Evaluation and Comparison of Effects of Different pH on Rate of Germination of Leguminous Plant Seeds of Cicer arietinum and Vigna radiate

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Abstract

Seed germination occurs only in optimal condition in presence of proper nutrient, sunlight and water. This statement holds correct when the pH of the given solvent for germination is about 6.5 – 7.5 with the mean pH is 7. The pH of the solution added to germinate the seeds, when either acidic or basic, hugely affects the rate of germination. This research article deals to investigate how change in pH of the solution affect the germination rate of two common seeds found in India, named Cicer arietinum, called Bengal gram and Vigna radiata. Ten fresh seeds of each type were added to ten different petri-dishes. All the germination conditions were kept same except the pH of the solution added during five experimental days. Solutions of pH 3.0, 4.5, 7.0, 9.2 and 10.0 were prepared and were added to individual petri-plates. The rate of germination of seeds present in each petri pate was observed for a period of five days and the rate was reported. pH 7 reported the best germination, followed by slightly acidic pH, 4.5. The germination rate showed at different pH showed P value less than 0.05 in ANOVA with 95% confidence interval, indicating the data is significantly different. Extreme acidity or alkalinity did not support growth or sprouting of the seeds and hence the seeds were spoilt without any trace of microbial attack.

Keywords: *Seed germination, Cicer arietinum, Vigna radiate, legumes, Bengal gram, Green moong, pH.*

1. Introduction

Seed is defined ideally as a matured ovule which is formed after fertilization. It contains embryonic materials required for growth of plant from the seeds and is covered by hard protective membrane. This protective hard covering is called seed coat [1]. The ovules present in structure of a flower are covered by protecting coating called integuments [2]. After fertilization, the ovary forms fruits, ovules forms seeds and integuments forms testa or the seed coat [3]. Dry indehiscent seed of the family of Gramineae are called grains and not seeds [4]. This is called caryopsis. Seeds have a microscopic opening called a micropyle that allows water to enter. Tegmen refers to the inner layer that lies beneath the testa. The embryo found inside seeds is made up of cotyledons, radicles, and plumules. Endosperm is present in the seeds. Certain seeds lack the endosperm. The scar formed by the seed breaking off the ovule stalk is called the hilum [5].

On the basis of number of cotyledon, there are two different type of seeds. These are namely monocotyledonous and dicotyledonous seeds. Monocotyledonous seeds like rice maize have single cotyledon in it. The cotyledon is quite thin with either very thin or no endosperm present in it. These seeds have prominent coleoptile and coleorhiza from where plumule and radicle emerge separately. Dicotyledonous seeds as the name suggest has two cotyledons attached with each other. These cotyledons can be found in seeds of peas, beans. They are usually fleshy because of the thick endosperm content in them and is rich nutritional source [6].

Seed germination is defined as the process by which a seed under influence of suitable optimal conditions, the seed coat ruptures to form radicle and plumule [7]. The radicle in turn grows to root while the plumule grows to shoot. The endosperm contains the nutrients and other nutritional requirement required by a seed to sprout and to grow to a sapling. This entire process can be called as sprouting of seeds. The seedlings are the most vulnerable stage of a seed growth. Seed to seedling and small plant are often prepared by nursery with specially designed conditions [8]. The growth of seedling from seed is called germination in case of angiosperms and gymnosperms. Similarly, for the growth of sporeling from spore in case of fungi, the process is also called germination. Three factors are required for successful germination: Water which penetrate to the seed by hilum. Water interacts with the endosperm and swells it up. This endosperm starts nourishing the embryo to start growing and emerges the plumule and radicle. Oxygen or air is required for optimal growth. It releases the energy for germination. Endosperm exhausts after the plumule is emerged. Beyond this stage the nourishment is obtained by the plant by photosynthesis. Temperature or warmth is required by the seeds for germination. Proper temperature allows the embryo to grow to plumule and radicle. Increase in temperature upto the maximum or optimal temperature is allowed. Beyond this temperature, the seed will enter dormancy stage and won't germinate. [9,10]

During agricultural investigations, it has been found that the crop plants are usually exposed to different temperatures, pH and salinity. These are called environmental stress. Due to the stressed condition, the rate of germination is hampered [11].

Seed dormancy can be defined as the condition, when in presence of harsh environment called environmental stress like high temperature, less rainfall, water scarcity, alteration in soil pH, the seed does not germinate. The testa and tegment remain unenterable for rupture that is required for germination [12], [13]. This ecological event ensures that the seed wont germinate when the probability of the seed germination is quite low. Even in presence of all normal condition, a seed requires certain period of time to germinate. This is called the period of dormancy. Presence of required water, air and temperature breaks a seed dormancy followed by its germination. During dormancy period, the embryo is being nourished by the endosperm. A seed can remain in stage of dormancy for several years before it starts germinating [14].

Based on the type of dormancy, the process of seed dormancy is classified into two categories called the embryo dormancy and coat dormancy. In the embryo dormancy, embryo does not form plumule or radical under harsh environment or for a few days even in presence of suitable conditions. Coat dormancy is the most common type of dormancy found in seed. In this case, the seed coat becomes hard and impenetrable and hence does not rupture in unfavourable conditions [15].

Based on the position of cotyledon seen during germination, the process of germination can be classified into two different types called the epigeal and hypogeal germination. As the name suggest ‘epi’ means above and ‘geal’ means ground. So, Epigeal Germination is the type of germination where elongation of hypocotyl is more rapid than the epicotyl. This means that the hypocotyl elongation brings the cotyledon above the soil with the plumule. Bean, tomato, pumpkin shows epigeal germination. All the leguminous plants show epigeal germination. Thus, the seedling grows in such a way that the cotyledons are pushed above the ground [16]. On the other hand, Hypogeal Germination is the complete opposite. In hypogeal germination, as the name suggests, ‘hypo’ means below, the cotyledon does not move up the soil and remains below. The epicotyl elongates more ensuring that the plumule moves up leaving back the cotyledons below the soil. Pea, mango, maize, rice, gram and groundnut have germination of this kind. If cotyledon is considered as a level, epicotyl is the axis that remains above the cotyledon or the plumule and hypocotyl is the axis portion that remains below the cotyledons or the radicle [17], [18], [19].

pH of a solution is the method to check the degree of acidity and basicity of a given solution. But pH is an important factor on which the rate of germination of a seed depend on. pH of the level 3 to 9 mainly facilitates seed germination [20], [21]. But pH of 7 is the best for seed germination. It is found at very high pH or a basic medium like 11 poppy seeds are unable to show a high rate of germination [22].
Two different seeds used are *Cicer arietinum* (Bengal Gram) and *Vigna radiata* (Mung beans). They both belong to same family called Fabaceae and the same order called Fabales [23, 24]. Both of these are highly nutritious food, which are grown in South Eastern Asian countries like India, Bangladesh, Pakistan and mainly in Indian Gangetic plains of West Bengal. The main reason of using these two types of seeds is:

a) They belong to same family
b) Due to high nutrition value they are consumed widely
c) Rapid rate of germination would ease the research

The effects of different pH on rate of germination of these two seeds are investigated in this paper.

2. Materials Required

2.1. Chemicals Required

All chemicals used were of laboratory and analytical grade. Chemicals were taken of company like EMPLURA and EMPARTA. Potassium dihydrogen phosphate (Emplura, Merck Life Science Private Limited), Phosphoric acid (Emplura, Merck Life Science Private Limited), ammonium acetate (Loba Chemie Pvt Ltd.), glacial acetic acid (Loba Chemie Pvt Ltd.), ammonium chloride (Emplura, Merck Life Science Private Limited) and ammonium hydroxide (Loba Chemie Pvt Ltd.) were used. Apart from these, laboratory prepared distilled water were used to perform the experiment.

2.2. Apparatus Required

Apparatus required includes five beakers of 200mL, thirty petri-plates, 5 pipettes of 5mL volume, Measuring cylinder. Instruments used were Digital Weighing Balance (Wensar Weighing Scales Limited – PGB200) and pH Meter (Systronics – 335). pH meter was used to determine pH of the buffer solution. Medical grade adsorbent cotton was used for this experiment.

2.3. Sample Required

The seeds of *Cicer arietinum* and *Vigna radiata* were procured from local plants cultivated in Champahati, West Bengal, India.

3. Procedure

3.1. Solution Preparation procedure

Preparation of buffer [25] of pH 3.0 was prepared by dissolving 0.34g potassium dihydrogen phosphate in water (90ml). Phosphoric acid is added to increase the pH to 3.0. The volume was made upto 100ml by adding distilled water to it. Universal indicator gives the colour reddish orange. pH meter read the pH to be 3.1 ± 0.21.

The buffer of pH 4.5 [25] was prepared by dissolving 7.7g ammonium acetate to 90mL distilled water. 7mL of glacial acetic acid was added to the solution to make the pH 4.5. The volume was adjusted to 100mL. Testing with universal indicator forms orangish-green colour. pH meter reading was 4.5 ± 0.1

Distilled water was prepared in the laboratory. The pH of the solution was reported to be 7.0. The universal indicator showed formation of green colour. Double distilled water was not used because it entirely eliminates any dissolved nutrient which would hamper the germination of the seeds. The pH meter reading was 7.0 ± 0.21

Buffer of solution pH 9.2 [25] was prepared by dissolving 13.4g of ammonium chloride to 60mL of water. Next, 16.8mL of ammonium hydroxide was added to the resulting solution.

The volume was made up to 100mL. This formed pH of buffer 9.2 and was indicated by bluish colour given by universal indicator. pH meter reading was 9.19 ± 0.02

Buffer of pH 10.0 [25] was prepared by dissolving 5.4g ammonium chloride to 20mL distilled water. 35mL of ammonium hydroxide solution was added and diluted to 100mL solution with distilled water. This gave pH 10.0. Universal indicator gave colour bluish-violet. The reading given by pH meter was 10.03 ± 0.11

3.2. Setup Preparation:

Ten different petri-plates were taken. Cotton of medical grade was used to cover the surface of petri-dishes. 50 different fresh seeds of *Cicer arietinum* and *Vigna radiata* were taken and ten seeds were added to each petri plate. The setup was arranged such that each set up contains two petri-plates containing ten seeds of *Cicer arietinum* and *Vigna radiata*.

Pipettes were used to withdraw different buffer solutions. For each buffer solution, a clean aseptic pipette was used to prevent contamination. First day, 10mL of buffer was added to soak the cotton. From the next day, 6mL of the buffer solution of each set was added to the petri-plate to allow germination to occur. The seeds were exposed to diffused sunlight during the early day time. Over-exposure was strictly controlled.

Replenishment of buffer after 24 hours was done continuously for five consecutive days. The observations were recorded in Figure 1..

Petri-plates containing *Vigna radiata* was kept on the beaker containing the buffer solution to prevent evaporation of the buffer, which would might affect the pH.

The entire set of experiment was repeated thrice.

3.3. Experimental Conditions

The research was performed in the year 2018 – May month in West Bengal, India. Average temperature was between 35 – 42oC at night and day respectively and humidity ranged from 45 – 51% in the period of experiment.

4. Observations

The seeds were observed each day to check any trace of germination. Continuous replenishment of buffer solution was done to ensure that the seeds do not remain dry. Sprouting or germination were observed and were noted. Figure 1-5 represents the germination from Day 1 to Day 5 for both the seeds used.

Figure 1. Germination of *Cicer arietinum* and *Vigna radiata* at different pH in Day 01

Figure 2. Germination of *Cicer arietinum* and *Vigna radiata* at different pH in Day 02

Figure 3. Germination of *Cicer arietinum* and *Vigna radiata* at different pH in Day 03

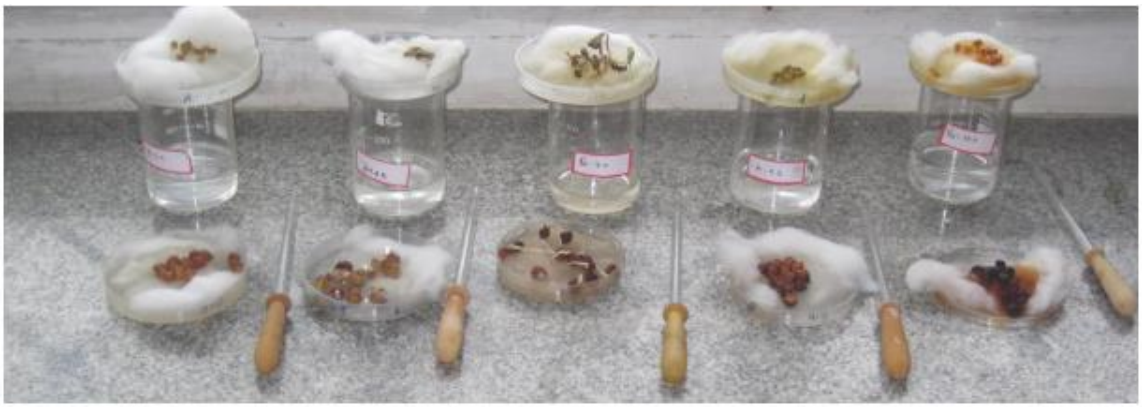

Figure 4. Germination of *Cicer arietinum* and *Vigna radiata* at different pH in Day 04

Figure 5. Germination of *Cicer arietinum* and *Vigna radiata* at different pH in Day 05

5. Results

The rate of germination of seeds present in each petri-dish which as supplied of buffer of different pH for five consecutive days were noted and tabulated in Table 1. ANOVA analysis was done for the observations obtained. The germination of *Cicer arietinum* seeds showed P-value of 0.020882, while for *Vigna radiata*, the P-value is 0.006362 which indicates that the data obtained with confidence interval 95% is significantly different.

Table 1. Rate of germination of the seeds of *Cicer arietinum* and *Vigna radiata* at different pH in 5 consecutive days (based on three times experiment) [n=3]

	No. of seeds germinated									
	Cicer arietinum					***Vigna radiata***				
	pH = 3.0	**pH = 4.5**	**pH = 7.0**	**pH = 9.2**	**pH = 10.0**	**pH = 3.0**	**pH = 4.5**	**pH = 7.0**	**pH = 9.2**	**pH = 10.0**
Day 00	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
Day 01	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
Day 02	0.00 ± 0.0	0.00 ± 0.0	4.00 ± 1.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.333 ± 0.577	5.667 ± 0.577	0.00 ± 0.0	0.00 ± 0.0
Day 03	0.00 ± 0.0	0.667 ± 0.577	7.667 ± 0.577	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	1.00 ± 1.0	8.333 ± 0.577	0.333 ± 0.577	0.00 ± 0.0
Day 04	0.00 ± 0.0	1.333 ±	8.667 ±	0.333 ±	0.00 ± 0.0	1.333 ±	1.333 ±	9.333 ±	1.333 ±	0.00 ± 0.0

		0.577	0.577	0.577		0.577	0.577	0.577	0.577	
Day 05	1.333 ± 0.577	1.333 ± 0.577	9.667 ± 0.577	1.00 ± 0.0	0.00 ± 0.0	1.667 ± 0.577	2.333 ± 0.577	9.667 ± 0.577	1.667 ± 0.577	0.00 ± 0.0
P value	**P = 0.020882**					**P = 0.006362**				

At the end of day 5, the percentage of seeds germinated at petri-plates supplied with buffer of different pH of *Cicer arietinum* and *Vigna radiata* are tabulated in Table 2.

Table 2. Percentage of seed germinated at Day 5 for both plant species (based on three times experiment)

Sl. No.	**pH**	**Percentage seed germination**		
		Cicer arietinum	***Vigna radiata***	
1	3.0	13.33 ± 5.773 %	16.667 ± 5.773 %	**P = 0.02088**
2	4.5	13.33 ± 5.773 %	23.33 ± 5.773 %	
3	7.0	96.667 ± 5.773 %	96.667 ± 5.773 %	
4	9.2	10 ± 0.00 %	16.667 ± 5.773 %	
5	10.0	0.00 ± 0.0%	0.00 ± 0.0%	

From the seeds which have reported to be germinated, the length of the plumule of each of three experimental sets were measured and reported in Table 3 shown below.

Table 3. Length of plumule seen in the germinating seeds

	Length of plumule germinated from the seeds (Average of germinated seeds) [in mm]									
	Cicer arietinum					***Vigna radiata***				
	pH = 3.0	**pH = 4.5**	**pH = 7.0**	**pH = 9.2**	**pH = 10.0**	**pH = 3.0**	**pH = 4.5**	**pH = 7.0**	**pH = 9.2**	**pH = 10**
Day 00	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
Day 01	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
Day 02	0.00 ± 0.0	0.00 ± 0.0	2.66 ± 0.577	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	1.16 ± 0.76	2.667 ± 0.577	0.00 ± 0.0	0.00 ± 0.0
Day 03	0.00 ± 0.0	1.16 ± 0.76	7.33 ± 1.54	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	2.33 ± 0.577	9.00 ± 1.00	1.333 ± 0.577	0.00 ± 0.0
Day 04	0.00	6.333	11.667	1.333	0.00	1.333	4.667	15.66	2.333	0.00

	± 0.0	±1.527	± 1.52	± 0.577	± 0.0	± 0.577	± 0.577	± 2.081	± 0.577	± 0.0
Day 05	1.333 ± 0.577	7.667 ± 0.577	19.667 ± 2.081	2.66 ± 1.52	0.00 ± 0.0	4.00 ± 1.00	9.333 ± 0.577	21.667 ± 1.527	2.333 ± 0.577	0.00 ± 0.0
P value	**P = 0.03317**					**P = 0.00588**				

The graphical representations of the rate of germination seen at end of Day 5 as a function of pH was plotted using Microsoft Excel 2019 and is illustrated in Figure 6 given below.

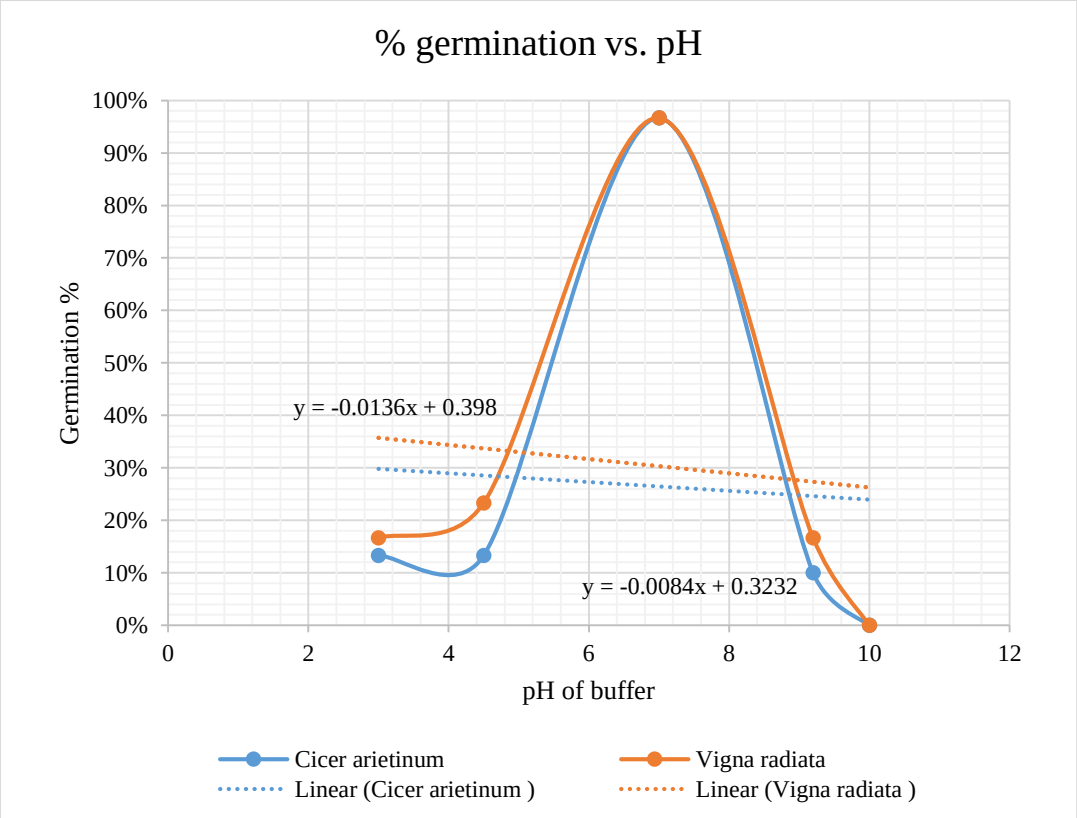

Figure 6. Graphical Representation of percentage germination vs pH of *Cicer arietinum* and *Vigna radiate*

The graphical representation illustrated in Figure 7 and Figure 8 signifies the statistical significance of the conducted experiment of *Cicer arietinum* and *Vigna radiata* respectively.

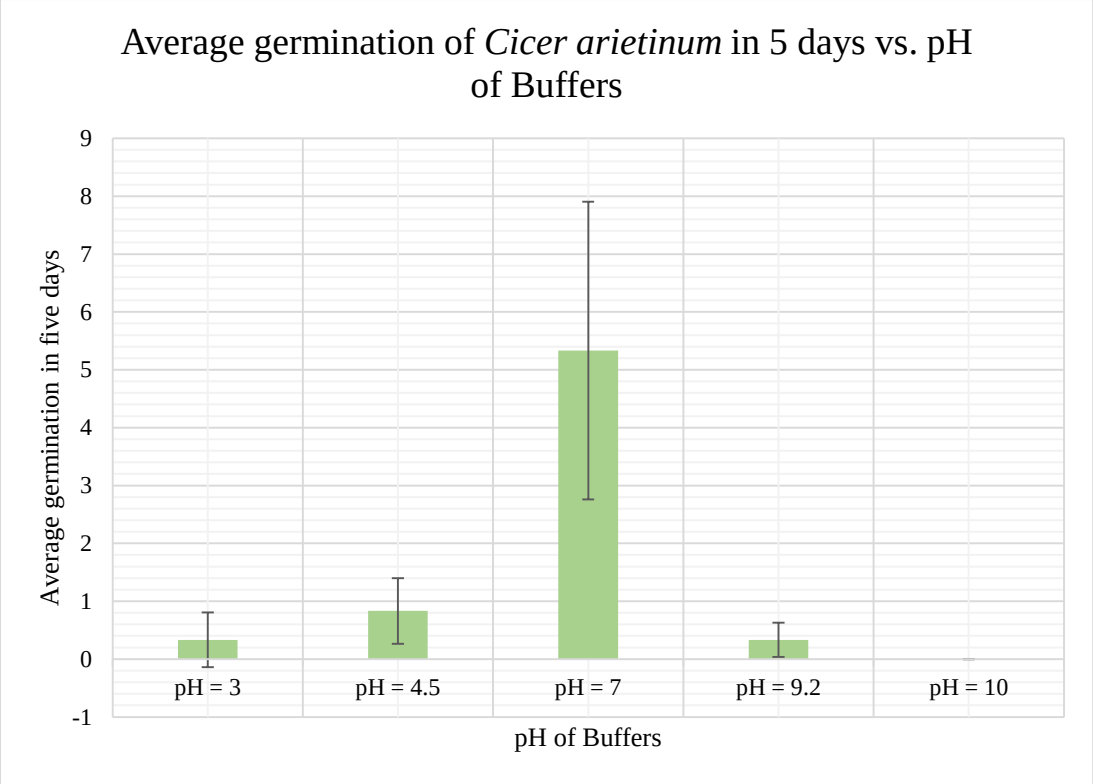

Figure 7. Statistical significance of the average germination of *Cicer arietinum* in five days

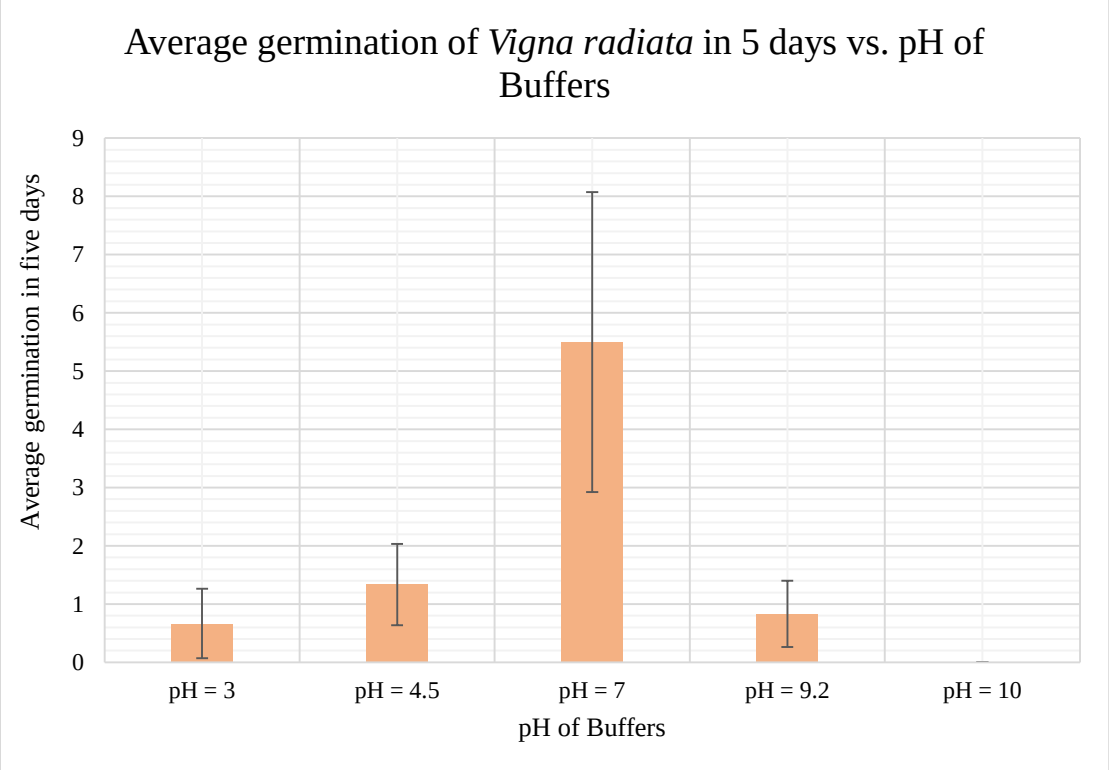

Figure 8. Statistical significance of the average germination of *Vigna radiata* in five days

The effect which pH has on plumule length is shown in Figure 9 at the end of Day 5 for both the seeds.

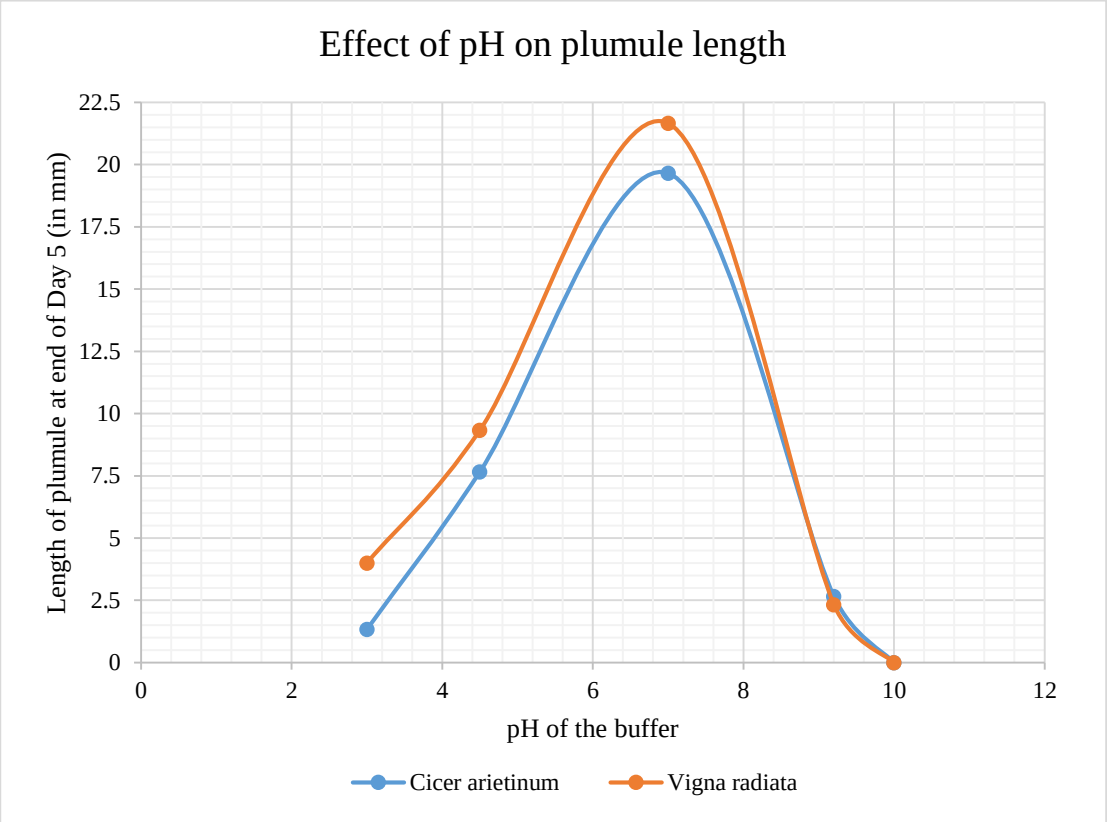

Figure 9: Effect of pH on length of plumule

6. Discussion

The observations and the results obtained explains, it was clear that both the seeds from the Fabaceae, namely *Vigna radiata* and *Cicer arietinum* showed maximum growth at pH 7, that is the neutral pH.

P-value less than 0.05 at 95% confidence interval of seeds of both the plants indicated that the difference in rate of germination is statistically significant. Hence, it obeys alternate hypothesis of research.

The correlation of germination of seeds and pH for *Cicer arietinum* at end of Day 5 can be represented in form of an equation y = -0.0084x + 0.3232, where 'y' is the percentage of seed germinated and 'x' is the pH of the solution added. The equation for rate of germination of *Vigna radiata* is y = -0.0136x + 0.398, 'x' being the pH of the buffer solution and 'y' is the percentage of seeds germinated.

The error bars found in the bar graph represented in Figure 3 and 4 are unequal in length, which represents no sign of error bar overlap. It is also a demonstration of statistical significance of the work done and indicates P value to be less than 0.05 at a confidence interval of 95%.

In all of the cases, most seeds remained dormant for the first two days. These period of dormancy makes sure that they collect nutrition for their growth. The dormancy is overcome after two-days exposure of optimum condition followed by rupture of the testa layer of the seed coat for germination [3].

From this study, it is also clear that both the seeds of Fabaceae family show epigeal germination as the hypocotyl grew first which pushed up the epicotyl out of the cotton film and then it gave rise to the new plumule. Thus, the cotyledons moved up the cotton film during sprouting and germination [16]. This is explained by rapid shoot of the plumule from the seeds as shown in Table 03.

It was clear that the plants also grew well in that of the acidic medium than the alkaline medium. This indicates their role of Nitrogen fixation to make the soil alkaline in nature. Being leguminous plants, they did not require any nutrient. They grow on soil with less Nitrogen content and causes Nitrogen fixation [26, 27]. Thus, the pH of soil increases after the growth of these plants. They are nitrogen fixers and can grow even in very low concentration of nutrients. So only cotton and pH solution was enough for the seeds to germinate. No other source of nutrition were required to be added to them.

The explanation to the observation that germination occurs best in slightly acidic to neutral soil can be:

1. These are leguminous plants and require Nitrogen fixing bacteria for Nitrogen fixation which grows best in slightly acidic to neutral soil. Thus, leguminous plant will prefer slightly acidic over alkaline pH to germinate. This concept is utilized in crop rotation done in agricultural field to increase soil fertility by leguminous plants.
2. Several enzymes act during the growth of radicle and plumule from the seed. These enzymes include amylase which breaks down sugar to provide enzymes; protease which breaks complex protein; lipase which hydrolyse fatty acid and glycerol to provide enzyme and cellulase which breaks down the cell wall of the seed cotyledons cell to emerge radicle and plumule [28].
3. Solubility of different nutrient in soil to be absorbed by the seed depend on the pH of the soil. In extreme pH, the solubility of nutrient gets less to nil. So, the seed does not get nutrient from soil and hence does not germinate [29].
4. Certain bacterial forms Indole acetic acid in the soil, which accelerates germination process. These bacteria thrive well in slightly acidic to neutral pH [30].

7. Conclusion

Difference in pH has significant effects on rate of germination of *Vigna radiata* and *Cicer arietinum* seeds. Acidic and alkaline pH condition does not allow growth of any fungus or other microbes on the seeds. Neutral pH is optimal for seed germination followed by mild acidic pH condition.

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