©2015 IJST. All rights reserved

http://www.ejournalofsciences.org

Potential of the Dark as a Factor affecting Seed Germination

Washa B. Washa
Mkwawa University College of Education
Private Bag Iringa Tanzania

ABSTRACT

An investigation was conducted between 1st February 2014 to 30th April 2014 in the Life science laboratories of Mkwawa University College of Education to justify the status of the dark of being a factor affecting seed germination. This investigation was important because a condition qualifies to be a factor affecting germination if have a potential ability to influence germination where as dark condition as many other findings in seed germination indicated. A split-split plot experimental design was employed where by germination condition (Dark and light) was regarded as a main plot, treatment of the medium by detergent (0%, 1% and 10%) was regarded as a sub plot while number of days (1-5) was regarded as sub-sub plot. A total of 600 seeds were used in the whole experiment. Number of seed germinated per condition, per treatment and per number of days was recorded and data were subjected to appropriate statistical analysis to get inferences. About 50% of seeds did not germinate, these comes from 1% and 10% from both type of seeds suggesting that detergent concentration hindered germination significantly. Seed germination was found to be significantly higher in the dark condition than in light condition at $P < 0.05$ ($P = 0.0028$, $t = 6.532$, $DF = 4$). Mean + SEM (Light = 1 ± 0.441, Dark = 2.6 ± 0.400) for the bean seeds. Results as conformed to other findings in seed germination suggest recommending dark as a factor affecting seed germination than it has been simplified.

Keywords: *Seed germination, seed anatomy, imbibitions, dark and light condition.*

1. INTRODUCTION

Plants produce seeds and seeds develop into new plants as a result the cycle continue to keep the plant species alive and continuity [1]. By using individual species life cycle, the tot potency of an individual species can be traced and genetically variation in various stages of growth and development of an individual species is the one which bring about variants within species and different families and groups in the plant kingdom [2]. One of the potential stages in the life cycle of plants and trees is seed germination [3] despite of other alternatives like cuttings, suckers, grafting, hyphae formed by sporeling of fungal spores and tissues culture [4]. Seed germination is a process in which a seed or spore awakens from dormancy and start to sprout [5]. Germination therefore refers to sprouting of a seed or resumption of plant growth from seed or growth of a sporeling from a spore, for example the growth of hyphae from fungal spores [6]. Dormancy is a condition at which a seed contains an embryonic plant in a resting condition [7]. Both seeds and spores can experience periods of dormancy which is often influenced by external factors as temperature and light [3]. Seeds and spores can be programmed to remain dormant or germinate by making the conditions unavailable or available for growth [7].

Factors affecting Seed Germination

Both internal and external factors from the environment are declared to affect seed germination. Hormones contained in various developmental stages of the seed and enzymes are some of the internal factors which in one way or another can affect seed germination [8]. The most important external factors which are declared to affect seed germination includes temperature, oxygen or air, water or moisture and sometimes light although most of literature in various research do declare that light have no effect on seed germination instead most of plants are reported to germinate earlier and successfully in the dark place compared to the light place but dark place is not declared in any published scientific research as a factor affecting germination [9]. Another external substance like gibberellic acid and other synthetic hormones which can affect germination significantly are also not reported to be factors affecting seed germination [10]. Many researchers also agree that soil, dormancy period, seed viability and thinness or thickness of the seed coat may affect seed germination and hence are factors for seed germination [10]

There is optimum or favorable range of temperature or heat for seed to germinate. Below optimum range germination is slow or hindered and above optimum range seed will not germinate because some seed enzymes which are protein in nature are denatured including the seed embryo. Some seeds are very sensitive to temperature while others can germinate in a wide range of temperature [11]. The optimum temperature to most of crops is between 25ºC to 40ºC. Few plant species can germinate below 25ºC to 4ºC while above

©2015 IJST. All rights reserved

40°C is extremely above the optimum which will not germinate the seed. To other plant seeds, the 4°C is a temperature for dormancy stage to the seed. The germination rate of seeds is directly proportional to the rise in temperature [11]. Mature seeds are extremely dry and need to take in a significant amount of water, relative to the dry weight of the seed, before cellular metabolism and growth can resume [8]. Amount of water to be take into the seed for germination depends on anatomical, physiological nature and plant species, other species need sparingly amount other needs little and others need large amount of water [12]. The uptake of water or moisture in seeds is called imbibition which leads to the swelling and the breaking of the seed coat [8]. When the seed imbibes water, hydrolytic enzymes are activated which breakdown these stored food resources to metabolically useful chemicals which give energy to the seedling to emerge from the seedling. Generally, talking on uptake of water in seeds is the same as talking on uptake of moisture [8].

Oxygen from the atmospheric air and or from the soil is needed in large quantities during germination of the seed [13]. At the dormant stage, the seeds respiratory rate is very low and so oxygen is required in very small quantities. Seeds sown deeply in soil fail to germinate because of little or lack of oxygen [13]. If soil conditions are too wet, an anaerobic condition persists and a seed may not be able to germinate. Oxygen oxidizes the stored food in the seed to release energy which will be used for other metabolic and hydrolytic processes in the seed and rapture of the seed coat [13]. Light requirements for seed germination vary with plant species. Some plants such as verbena and phlox require darkness to germinate which is not regarded as a factor while other plants as begonias and geraniums require a good amount of light which is considered as a factor and other plants will germinate in either the light or the dark place [9]. The most scientific reason as to why seeds do not germinate in light is that light is reported to decompose carbonic acid gas, expel oxygen which is a germinating factor and fixe the carbon, thus hardening all parts of the seeds which prevent vegetation [9]. Darkness have no effect to carbonic acid gas and the oxygen remain undisturbed to favor germination and hence is more correct to state darkness as a germinating factor and light as a hindering factor. Little plant species which their seeds germinate in light are normally planted near the surface of the soil to receive an adequate amount of light [9]. Other researchers ague that dark place is just a condition for germination but do not clarify as to why or how is a condition while others ague that dark place is just the absence of light but light is reported to have no effect on seed germination instead darkness is reported to influence germination [9]. The main concern of this research is to justify the potential of darkness as a factor affecting seed germination and not as only the condition or absence of light as other researchers tends to simplify.

Seed Anatomy

A seed is composed of three generations of plant tissues including the sporophyte that produce an immature seed known as ovule and the gametophyte that develop inside the ovule to produce the ova. The third plant tissue of the seed is the new sporophyte embryo. Endosperm is a nutritive substance of the seed upon which the seed embryo feeds as it develops [3]. Despite of these anatomical structures of seeds in high plants, seed morphologies differ from one plant species to another, there are small and big seeds, thin, flat, light. Papery, dehiscent and indehiscent, smooth and hard coat seeds [14]. Some plants such as *D. melanoxylon* have adapted to root suckers regeneration due to difficult of their seeds to germinate [15].

Seed germination stages

Seed germination starts with activation stage in which there is imbibition, an uptake of water by the seed which causes the seed to increase in volume [1]. Respiration increase, and various enzymes involved in endosperm digestion and other functions are manufactured. The cells of the embryo elongate and the radical began to grow [1]. Digestion and translocation is the second stage in seed germination in which metabolic activity increases dramatically, protein synthesis begins, and endosperm is metabolized. Enzymes soften and loosen the cell walls in preparation for further cell elongation and volume increase. Nutrients from the endosperm is mobilized and transported to areas of growth [1]. Seedling growth is the last stage in seed germination in which there is rapid cell elongation and cell division. At this stage, the radical emerge from the seed coat and the embryo can assess water and nutrients from the environment [1]

2. OBJECTIVE OF THE RESEARCH

To determine the potential of the dark as a factor affecting seed germination.

Research question

What is the effect of dark in the germination of bean and maize seeds?

Hypotheses

I predict germination in dark will be as twice as compared to germination in the light place.

3. METHODS AND MATERIAL

Study area

All activities for this research were conducted at the Life sciences laboratories of Mkwawa University College of

©2015 IJST. All rights reserved

Education, a Constituent College of the University of Dar es Salaam located in Iringa, Tanzania. A criterion for selecting this place is easy of attending the laboratory works since is my working place but also the southern highlands of Tanzania including Iringa has favorable environmental conditions for seed germination. Experiments were conducted between 1st February 2014 to 30th April 2014 (3 months).

Materials

1. Masking tape and/or Marker, **2.** Scissors, **3.** 3 plastic bottle/container, **4.** Forceps
5. Paper Towels **6.** Metric Ruler **7.** 15 seeds each, **8.** Distilled water **9**. Measuring Cylinder 100 ml **10**. 1% detergent solution, **11**. 10% detergent solution, **12**. Paraffin/Cling/Aluminum Foil **13.** Graph paper

Procedure:

1. Label the 3 container: Control (DW), 1% Solution (DT_1) and, 10% Solution (DT_2). **2.** Cut 6 square pieces of paper toweling to fit each container. **3.** Place 2 squares in each container. **4.** Distribute 5 seeds on each side of the paper towel between the plastic and towel. **5.** In the control container add 25 ml of distilled water completely moistening the paper towel. **6.** In the 1% solution container add 25 ml of 1% detergent solution making sure to completely moisten the towel. **7.** Do the same to the 10% solution container by adding 25 ml of 10% detergent solution. **8.** Make sure all containers are sealed tightly. **9.** Place the container in a dark warm place. **10.** Examine the containers daily for 5 days. Record any changes that might have occurred. If the roots are visible the seed is considered germinated. **11.** Record the date as in the Tables 1-9 below. Tables are designed to fit a Split-split plot experimental design (Dark and Light) are main plots, Treatments (0%, 1% and 10%) are sub-plots while days (1-5) are Sub-sub plots. **12**. Do not allow the towels to dry out. Moisten each container with the appropriate solutions in equal amounts. **13.** Measure the root growth of each seed daily from the time it appeared. **14.** Graph the data from the table using the colored Pencils/pens to represent each of the containers. The experiment with procedures number 1-14 were repeated 10 times as replicates within 3 months from February to April 2014 and average number of germination were recorded for statistical analysis.

Data Analysis

Data recorded include average number of seed germinated per day, average number of seed germinated per seed number and average number of seed germinated per germination condition (light or dark). Data were subjected to appropriate statistical analysis to get inferences. Tables and graphs were used to present data for easy comparison, discussion, recommendation and conclusion.

Table 1: Concentration 0%

	Average Bean Seed Growth in Light					Average Bean Seed Growth in Dark				
	Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5
Seed 1	0	0.2	1.5	2.1	2.5	0	1	2.1	4.0	5.5
Seed 2	0	0	0.7	0.9	1.4	0	0	0.1	1.2	2.0
Seed 3	0	0.5	2.1	2.9	3.3	0	0	1	0.3	1.0
Seed 4	0	0	0	0.4	1.0	0	0.3	0.9	1.4	2.7
Seed 5	0	0	0.6	1.4	1.9	0	1.5	2.7	5.5	8
Average	0	0.14	0.98	1.54	2.02	0	0.56	1.36	2.48	3.84

Table 2: Concentration 1%

	Average Bean Seed Growth in Light					Average Bean Seed Growth in Dark				
	Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5
Seed 1	0	0	0	0	0	0	0	0	0	0
Seed 2	0	0	0	0	0	0	0	0	0	0
Seed 3	0	0	0	0	0	0	0	0	0	0

©2015 IJST. All rights reserved

Seed 4	0	0	0	0	0	0	0	0	0	0
Seed 5	0	0	0	0	0	0	0	0	0	0
Average	0	0	0	0	0	0	0	0	0	0

Table 3: Concentration 10%

	Average Bean Seed Growth in Light					Average Bean Seed Growth in Dark				
	Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5
Seed 1	0	0	0	0	0	0	0	0	0	0
Seed 2	0	0	0	0	0	0	0	0	0	0
Seed 3	0	0	0	0	0	0	0	0	0	0
Seed 4	0	0	0	0	0	0	0	0	0	0
Seed 5	0	0	0	0	0	0	0	0	0	0
Average	0	0	0	0	0	0	0	0	0	0

Table 4: Concentration 0%

	Average Maize Seed Growth in Light					Average Maize Seed Growth in Dark				
	Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5
Seed 1	0	0	0.2	0.6	1.0	0	0	2.3	4	6
Seed 2	0	0	0.15	0.3	0.7	0	0	1	2	4
Seed 3	0	0	0.1	0.2	0.4	0	0	1	2.1	4.3
Seed 4	0	0	0	0.1	0.3	0	0	1	2	4
Seed 5	0	0	0	0	0.1	0	0	0.7	1.2	2
Average	0	0	0.09	0.24	0.5	0	0	1.2	2.26	4.06

Table 5: Concentration 1%

	Average Maize Seed Growth in Light					Average Maize Seed Growth in Dark				
	Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5
Seed 1	0	0	0	0	0.3	0	0	1	2	3
Seed 2	0	0	0.5	1.1	1.7	0	0	1	2	3.1
Seed 3	0	0	0.3	0.8	1.2	0	0	1	2.1	3.3
Seed 4	0	0	0	0	0.2	0	0	0	0	0
Seed 5	0	0	0	0	0	0	0	0.4	0.7	1.2
Average	0	0	0.16	0.38	0.68	0	0	0.68	1.36	2.12

Table 6: Concentration 10%

	Average Maize Seed Growth in Light					Average Maize Seed Growth in Dark				
	Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5
Seed 1	0	0	0	0	0	0	0	0	0	0
Seed 2	0	0	0	0	0	0	0	0	0	0
Seed 3	0	0	0	0	0	0	0	0	0	0
Seed 4	0	0	0	0	0	0	0	0	0	0
Seed 5	0	0	0	0	0	0	0	0	0	0
Average	0	0	0	0	0	0	0	0	0	0

Table 7: Compared differences in germination between Light and Dark across conc. and days

©2015 IJST. All rights reserved

	BEAN SEEDS						MAIZE SEEDS					
	Light Condition			Dark condition			Light condition			Dark condition		
Days	0%	1%	10%	0%	1%	10%	0%	1%	10%	0%	1%	10%
1	0	0	0	2	0	0	0	0	0	0	0	0
2	0	0	0	2	0	0	0	0	0	0	0	0
3	1	0	0	2	0	0	0.09	0.16	0	1.2	1.7	0
4	2	0	0	3	0	0	0.24	0.38	0	2.5	2.5	0
5	2	0	0	4	0	0	0.5	0.68	0	4.46	4.46	0

Table 8: Temperature recorded in Light

DAY	MORNING	AFTERNOON	EVENING	NIGHT	AVERAGE
1	22	29	32	16	19.8
2	21	30	35	16	20.4
3	20	28	33	17	19.6
4	21	28	33	17	19.8
5	21	28	33	17	19.8

Table 9: Temperature recorded in Dark

DAY	MORNING	AFTERNOON	EVENING	NIGHT	AVERAGE
1	22	23	22	17	16.8
2	21	24	23	16	16.8
3	21	23	23	17	16.8
4	21	24	23	16	16.8
5	21	24	23	16	16.8

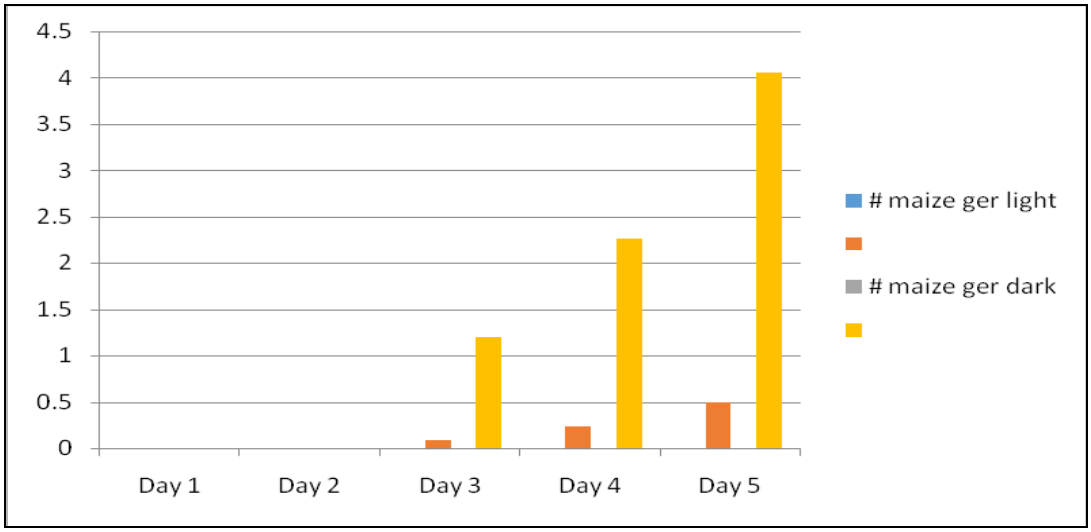
4. RESULTS

Statistical computation from Table 7 was done to get mean, standard error of mean, paired t test, DF and P vales. Table 7 is a result of data summation from Table 1- 6. Figure 1-6 are representative sample of slides to represent germinated seeds and those not germinated. A control experiment (0%) or distilled water for the bean seeds in light against dark condition indicated bean germination to be significantly higher in the dark condition than in light condition at $P < 0.05$ ($P = 0.0028$, $t = 6.532$, $DF = 4$). Mean + SEM (Light = 1 ± 0.441, Dark = 2.6 ± 0.400). The results conform to the findings which report light to decompose carbonic acid gas, expel oxygen and fixe the carbon, thus hardening all parts of the seeds which prevent vegetation [9]. This finding also report darkness to have no effect to carbonic acid gas and the oxygen remain undisturbed to favor germination. Recorded temperature (maximum 17^{o}C in dark and 20^{o}C in light) is contrally to the optimum temperature required for seed germination (Table 8 and 9) implying that decomposition of carbonic acid in light condition suppresses other germination conditions in light. Observation of recorded data from Table 1, 2, 3 and 7 also conform to and support the statistical results as also supported by graph 3. A control experiment

©2015 IJST. All rights reserved

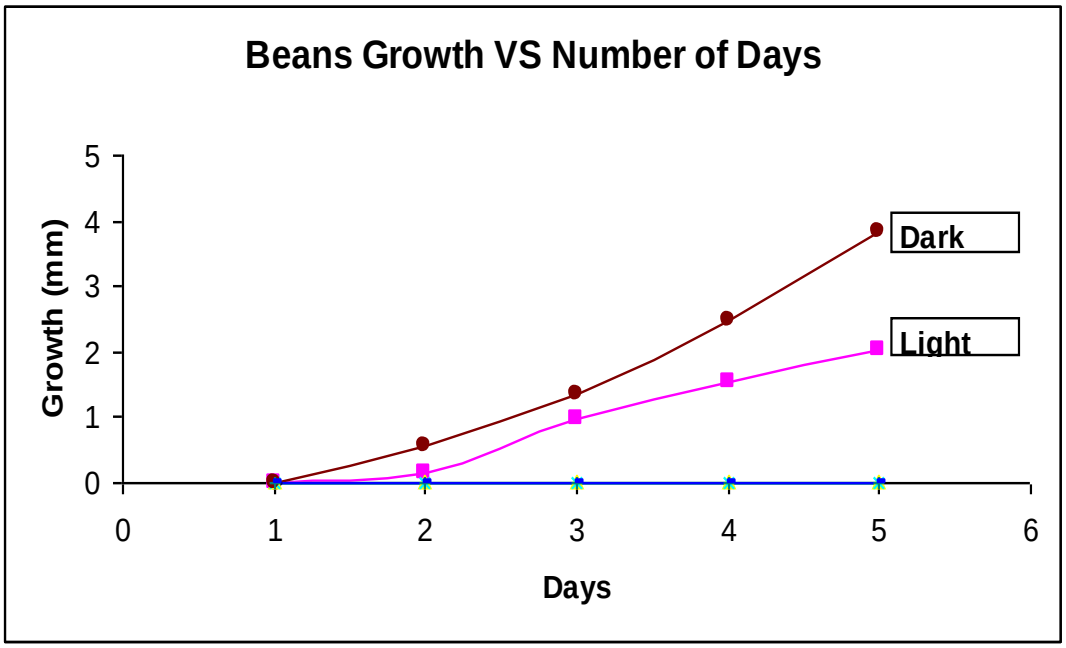
(0%) or distilled water for the maize seeds in light against dark condition indicated maize germination to have difference between light and dark condition but not statistically significant at P > 0.05 (P = 0.1235, t = 1.4465, DF = 4). Mean + SEM (Light = 0.166 ± 0.094, Dark = 1.64 ± 0.851). The results conform to findings which suggest that dormancy period, seed viability and thickness of the seed coat may affect seed germination [10].

Seed anatomy differences between bean and maize seed is another factor which might have influenced the results but also effect of factors to germination of seeds differs in different plant species [9]. Observation of recorded data from Table 4, 5, 6 and 7 also conform to and support the statistical results as also supported by graph 1, 2 and 3. Statistical computation from Table 7 for the maize germination in contaminated water (1% Light and 1% Dark) indicated no significant difference in maize germination under 1% treatment at P > 0.05 (P = 0.1051, t = 2.088, DF = 4). Mean + SEM (Light = 0.24 ± 0.129, Dark = 1.74 ± 0.844). The results suggest that detergents as well as minerals in soil hinders early and germination percentage depending on plant species but also concentration of the hindering factor that is why germination percentage between 1% and 10% of both bean and maize differs although not statistically significant as also can be supported by recorded data in Table 5. Results also suggest that the higher the amount of contamination (detergent) hindering effect increases that is why there was no germination at all in all petri-dishes under 10% compared to 1% in whatever conditions (See Table 2, 3, 5 and 6). A way forward can be traced from seeds germinated in a contamination free moisture (0%) reflected by data from Table 1, 4 and 7 in which a power to influence germination between dark and light can be easily and vividly compared not necessarily statistically but also a ray man can compare. Both statistical data and data presented on tables indicate strong effects attributed by dark condition for seed germination suggesting that, dark is not only the environment or condition for germination or absence of light as other researcher used to simplify but it qualify to be listed as a factor affecting seed germination.

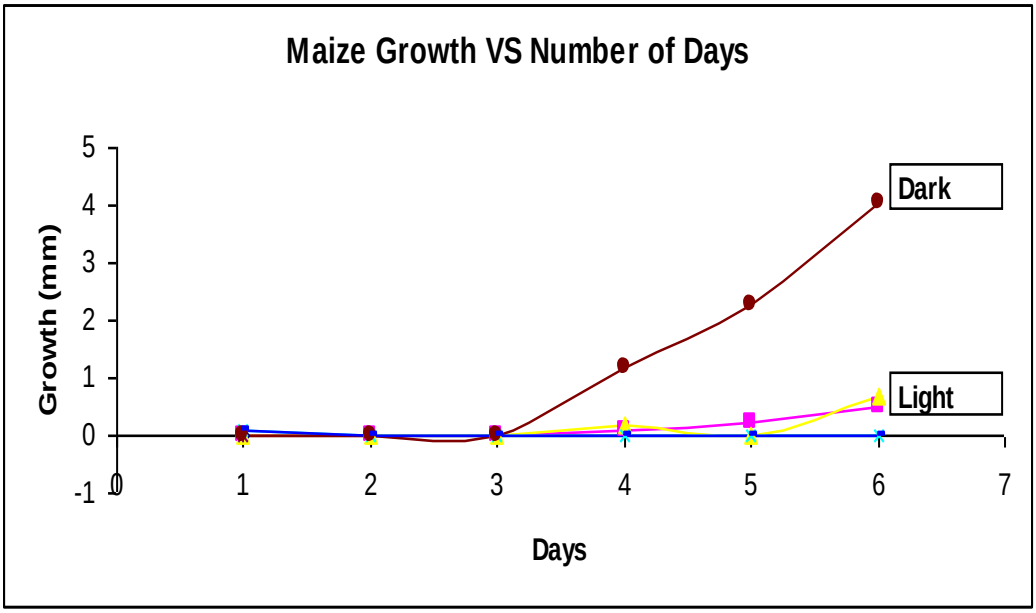


Graph 1: Effect of dark on seed germination (Produced from Table 4 above)

©2015 IJST. All rights reserved



Graph 2: Beans growth-Number of Days



Graph 3: Maize growth-Number of Days

©2015 IJST. All rights reserved



Figure 1: 1% Beans and maize in the. dark-Day 2 Not germinated



Figure 2: 1% Beans and maize the dark-Day 3. Maize germinated



Figure 3: Control Beans and Maize in. dark-day 4. Maize and Beans germinated



Figure 4: Control Beans and Maize in dark-day 5. Maize and Beans germinated



Figure 5: Control Beans in dark-day 6.Beans germinated



Figure 6: Control Beans in dark-day 7.Beans germinated

5. CONCLUSION AND RECOMMENDATION

There are many investigations have been conducted on factors affecting plants seed germination. These investigations declare that may plant species germinate significantly in dark than light condition but they don't include dark in the list of factors for seed germination as have been justified in this research. Let justification of this

©2015 IJST. All rights reserved

research convince the body of science (Botanist) declare dark condition as a factor affecting seed germination.

Acknowledgement

I have the pleasure to acknowledge all people who contributed to conduction of the investigation and finally production of the findings. Special acknowledgements are given to Dr. Mganga N from MUCE for statistical analysis, and secondly to Mr. Halid and Mr Tukay from MUCE for laboratory technical assistance.

REFERENCES

[1]. Newman S, (2009). Growing Plants from Seed; Colorado State University Extension;

[2]. Roca, M.G.; Arlt, J.; Jeffree, C.E.; Read, N.D. (2005). "Cell biology of conidial anastomosis tubes in Neurospora crassa". *Eukaryotic Cell* **4** (5): 911–919. doi:10.1128/EC.4.5.911- 919.2005. PMC 1140100. PMID 15879525.

[3]. The Seed Biology Place (2009). Seed After-Ripening: Dormancy Release and Promotion of Germination;

[4]. Diane Relf (2009). Plant Propagation from Seed Virginia Cooperative Extension;

[5]. Derek Bewley, J.; Black, Michael; Halmer, Peter (2006). "The encyclopedia of seeds: science, technology and uses Cabi Series". *CABI*. p. 203. ISBN 0-85199-723-6. Retrieved 2009- 08-28

[6]. Takayama S, Isogai A (2005). "Self-incompatibility in plants". *Annu Rev Plant Biol* **56**: 467–89. doi:10.1146/annurev.arplant.56.032604.144249. PMID 15862104

[7]. Robert L. Geneva, (2009). Seed Dormancy in Commercial Vegetable and Flower Species; University of Kentucky;

[8]. Raven, Peter H.; Ray F. Evert, Susan E. Eichhorn (2005). *Biology of Plants, 7th Edition*. NewYork: W.H. Freeman and Company Publishers. pp. 504–508. ISBN 0-7167-1007-2.

[9]. Neff, M. N., L. Sanderson, and D. Tedor (2009). Light-mediated germination in lettuce Seeds: Resurrection of a classic plant physiology lab exercise. American Biology Teacher 71(6): 367-370.

[10]. Finch-Savage, W. E. and G. Leubner-Metzger (2006). Seed dormancy and the control of germination. New Phytologist 171: 501-523.

[11]. Michael M. Case (2005). Determining the Effects of Water Temperature and Exposure Terms on Seed Germination; California State Science Fair;

[12]. Washa, B.W and Nyomora, A. M. S. (2012). The effect of moisture and seed treatment on the In-situ and ex-situ regeneration of *Dabergia melanoxylon* (African Blackwood) in Pugu Forest reserve. *HURIA Journal*, **10**: 56-70.

[13]. Koning, R. E. (1994). Seeds and Seed Germination. *Plant Physiology Information Website*. http://plantphys.info/plant_biology/seedgerm.shtml.

[14]. Washa, B.W; Nyomora, A.M.S and Lyaruu H, V. (2012). Improving Propagation. success of *D.melanoxylon* (African Blackwood) in Tanzania. I: Characterization of mycorrhizal associated with *Dalbergia melanoxylon* (African Blackwood) in Tanzania. *Tanzania Journal of Science*. **38**: (1). 35-42.

[15]. Washa, B.W; Nyomora, A.M.S and Lyaruu H, V. (2012). Improving Propagation Success of *D.melanoxylon* (African Blackwood) in Tanzania. II: Rooting ability of root and stem cuttings of Dalbergia melanoxylon (African Blackwood) in Tanzania. *Tanzania Journal of Science*. **38**: (1). 43-53

[16]. Washa, B. W. (2008). Dependence of *Dalbergia melanoxylon* Natural Population on Root Suckers Regeneration. *Journal of African affairs, Hankuk University of Foreign studies*, Seoul, Korea. **24**:177 – 198.