Materials Today: Proceedings xxx (xxxx) xxx

Contents lists available at ScienceDirect

Materials Today: Proceedings

journal homepage: www.elsevier.com/locate/matpr

Stimulation of *Pithecellobium dulce* (jungle jalebi) seed with electromagnetic exposure and its impact on biochemical parameter and growth

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ARTICLE INFO

Article history:
Received 3 November 2020
Received in revised form 16 January 2021
Accepted 21 January 2021
Available online xxxx

Keywords:
Pithecellobium dulce
Electromagnetic field
Physical dormancy
Biochemical parameters

ABSTRACT

The productivity of crops is an important indicative parameter for the prosperity of the Nation. Production of the crop is directly related to the germination of seed. The effect of stimulation with pre-sowing electromagnetic field treatments on *Pithecellobium dulce* seed to break physical dormancy is being explored in the present study. The uniform seeds were exposed to the electromagnetic field of 500 G for 10, 20, 30, and 40 min respectively which are optimized under the varying electromagnetic condition in the range of 100, 200, 300, 400, 500, and 600 G for a fixed time of 20 min. Non exposed seeds were used as the control seeds. The treated and control seeds were sown in separate trays in the same laboratory conditions. The effect of electromagnetic exposure on the seeds was investigated and compared with the observation of vital parameters of seeds - germination percentage (GP), relative germination percentage (RGP), relative root growth (RRG), relative shoot growth (RSG), germination index (GI), vigor index (VI). Biochemical parameters reducing sugar, total Protein, amylase activity were calculated by standard methods. The result indicates that the stimulation of seeds with a 500G for 30 min of exposure favors the growth of the plant as compared to another set of experimental conditions. Based on the result it can be concluded that pre-sowing electromagnetic treatment may have the potential to improve the productivity of crops by enhancing germination and seedling growth in a chemical-free, damage-free and sustainable procedure at the cultivator level.

Selection and peer-review under responsibility of the scientific committee of the Second International Conference on Recent Advances in Materials and Manufacturing 2020.

1. Introduction

Pithecellobium dulce belongs to the family of Fabaceae and commonly known as Jungle Jalebi. It is an evergreen medium-sized, spiny tree. Each part of the plant has vast nutritional and medicinal important properties. It is widely used in Ayurvedic medicines and home remedies. The bark and pulp of it used against gum ailments, toothache, and hemorrhage. Bark extract is used against dysentery, diarrhea, and constipation [9]. Extracts of leaves are used for gall bladder ailments and to prevent miscarriage. Grounded seeds are used to cleanse ulcers. Many studies on it resulted in its antioxidant, anti-inflammatory, anti-diabetic, and anti-cancer properties [13,20]. Nutritional values in plants stuffed with essential vitamins, amino acids, and minerals [7]. Due to its valuable quality in agroforestry, researchers pay keen interest in germination and cultivation of it. Germination from seeds takes time of more than 2 weeks. Delayed germination is also known as seed dormancy. Seed dormancy has been described as one of the least understood phenomena in seed biology [17]. The ability of the seed to delay their germination until the time and place are right is an important survival mechanism in the plant [21]. But it causes a delay in production. No pre-conditioning before sowing of seed is found successful in that. Soaking of seeds reduces germination and heating of seeds kills the seeds. It was observed that using different methods for breaking dormancy in seeds of Pithecellobium dulce. Methods taken were immersion of seed at 80 °C, chemical treatment of concentrated sulfuric acid for five minutes, and mechanical scarification with sandpaper**.** Among all mechanical scarification was found good but it damages the germination point of the seeds [6]. Thus it is of utmost necessary to develop a method that may helpful for fast germination and growth of the *Pithecellobium dulce*

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https://doi.org/10.1016/j.matpr.2021.01.649
2214-7853/
Selection and peer-review under responsibility of the scientific committee of the Second International Conference on Recent Advances in Materials and Manufacturing 2020.

seed without any damage and harm to seeds. Literature shows that methods available for breaking physical dormancy on the other seeds are chemical, thermal, and magnetic, and water imbibition methods. The effect of temperature on the germination of *Cucurbita pepo* seeds has also been reported, the temperature ranges taken for the study was 10–35 °C for 14 days in an incubator [23]. The effect of chemicals KNO_3 and $CaCl_2$ on the germination of melon seeds under laboratory conditions. Melon seeds were soaked in 1, 2, and 3% solutions of KNO_3 and $CaCl_2$ for 24 h [10]. The effect of exposure of stationary magnetic fields on maize seeds under laboratory conditions. They used two magnetic field strengths; 125mT and 250mT, and exposure time varied from 10 min to 24 h for each batch and observed its germination studies [11]. Previous studies also support these findings of the effect of static magnetic fields on the growth of crop of *Helianthus annuus*. Magnetic fields applied are 50, 100, 150, 200, and 250 mT for time duration 1, 2, 3, and 4 hr. It was concluded that growth on 200 mT for 2 h was better [3].The effect of magnetic field treatments on the growth of maize seeds was studied. They studied the growth of the plant, leaf water status, system of photosynthesis, and enzyme. These have been experimented with under greenhouse conditions. The seeds were exposed to static MFs of 100 and 200 mT for 2 and 1 h, respectively [1]. Another theory stated the effect of the pulsed magnetic field on Glycine max. A pulsed magnetic field of 1500 nT was injected on seeds up to 20 days and soil microbial populations and germination effect on plant observed [18]. The above-discussed methods have certain limitations. The Chemical-based method is costly and traces of chemicals get entered into the food chain. The presence of chemicals even at the minute level gives adverse effects to the environment. Thermal and mechanical abrasion methods cause damage to the seeds in bulk. The water imbibition method cannot sow the seed very next day, as imbibed seeds were not possible to store for a long time. Modern agricultural efforts are now in search of a competent ecology tool based on physical treatment of seeds to enhance the germination, seedling strength and crop yield [5] Researchers consider that magnetic and electromagnetic treatment can affect chemical reactions by altering electron spin location and in this way they may have the potential to cause a biological effect [17,24]. Based on the above facts present study is designed to investigate the effect of pre-sowing electromagnetic exposure on the seeds and its effect on parameters of seeds - seedling growth, plant physiology, reducing sugar, total protein, and amylase activity. Treated seeds of *Pithecellobium dulce* were grown under laboratory conditions and analyzed.

1.1. Collection of seed

The seed was collected from the government authorized shop. Only mature and uniform size seeds were used for electromagnetic exposure. A lot of 50 seeds were selected and stored in sealed paper bags after drying in shade. The moisture content in dry seeds was 8.5%.

1.2. Electromagnetic stimulation

Electromagnetic shocks provided to the seeds by the electromagnetic stimulator as shown in Fig. 1 it is assembled for the present study. A count of 50 healthy and uniform seeds of *Pithecellobium dulce* was exposed. To optimize the experimental conditions exposure on the seeds was done in two steps. The optimization steps are:

a) Under varying electromagnetic Induction - In the range of 100, 200, 300, 400, 500, and 600 G of electromagnetic Induction given in each seed lot for a fixed time of 20 min.

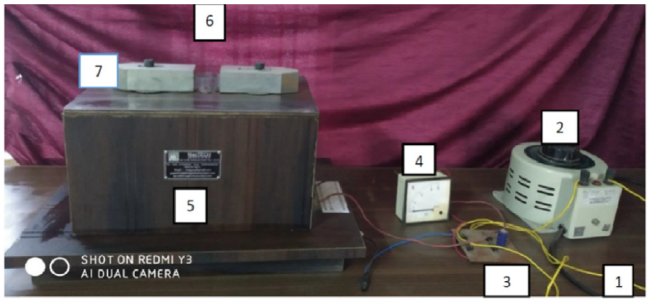

Fig. 1. Electromagnetic Simulator assembled setup.

b) Under varying time induction – In this condition duration of 10, 20, 30, and 40 min were selected to expose seeds on a fixed electromagnetic induction 500 G.

The components of the electromagnetic simulator are:

1 Current Supply- 230-volt single-phase AC
2 Dimmer sate- Control the current flow
3 Converter- Converts the current AC to DC
4 Ammeter- Measure the current supply
5 Magnetic Field Stimulator- Mug Tech association with variable magnetic strength (1 kilo Gauss to 10 kilo Gauss)
6 Sample holder
7 Bar magnet

1.3. Seed propagation and culture practices

The size of the soil container used for the experiment was 30 cm long, 20 cm wide, and 8 cm in depth. Pots were filled with garden soil up to 5 in.. Row to row and seed to seed distance was 1to 1.5 in.. For germination seeds were seeded in a pit of the depth of 1.5–2.5 cm and water irrigated twice daily. Experimental setup under laboratory conditions is shown in Fig. 2.

1.4. Germination parameter

Each experiment had a randomized complete block design with two replicates and with 50 seeds per pot. Germination counts were made every day for 3 weeks. A seed was considered germinated when the trip of the radical 2 mm had grown free of the seed. The germination parameter was calculated as described by The Association of official seed Analysis 1990 [14]by the following formula:

Percentage of Relative Root Growth (RRG %): It measures the growth efficiency of the plant

$$\mathrm{RRG}\% = \frac{\mathrm{Meanrootlengthinthesoil}}{\mathrm{Meanrootlengthincontrol}} \times 100$$

Germination index (GI): GI is a synthetic measure designed to reflect the synthesized germination ability including germination rate and germination of seed.

$$\mathrm{GI}\% = \frac{\mathrm{RSG} \times \mathrm{RRG}}{100}$$

Vigor Index (VI): To assess the vigor, the length of the root and shoot of each seedling was measured. The Vigor index was calculated using the formula.

VI = Total seedling length (Root + Shoot) × Percentage of germination

The germination parameter was calculated by the standard formula proposed by the International Seed Testing Association (ISTA). Seed viability is 100% detected by standard tetrazolium test

Fig. 2. Plant cultivation set up in laboratory.

[16]. Chemical Reaction of Tetrazolium test may be written as below and test performed on experimental seeds are shown in Fig. 3.

$$2,3,5-\text{Triphenyl2H}-\text{TetrazoliumChloride}\frac{2H+2C}{Formazanred}\rightarrow \text{Dehydrogenases}$$

1.5. Biochemical parameter

Changes in biochemical parameters indicate the germination and dormancy stage of the seeds. Biochemical parameters reducing sugar, total Protein, amylase activity are determined and interpreted.

Reducing sugar was measured by the standard DNSA method, which detects the free carbonyl group (C = O) of reducing sugars [4]. Glucose as reducing sugar reduces 3,5- dinitro salicylic acid at alkaline solution into 3 amino 5 nitro salicylic acid, color changes into brown. Chemical reaction is given below in Fig. 4:

Total protein content was performed by the standard Lowry method [12]. the total protein concentration is exhibited by a color change of the sample solution in proportion to protein concentration, which can then be measured using colorimetric techniques. A chemical reaction is shown in Fig. 5. The determination of protein concentration lies in the reactivity of peptide nitrogen with copper ions under an alkaline condition in which the sample color changes.

Total carbohydrate content was measured by the standard Anthrone assay method (Ruth [19]. Carbohydrates are dehydrated with concentrated H_2SO_4 to form furfural a green color complex that can be measured through a colorimeter. A chemical reaction is represented in Fig. 6.

Fig. 3. Seed viability test by tetrazolium chloride.

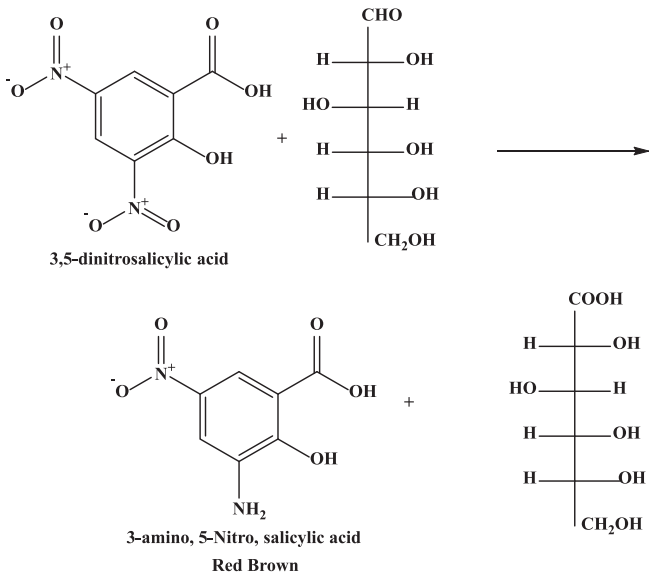

Fig. 4. Chemical reaction of reducing sugar estimation by DNS method.

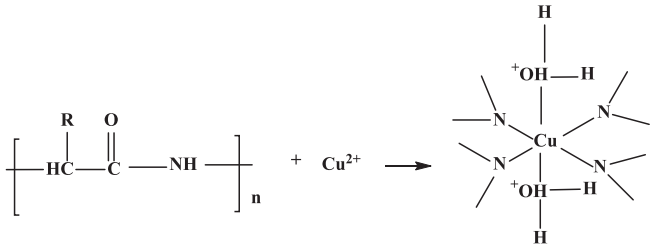

Fig. 5. Chemical reaction of total protein estimation by Lowry method.

2. Results and discussion

Seed dormancy is a physiological phenomenon in plants, which is caused by external or internal factors and prevention of seeds germination, it elongates the period of the crop cycle. Seed dormancy is broadly divided into two types' embryonic dormancy and physical dormancy. Embryonic dormancy is a genetic feature of the seed so nothing will do externally. Physical dormancy is a morphological feature of seed. It occurs in seeds due to the formation of an impervious layer on the outer surface while maturation and drying of the fruits and seeds. In normal times enzymes and bio-chemicals present inside the seeds are staying in inactive forms. Because the outer layer of the seed prohibits the exchange of moisture and gases inside the seed from the environment.

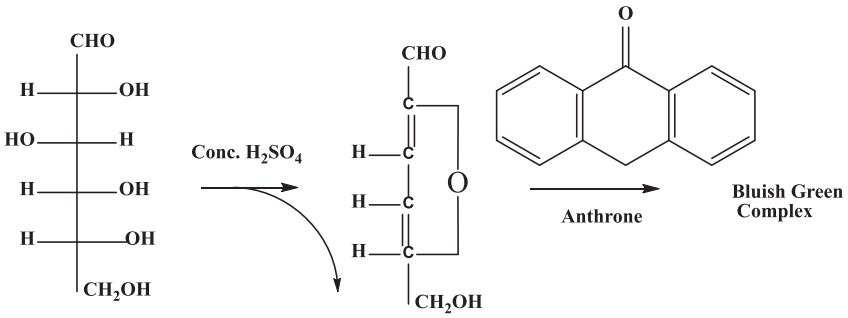

Fig. 6. Chemical reaction of total carbohydrate estimation by Anthrone assay.

During germination, these enzymes and bio-chemicals get activated in presence of moisture and gases, thus the germination process is initiated inside the seeds. But in the case of a hard seed coat exchange of moisture and gases inside the seed from the environment get blocked. Due to the unavailability of these environmental activating agents seed left in the ground in unresponsive stage till then the possibility of exchange of water and gases. Thus the time required for germination gets stretched and germination periods of seed become long. Such time delaying factor gives a negative impact on the productivity of the plant. Other varieties of physical dormancy are hard seed coat, immature and rudimentary embryo, and inhibitors materials. Because of this reason, the main focus of the researches is only towards breaking of Physical dormancy instead of Embryonic dormancy. Due to electromagnetic exposure on the surface of the seed forms minute cracks on the surface of the seed coat. These minute hair cracks make it permissible for the exchange of gases and water from the environment to the seeds. Enzymes and biochemical get triggered due to the exchange of these vital factors and the process of germination inside the seed gets activated. For accuracy and optimization of the proposed study, experiments were performed in two sets. First at fixed time duration 20 min but varying electromagnetic field induction on the seeds, values are listed in Table 1. It is observed among values of all electromagnetic fields that Germination Percentage increases with increasing field strength of electromagnetic stimulation. Germination Percentage increases with increasing from 100G onwards. At 500 G electromagnetic field gives better results and at a high field, it goes towards declination on the germination of seeds.

Germination parameters: Based on the result obtained from Table 1 further experiments were performed on *Pithecellobium dulce* seeds at 500 G electromagnetic induction. The time chosen for exposure was 10, 20, 30, and 40 min. Germination parameters concerning time are listed in Table 2. The growth rate is considered as one of the important parameters to enhance seed germination. For that Germination index (GI %), Relative root growth (RRG %) Relative shoot growth (RSG %) parameters of seeds are compared. Table 2 summarizes that a better result was obtained at 30 min.

Plant height, the diameter of the stem, radius of stem, seed vigor index, and germination percentage parameters are listed from Figs. 7 to 10. Comparisons of germination parameters between control and electromagnetic treated seeds were observed through the figures. A controlled set of seeds have less germination percentage, seedling growth, diameter, and radius of the plant

Table 2
Germination Parameter of *Pithecellobium dulce* treated with 500 G electromagnetic field under varying time period.

Characteristics	Germination index (GI %)	Relative root growth (RRG %)	Relative shoot growth (RSG %)
10 min	100.02	98.04	104.56
20 min	105.12	100.00	113.15
30 min	147.35	150.00	109.30
40 min	109.92	130.00	106.92

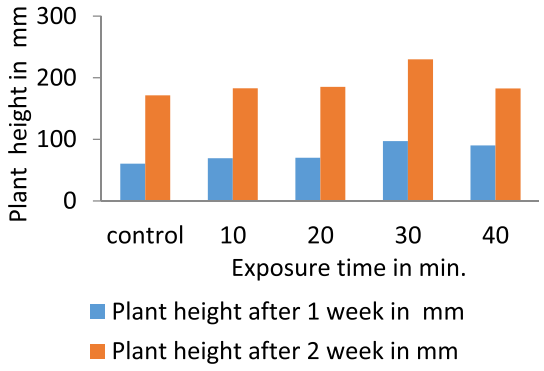

Fig. 7. Plant height of *Pithecellobium dulce* before and after Electromagnetic Exposure.

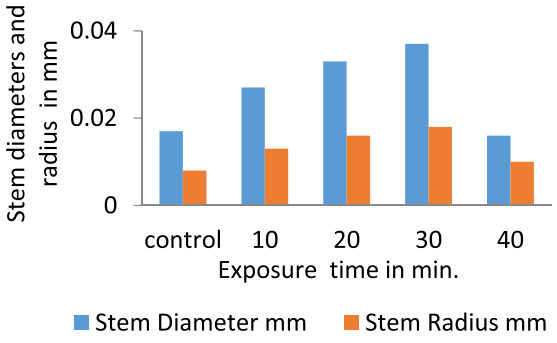

Fig. 8. Stem diameters and radius of *Pithecellobium dulce* before and after Electromagnetic Exposure.

Table 1
Germination Percentage of *Pithecellobium dulce* treated for 20 min underexposure of varying an electromagnetic field.

Electromagnetic field (G)	100	200	300	400	500	600
Germination Percentage	70	71	79	80	94	84

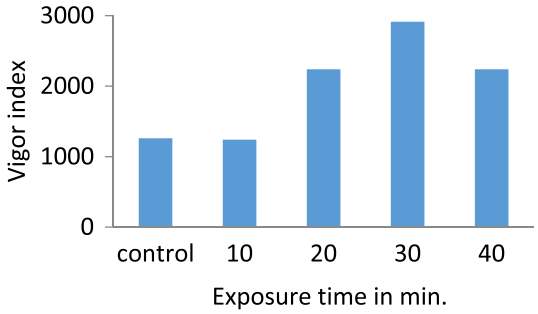

Fig. 9. Vigor index of *Pithecellobium dulce* before and after Electromagnetic Exposure.

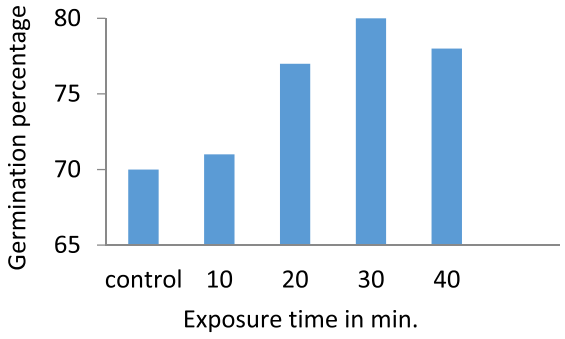

Fig. 10. Germination percentage of *Pithecellobium dulce* before and after Electromagnetic Exposure.

stem, and vigor index were identified. If comparisons are made among treated seeds of 10, 20, 30, and 40 min of electromagnetic induction. The noticeable increase by increasing the exposure time on the seeds gives a favorable effect on the germination and growth parameters. Parameters promoted by the electromagnetic exposure indicate the alterations in the growth pattern of seedling and shows a better result at 30 min exposure.

Biochemical analysis: The level of biochemical present inside the seed before and after stimulation was determined by standard methods as available in the literature[15,2,22]. Reducing sugar, total protein, amylase, and total carbohydrates are plotted in Fig. 11. Changes in the concentration of biochemical give an important indication to start the germination process in any seed sample. During electromagnetic stimulation on seed, minute cracks develop on the surface of the seed coat. Cracks make it possible to exchange gases and moisture through the environment. This vital exchange triggers the hormones responsible for the germination inside the seeds.

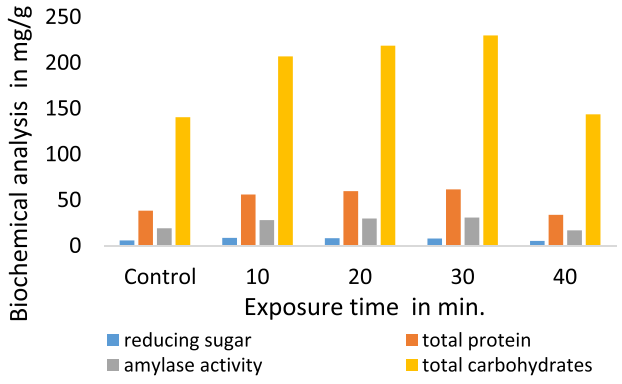

Fig. 11. Presence of reducing sugar, total protein, amylase activity, and total carbohydrate on *Pithecellobium dulce* before and after Electromagnetic Exposure.

Reducing sugar has played a great physiological role during the germination process and it gives the signal for germination signals. Total protein Hydrolysis of stored proteins produced free amino acids, which support protein synthesis in endosperm and embryo and so proceeding of germination process. Amylase hydrolyzes the starch into sugars, which provide the energy for the growth of roots and shoots. The increased concentration of amylase after stimulation is observed as compared to control, it favors the starting process of germination. The total carbohydrate increased to a maximum during germination, subsequently decreased in amount during maturation [8]. The significant increase in reducing sugar was observed. Its trend is much favorable with 500 G for 30 min stimulation than treated with 500 G for 10, 20, and 40 min. a similar pattern of changes observed in total protein, amylase activity, and total carbohydrate. These parameters also increase in treated seed instead to control one.

3. Conclusion

Electromagnetic waves create minute cracks on the surface of seeds while exposed. Cracks on the seed coat make it permeable to environmental gases and moisture for the seed. The combined effect of moisture and gases triggered the enzymes which are responsible for bio-chemicals changes, related to seed germination. Results of the experiment may conclude that specific time 30 min and specific electromagnetic field exposure 500 G are desired for the treatment of seed to get a better effect. In controlled conditions germination parameters and growth of the plants are less whereas exposure to the high electromagnetic field and long duration of exposure adversely affects the germination and growth. Increased quantity of reducing sugar, total protein, and amylase activity in treated seed confirms the above finding that physiological changes get initiated inside the seed for germination. Through the proposed technique farmers may able to treat a lot of seeds without any harm of chemicals, damage of plumule, and use of huge manpower. Early germination of the seed helps in crop production and crop rotation quietly and safely. Thus, it may be concluded that electromagnetic field exposure technology in seeds, may have the great potential to give a significant improvement in crop germination, enhancement of productivity, and better plant growth for future aspects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors are thankful to the management of Rungta College of Engineering and Technology for providing laboratory facilities.

References

[1] A. Anand, S. Nagarajan, A.P. Verma, D.K. Joshi, P.C. Pathak, J. Bhardwaj, Pretreatment of seeds with static magnetic field ameliorates soil water stress in seedling of maize (Zea mays L.), Indian J. Biochem. Biophys. 49 (2012) 63–70.
[2] A. Cotado, M.B. Gracia, S. Munne-Bosch, Physiology seed dormancy increases at high altitude in Pyrenean saxifrage (Saxifraga longifolia lapeyr), Environ. Exp. Bot. 171 (103929) (2020) 1–9.
[3] A. Vashisth, S. Nagarajan, Effect on germination and early growth characteristics in sunflower (Helianthus annuus) seeds exposed to exposed to static magnetic field, J. Plant Physiol. 167 (2010) 149–156.
[4] B. AdesolaOgyndipek, K.T. Sangosanya, B.D. Akintola, A. Oluwa, E. Hassan, Comparative study on the biosorption of Pb(II), Cd(II), and Zn(II) using lemongrass(Cymbopogon citratus): kinetics, isotherms, and thermodynamics, Chem. Int. 2 (2) (2016) 89–102.

[5] C.C. baskin, Jerry M. Baskin, Germination ecology of seeds physical dormancy, Seeds 2 (2014) 145–185.
[6] F.E.C.B. Pereria, P.G. Isaias, B.T. Salvador, P.B. Clarisse, Dormancy breaks in Pithecellobium dulce (Roxb.) Benth, Seeds, Semina: ciencias Agrarias, Londrina 36 (2015) 165–170.
[7] G. Srinivas, H.P. Geeta, A.S. Kumar, J.N. Champawat, A review on Pithecellobium Dulce A potential medicinal tree, Int. J. Chem. Studies 6 (2) (2018) 540–544.
[8] K.S. Amuti, C.J. Pollard, Soluble Carbohydrates of dry and developing seeds, Phytochem. 16 (5) (1977) 529–539.
[9] K.V. Kulkarni, V.R. Jamakhandi, Medicinal uses of Pithecellobium dulce and its health benefits, J. Pharmacogn. Phytochem. 7 (2) (2018) 700–704.
[10] M. Farooq, S. Basra, H. Rehman, N. Ahmad, B. Saleem, Osmopriming improves The germination and early seedling growth of melons(Cucumis melo L.), Pak-J Agric 44 (2007) 529–536.
[11] M. Florez, M.V. Carbonell, E. Martinez, Exposure of maize seed to stationary magnetic field: effect on germination and early growth, Environ. Exp. Bot. 59 (2007) 68–75.
[12] M. Iqbal, Z. Haq, Y. Jamil, M. Ahmad, Effect of pre-sowing magnetic treatment on properties of pea, Int. Agrophysics 26 (2012) 25–31.
[13] M. Kumar, K. Nehara, J.S. Duhan, Phytochemical analysis & antimicrobial efficacy of leaf extract of Journal of Pharmacognosy and Phytochemistry Pithecellobium Dulce, Asian J. Pharm. Clin. Res. 6 (1) (2013) 70–76.
[14] N. Gupta, M. Agrawal, D. Pal, Physico-Chemical analysis of municipal solid waste on germination and plantlet enhancement of groundnut (Arachis Hypogaea) seeds, J. Indian Chem. Soc. 97 (2020) 989–992.
[15] P. Singh, M. Agrawa, Impact of electromagnetic treatment on seed germination and growth of cucurbita pepo and Cucumis melo, AIP Conf. Proceed. (2019), 070003-1-070003-7.
[16] Q. Manzoor, R. Nadeem, M. Iqbal, R. Saeed, T.M. Ansari, Organic acid pretreatment effect on Rosa bourbonia phyto-biomass for removal of Pb(II) and Cu(II) from aqueous media, Bioresour Technol 123 (2013) 446–452.
[17] R.S.D.S. Venacio, Overcoming dormancy of Senna multijuga seeds with an ultrasonic probe thecomparison with ultrasound and sulfuric acid baths, Cienc. Rural 49:9.1-7 (2019).
[18] R. Radhakrishnan, B.D.R. Kumari, Pulsed magnetic field treatment enhances soybean plant growth, Indian J. Biochem. Biophys. 50 (2013) 312–317.
[19] Ruth Finkelstein, Wendy Reeves, Tohru Ariizumi, Camille Steber, Molecular aspects of seed dormancy, Annu. Rev. Plant Biol. 59 (2008) 387–415.
[20] S.K. Murugesan, D.K. Lakshmanan, V. Arumugam, R. AnufAlexander, Nutritional and therapeutic benefits of medicinal plant Pithecellobium dulce (Fabaceae): A review, J. Appl. Pharmaceutic. Sci. 9 (7) (2019) 130–139.
[21] S. Munawar, M. Naeem, H.H. Ali, M. Jamil, M. Iqbal, M.Q. Nazir, R.M. Balal, M.E. Safdar, Seed dormancy breaking treatment for africanpurslane (Zaleya pentandra), Plant daninha 33 (4) (2015) 623–629.
[22] S. Sevengor, F. YasarKusvuran, S. Ellialtioglu, The effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidative enzymes of pumpkin seedling, African J. Agricult. Res. 6 (21) (2011) 4920–4924.
[23] S.Z. Salmasi, Study of cardinal Temperatures for pumpkin (Cucurbita pepo) seed germination, J. Agronomy 5 (2006) 95–97.
[24] W. Abbas, T.H. Bokhari, I.A. Bhatti, M. Iqbal, Degradation study of disperses red F3BS by gamma radiation/H_2O_2, Asian J. Chem 27 (2015) 282–286.