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Full Length Article

Effect of Electric and Magnetic Treatments on Germination of Bitter Gourd (*Momordica charantia*) Seed

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Abstract

Bitter gourd (*Momordica charantia*) is a medicinal plant which has potential benefit in diabetes and other diseases. After being treated with electric and magnetic fields, there is an increase in the yield of bitter gourd. The seeds of bitter gourd were exposed to 300, 500, 700 and 1000 V/cm electric field for 20 min. The results showed that electric field stimulates germination of bitter gourd seeds positively at low levels and has a resonating effect at 500 V/cm. At higher electric fields, the germination was decreased sharply. Seeds of bitter gourd were also exposed in batches to static magnetic fields of 0.07, 0.12, 0.17 and 0.21 T for 20 min. The seeds respond differently when exposed to a magnetic field, showing a continuous positive stimulation under all applied fields. Bitter gourd supports the magnetic time model based on the following equation: B = 174.02 g(r)- 1.815.

Keywords: Magnetic time model; Water absorption; Stochastic model; Medicinal plant

Introduction

Some traditional medicines are derived from medicinal plants, minerals and organic matter (Grover *et al.*, 2002). India is the biggest producer of medicinal plants around the world (Seth and Sharma, 2004). *Momordica charantia*, also known as bitter gourd, bitter melon and Karela, belongs to the Cucurbitaceae family and is commonly consumed as a food (bitter flavouring) in India. It has been traditionally used to treat diabetes (Lana and Julia, 2012). Its leaves and stems are used to cure diseases due to its hypoglycaemic effect (Fernandes *et al.*, 2007), and contain charantin, a steroidal glycoside; vicine, a glycoalkaloid and polypeptide 'p', a 166 residue insulinomimetic peptide. Its hypoglycaemic activity is based on a structural similarity to insulin and stimulation of insulin secretion by the pancreas (Lana and Julia, 2012). It is also used to treat inflammation, colds, hypertension, sore throats, menstrual problems, tuberculosis, fever, anaemia, kidney stones, parasites, malaria, anorexia, liver problems, vomiting and headache (Lana and Julia, 2012). Punjab Agricultural University (PAU) Ludhiana conducted a survey regarding the consumption of bitter gourd in Punjab (India) and showed an interesting trend (Avrdc, 2012). Out of the 400 surveyed consumers, the majority liked bitter gourd even for its bitter taste. Almost all consumers knew the health benefits of the bitter gourd, out of which the highest number of response was for blood-glucose management. In view of increasing demand of this medicinal plant, it is imperious to increase its production. In general, synthetic inputs such as fertilizers are used to increase this crop yield, but excessive use of chemicals do pose a potential risk to humans and side effects to the environment. So there is a need to use new technologies to increase food production. Pre-sowing electric and magnetic treatment of seeds is one of the cost effective new technological methods to enhance this crop yield with no side effects to the environment.

There is controversy over the effects of weak electromagnetic field on living organisms and there is a lack of understanding regarding how electric or magnetic fields affect the living system. The influence of an artificially inducted electromagnetic field (EMF), which affects human beings directly and indirectly through the environment, especially in agriculture, is still unknown (Pietruszewski *et al.*, 2007). Exposure to an electric field increases negative charge on the cell surface, whereas exposure to magnetic field decreases the hydrophobic character of the cell surface (Marron *et al.*, 1988). Both magnetic and electric fields significantly affect seed germination, but the energy needed to induce stimulation with a magnetic field is lower than the energy needed with an electric field (Blank, 1995). The effects of magnetic field treatment on plants have been studied by various researchers since the 19th century (Flórez *et al.*, 2007) to enhance the germination rate, seedling vigour and growth at the later stages of plant development (Marks and Szecówka, 2010). The stimulation of seeds before sowing with static and variable magnetic fields has a positive effect on the germination speed and subsequent, seedling

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growth (Martínez *et al.*, 2009; Mahajan and Pandey, 2012, 2014). The literature indicates that electrically treated seeds result in better seedling growth, stem height and root length compared with non-exposed seeds (Kiatgamjorn *et al.*, 2002). *M. charantia* is studied here to explore its behaviour in the presence of static electric and magnetic fields. The objectives of the present research is to find the level of electric field which stimulates bitter gourd seeds, to explore the germination of electrically treated seeds in the off-season and to find the behaviour of magnetic field on the germination of bitter gourd seeds.

Materials and Methods

The bitter gourd variety SEJAL of *M. charantia* obtained from Tropica Seeds (P) Ltd Bangalore was used to study the effects of electric and magnetic fields on the seed germination parameters. The temperature range for best germination of bitter gourd is 24–27°C.

Magnetic and electric field generators were used to deliver a variable horizontal magnetic field (up to 750 mT) and variable vertical electric field (up to 13 kV/cm). A constant DC supply source (0–45 V/0–7.5 A) was provided to the electromagnet of the magnetic field generator. Flat-faced pole pieces with a diameter of 7.5 cm were cylindrical in shape. The gap between the magnetic poles varied from 5 to 10 cm. A digital Gauss meter was used to check the field strength between the pole pieces. Three replications of a set of four samples (each sample comprising 40 seeds at 27°C) were placed in the plastic container to expose to magnetic field of 0.07, 0.12, 0.17 and 0.21 T for 20 min between the pole pieces of the electromagnet.

In the electric field generator, two circular aluminium electrodes with a diameter of 8 cm were used. The circular electrodes were arranged with an adjustable gap between them. A low-voltage variable DC supply (0–13 kV) was used to set-up the vertical electric field between two circular aluminium electrodes. To provide the electrical treatment, a single layer of bitter gourd seeds was loaded in between the two circular electrodes. High-density polyethylene layers were used above and below the seed layer to avoid directly contacting the seeds with the electrodes. No heating effect was observed during the experiment. Three replications of five samples (four treated and one control), with each sample comprising 40 seeds was exposed to 300, 500, 700 and 1000 V/cm electric field for 20 min. A slightly off temperature (34°C) was chosen for the electrically treated bitter gourd seeds to determine the field effects under off-season germination conditions. The distance between the two electrodes was maintained at 1 cm.

All magnetically and electrically exposed bitter gourd seeds from each sample were stored in between two thin wet cotton cloth layers. Seeds were shifted instantly after the treatment. Each cotton layer was then transferred onto a separate sponge bed sheet with a thickness of 1.8 cm and stored inside a transparent plastic box ($20 \times 13 \times 4$ cm^3) with a lid. In each box, the sponge bed sheets were dampened with an equal amount of water. All external conditions that could affect the germination were ensured to be the same for each sample during the germination process (boxes were kept in germinator to maintain constant temperature for germination). The number of germinated seeds was counted after each fixed interval of time. A seed was considered to be germinated when the radicle emerged and was longer than 2 mm.

The electric field intensity, E_{av}, can be calculated as follows (Kiatgamjorn *et al.*, 2002):

$$E_{\max} \equiv \frac{U}{d\eta} \qquad (1)$$

$$\eta \equiv \frac{E_{av}}{E_{\max}} \leq 1 \qquad (2)$$

$$E_{av} = \frac{U}{d} \qquad (3)$$

Where, U is the voltage between electrodes (V), d is the distance between electrodes (cm), E_{max} is the maximum value of the electric field intensity (V cm^{-1}), E_{av} is the average value of the electric field intensity (V cm^{-1}) and η is the field utilisation factor (dependent on the geometry of the electrodes). The examination of water uptake during the initial stages of germination is necessary to understand the process of seed germination. The changes in the water status during imbibition by the seeds influence the subsequent development and growth of the plants (McDonald, 1999). Water uptake can be measured by weighing the seeds before and after soaking at a definite time interval. The relative water absorption, *w*, can be calculated as $w = ([w_2 - w_1]/w_1) \times 100$ (Nizam, 2011), where w_1 is the initial seed weight and w_2 is the seed weight after absorbing water over the time interval *t*). The number of seeds remaining in the sample at any time *t* can be measured using the following stochastic model for a two-state germination process: $E[M_1(t)] = M_0 \exp(-\lambda_1 t)$ (Tseng and Hsu, 1989). The mean germination time $\bar{t}$ (Ranal and Santana, 2006; Salehzade *et al.*, 2009) can be calculated as follows:

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i \times t_i}{\sum_{i=1}^{k} n_i} \qquad (4)$$

Where, t_i is the time from the start of the experiment to the ith observation (hour), n_i is the number of seeds germinated by the ith observation and t_k is the last time to evaluate germination for the kth observation.

The reformulated Malthus-Verhulst function (Pietruszewski, 2001; Pietruszewski and Kania, 2010) can be used to determine the germination capacity (%) at any instant *t* as follows:

$$N_g(t) = N_k N_i / [N_i + (N_k - N_i) \exp\{-\alpha N_k (t - t_o)\}] \quad (5)$$

Where, $N_g(t)$ is the number of seeds that germinated within time *t*, N_k is the final number of germinated seeds, α is the germination rate coefficient and t_0 is the initial time of germination.

In equation (1), η is the field utilisation factor (dependent on the geometry of the electrodes). In the present set-up, $\eta \cong 1$.

Results

The water content absorbed as a percentage by electrically treated bitter gourd seeds differs significantly at all imbibition time from the control group (Fig. 1a) under the 500 and 1000 V/cm electric fields, as given by the following equations: $W_{control} = 10.804 \ln(t) + 1.8548$ ($R^2 = 0.9972$), $W_{500\,V} = 10.545\ln(t) + 4.9615$ ($R^2 = 0.9957$) and $W_{1000\,V} = 10.175\ln(t) + 2.0567$($R^2 = 0.9889$). The seeds showed a wide range in their germination capacity between the electrically treated and control groups (Fig. 2a). The germination capacity of the electrically treated seeds differs considerably from control group for both 500 and 1000 V/cm treatments (Fig. 2a). The stochastic model and experimental data support a high retardation in the germination capacity under the 1000 V/cm electric field (Fig. 3a) and small positive growth at 300 and 500 V/cm. The water absorption of untreated seeds in their best season (27°C) is compared with the water absorption of the same seeds in off season (34°C) with and without the 500 V/cm treatment (Fig. 4). With the electric treatment (Fig. 5a), all time values (e.g., mean germination time and transition time) showed a decrease between 300 and 500 V/cm and an increase at 1000 V/cm. It implies that there is improvement in mean germination time and transition time between 300 and 500 V/cm, whereas at 1000V/cm electric field the seeds took longer time to germinate. The result is in coincidence with water absorption curve (Fig. 1a). The germination rate, which is the reciprocal of the transition time, increases linearly with the applied magnetic field, as given by the following equation: B = 174.02g(r) - 1.815. The magnetic-time constant is 174.02 Th (Fig. 6). There is a continuous increase (Fig. 1b) in the seed water content absorption (as a percentage) for the

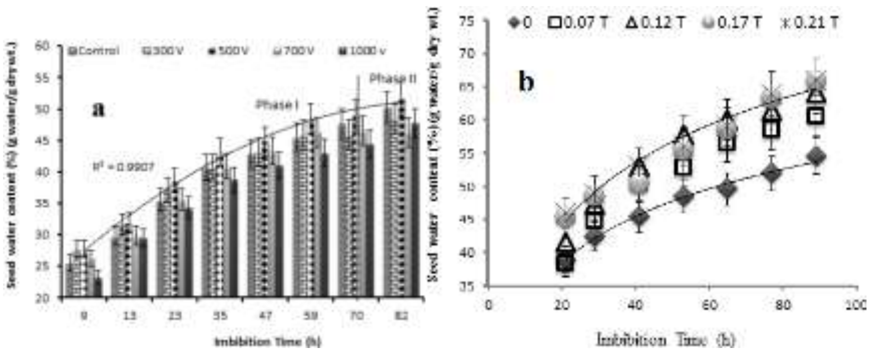

Fig. 1: Seed water content absorbed for electrically and magnetically treated seeds with imbibition time at different electrical and magnetic intensities

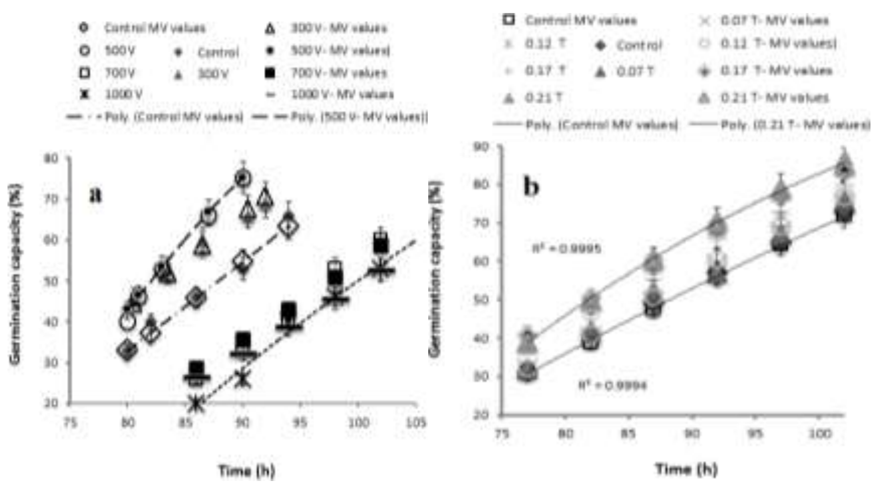

Fig. 2: The growth of germination capacity in percentage for electrical and magnetically treated seeds

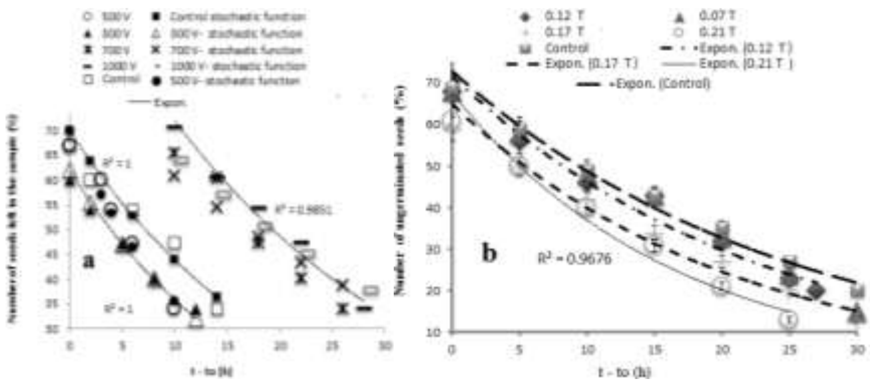

Fig. 3: Exponential curves showing the matching of Stochastic model with experimental data for electrical and magnetically treated seeds

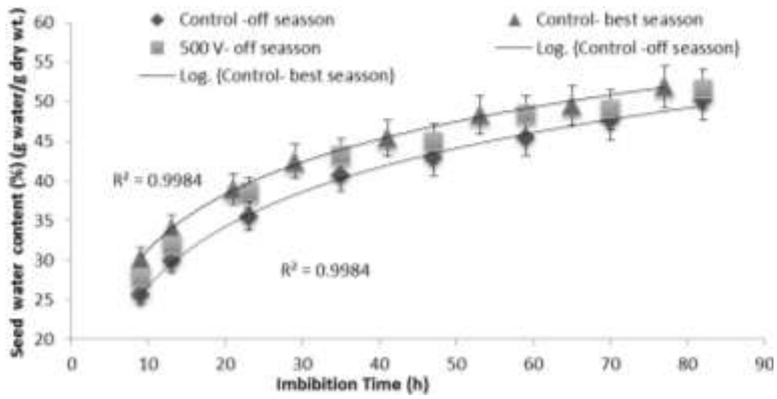

Fig. 4: Comparison of water absorption of untreated *Momordica charantia* seed in its best season (27°C) with water absorption in off season (34°C) and treated *M. charantia* seeds in its off season germination at 500 V

magnetically treated seeds compared with the control. The highest absorption is observed at 0.21 T, as given by the following equations: $W_{control} = 10.25 \ln(t) + 7.6401$ (R^2 = 0.9895) and $W_{0.21\,T} = 13.864 \ln(t) + 2.466$ (R^2 = 0.9692). For the magnetically treated seeds, the reformulated Malthus-Verhulst equation and experimental data show a continuous increase in the seed germination capacity (as a percentage) compared with the control (Fig. 2b). The number of seeds remaining (Fig. 3b) in the sample decreases exponentially with time. The trend of the exponential curve differs with the change in the magnetic dose. The largest decrease is observed at 0.12 T. There are very small improvements in the mean germination time, transition time and time taken for the first seed to germinate with the magnetic-field treatment (Fig. 5b). The magnetic treatment results in a linear increase in the germinating coefficient (Fig. 7) with an increasing magnitude of the magnetic field.

Discussion

When the seeds are placed in an electric field at a value of E_o (between the electrodes), then the magnitude of the electrostatic field inside the seed drops to the value E, depending upon the relative permittivity, $\varepsilon_{r(seed)}$, of the seed (Pietruszewski *et al.*, 2007), which can be determined by the composition and tissue structure of the seed. The actual permittivity, ε, is then calculated by multiplying the relative permittivity by the electric constant, ε_o (vacuum permittivity): $\varepsilon = \varepsilon_o\, \varepsilon_{r(seed)} = \varepsilon_o (1+\chi)$, where χ is the electric susceptibility of the seed. The electric susceptibility indicates how much a medium polarises in response to an electric field. Seeds become polarized in the presence of an electric field. Upon removal of the applied field, the seeds retain some polarisation, known as remnant polarisation. When these seeds come into contact with the water dipoles, an interaction between the seed dipoles and water dipoles occurs. This interaction affects the water uptake by the seed, which further affects the germination time and germination rate. The dipole-dipole interactions of the *M. charantia* seeds are optimised at 500 and 1000 V/cm as the water content absorbed in the presence of these electric fields differs significantly from control (Fig. 1a). As the relative water absorption is increased at 500 V/cm and decreased at 1000 V/cm, the germination capacity is also increased at

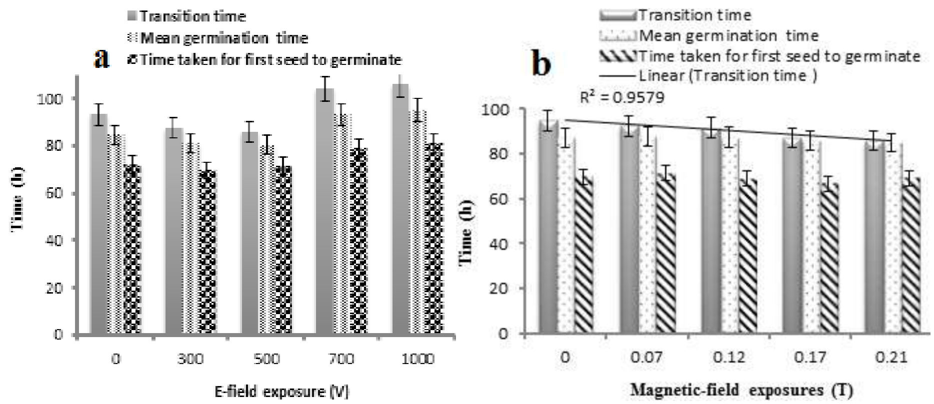

Fig. 5: The variation of mean germination time, transition time and time taken for first seed to germinate for electrical and magnetically treated seeds

500 V/cm and decreased at 1000 V/cm (Fig. 2a, 3a). These

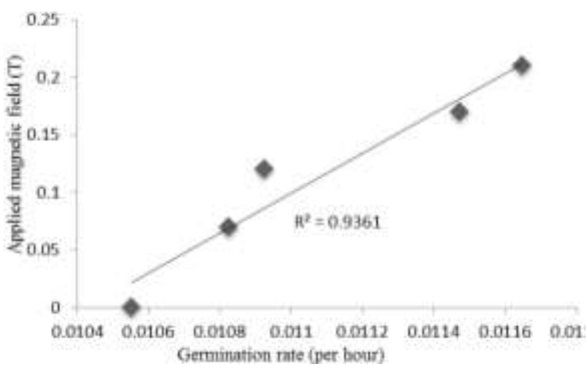

Fig. 6: Germination rate is increasing linearly with applied magnetic field with equation: B = 174.02g(r) - 1.815

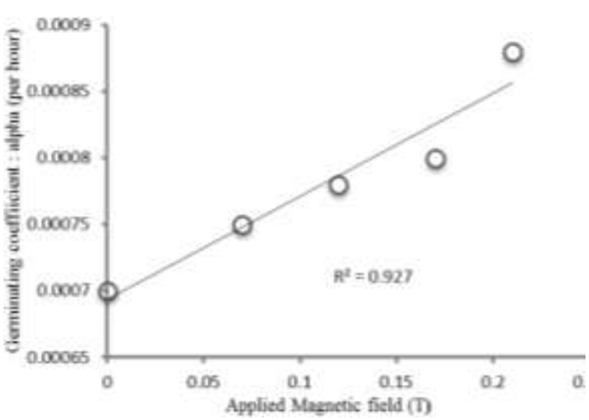

Fig. 7: There is smooth (linear) increase in the value of germinating coefficient with applied magnetic field

results showed that information on the germination capacity can be retrieved from the water absorption curves. In the off-season, the water absorption is lower than the best season. Interestingly, in the off-season, the electrically treated seeds (500 V/cm) used for germination provided almost the same amount of germination as for control seeds in their best season.

Aladjadjiyan (2010) showed that seeds exhibit paramagnetic behaviour. The magnetic moments of seed particles in an external magnetic field orient themselves in the direction of the field. The impact of the magnetic field on the seeds results in an increase in their energy (Aladjadjiyan, 2010). This distribution of energy to the seed particles accelerates the metabolism, consequently resulting in better germination. Bhatnagar and Dev (1978) showed that the coefficient of the velocity of germination and the germination capacity were linear functions of the magnetic field. Mahajan and Pandey (2012) formulated the magnetic-time model ($B + H_g = \theta_B(g)\, g(r)$) and experimentally verified that the germination rate for a given seed fraction or percentage was a linear function of B above H_g, where H_g is the base value in the units of the magnetic field and the germination rate attains some minimum value (but not zero). *M. charantia* supported this model by generating the equation, B = 174.02 g(r) - 1.815 (Fig. 6). The magnetic-time constant and H_g are 174.02 Th and 1.815 T, respectively. A high value of the magnetic-time constant signifies a reduced effect of the magnetic field on *M. charantia* germination. There is an increase in water absorption (Fig. 1b) with an increasing magnetic field, following the equation $w = C_1 \times \ln(t) \pm C_2$, with $R^2 > 0.9$ for all curves (where w is the percentage of water uptake by the *M. charantia* seeds relative to the seed dry weight and C_1 is the water uptake coefficient). The experimental data showed fit well in the re-formulated Malthus-Verhulst equation, $N_g(t) = N_k N_i / [N_i + (N_k - N_i) \exp\{-\alpha N_k (t-t_o)\}]$, showing an increasing trend in the germination with an increasing magnetic field (Fig. 2b). The number of seeds remaining in the sample at any time t of the different samples exposed to different doses of the magnetic field fit well in the stochastic model, $E[M_1(t)] = M_0 \exp(-\lambda_1 t)$

for a two-state germinating processes (Fig. 3b). This supports that seed germination is a two state process because all data (the number of seeds remaining in the sample at any time *t* of any dose value) satisfies exponential behaviour, which is required by stochastic model of two-state germinating processes (Fig. 3b). Conversion from the un-germinated to the germinated state depends upon the transition time. The mean germination time, transition time and time taken for the first seed to germinate is slightly improved with the application of a magnetic field, showing a linear trend (Fig. 5b) that further supports the magnetic time model. Aladjadjiyan and Ylieva (2003) exposed tobacco seeds to magnetic field of 0.15 T for 10, 20 and 30 min and found a linear increase in the germination capacity (G) with increasing exposure time, as given by the equations $G= 0.088t + 0.215$ and $G = 0.0745t +0.3$ for non-soaked and preliminarily soaked seeds, respectively. They also explained that the magnetically treated seeds stimulate the process of germination because mitochondria possess paramagnetic properties similar to those found in chloroplasts.

Using the re-formulated Malthus-Verhulst equation, the germinating coefficients are calculated for the electrically and magnetically treated seeds. There is a linear increase in the value of the germinating coefficient with the applied magnetic fields (Fig. 7). In the electrically exposed seeds, there is an improvement in the value of α (germinating coefficient) from 0 to 500 V/cm; thereafter, it decreases and attains a minimum value at 1000 V/cm ($\alpha_{control} = 0.0009h^{-1}$, $\alpha_{300V} =0.00095$ h^{-1}, $\alpha_{500V} = 0.0014$ h^{-1}, $\alpha_{700V}= 0.00079$ h^{-1}, $\alpha_{1000V} = 0.0007$ h^{-1}). The data showed that an electric field stimulates germination of bitter gourd seeds positively at lower levels and that a resonating effect (maximum energy transfer) is observed at 500 V/cm. At higher levels of the electric field, the effect becomes negative. The seeds respond differently when exposed to a magnetic field; they show continuous positive stimulation at all applied field values.

Conclusion

The electric field stimulates germination of bitter gourd seeds positively at lower levels and has a retarding effect on germination at higher levels. The behaviour of a magnetic field on seed germination is altogether different from that of an electric field, showing a continuous positive stimulation at all applied values. The pre-treated bitter gourd seeds support the magnetic-time model.

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