Experiments on The Effects of Reagants and Electricity on Seed Germination Growth and Viability

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Abstract:

Most plants develop from seeds. Dry seeds under dormant condition show no sign of life. Only on the availability of stimulants like water, an adequate temperature etc., do seeds undergo biochemical reactions and develop in to plants. Generally the first and most important thing for seeds to germinate is the availability of an optimum quantity of water. Under all circumstances seeds are soaked in '*plain*' water before germination. They swell up, their surfaces become smooth and slowly radicles protrude. Various chemical changes occur within a seed during germination. Now these reactions may be altered by use of reagents. If the chemical natures of the constituents of seed layers are known, it may be possible to change the chemicals by suitable reactions. For E.g. if aldehydic group be present, it may either be oxidized or reduced. But if this occurs the entire process of germination and plant growth '*should*' be affected. In this paper chemical effects on seed germination have been expounded in detail.

Keywords: Seed; Germination; Radicle; Oxidation

Introduction:

Germination of seeds is a natural phenomenon which prevents extinction of plant species. From the agricultural point of view germination is important because it is the basis of crop production. Use of disease resistant varieties along with the extensive use of chemical fertilizers have enhanced yield in the recent years. Before plantation seeds are either soaked in water for a predetermined time or they may be scattered directly on wet soil to facilate germination. The process to be chosen depends on the nature of the seed. Dry seeds contain 10-15% water. Low levels of respiration have been reported in dry seeds. During imbibitions in 15 minutes is the reformation of keto acids from amino acids by deamination and transamination. Whatsoever the case may be the *prerequisite of seed germination is water*. The source of water is either soil moisture or it may be supplied from ponds, lakes etc. Though the water may contain dissolved minerals in it, *the germination process is unaffected due to their quantity being minute*. Now if chemicals are present in considerable amounts, would the seeds still germinate? The following questions are self-assertive:-

- The chemical may induce side reactions within the seed. So in a way the chemical may be harmful.
- The excess addition of electrolytes may render the solution hypertonic so there may be a chance of plasmolysis.

  If the germinated seeds are planted in soils with excess of salts would the plants still flourish? What would be the effects on plant growth?

Before proceeding further a general idea regarding the composition of a seed is essential.

<u>Seed Coat-</u> It is easily permeable and in no way delays germination. It consists of four layers viz.

I. The outer portion of the pericarp, consisting of one or more layers of cells whose walls contain some pectic substance. Glucose is present in the pericarp.

II. The inner epidermis of the pericarp in which lignification has occurred.

III. The lignified testa.

IV. The suberized remnant of the nucellar tissue.

Endosperm- Contains large amount of starch (carbohydrates), proteins like glutenin and gliadin minute quantities of fats and some minerals.

Embryo- Contains carbohydrates like sucrose, enzymes like peroxidase, and catalase minerals like potassium and magnesium.

Generally the seed coat is permeable. This is evident from the fact that large quantities of water are imbibed by the seed resulting in its swelling. Many chemicals are encountered in seeds like glucose, starch, cellulose, sugars enzymes etc. Their percentage varies according to the nature of the seed.

Various compounds are present within the seed among which number cellulose, glucose, starch, proteins, enzymes, sugars etc. Now looking in to these compounds one by one

i. Cellulose: - Bearing a formula $(C_6H_{10}O_5)_n$, it is a poly sachharide consisting of a linear chain of several hundred to many thousands of β (1→4) linked D-glucose units. CH_2OH group is susceptible to attack by oxidizing agents.

ii. Glucose: - Contains one –CHO and one CH_2OH group. These are easily oxidizable using agents like HIO_4, HNO_3 etc. While on oxidation using periodic acid it yield a mixture of formic acid and formaldehyde, on using nitric acid it gives glucaric acid (saccharic acid).

iii. Starch: - Contains 20% water soluble amylase along with 80% insoluble amylopectin. Amylose contains –CH_2OH group which may get oxidized, though *the reactions may be slow due to its long chain structure and presence of neighboring groups*.

iv. Sugars: - May be in the form of fructose, sucrose etc. These may exhibit characteristic reactions of sugars.

v. Proteins and amino acids: - These are viable to attack in the COO^- and NH_3^+ groups. Strong conditions may alter the structure of protein.

vi. Enzymes:- Presence of external chemicals can retard or accelerate their activity, the determination of which requires careful observation.

Chemicals and Apparatus required:

1. Pea, Mung, Lentil and Gram (purchased from a grocer)
2. Oxalic acid crystals
3. Ammonium sulfate
4. Sodium Chloride
5. Sucrose (Ordinary table sugar)
6. Monosodium glutamate

7. Potassium Permanganate
8. Dilute sulfuric acid.
9. Muriatic acid
10. Hydrogen peroxide (6% solution)
11. Iron, Aluminum, Zinc, Copper
12. 5V D.C source
13. 4W LED lamp operating from 230V source
14. 100W incandescent lamp
15. Merbromin 2% solution
16. Methylene blue stain solution
17. Tea leaves extract
18. Flour
19. Sodium hydroxide .1N solution
20. Kerosene oil
21. Acetone
22. Absolute alcohol
23. Mustard oil
24. Boric acid I.P

Experimental:

The choice of seeds was *crucial* because *germination time* and *availability* were important factors. For the experiments Pea (*Pisum Sativum*), Mung bean (*Vigna Radiata*), Gram (*Cicer Aritinum*) and Lentil (*Lens Culinaris*) were chosen. The reason behind choosing the above were:-

I. Short time required for germination (≤24 hours).

II. Rapid growth

III. Large availability and low cost.

The above seeds (in dry condition) when soaked in tap water for 8-10 hours swell up by imbibing water and the surface becomes smooth. The water is then removed and the wet seeds are covered and kept in the dark. After about a day radicles are observed. When planted in soil, they develop into plants. Under favorable conditions the plants bear fruit. This is the commonest method practiced for the production of plants. When large scale production is required, the dry seeds are often scattered on wet soil by broadcasting method. The moisture which is the prerequisite of germination comes from the soil itself. However under any circumstance if the seeds are soaked in water beforehand, the entire process gets accelerated.

To start with a simple experiment similar to the one adopted by farmers for the cultivation of crops was tested. Ten pieces each of pea, gram, mung bean, and lentil were soaked in 100ml. water for 8 hours. The period chosen was exactly half of the 16 hour soaking period by which imbibition is supposed to reach completion[1]. After completion of 8 hours, the water was thoroughly rinsed and the seeds were covered. After another 10 hours emergence of radicles was observed. These were allowed to grow for another 10 hours and were planted in untreated soil sprinkled with water. Very quickly all the 40 seeds (10 from each sample) developed in to healthy plants. It was also observed that *mung beans display the fastest rate of germination and a few germinated during the soaking period itself*. So the entire *process of germination was complete within 12 hours for mung and 28 hours for the remaining samples*. The plants were kept under diffused sunlight not enough to scorch them.

The effect of salinity on the germination and growth[2] has already been studied. It has been found that high salinity is harmful for the development of plants in terms of height, length of root, no. of leaves, leaf dry mass and root dry mass.

Previously it has been stated that the seed coat is permeable. A simple experiment was performed to test it.

Five seeds of each sample were soaked in turn in kerosene oil, mustard oil, acetone and absolute alcohol for 24 hours. Apparently no absorption occurred. The seeds retained their crooked structure as is expected when they are dry. The surfaces of the seeds soaked in kerosene oil mustard oil became oily but those immersed in acetone and absolute alcohol remained normal. After the completion of 24 hours, the seeds were immersed in water for 10 hours, the water was removed and the seeds were covered to induce germination. Within the next 30 hours they germinated. They were planted and developed into healthy plants. So it may be inferred that *nonionic liquids like mustard oil, alcohol etc have no apparent effect on seed germination and viability*.

Experiment no. 1

Pea seeds were soaked in a solution of $KMnO_4$ acidified with a few drops of dilute H_2SO_4 for 8 hours. The solution gradually faded in color indicating oxidation in some form. After 8 hours the seeds were properly washed with cold water and left in the air for half an hour. These were then covered and kept in the dark to allow germination. After about 15 hours emergence of radicles was observed. These were planted in moist soil. The cotyledons showed rapid growth. From after two days potassium permanganate solution was added to the soil in doses of 5-10drops at a time and this was done 3 times a day. The plants were not kept under sunlight. They were irradiated by a 4W LED instead for 4 hours a day. The plants showed very rapid growth. *Within four hours shoot length increased by 2.5mm under constant irradiation*.

Pea seeds were ground in to powder using a mortar and pestle. The powder was treated with acidified potassium permanganate. It slowly turned colorless indicating oxidation of the powder. The same must have occurred when the seeds were soaked in acidified potassium permanganate solution.

Experiment no. 2

Concentrated tea leaf extract was used to soak pea and gram seeds in separate beakers for 10 hours. They were washed with cold water and covered. They germinated and were planted in soil enriched with the same tea leaf extract. The plants were irradiated in turn by a 4W LED and a 25W GLS lamp. The plants showed very rapid growth. Doses of tea leaf extract were added to the soil instead of water to keep it moist.

A sample of the same tea leaf extract that was used for soaking the seeds was treated with acidified $KMnO_4$ which turned colorless indicating oxidation of the extract.

Experiment no. 3

Pea seeds were soaked in a solution of strong sodium carbonate for 10 hours. The seeds imbibed and swelled up as usual. They were washed and kept in air for half an hour. After this they were covered and allowed to germinate. After 20 hours it was found that the seeds had shrinked and only 50% seeds had germinated. When planted in soil they showed retarded growth. The shoots were lean and the plants unsteady. They did not survive long.

Experiment no.4

Gram seeds were soaked in a solution of .1N NaOH for 10 hours. They were washed with cold water and kept covered for 20 hours. The seeds shrinked and did not germinate. After 1 day black particles were observed on the surfaces of the seeds. So clearly NaOH had damaged the cells and allowed the growth of microbes.

Experiment no.5

Pea seeds were soaked in a strong sucrose solution and allowed to rot. The *principle behind this was that sugar and pea being both biodegradable should be acted upon by microbes and broken down in to simpler substances*. As expected the solution rotted within two days. It was allowed to stand for another 5 days till black scummy mass floated on the surface of the solution. This solution has been referred to as the '*extract*'. Pea, gram and mung were soaked in 80ml water '*poisoned*' with 10 drops of the extract. The soaking period was 10 hours. They were then allowed to germinate. *Emergence of radicles was observed 15 hours except for mung where this period was only 6 hours*. The same experiment was repeated, this time with 20 drops of extract. Every seed germinated. They were planted in soil enriched with the extract. They developed in to very healthy plants.

Experiment no. 6

Pea, gram and mung were soaked in s solution of 1% boric acid for 10 hours. The seeds swelled up and the surfaces became smooth. The seeds were rinsed and left in the open for half an hour. After this they were covered to induce germination. After another 10 hours emergence of radicles was observed. These were planted in soil moistened with the same 1% boric acid solution that was used for soaking the seeds. They rapidly developed in to healthy plants. The soil was kept moist by periodic addition of 1% boric acid solution in drops. The plants were irradiated using a 4W LED emitting 350lumens for 4 hours a day.

Experiment no. 7

Mung was soaked in a solution of 2% NaCl along with a few drops of methylene blue so as to render the solution blue for 8 hours. The seeds developed a blue hue. They were covered after rinsing the solution. Very healthy radicles emerged. They were planted in soil enriched with the same solution. Very rapidly they developed in to healthy plants. The same experiment was repeated using pea. They yielded similar observations and results.

Experiment no. 8

Mung was soaked in 6% hydrogen peroxide to a depth such that the top of the seeds were in the peroxide air interface. A lot of bubbling was observed. The green color of the seeds faded and they seemed bleached. This was certainly due to oxidation by nascent oxygen. After 8 hours even before the removal of the solution, tiny radicles were observed. They were allowed to grow and were planted in soil. They developed in to very healthy plants. But gram treated by the same method showed contradictory results and showed no germination. The surfaces of the seeds were hued black and they shriveled.

Experiment no. 9

Mung was soaked in pure lemon juice for 10 hours. The seeds did not swell up indicating no absorption. When left in the open air after the post soaking period, no change in physical appearance of the seeds was noticed. They were now dipped in water. Still no absorption occurred. The seeds were scattered over wet soil. Slowly they dried up and shrinked. So clearly lemon juice having 6%citric acid by composition had destroyed the imbibing capacity of the seeds.

Experiment no.10

Pea was soaked in M/50 solution of oxalic acid for 12 hours. The seeds swelled up indicating absorption. They were washed with water and kept covered to allow germination. But no germination occurred. Instead white particles were observed on the surfaces of the seeds. The seeds became '*sticky*'. Mung treated by a similar method germinated and developed in to plants.

Experiment no. 11

Strong ammonium sulfate solution was used to soak pea seeds for 14 hours. The seeds imbibed as usual. These were washed with water and covered. No germination occurred. White sticky mass formed on the seed surfaces instead.

Besides conducting these experiments for studying seeds germination and growth, several '*generations*' of plants were grown to study the overall effect of soil chemicals and electric current on plant growth and development. The use of the word generation here is improper because no study was made on the offspring. Moreover the same species of plant was not grown for two successive generations.

For these experiments two separate pots of maximum diameter 7cm and minimum diameter 4.5cm were filled with equal quantities of soil. One was labeled 'SET UP A' while the other 'SET UP B'. Consequently the first generation of 'SET UP A' was named 'Generation 1A' and so on.

Generation 1A

Pea seeds were soaked in plain water for 10 hours and were covered. After germination they were planted in soil. The soil was kept moist by periodic addition of small doses of water. The plants were irradiated using a 4W LED emitting 350lumens at an average of 90 minutes a day. The light was connected in series with the soil and the soil was used as a conducting medium so as to pass 30mA A.C current through the soil. After 3 days 5 ml of 6% H_2O_2 was added to the soil and a lot of bubbling was observed. 24 hours hence M/20 solution of potassium permanganate was added in doses of 3ml at a time thrice a day. The current was increased to 100mA by replacing the LED with an incandescent lamp. The leaves started yellowing and drooping. They lasted for a total of 10 days.

Generation 2A

Gram seeds were soaked in a solution of monosodium glutamate prepared by dissolving 5g of the solute in 100ml water along with a few drops of methylene blue. The idea behind this was to test whether the compound would poison the seeds in any way. The seeds shriveled up but germinated, nevertheless. They were planted in the soil. The plants were irradiated using a 4W LED emitting 350 lumens. 4.5-6mA D.C was passed through the soil using iron electrodes. The anode gradually corroded and a brown scar mark was observed around the anode. So iron was slowly dissolved in the soil. The plants were quite healthy and showed rapid growth.20 ml of M/20 potassium permanganate was added to the soil. After 2 days 20 ml water with 5 drops of methylene blue was added. 20 ml strong tea leaf extract was also added to the soil. The plants were very healthy. They lasted for 20 days.

Generation 3A

Mung was soaked in strong sucrose solution energized from the output of electronic ballast (used in fluorescent tubes). The solution became hot and the temperature increased to 60˚C. The solution developed a yellow hue. The seeds dried up once the solution was removed. On stimulating by a few drops of water they germinated and were planted. Very healthy plants developed. The soil was energized using 230V-50Hz A.C using a 2.5μF capacitor in series. The plants developed very rapidly. Their life span was 20 days.

Generation 4A

Mung was soaked in a solution prepared by dissolving .2g $KMnO_4$ and 5g ammonium sulfate in 100ml water. Only 50% seeds germinated. They were planted in soil and M/20 potassium permanganate (20ml) was added to the soil. Very healthy plants were obtained. They were irradiated by a 4W LED. The plants lived for 15 days.

Generation 1B

50ml. M/20 $KMnO_4$ solution was added to soil. Dry mung was scattered over this wet soil. The soil was energized from a 230V-50 Hz A.C source with a 2.5μF capacitor in series. An additional earth electrode was also buried in the soil. All the electrodes were made of copper having a crossection of $1.5mm^2$. Emergence of radicles was observed in less than 48 hours. The plants were irradiated using a 4W LED emitting 350 lumens. 40 ml of M/20 potassium permanganate was added to the soil in doses of 10 ml at a time 4 times a day. Strong tea leaf extract was also added. Plants flourished. They lasted for 20 days.

Generation 2B

10 drops of lemon juice was diluted to 25ml and was added to the soil. Zinc and iron electrodes were buried in the soil. Pea seeds were soaked in M/20 potassium permanganate for 12 hours and in water for another 45 minutes. These were allowed to germinate and were planted in soil. They developed in to very healthy plants. As usual they were irradiated from a 4W LED. The plants survived for 25 days.

Generation 3B

Pea seeds were soaked in water for 12 hours and allowed to germinate. 50ml M/20 $KMnO_4$ was added to the soil and the germinated seeds were planted in this soil. 5V D.C was applied to the soil using Al electrodes at an average of 4 hours /day. Concentrated sugar solution (50ml) was added to the soil after 5days. 25ml water, sucrose and flour were allowed to rot. The rotten solution was added to soil. Ammonium sulfate solution was added to the soil. The plants lasted for 20 days.

Generation 4B

Pea seeds were soaked in solution of 100 ml water and 5 drops of 2% merbromin ($C_{20}H_8Br_2HgNa_2O_6$) for 9 hours. This is an oraganomercuric compound and a fluorescein. Mercury being toxic *might* affect germination and retard plant growth. During the soaking period the solution was supplied with 5V D.C using aluminum electrodes which caused electrolysis of the solution. The seeds were stained red. They were planted in the soil. Pre germinated gram seeds were also scattered over the soil and the plants were irradiated by a 4W LED. The seeds germinated in their due course. Apparently Hg had no effect.

Observations: The results of the experiments have been tabulated below, the parameters of comparison being time required for radicle emergence, germination percentage, and nature of the radicles (healthy or unhealthy) and maximum height attained.

Table no. 1

Seed used	Solution used	Time of immersion in hours	Time required for emergence of radicles in hours	% of germination	Nature of radicles	Max. height in cm.
Pea	M/20 $KMnO_4$ acidified with dilute H_2SO_4	8	15	100	Very healthy	29
Gram	Conc. Tea leaf extract	10	15	100	Very healthy	29
Pea	Conc. Na_2CO_3	10	20	< 50	Unhealthy	10
Gram	.1N NaOH	10	20	0	—	—
Pea, gram and mung	Water poisoned with extract	10	15-20 hours for pea and gram, 6 hours for mung	100	Very healthy	27.5
Pea, gram and mung	1% boric acid	10	10	100	Very healthy	29.5
Mung and pea	2% NaCl with few drops of methylene blue	8	10	100	Very healthy	28
Mung and Gram	6% H_2O_2	8	0 for mung, no germination for gram	100 for mung, 0 for gram	Very healthy for mung	26 (for mung)
Mung	Pure lemon juice	10	No germination	0	—	—
Pea and Mung	M/50 oxalic acid	12	No germination for pea, 0 for mung	70 for mung, 0 for pea	Healthy for mung	26 (for mung)
Pea	Strong ammonium sulfate	14	No germination	0	—	—
Pea	Merbromin solution excited by passage of 5V D.C	9	15	100	Healthy	24

It has been observed that irradiation by LED cool daylight/6500K caused very rapid growth. Addition of $KMnO_4$ drop wise to the soil proves to be highly beneficial for the growth of pea. Dissolution of aluminum slowly in to the soil by electrolysis also increased growth and helped plants flourish rapidly. Constant excitation from a 230V-50Hz source using a capacitor in series is also beneficial.

Results and Discussions:

Seeds germinate as a result of complex biochemical reactions occurring within the seeds stimulated by the availability of water and a prerequisite temperature. Chemicals affect the reactions in various ways. Only a few have dealt with in this paper. The following important results have been summarized:—

I. Acidified M/20 solution of $KMnO_4$ leads to excellent germination rates in legumes (in peas, gram and mung). Its addition in regulated doses to the soil leads to rapid growth of plants.

II. If the plants (as mentioned above) are irradiated by LED lamps, the growth is accelerated. This prevents the risk of the plants from getting scorched in direct sunlight.

III. Ammonium sulfate though an excellent fertilizer totally retards seed germination. The percentage of germination decreases significantly. The few seeds that germinate display weak and lean radicles that do not show growth.

IV. Oxalic acid allows '*preferential*' germination. While mung germinates in a solution of oxalic acid others do not.

V. Alkaline solutions like sodium hydroxide and sodium carbonate totally retard germination. They assist in the growth of fungi instead.

VI. Hydrogen peroxide allows the germination of mung but retards the germination of other legumes (pea and gram).

VII. Pure lemon juice having 6% citric acid disallows germination. The seeds don't absorb. However lemon juice diluted with water causes germination and rapid growth.

VIII. No absorption of organic solvents like acetone, absolute alcohol and kerosene is noticed. But soaking in these solvents does not damage the seeds either. On soaking the seeds back in water the seeds absorb water and germinate.

IX. Application of electricity to the solution during the soaking period causes electrolysis of the solution but has no apparent effect on the germination process. However if copper or iron electrodes are used insoluble metal hydroxides on the seeds surfaces hinders absorption and consequently retards germination.

X. Application of low voltage D.C at 5-12V and 5-10mA to the soil using aluminum electrodes leads to slow dissolution of electrodes in the form of Al^{3+} in to the soil. It proves highly beneficial for the growth of plants.

XI. Compound of boric acid, merbromin and methylene blue have no significant effect on the germination.

XII. Best results are obtained if the soaking period is ≈8hours and the seeds are soaked in plain water for 15 to 20 minutes during the post soaking period.

XIII. Solutions of strong acid like HCl just like strong bases (discussed earlier) have a detrimental effect on seed germination. Concentrations of ≥.01M may be harmful.

XIV. High voltage A.C (230V-50Hz) in '*short pulses*' to the soil renders the soil hot and assists in destroying harmful bacteria and helps in plant growth.

Compounds like potassium permanganate are inexpensive and easily available in the market. Careful application can significantly increase the yield of legumes. Also the legumes do not require sunlight and a low power LED serves the purpose. Hence the plants may be cultivated under LED without compromising with growth and yield. However GLS lamps consume more power and produce more heat resulting in yellowing and consequent drying up of the leaves and are not useful for growth. It has been observed that tea leaf extract is highly beneficial; for germination and growth. The extract is 80-90% more effective as compared to ordinary leaves used as manure. Occasional addition of boric acid proves helpful. Zinc, iron and aluminum electrodes buried in soil causes their slow leaching in to the respective ions increasing growth.

Conclusion:

Addition of chemicals e.g. potassium permanganate in predetermined doses can significantly affect the germination rate and growth of leguminous plants like pea, mung and the like. These may also prevent the growth of harmful microbes and hence enhance productivity. $KMnO_4$ works best under these situations owing to its oxidizing nature. Irradiation from a LED placed at a short distance about 1 inch horizontally from the plant causes very rapid growth and eliminates the dependence on sunlight. Slow dissolution of zinc and aluminum ions in to the soil also increases yield to a great extent. For this electrolysis at low voltage and 5-10mA current is highly useful. However current should not exceed 20mA as it would lead to very fast dissolution of ions and increase their concentration in the vicinity of the roots causing plasmolysis. The voltage should be maintained at 5-10V.

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