



P-ISSN: 2349-8528

E-ISSN: 2321-4902

[www.chemijournal.com](http://www.chemijournal.com)

IJCS 2021; 9(1): 1141-1144

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Received: 27-11-2020

Accepted: 30-12-2020

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## Effect of pulsed magnetic field on seed borne pathogen and germination of tomato

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DOI: <https://doi.org/10.22271/chemi.2021.v9.i1p.11380>

**Abstract**

Effect of Pulsed Magnetic Field (PMF) treatment on the control of *Alternaria solani* (Ellis and Martin) Jones and Grout causing Early blight of tomato (*Solanum lycopersicum* L.) cultivar PKM 1 was studied. The seeds pre-inoculated with *A. solani* were 1500nT PMF treated with frequency of 10 Hz for 5 h for varied durations of 0, 5, 10, 15, 20 and 25 days. PMF treatment significantly reduced pathogen incidence and increased both germination and plant growth in comparison with the control. Experimental results indicated that all the treatments were significantly different and the seeds treated for 20 and 25 days showed the minimum percent disease incidence (71%) with highest percent disease inhibition over control (29%). It also recorded the highest plant biometrics viz., germination (81%), speed of germination (7.74), root length (13.37 cm), shoot length (6.45 cm) and vigour index (1606). Control recorded the minimum plant biometrics viz., germination (61%), speed of germination (7.27), root length (12.92 cm), shoot length (6.17 cm) and vigour index (1164).

**Keywords:** Pulsed magnetic field, tomato, *Alternaria solani*, early blight

**Introduction**

Modern agricultural efforts are now in search of an efficient eco-friendly production technology based on physical treatment of seeds to increase the seedling vigour and crop establishment. 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. Pre-sowing seed treatment with PMF can be used in practical agriculture to improve the plant growth and yield (Shine *et al.*, 2011)<sup>[36]</sup>. Magnetic field has been found to improve food reserve utilization and help for better absorption and assimilation of nutrients by plants (Kavi, 1977)<sup>[22]</sup> and photosynthetic activities (Lebedev *et al.*, 1977)<sup>[24]</sup>. Various researchers have studied and reported that vegetable and grain crops seed treated by magnetic field showed high performance in terms of germination, plant growth, height, seed mass per spike as well as shoot and root length and total fresh and dry masses, chlorophyll contents, enzymatic activities and yield (Iqbal *et al.*, 2013; Jamil *et al.*, 2013; Naz *et al.*, 2012; Perveen *et al.*, 2011; Zia-ul-Haq *et al.*, 2012)<sup>[18, 20, 27, 30, 38]</sup>. Different researchers presume that magnetic field can effect chemical reactions by altering electron spin location and in this way they have potential to cause biological effects (De Souza *et al.*, 2014)<sup>[8]</sup>. Electro magnet induced physiological and biochemical changes in biological objects. Water assimilation and intensified photosynthesis collectively enhance the seed germination and growth (Podleoeny *et al.*, 2004)<sup>[31]</sup>. Harichand *et al.*, (2002)<sup>[17]</sup> reported that exposure of magnetic field increased plant height, seed weight per spike and yield of wheat. Aladjadjiyan (2002)<sup>[3]</sup> observed that the magnetic field stimulated the shoot development of maize and led to an increase in germinating energy, germination, fresh weight and shoot length. The fungus *Alternaria solani* (Ellis and Martin) Jones and Grout cause early blight disease, is a major disease of tomato causing considerable yield loss. It being the seed borne pathogen it is necessary for efficient elimination of this pathogen to improve the seed quality and crop yield. Based on the available information current work was designed to study the efficacy of PMF treatment on disinfection, germination and vigour of tomato cultivar PKM 1.

## Materials and Methods

Effect of Pulsed Magnetic Field treatment on the control of *Alternaria solani* (Ellis and Martin) Jones and Grout causing Early blight of tomato (*Solanum lycopersicum* L.) cultivar PKM 1 was studied by following the below methodologies.

### Isolation of pathogen and pathogenicity test

The pure culture of *Alternaria solani* was obtained by single spore isolation method and sub culture was used for pathogenicity test by following Koch's postulate. The pathogenicity test was carried by pre-inoculation with spore suspension and homogenized mycelial bits of *A. solani* on foliage of 30 days old plants of PKM 1 cultivar of tomato. After inoculation, the symptoms appeared on inoculated leaves as brown, oval or angular necrotic spots with concentric rings and surrounded by a border of yellow host tissue. The fungus was re-isolated and purified culture from these artificially infected leaves was similar to that of original culture. The plants which were not inoculated with the fungal spore suspension did not show any symptoms of the disease. Thus pathogenicity on tomato was confirmed. (Prathima *et al.*, 2018)<sup>[33]</sup>.

### Seed inoculation

Tomato seeds were immersed in a solution of 10% commercial bleach with Tween20 (1 drop/100 mL of solution) for 15 min, followed by 2 rinses with sterile distilled water for 5 minutes. After that, they were immersed in hydrogen peroxide (33%) for 10 min and rinsed three times with sterile distilled water for 10 minutes. Later, seeds were stirred in water for 24 h and then air-dried in a laminar flow cabin for at least 2 h. Spores of *A. solani* were obtained by scraping a 7 day old culture of a fungus colony growing on PDA at 25 °C, adding 0.5% KCl + 1 drop of Tween20 and filtered through two layers of cheese cloth. The concentration was adjusted to give a spore suspension of 10<sup>3</sup> spores/ $\mu$ L. Seeds were immersed and stirred in this spore suspension for 30 s and then air dried used for sonication treatment. (Evira-Recuenco *et al.*, 2015)<sup>[9]</sup>.

### Pulsed Magnetic Field treatment

The 100 g seeds kept at the geometric centre of coil assemblies were subjected to 10.0 Hz at 1500 nT as per the method described Leelapriya *et al.*, (2003)<sup>[25]</sup>. Exposure to PMF was for 5 h per day for varied durations of 0, 5, 10, 15, 20 and 25 days and control seeds were kept under the similar condition in the absence of the PMF. The magnetic treatment of seeds was applied using an electromagnet highly improved version of the classical Helmholtz coil. This consisted of four-coil system that are made up of two larger (inner) coil frames and two smaller (outer) coil frames. The four coils have the same number of turns and connected in series-aiding configuration. The four coils are mounted co-planar and co-axial. The ratios between the radii of inner and outer coil frames and the spacing between them are pre-determined according to the parametrical equations of Fanselau (1929)<sup>[10]</sup> and Brauenbeck (1934)<sup>[6]</sup>.

### Standard Blotter Method

Detection of seed borne fungi in seed samples was done by following ISTA procedures. In this method, three layers of blotter paper was soaked in sterilized water and placed in the petri plates. 100 seeds were sterilized in 0.2% Sodium hypochlorite solution for 2 to 3 minutes and seeds taken

randomly from each sample and were placed in petri plates and incubated for seven days in the laboratory under alternating cycles of 12 hrs light and 12 hrs darkness. The incubated seeds were examined under stereo binocular microscope to ascertain the presence of fungi. (ISTA, 1993)<sup>[19]</sup>.

### Speed of germination

Four replicates of twenty five seeds each were used to test the speed of germination of seeds. The seeds showing radicle protrusion were counted daily from second day of sowing until seventh day. From the number of seeds germinated on each day, the speed of germination was calculated using the following formula and the result was expressed in number (Maguire, 1962)<sup>[26]</sup>.

### Germination test

Four replicates of 100 seeds were uniformly placed on standard germination paper roll-towel medium and kept in germination room maintained at 25 $\pm$  2°C and 90  $\pm$ 2 per cent relative humidity. After 14 days, the seedlings were evaluated as total number of normal seedlings and germination as percentage. (ISTA, 1993)<sup>[19]</sup>

### Root length and Shoot length

On fourteenth day, ten normal seedlings per replication from roll towel medium were carefully removed at random from each treatment. The root length was measured from the base to the top of the primary root and the shoot length was measured from the base of the shoot to tip of primary leaf and the mean value was calculated and expressed in cm. (ISTA, 1993)<sup>[19]</sup>

### Vigour index

The Vigour index was calculated and compared by adopting the following formula and expressed as whole number. (Abdul-Baki and Anderson, 1973)<sup>[1]</sup>

### Statistical analysis

The data obtained from various experiments were analysed statistically by adopting the procedure described by Panse and Sukhatme (1985)<sup>[29]</sup>. The laboratory experiments were laid out in completely randomized design (CRD). The data recorded on per cent values were arc-sine transformed before analysis and the critical differences (CD) were calculated at 5 per cent probability level.

### Results and Discussion

Standard blotter method for detection of seed borne fungi in seed samples was done by following ISTA procedures. The results indicate that all the treatments were significantly different with varied level of pathogen inhibition compared with control. Among the different PMF treatments tomato seeds treated for 20 and 25 days performed better, both the treatments found to be in same group, 25 days treatment does not have any significant increase over 20 days. PMF treatment for 20 days recorded the minimum percent disease incidence (71.16%) with highest percent disease inhibition over control (28.84%) (Table 1). It also recorded the highest plant biometrics *viz.*, germination (81%), speed of germination (7.74), root length (13.37 cm), shoot length (6.45 cm) and vigour index (1606). Control recorded the minimum plant biometrics *viz.*, germination (61%), speed of germination (7.27), root length (12.92 cm), shoot length (6.17 cm) and vigour index (1164) (Table 2).

**Table 1:** Effect of Pulsed Magnetic Field treatment on tomato cv. PKM 1 seeds inoculated with *Alternaria solani*

Pulsed Magnetic Field (5h/day)	SBM infection (%)	Per cent Inhibition over control (%)	Speed of germination	Per cent increase over control (%)
5 Days	90.23	9.77	7.32	0.69
10 Days	87.72	12.28	7.47	2.75
15 Days	79.84	20.16	7.59	4.40
20 Days	71.29	28.71	7.71	6.05
25 Days	71.16	28.84	7.74	6.46
Control	100.00	—	7.27	-
SEd	1.09	0.41	0.16	0.06
C D (P = 0.05)	2.37	0.92	NS	0.13

\*SBM – Standard Blotter Method

**Table 2:** Effect of Pulsed Magnetic Field treatment on biometrics of tomato cv. PKM 1 seeds inoculated with *Alternaria solani*

Pulsed Magnetic Field (5h/day)	Germination (%)	% increase over control (%)	Shoot length (cm)	% increase over control (%)	Root length (cm)	% increase over control (%)	Vigour Index	% increase over control (%)
5 Days	70	14.75	6.21	0.65	13.09	1.32	1351	16.07
10 Days	73	19.67	6.27	1.62	13.17	1.93	1419	21.92
15 Days	77	26.23	6.38	3.40	13.26	2.63	1512	29.92
20 Days	81	32.79	6.43	4.21	13.37	3.48	1604	37.78
25 Days	81	32.79	6.45	4.54	13.38	3.56	1606	37.99
Control	61	-	6.17	-	12.92	-	1164	-
SEd	1.55	0.54	0.15	0.07	0.37	0.06	27.13	0.78
C D (P = 0.05)	3.37	1.21	NS	0.16	NS	0.13	59.10	1.75

The positive effect of PMF on seed germination was reported by many researchers (Vashisth, and Nagaraja, 2010; Shine *et al.*, 2011; Chen *et al.*, 2011) [37, 36, 7]. Florez *et al.* (2007) [12], observed that total length of maize plant was greater than control plants of maize exposed to magnetic field. The polypeptide distribution and translational pattern were altered in bacterial and eukaryotic cells in response to different levels of electromagnetic stress (Blank *et al.*, 1994; Goodman *et al.*, 1994) Novitsky *et al.* (2001) [5, 5, 28] had shown the two fold increase of protein contents in onion leaves subjected with magnetic field. Magnetic stimulation of broad bean and pea seeds increased seedling emergence resulting in higher pod number and seed yield (Podlesny *et al.*, 2005) [32]. The possible mechanism would be a change in the electrostatic balance of the plant system at the cell membrane level, as it is the primary site for action of any inhibition or enhancement of plant growth at extremely low frequency electromagnetic fields on biological systems (Adey, 1988) [2]. Galland and Pazur (2005) [13] described the effects of magnetic field by radical pair mechanism and ion cyclotron resonance mechanisms.  $\alpha$ -amylase plays an important role in hydrolyzing the starch into simple sugars, which provide the energy for the growth of roots and shoots (Kaneko *et al.*, 2002) [21]. Increase of acid phosphatase activity could be related to either de novo synthesis of protein or activation of protein (Garcia *et al.*, 2004) [14].

Vashisth and Nagarajan (2010) [37] reported the enzyme activities of  $\alpha$ -amylase, dehydrogenase and protease were significantly higher in treated seeds of sunflower in contrast to control. It is supposed that magnetic field influences the structure of the cell membrane and increases their permeability and ion transport in the ion-channels which affects the metabolic pathway activity (Labes *et al.*, 1993) [23]. Increased uptake of Ca ions in rice seedlings grown from seeds exposed to pulsed magnetic field was found responsible for better leaf growth, meristematic tissues in stems and roots (Saktheeswari and Subrahmanyam, 1989) [34]. All these above changes also make the seedlings vigorous to avoid the pathogen incidence by increased metabolism and secondary metabolites production to enhance the growth and yield.

Production of free radicals in the germinating seeds has been associated with change from quiescent stage to a metabolically active state that explains for faster germination (Podlesny *et al.*, 2005) [32]. Free radicals are also involved in the elongation of plant organs and their generation is a developmentally regulated process (Schopfer, 2001) [35]. This observation indicates that the internal energy of the seed responds positively when there is an appropriate combination of magnetic field and exposure time (Bhatnagar and Deb, 1977) [4]. Such enhanced performance of seeds exposed to magnetic fields has been reported previously by workers dealing with various crop seeds (Aladjadjiyan, 2002; Fischer *et al.*, 2004; Florez *et al.*, 2007) [3, 11, 12].

### Conclusion

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. Pulsed Magnetic Field treatment is a new promising technology for managing seed borne and other phyto-pathogens.

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