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Research Article

ASSESSMENT OF THE EFFECT OF PULSATING ELECTROMAGNETIC FIELDS ON BIOCHEMICAL AND MORPHOLOGICAL PARAMETERS CHANGES OF *BRASSICA JUNCEA (MUSTARD SEEDS)*

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ABSTRACT
In order to study the effect of pulsed magnetic field on the morphological and biochemical features of *Brassica juncea* the possible involvement of pulsed magnetic pretreatment in physiological and biochemical factors in *Brassica juncea* was investigated. Treatments such as seeds without exposure to pulsating magnetic field (control), seeds with magnetic field 0.01 HZ ± 12000nT, current 10 mA for 5 hours per day for 15 days (test) were carried out. According to results obtained with *Brassica juncea* it can be said that the 0.01Hz PMFs remarkably improved the fresh weight of shoots and roots, leaf area and plant height from seedlings from magnetically-exposed seeds compared to the control, increased the total soluble sugar, total protein and amino acid contents also. The leaf chlorophyll a, b and total chlorophyll were higher in PMF pretreated plants, as compared to other treatments. In addition, the activities of α-amylase and β- amylase enzymes were increased. The results confirmed that the intensity of the magnetic field had effects on the growth parameters and biochemical composition.

Keywords: *Chlorophyll a, Chlorophyll b, α- Amylase, β- Amylase, Magnetic Field, Brassica juncea, Protein*

INTRODUCTION
Optimal physical methods for plant growth stimulation can lead to less damage effects on the environment (Putincev and Platonova, 1997). Studies of interactions between magnetic field and biological systems classify them into two categories which define the source or the origin of the magnetic field 1. Geo- magnetic interactions 2. Man –made field interactions. The concept of geomagnetic field and biological systems define that the earth itself is a big magnet. The earth has to be a giant magnetized sphere, with the north seeking pole near the geographic north -pole and the south seeking pole near the geographic south- pole. Geomagnetic field has exerted great influence on biological evolution systems. It has played a great role in organic evaluation (Friedmann *et al.,* 1963; Sankar Narayan, 1989). Man- made magnetic field namely static magnetic field and time varying magnetic field differ in the fact that strength and polarity of the later varies with time. Magnetic fields have had uses in agriculture of ancient and modern society. The advent of modern technological systems in electric and electronic equipment has, in recent years, increased both interest and concern about the effects of electromagnetic and magnetic field on plants. Information of the mechanisms of the action of the magnetic field on various biological systems such as cells and tissue of plants, animals and microorganisms, may be effectively used as a means of regulating biological activity and removing unfavorable compounds from the bio systems (Gholami and Sharafi, 2010). The use of a magnetic field creates a stress condition for plant growth stimulation just as environmental stresses like salinity, drought, UV light, heat and chilling cause. Physical methods are frequently used in seed germination and growth stimulation (Aladjadjiyan, 2007).
The main aim of agriculture is to prepare seeds for sowing and seeds which have a positive influence on considerable acceleration and uniformity of seeds germination and other features as well as on obtaining abundant good-quality yield. Nowadays magnetic field is used as a physical method in agriculture (Delibaltova and Ivanova, 2006). Treatments of seed with magnetic field have reported an increase in the performance of crop plants (Das and Bhattacharya, 2006). Exposure of seeds to magnetic field is one of the affordable physical methods to enhance post germination and development of crop plants (Vashisth

and Nagarajan, 2008). The effect of magnetic field treatment on seeds showed that germination depends on the induction of the magnetic field strength and frequency, exposition of samples (period sample is exposed to magnetic field) and the sample species of plants (Vashisth and Nagarajan, 2010). The aim of the present study was to evaluate the effect on the germination of mustard seeds (*Brassica juncea*) by magnetic treatment in exposing the seeds to PMF at 0.01Hz and PMF at ± 12000 nT. The intensity of the magnetic field induces changes in the morphological, biological and variations in the enzymatic activities.

MATERIALS AND METHODS

Seeds of *Brassica Juncea* have been used for investigating the influence of the magnetic field on the development of plant. Seeds were exposed to pulsed magnetic field in a solenoid enclosure. Solenoid enclosure has been chosen because of the good length of the solenoid, highly uniform and very intense magnetic field. In this study, solenoid coil was used at the middle of the magnetic field = 1256.5 nano tesla per milli ampere of current generated. The two ends of the solenoid will have a field equal to half the central value. The healthy uniform 10gms of dry seeds were selected and seeds were kept at the geometric center of coil assemblies. Exposure was (0.01 HZ ± 12000 nT) 5 hours per day for 15 days. Seeds without exposure to pulsating magnetic field served as control. The experiments have been performed under laboratory conditions.

Seed Germination and Seedling Development

Magnetic field pretreated and control seeds were surface sterilized with 1% NaOCl (w/v) for 5min, washed 3 times with distilled water and then propagated in pots containing soli and sand mixture (1:2). The pots were maintained under natural photoperiod with 35% (w/w) soil moisture content. Seed germination was observed after one week, and germination seedlings were uprooted and measured the length, fresh and dry weight of 10 days for both control and treated seedlings (figure 1).

Figure 1: Pot Culture Studies of Mustard Plant

Pigment Contents (Chlorophyll a, Chlorophyll b) (Arnon, 1949)

The photosynthetic pigments e.g., chlorophyll a and b were extracted in 0.5ml of chilled 80% acetone by grinding the leaves of salt treated seedlings in a chilled mortar and pestle. The homogenate was centrifuged at 5000 rpm for 10 minutes. The supernatant was taken at 645 nm and 663 nm. Different pigments were estimated using the formula as given below:

Total Chl (a and b) (mg/l) = {20.2*(A 645)} + {8.02*(A663)}
Chl a (mg/l) = {12.7*(A663)} + {2.69*(A645)}
Chl b (mg/l) = {22.9*(A645)} - {4.68*(A663)}

Estimation of Carbohydrates

Estimation of carbohydrates content was determined by Anthrone reaction by using spectrophotometer (Carrol *et al.,* 1956). Briefly, 1gm mustard seeds were taken and crushed thoroughly in mortar and pestle. 5ml of distilled water was used to dissolve the fine powder. Then, the solution was centrifuged at 5000 rpm for 10 minutes. The supernatant was diluted with the same extract solvent at a suitable concentration for assaying carbohydrate content. 0.5ml and 1.0ml of diluted extraction was introduced into 0.5ml test tube. Then, 4.0 ml of anthrone solution was added and the content was kept at room temperature for 10

minutes and then cooled. The colour was developed and the absorbance was measured at 620 nm using red filter. A standard graph was drawn by plotting (concentration of standard glucose solution in X axis and the optical absorbance in Y axis). From the graph the amount of carbohydrate present in the mustard seeds was calculated.

Estimation of Total Proteins

Total phenolic content were determined by spectrophotometric method using folin – ciocalteau reagent. This method is described by (Lowery *et al.,* 1951). 1gm of fine powder of mustard seed was weighed accurately and dissolved in a limited quantity of water. 5.0ml of 50% trichloro acetic acid was added and centrifuged at 12,000 rpm for 20 min.

The supernatant liquid was discarded. Then, the precipitate was made upto 100 ml with distilled water. 0.5 ml and 1.0 ml of diluted extraction was transferred into two separate 5.0 ml test tubes. Working standard Bovine albumin solution was prepared.

From that 0.2 to 1.0 ml was transferred into series of test tubes. 1.0ml of distilled water and 4.5 ml of alkaline copper sulphate reagent was added for all the test tubes. After 10 minutes 0.5 ml of folin – ciocalteau reagents were added and allowed standing for 30 minutes. Absorption was measured at 640 nm in a shimadzu UV-VIS spectrophotometer.

Determination of Total Free Amino Acid

Methanolic extract of mustard seed was prepared. 1 gm of mustard seedlings was taken then 10 ml of 80% methanol was added. Then, it was centrifuged at 7000 rpm for 10 minutes. The supernatant liquid was used as sample source. Then, 5gm of glycine was dissolved in 10 ml of distilled water. This solution was used as working standard. 0.2 to 1.0 ml of standard solution was transferred into series of test tubes. 2.0 ml of test solution was transferred into individual test tube. 2.0 ml of distilled water was maintained as blank. 0.1 ml of 80% phenol was added to all the test tubes. Then, these test tubes were kept in a boiling water bath for 5 minutes.

Then, 2.0 ml of 0.5% ninhydrin was added, mixed well and these were kept in a boiling water bath for 10 minutes. The solution was cooled down to room temperature. Then, the volume of each tube was made up to 10 ml with 60% ethanol. The bluish violet colour was developed and the readings were measured at 575 nm against reagent blank.

Estimation of DNA (Burton, 1956)

100 mg of DNA was dissolved in 50 ml of saline water. This solution was used as a standard solution. Different volumes of DNA solution were pipetted out (0.2 to 1 ml) and transferred into series of test tubes. Trichloroacetic acid extract of mustard seed was used as a sample. Sample solutions were transferred into a test tube. All the test tubes were made up to 3 ml with distilled water. 3.0 ml of distilled water was taken in a test tube to act as a blank. 5.0 ml of diphenyl amine reagent were added to all the test tubes then mixed well and heated on a water bath for 10 minutes. Optical density was measured at 595 nm.

Determination of Enzyme Activity of Amylase (α, β)

Assay of α- Amylase (Jayaraman, 1996; Plummer, 1998)

Extraction of α- Amylase from Mustard seeds

100 mg of fresh leaf samples from 15 days old plant was ground in 5.0 ml of distilled water. Then, it was centrifuged at 3000 rpm for 10 minutes and kept at 70˚C for 5 minutes to inactive β – amylase. This solution serves as the enzyme extract for further analysis. 100 µg/ml concentration of working standard maltose solution was prepared. 0.2 to1 ml of standard solution was transferred into the series of test tubes. 1.0 ml of test solution was transferred into two test tubes separately. Then, 1.0 ml of distilled water was added to all the test tubes, 1.0 ml of 0.1 m citrate buffer of pH 5.0 and 0.5 ml of 2% soluble starch was added.

Then, this solution was kept at 30˚C for 10 minutes. Then, 2.0 ml of DNS reagent was added and kept in a boiling water bath for 5 minutes. After cooling, the volume was made up to 10 ml with distilled water. A blank was maintained simultaneously with 1.0 ml of distilled water and the above said reagents were added. The absorbance was measured at 540 nm against reagent blank.

Assay of β- Amylase

Extraction of β- Amylase from Mustard seeds:

100 mg of fresh leaf samples from sprouted seedlings were ground in 5.0 ml of distilled water. Then, it was centrifuged at 3000 rpm for 10 minutes. This solution serves as the enzyme extract for further analysis. 100 µg/ml concentration of working standard maltose solution was prepared. 0.2 to 1 ml of standard solution was transferred into the series of test tubes. 1.0 ml of test solution was transferred into two test tubes separately. Then, 1.0 ml of distilled water was added to all the test tubes, 1.0 ml of 0.1 m citrate buffer of pH 3.4 and kept at for 5 minutes to inactive α- amylase. Then, 2.0 ml of 2% soluble starch was added and kept 30˚C for 10 minutes. Then, 2.0 ml of DNS reagent was added and kept in a boiling water bath for 5 minutes. After cooling, the volume was made up to 10 ml with distilled water. A blank was maintained simultaneously with 1.0 ml of distilled water and the above said reagents were added. The absorbance was measured at 540 nm against reagent blank.

RESULTS AND DISCUSSION

The effects of electromagnetic radiation, fields (ionizing and non- ionizing) on biological system are a major research topic today. The numbers of artificial man made ones of EM energy are increasing by leaps and bounds with a consequent need for assessment of safety norms and risk factors, which deserves detailed investigations. Research efforts are mainly addressed to the basic principle of coupling between EM energy and biomolecular structures. Microscopic sites, worth being studied are protein channels inside the membrane. EM fields perturb this gating and affect the whole physiological behaviour. Different investigators have proposed evidence of alterations in the rate of metabolic processes, by preferential slowing down of chemical transformations on the basis of relative magnetic susceptibilities of reactants and end products or by changing the activity of enzymes through bringing about greater order at either an intra or intermolecular level. In the present investigations, the mustard seeds, exposed to PMF (test) and control seeds unexposed to PMF were analyzed for certain morphological and biological parameters. The report of the investigation of estimation of biological parameters like carbohydrate, total protein, total free amino acid, nucleic acid and enzyme activity (α, β amylase) showed that there was an increase in the test amount when compared to the control.

In carbohydrate Anthrone reaction was used for the determination of carbohydrates. It is the basis of a rapid and convenient method for the determination of hexoses, aldopentoses, and hexouric acids, either free or present in polysaccharides. The main principle of this reaction is that carbohydrates are dehydrated by concentrated sulphuric acid to form furfural. Furfural condenses with anthrone to form a blue colour complex which is measured by spectrophotometric method. The total carbohydrates content present in mustard seeds both in control and test was found to be 3 mg/g and 4 mg/g respectively. The increase in carbohydrate content was 1mg/g when compared to control. The utilization of starch during germination was more in the PMF treated seeds than in the control seeds. During the process of germination there is mobilization of reserve food material from the cotyledon to the embryo for the nutrition of the radical and plumage. The utilization of starch during germination increases, then growth is also faster. The experiments tend to prove that the PMF hasten physiological processes like germination and growth of seedlings (Saktheswari and Saradhasubramanyam, 1987).

The total protein content was determined by using Folin – Ciocalteau reagent. It is a quite complex reagent and it contains phosphomolybdic acid and tungstate. The aromatic amino acids like tyrosine and tryptophan present in proteins give dark blue colour with this reagent. The colour formation is due to the reaction of alkaline copper with proteins and the phosphomolybadate is reduced by tyrosine and tryptophan present in proteins. The intensity of colour depends on the amount of aromatic acids. The protein content of mustard seeds present in control and test were found to be 8 mg/g and 11.8 mg/g respectively. The increase in protein content was 3.8 mg/g when compared to control. Amino acids contents are routinely estimated by Ninhydrin method. It reacts with amino acid and produce purple coloured complex. The free amino acid content in control and test were found to be 4 mg/g and 7.3 mg/g respectively. The increase in amino acid content was 3.3 mg/g when compared to control. The low

strength of magnetic field (1800nT) used in the treatment of seeds gives beneficial effect in the total protein and total aminoacid content in leaf of young seedling. Estimation of DNA content was used by diphenylamine reagent. The content of DNA also increases in the test when compared to the control. The possible mechanism for the interaction of EM fields with cell membrane, have explained that the electric fields as well as magnetic field stimulate transcription and both fields would interact with DNA directly (Blank and Goodman, 1997). The reports were shown in table 1 and figure 2.

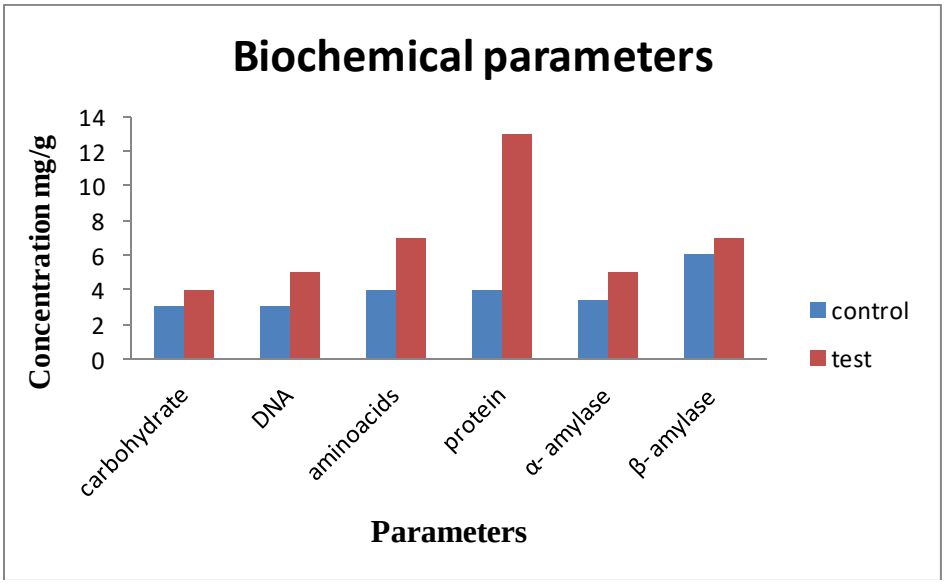

Figure 2: Estimation of Biochemical Parameters

Table 1: Reports of Biochemical Parameters

Name of the Parameters	Control (mg/g)	Test (mg/g)
Carbohydrate	3±0.44	4±0.63
DNA	3±0.67	4.4±0.58
Amino acid	4±0.5	7.3±0.6
Protein	8±0.76	12±0.87
α- amylase	3.4±0.48	5±0.3
β-amylase	6±0.64	7±0.61

Enzyme activity of amylase was determined by using alkaline Dinitro salicylic acid solution. Amylase is a hydrolytic enzyme, which breaks down many polysaccharides: for example starch converted to yield maltose. The product maltose is colourless and could not be estimated directly. However, it could be converted to coloured product by introducing chromophore groups like alkaline Dinitro salicylate which react with maltose to produce orange colour. The amylase enzyme (α, β) activity was increased in the test when compared to that in control. The result showed the mechanism of action of enzyme (α, β). The starch was hydrolysed to monosaccharide which nourishes the growing embryo. The PMF hastens the starch hydrolysation during germination and α-amylase activity. The PMF energy was used to increase the uptake and water absorption, chlorophyll content in the leaves, the cell division in the root, number of root hairs helping better water absorption, number of veins responsible for water and mineral salt translocation. Thus, PMF leads to better yield of plants. Estimation of total chlorophyll content was determined by using acetone extract of leaves. Total chlorophyll content and chlorophyll a, chlorophyll b in mustard leaves was found to be increased. The total chlorophyll content in test and control were found to be 56 mg/l and 48 mg/l respectively. The total chlorophyll content has increased by 18 mg/l in test compared to that in control. The report was shown in table 2 and figure 3.

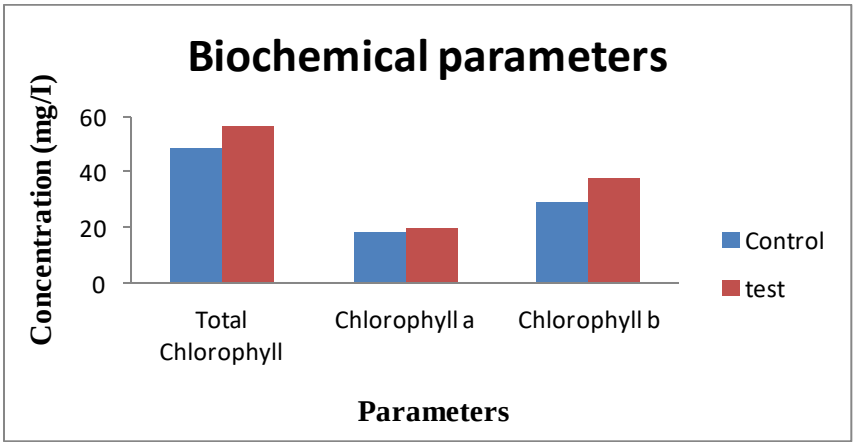

Figure 3: Estimation of Chlorophyll Content

Table 2: Reports of Chlorophyll content

S.No	Name of the parameter	Control (mg/l)	Test (mg/l)
1	Total Chlorophyll	48.32±0.04	56.52±0.06
2	Chlorophyll a	18.13±0.023	19.54±0.058
3	Chlorophyll b	29.22±0.42	37.62±0.59

It proved that PMF increases chlorophyll content. While studying morphological parameters the results showed that when the seeds were exposed to PMF increased the germination percentage, length of root, length of the shoot, growth and yield of the mustard seeds in the test when compared to that in control. This is evidently supported by a study conducted by (Livingston, 1996). The present study proves, the orientation of bar magnet towards the east-west direction was more effective to promote shoot growth than the roots under electromagnetic field of 4000 G. The optimum duration of treatment for maximum effect was 40 sec. The cell division increases the growth of the plants in the test when compared to that of control. Bhatnagar *et al.*, (1978) reported increase the number of parenchyma cells led to increased rate of cell division. The large number of root hair caused better water absorption. The growth of root was simple compared with the growth of stem. The stem has no nodes. Auxin accelerated very early growth in the tip of the root. The experiment has indirectly proved that PMF increases the hydrolysation of starch during germination, due to the activity of α – amylase. The α – amylase activity was directly related to auxin activity. The fresh weight of root and shoot were found to be 0.3 mg/g, 0.5 mg/g respectively. The result showed an increase in the weight of root and shoot in the test compared to that in control. The present study showed the plant growth was more when compared to that in control due to the cell division and proliferation in the test. Kumlin *et al.*, (1998) evidently reported that a short term magnetic field exposure affects the polyamine metabolism in-vivo affecting molecular pathways leading to cell growth and proliferation. Similarly, many in-vitro studies have reported magnetic field induced alterations in cell proliferation and activities of growth related enzymes such as ornithine decarboxylase or protein kinase (Byus *et al.*, 1987; Blackmann *et al.*, 1993). The result of the experiment showed an increase in growth rate, shoot and root length in test when compared to that in control. The results were shown in figure 4 and table 3.

Table 3: Reports of Morphological Parameters

Name of the Parameters	Control	Test
Weight of the Shoot (g)	6.0	7.0
Weight of the Root (g)	1.0	1.5
Length of the Shoot (cm)	11	15
Length of the Root (cm)	1.0	2.5

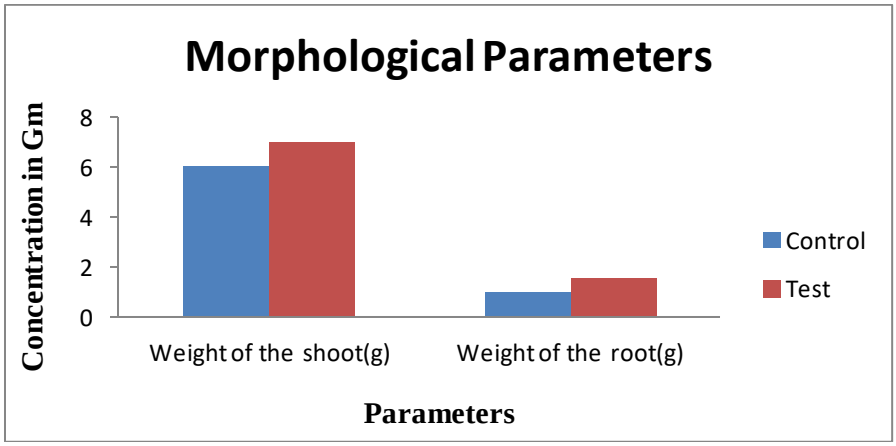

Figure 4: Length of Shoot and Root of Mustard Plant

The report was evidently proved by (Martinez *et al.*, 2001). He reported that chronic exposure of plants provided maximum total length and weight. This was shown in figure 5.

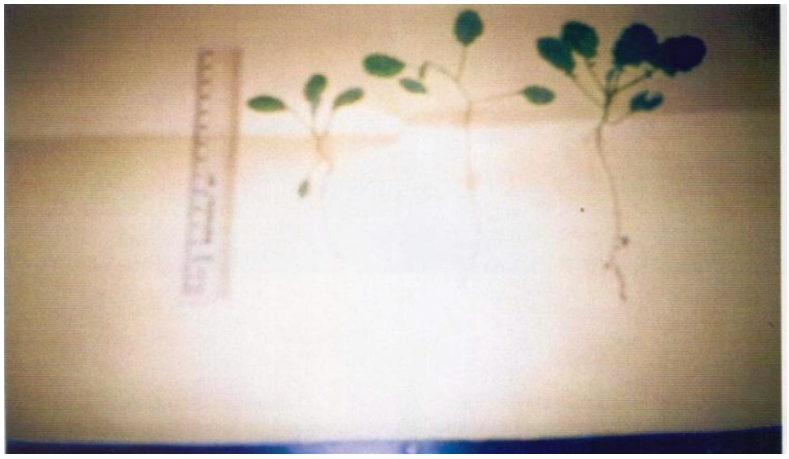

Figure 5: Shoot and Root Length of Mustard Plant

The result of the present study of the mustard seeds after exposure to PMF, the morphology and biochemical changes were seen to increase in the test when compared to control.

Conclusion

In conclusion, the exposure of mustard seeds to magnetic field revealed the stimulatory influence on the plants: increase in the length of root and shoot, increase the growth and yield of mustard seeds, increase of the average plants height and increase in total chlorophyll content. From this study it confirms that the magnetic field showed positive effect on the test plant both morphologically and biochemically. We hope to draw the attention of scientists to this interesting research study on, seed germination improvement and growth parameters by magnetic field, in order to reduce the use of insecticide, underground pollution and lastly minimize the production costs and increase farmer's earning by recognizing of optimal frequency and exposure time of magnetic field.

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