity was determined. The enzymatic activity using different frequencies in both seed lots is depicted in Table 2. It increased in the seeds exposed to 50 Hz and 100 Hz. Frequency of 50 Hz accelerated α-amylase activity from 3.3 moles $min^{-1}g^{-1}$ to 4.5 moles $min^{-1}g^{-1}$ in fresh lot and from 2.9 moles $min^{-1}g^{-1}$ to 3.8 moles $min^{-1}g^{-1}$ (control) in revalidated lot. There was 36.36% and 31.03 % increase in enzymatic activity in both lots respectively, over control seeds. The per cent of enzymatic activity is presented in Fig. 2. Similarly, enzyme activity at 100 Hz also increased steadily from 3.3 moles $min^{-1}g^{-1}$ (control) to 4.6 moles $min^{-1}g^{-1}$ in fresh lot of seeds and from 2.9 moles $min^{-1}g^{-1}$ (control) to 3.9 moles $min^{-1}g^{-1}$ in revalidated seed lot. There was 39.39% and 34.34% in both lots of seed over the control seeds. Likewise, at lower frequencies

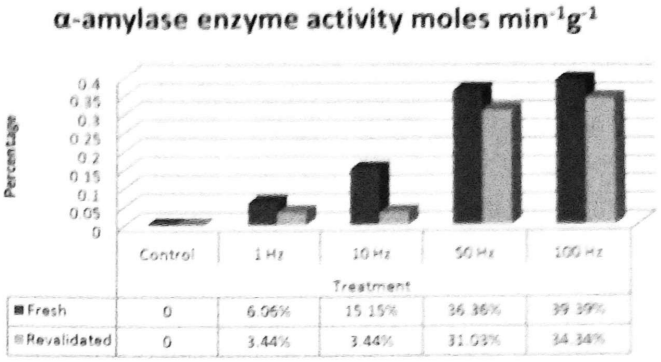

Fig. 2. Percentage of amylase activity in mungbean treated at 1.0 Hz, 10Hz, 50Hz and 100Hz frequencies

of 1 Hz and 10 Hz, the α-amylase activity marginally increased from 3.3 moles $min^{-1}g^{-1}$ (control) to 3.5 moles $min^{-1}g^{-1}$ and 3.8 moles $min^{-1}g^{-1}$, respectively in fresh lot of seeds and from 2.9 moles $min^{-1}g^{-1}$ (control) to 3.0 moles $min^{-1}g^{-1}$ and 3.0 moles $min^{-1}g^{-1}$ in revalidated seed lot correspondingly.

Free amino acid content

The pre-sowing seed exposures with PEMF frequency 50 Hz enhanced free amino acid amount. It was improved from 53.14 mg/g to 55.65 mg/g. There was 4.72% increase in the free amino acid amount over the untreated seeds in fresh lot of seeds as shown in Fig. 3. In case of revalidated lot, with 50 Hz frequency, the free amino acid content was enhanced from 52.26 mg/g to 53.30 mg/g. This resulted in 1.99% increase in free amino acids over untreated seeds in revalidated lot. Similarly 100 Hz frequency also enhanced free amino acids from 53.14mg/g to

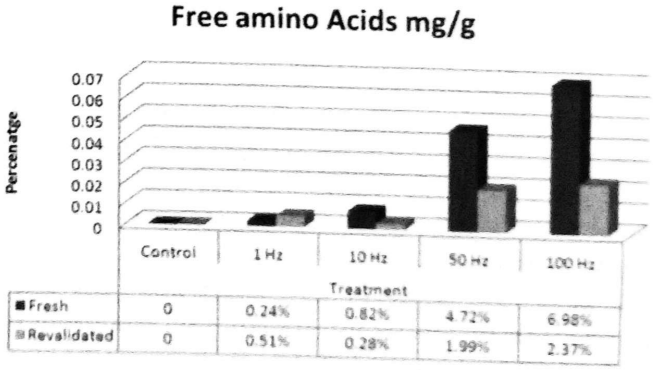

Fig. 3. Amino acid percentage of mungbean at 1.0 Hz, 10Hz,50Hz and 100Hz frequency treatment

56.85mg/g. There was 6.98% rise in free amino acids in fresh lot over the control. On the other hand in revalidated seed lot, at 100 Hz frequency, the free amino acid content was augmented from 52.26 mg/g to 53.5 mg/g and it comprised 2.37% increase in content over control. Similarly, 1 Hz and 10 Hz, gradually elevated the quantity of free amino acids from control values of 53.14mg/g, 52.26mg/g to 53.27 mg/g, 53.58mg/g and 52.53mg/g and 52.41mg/g respectively in the fresh and revalidated seed lots.

The studied biochemical quality parameters *i.e.* total soluble sugars, enzyme activity and free amino acids were influenced by the PEMF in mungbean. All PEMF treatments indicated positive results over the control. In a similar study conducted by Florez *et al.* [14], electromagnetic fields enhanced enzymatic activity and protein content that resulted in increased biomass in plants. Likewise fresh and dry weight and mineral accumulation were also increased by pulsed magnetic fields [15]. The good effect of magnetic field can be found in synthesis of free amino acids, cell production, enzyme activity and nucleic acid content [16]. Magnetic fields pierce cell membranes and cell wall, resulting in influx of biochemical reactions [17]. Magnetic treatments enhance seed vigour by influencing the biochemical processes, which stimulate the activity of proteins and enzymes [18]. Most of the biochemical systems involve a radical pair mechanism, where a pair of free radicals is either produced or consumed. The magnetic field influences the inter conversion of singlet and triplet spin states of those free radical pair that stimulates activity of proteins and enzymes [19].

Statistical analysis

The PEMF treatments improved the seed quality in terms of increased germination per cent, total soluble sugars, α-enzyme activity and free amino acid content. These factors are very important and responsible for seed germination. The treated seeds indicated better germination per cent than the untreated seeds in both seed lots. The data of the parameters were statistically analyzed to find the correlation of these parameters towards germination. The co-relation was found using sigma plot software version 5.1. A significance level of 0.05 was applied for all statistical tests. The statistical analysis indicated the significant relationship of treatments with free amino acids, total soluble sugars and enzyme activity. The analysis indicates that there was a positive correlation among the biochemical parameters towards germination in fresh and revalidated seed lot. This positive correlation is presented in Table 3 and Table 4.

In case of fresh seed lot, correlation coefficients and P values of treatments and biochemical, parameters are increasing together thus resulting in significant relationship Revalidated seed lot also has a significant positive relationship. It is found that the correlation coefficient of enzyme activity was increasing and that of germination per cent was decreasing, thus resulting in negative correlation. The seeds exposed to 50 Hz and 100 Hz have strong significantly higher values of total soluble sugars, á-amylase activity and free amino acid content, along with germination percent as shown in Table 2.

When different frequencies were statistically analyzed, it was found that 50 Hz and 100 Hz

Table 3. Pearson Product Moment Correlation in fresh seed lot

	Total soluble sugars	Enzyme activity	Amino acids content	Germination per cent
Treatments	0.957 0.0106	0.932 0.0212	0.884 0.0467	0.971* 0.00588**
Total soluble sugars		0.996 0.000279	0.939 0.0179	0.995* 0.000456**
Enzyme activity			0.951 0.0128	0.983* 0.0026**
Free amino acids content				0.904* 0.0352**

*Correlation Coefficient **P Value. The pair(s) of variables with positive correlation coefficients and P values below 0.050 tend to increase together. For the pairs with negative correlation coefficients and P values below 0.050, one variable tends to decrease while the other increases. For pairs with P values greater than 0.050, there is no significant relationship between the two variables.

Table 4. Pearson product moment correlation in revalidated seed lot

	Total soluble sugars	Enzyme activity	Amino acids content	Germination percent
Treatments (1Hz, 10Hz, 50Hz, 100Hz)	0.968 0.00685	0.909 0.0323	0.985 0.00223	0.948* 0.0139**
Total soluble sugars		0.885 0.046	0.968 0.00682	0.993* 0.000704**
Enzyme activity			0.962 0.00878	0.83* 0.0817**
Free amino acids content				0.94* 0.0177**

*Correlation Coefficient **P Value. The pair(s) of variables with positive correlation coefficients and P values below 0.050 tend to increase together. For the pairs with negative correlation coefficients and P values below 0.050, one variable tends to decrease while the other increases. For pairs with P values greater than 0.050, there is no significant relationship between the two variables.

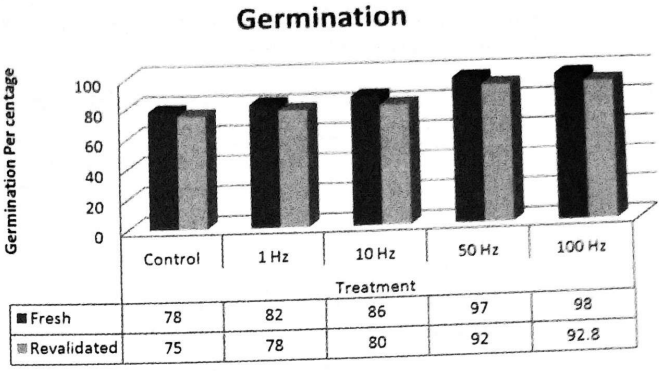

Fig. 4. Percentage of germination rate resulted by different frequencies of PEMFs in mungbean

frequencies gave best results in terms of free amino acids, amylase and total soluble sugars. However, revalidated seed also indicated seed quality enhancement in terms of enhanced free amino acids, soluble sugars and amylase activity as presented in Fig. 4. It was found that germination of the seed after pulsed electromagnetic field exposure with different frequencies was enhanced as compared to untreated seeds.

PEMF increased the free amino acid content and hence proteins, which are the key components for seed germination. Seed coat of mungbean is hard thus resulting in less imbibition process, however, after EMF seed exposure, water intake increased resulting in higher germination. This water intake, facilitated mobilization of the storage reserves *i.e.* total soluble sugars-carbohydrates, through the hydrolysis process. This facilitated the enhanced efficiency of free amino acids and α-amylase which culminated in high germination and seed quality [20]. From this experiment, it is apparent that the biochemical environment of key components involved in the seed germination of mungbean, is strongly influenced by the pulsed electromagnetic fields. The positive co-relation among the biochemical parameters and germination indicated the significant relationship of seed quality and biochemical parameters. Therefore, it can be recommended that seed quality of revalidated seed of mungbean can be invigorated using PEMF of 50Hz or 100Hz.

CONCLUSION

Present findings indicated the potential of 50 Hz and 100 Hz PEMF treatment to invigorate the seed quality of fresh as well as revalidated mungbean due to increased total soluble sugars, α-amylase enzymatic activity and free amino acid content. Therefore, it was concluded that the mungbean seed may be given an exposure of 50 Hz or 100 Hz of PEMF before sowing, to improve its quality.

REFERENCES

1. RISHI N (2009). Significant plant virus diseases in India and a glimpse of modern disease management technology. *Journal of General Plant Pathology*, **75**: 1-18.

2. SITAL JS, JS MALHOTRA, S SHARMA AND S SINGH (2011). Comparative studies on biochemical components in mungbean (*Vigna radiata* (L.) Wilczek) varieties cultivated in summer and kharif seasons. *Indian Journal of Agricultural Biochemistry*, **24**: 68-72.

3. CAKMAK T, R DUMLUPINAR AND S ERDAL (2010). Acceleration of germination and early growth of wheat and bean seedlings grown under

various magnetic field and osmotic conditions. *Bioelectromagnetics*, **31**: 120-9.

4. DE SOUZA A, GARCÍD, L SUEIRO, F GILART, E PORRAS AND L LICEA (2006). Pre-sowing magnetic treatments of tomato seeds increase the growth and yield of plants. *Bioelectromagnetics*, **27**: 247-57.

5. NIMMI V AND G MADH (2009).Effect of pre-sowing treatment with permanent magnetic field on germination and growth of chilli (*Capsicum annum*. L.). *International Agrophysics*, **23**: 195-8.

6. AUDUS LJ (1960). Magnetotropism: A new plant growth response. *Nature*, **185**: 132-4.

7. PITTMAN UJ (1977). Effects of magnetic seed treatment on yields of barley, wheat and oats on Southern Alberta. *Canadian Journal of Plant Science*, **57**: 37-45.

8. BHARAMARAJ B, H RAVI, NK BIRADAR PATIL, DP BIRADAR AND AT VILAS (2014). Effect of pulsed electromagnetic field treatment on seed quality enhancement and storage in soybean. *Seed Research*, **42**(2): 192-200.

9. ISTA (2008). International rules for seed testing. *Seed science and technology*, pp. 164.

10. DUBOIS MK, N GILLIES, JK HAMILTON, REBERSPA AND F SMITH (1956). Colorimetric method for determination of sugars and related substance. *Analytical Chemistry*, **28**: 350-6.

11. JAYARAM J (1996). Laboratory manual in biochemistry 5th reprint, pp: 53, 79, 121-2, New age international limited publishers, New Delhi.

12. LEE YP AND T TAKAHASHI (1966). An improved colorimetric determination of amino acids with the use of ninhydrin, *Analytical Biochemistry*, **14**: 71-77.

13. RADHAKRISHNAN R AND BDR KUMARI (2013). Influence of pulsed magnetic field on soyabean seed germination, seedling growth and soil microbial population, *Indian Journal of Biochemistry and Biophysics*, **50**: 312-7.

14. FLOREZ M, MV CARBONELL AND MARTINEZE (2007). Exposure of maize seeds to stationary magnetic fields: Effects on germination and early growth. *Environmental and Experimental Botany*, **29**: 68-75.

15. RADHAKRISHNAN R AND RB KUMARI (2012). Pulsed magnetic field: A contemporary approach offers to enhance plant growth and yield of soybean. *Plant Physiology Biochemistry*, **51**: 139-44.

16. STANGE BC, RE ROWLAND, BI RAPLAY AND JV PODD (2002). ELF magnetic fields increase amino acid uptake into *Viciafaba* L. roots and later ion movement across the plasma membrane. *Bioelectromagnetics*, **23**: 347-4.

17. CAKMAK T, R DUMLUPINAR AND S ERDAL (2010). Acceleration of germination and early growth of wheat and bean seedlings grown under various magnetic field and osmotic conditions. *Bioelectromagnetics*, **31**: 120-9.

18. VASHISTH A AND S NAGARAJAN (2010). Effect on germination and early growth characteristics in sun flower (*Helianthus annuus*) seeds exposed to static magnetic field. *Journal of plant Physiology*, **167**(2): 149-56.

19. SALIKHOV KM, YN MOLIN, RZ SAGDEEV AND AL BUCHACHENKO (1984). Spin Polarization and magnetic effects in radical reactions. Amsterdam: Elsevier.

20. KALININ LG, IL BOSHKOVA, GI PANCHENKO AND KOLOMI CHUKSG (2005). The influence of low and high-frequency electromagnetic fields on seed. *Biofizika*, **50**: 361-6.