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Effects of Seed Treatment on the Germination of *Crotalaria verrucosa* L.

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Authors' contributions

This work was carried out in collaboration between both authors. Authors KO and IGE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author KO managed the analyses and literature searches of the study. Both authors read and approved the final manuscript.

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ABSTRACT

The study was conducted to ascertain the type of dormancy and evaluate various methods for breaking the dormancy of *Crotalaria verrucosa* L. seeds. The seed coat anatomy was also carried out to determine the relationship between the seed coat and the nature of dormancy. The seeds were subjected to the following treatments: immersed in 50%, 70% and 100% sulphuric acid (H_2SO_4) and hydrochloric acid (HCl) for 2, 5, 10, 15, 20 and 30 minutes respectively, soaked for 24 hours in 10 mM, 50 mM, 100 mM, 1000 mM sodium nitrate ($NaNO_3$) and soaked in hot distilled water (50℃, 70℃ and 100℃) for 2, 5, 10, 15 and 2 0 minutes. The treatments were replicated four times. Germination increased significantly after treatment with concentrated H_2SO_4 for 20 minutes (87.5%), concentrated HCl for 30 minutes (47.5%), 100℃ distilled water for 20 minutes (72.5%) and 50 mM $NaNO_3$ (62.5%). All the treatments increased the germination of *C. verrucosa* seeds significantly at *P=.05* when compared with the control (12.5%). However, treatment with concentrated H_2SO_4 for 20 minutes was most efficient in promoting germination (87.5%). Anatomical analysis of the seed coat revealed several layers of water and gas impermeable sclerenchymatous tissues (macroslereids, osteosclereids and cuticule). From the result obtained, the type of dormancy exhibited was found to be physical or exogenous in nature.

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Keywords: Dormancy; anatomy; Crotalaria verrucosa; scarification; seed treatment.

1. INTRODUCTION

Crotalaria verrucosa L., commonly known as blue rattlepod or blue rattle snake, is a species of flowering plant belonging to the legume family, Fabaceae, subfamily Faboideae. The plant is found in Asia, Africa and America regions. *C. verrucosa* is a perennial shrub growing to about 50 – 100 cm in height. The leaves, 5 – 15 cm long, are ovate-rhomboid or ovate-deltoid, and obtuse. The flowers are bluish-purple and 15 – 20 cm long. The pods are 2.5 – 3 cm long, oblong, inflated, black and hairy [1]. *Crotalaria verrucosa* grows on fallow fields, on marshy grounds, along rivers and roads, grassland and sparse forests; at elevations up to 1200 m. *Crotalaria verrucosa* is a potential ornamental and is sometimes grown as green manure, forming a symbiotic relationship with certain soil bacteria which form nodules on the root and fix nitrogen. The leaf extract is applied to soothe skin allergies. Studies suggested that the extract possess strong antipyretic, antidiabetic and central nervous system depressant activity diuretic [1,2]. The leaves of *C. verrucosa* are expectorant [3]. It was reported that thin layer chromatography (TLC) profiling of the extracts of *C. verrucosa* showed a wide array of compounds like alkaloids, flavonoids, tannins, saponins, glycosides, triterpenoids, phenols, steroids, coumarins, cardiac glycosides and phytosterols [4]. Water and methanolic extracts of *C. verrucosa* seeds showed maximum inhibition effects against human pathogenic bacteria, *Bacillus subtilis* and *Pseudomonas aeruginosa* [5]. The ethanolic leaf extract of *C. verrucosa* inhibited the cascades of inflammation, supporting its traditional use as anti-inflammatory [2]. The two major methods for propagation of *C. verrucosa* are by *in-vitro* [6] and by seed.

Seeds are the primary means of dispersal and perpetuation of species of flowering plants [7]. Seed dormancy is a common condition found in many species. It is common among members of the Fabaceae family [8,9,10,11,12,13]. It is an adaptation that allows a species to determine the timing of germination for seeds in a population [14,15]. Some species use environmental signals to synchronize germination for most seeds at a particular time of the year. Other species are adapted for asynchronous germination over an extended time. This allows periodic germination and the establishment of a persistent seed bank. Domestication and mass production of crop plants has led to the reduction or elimination of seed dormancy to fit cropping schedules [16]. This is achieved through several seed treatment methods depending on the type of dormancy.

Dormancy is an innate state of arrested growth that occurs across all life forms [17,18]. Exogenous dormancy can be eliminated by several physical and chemical scarification methods. Chemical scarification has been demonstrated to be effective on seeds of related species such as *Crotalaria retusa* [19]. *Abrus precatorius* has been found to exhibit a form of dormancy known as hard seededness due to its leathery testa, leading to water and oxygen impermeability; mechanical scarification proved most effective in enhancing germination [20]. Seed treatment has been demonstrated in unrelated species: Leaching in running water for 12 hours was found to release the dormancy in the seeds of *Occimum gratissimum* [21]. Seeds with hard seed coats are not only more resistant to environmental factors, but also preserve their shape and structure a long time [22]. The seed coat is the external protective covering of the seed [14].

There is need for the elimination of dormancy in *C. verrucosa* seeds in order to ensure maximum yield and uniform stand establishment. Hence, the study, therefore, seeks to determine the effects of chemical scarification on the germination of *C. verrucosa* seeds and examine the seed coat anatomy.

2. MATERIALS AND METHODS

The seeds of *C. verrucosa* were collected from the International Institute of Tropical Agriculture (IITA), Ibadan Nigeria and properly identified by the Curator of University of Port Harcourt Herbarium. The floatation test was done to ascertain the intactness of the seeds' embryo. The seeds were pretreated in 50%, 70%, 100% sulphuric acid (H_2SO_4) and hydrochloric acid (HCl) for 2, 5, 10, 15, 20 and 30 minutes respectively, hot water at 50℃, 70℃ and 100℃ for 2, 5, 10, 15 and 20 minutes, and sodium nitrate ($NaNO_3$) at 10 mM, 50 mM, 100 mM and 1000 mM for 24 hours. Alongside the treatments, a control experiment was also setup in which the seeds were germinated without any form of pretreatment. These were left under the temperature interval of 25℃ and 35℃.

For each treatment, four replicates of 10 seeds per replicate were plated in petri dishes lined with Whatman filter paper and moistened with 4 ml of distilled water. The seeds were observed daily and watered as appropriate. Germination counts were taken daily and final count was taken after 14 days. Germination was marked by the protrusion of the radicle. The germination percentage and germination rate were calculated. All experiments were conducted in a completely randomized design. The data obtained were subjected to statistical analysis using Microsoft Excel version 2013. The analysis of variance (ANOVA) and least significant difference (LSD) were calculated.

Seed coat anatomy of *C. verrucosa* was determined as follows: Dry mature seeds from the specimens were fixed in FAA (formalin, acetic acid and alcohol) for 12 hours. Thereafter, the specimens were dehydrated in series of different percentages of ethanol (30% and 50%), and stored in 70% ethanol. The specimens were hand-sectioned according to the methods outlined by [23]. The sections were stained in 1% Safranin red for two minutes, counter-stained with Alcian blue, mounted on a slide, viewed under microscope and micro-photographed using Leica WILD MPS 52 microscope camera on Leitz Diaplan microscope. This method is as modified by Metchalfe and Chalk [24].

3. RESULTS AND DISCUSSION

3.1 Hydrochloric Acid Treatment

Seeds of *C. verrucosa* presoaked in 100% HCl for 30 minutes increased germination percentage significantly when compared to control, 50% and 70% HCl treatments (Fig. 1). There was no significant increase in germination rate after 20 minutes treatment in 100% HCl. There was no significant difference *P*=.05 in germination rate between 5 – 20 minutes treatment in 100% HCl (Table 1). Hydrochloric acid at 50% concentration was found to induce germination of dormant seeds of *Parkia biglobosa* [25]. Viability of *Crotalaria* seed decreases among the different seed coat colours [26]. Germination can be affected, from chemicals that get into the seed, after seed soaking to soften the hard seed coat, from plant growth regulators [27,28,29,30] and stress factors [31,32].

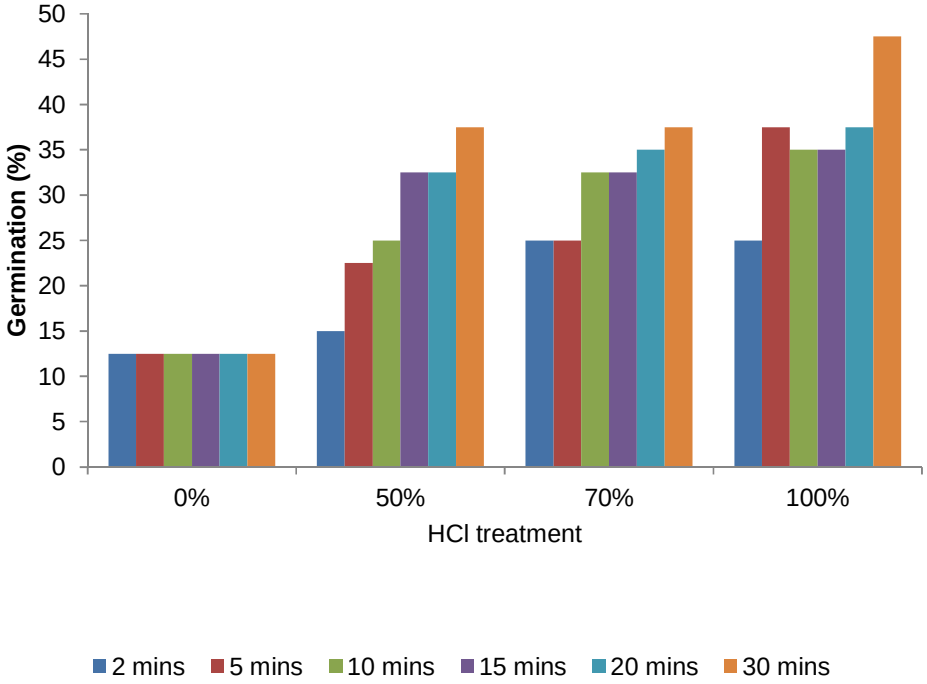

Fig. 1. Germination percentage of *C. verrucosa* seeds after a period of HCl (0%, 50%, 70% and 100%) treatment

3.2 Sulphuric Acid Treatment

Seeds of *C. verrucosa* presoaked in 100% H_2SO_4 for 20 minutes gave the highest germination percentage when compared to other treatments (Fig. 2). Germination was found to increase significantly after 5 minutes for each H_2SO_4 treatment. Germination rate also differed significantly at *P*=.05 between 10, 20 and 30 minutes treatments in 100% H_2SO_4 (Table 1). Chemical scarification using sulphuric acid has been found effective in elimination of dormancy in species such as *Senna obtusifolia* [13], *Crotalaria senegalensis* [12]. In some species that exhibit exogenous forms of dormancy such as *Cassia fistula*, a combination of mechanical and chemical scarification (treatment with concentrated H_2SO_4) was most effective in enhancing germination [8,10,33,34].

3.3 Hot Water Treatment

Hot water treatments at 50℃, 70℃ and 100℃ improved germination percentage of *C. verrucosa* significantly when compared to the control (Fig. 3). There was significant difference at *P*=.05. However, there was no significant increase in germination rate after 2 minutes for other treatments (Table 1). Every plant species has its own temperature requirement for germination [35]. The work of Shaban [36] showed that for maximum germination to be obtained, temperature has to be constant. The optimum temperature for the germination of the seeds of most plant species is between 30℃ and 40℃ [37]. Hadas [38] stated that germination of seeds will not occur below minimum or above maximum temperatures respectively. Akobundu et al. reported that germination percentage of *C. verrucosa* presoaked in hot water decreases with time interval [26].

3.4 Sodium Nitrate Treatment

Seeds of *C. verrucosa* soaked in 50 mM $NaNO_3$ for 24 hours were found to increase germination percentage when compared to the other treatments (control, 10 mM, 100 mM and 1000 mM) as shown in Fig. 4. There was significant difference in germination rate at *P*=0.05 between 50 mM treatment and other treatments. However, there was no significantly increase in germination among control, 10 mM, 100 mM, 1000 mM treatments (Table 2). Saberi et al. reported that KNO_3 increased the germination percentage of dormant *Citrullus colocynthis* seed by 50% when compared to control [39].

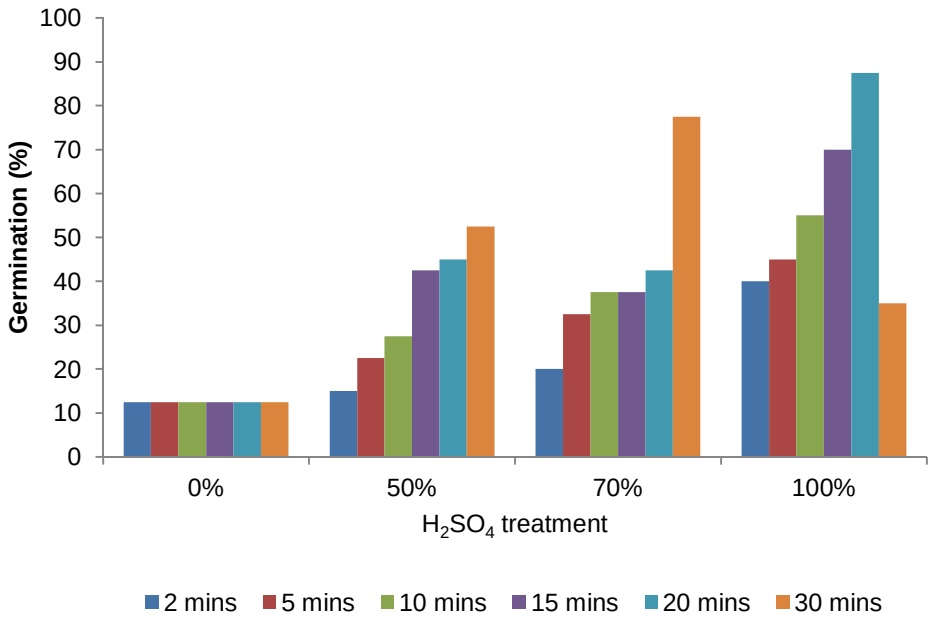

Fig. 2. Germination percentage of *C. verrucosa* seeds after a period of H_2SO_4 (0%, 50%, 70% and 100%) treatment

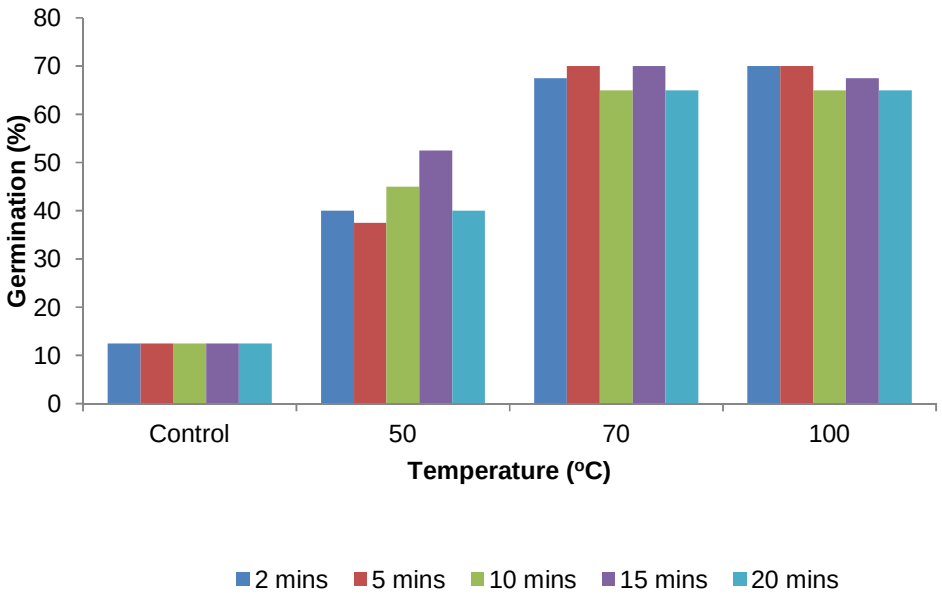

Fig. 3. Germination percentage of *C. verrucosa* seeds subjected different water temperature (50℃, 70℃ and 100℃) treatment

Table 1. The germination rate of *Crotalaria verrucosa* seeds in different period of scarification treatments

Time	H_2SO_4			HCl			H_2O		
(mins)	50%	70%	100%	50%	70%	100%	50°C	70°C	100°C
Control (0)	1.25^{e}	1.25^{d}	1.25^{e}	1.25^{e}	1.25^{e}	1.25^{e}	1.25^{b}	1.25^{b}	1.25^{b}
2	1.50^{de}	2.00^{cd}	4.00^{d}	1.50^{cde}	2.50^{bcd}	2.50^{cd}	7.00^{a}	6.75^{a}	7.00^{a}
5	2.25^{cd}	3.25^{bc}	4.50^{cd}	2.25^{bd}	2.50^{bcd}	3.75^{bc}	6.75^{a}	7.00^{a}	6.75^{a}
10	2.75^{c}	3.75^{b}	5.50^{c}	2.50^{bc}	3.25^{ad}	3.50^{bcd}	6.50^{a}	7.00^{a}	6.50^{a}
15	4.25^{b}	3.75^{b}	7.00^{b}	3.25^{ab}	3.25^{ac}	3.50^{bc}	6.75^{a}	7.00^{a}	6.75^{a}
20	4.50^{ab}	4.25^{b}	8.75^{a}	3.25^{a}	3.50^{ab}	3.75^{ab}	7.25^{a}	6.50^{a}	7.25^{a}
30	5.25^{a}	7.75^{a}	3.50^{d}	3.75^{a}	3.75^{a}	4.75^{a}			
LSD	0.89	1.71	1.45	1.00	1.02	1.11	1.31	1.14	1.31

Values with the same superscript alphabet are not significantly different

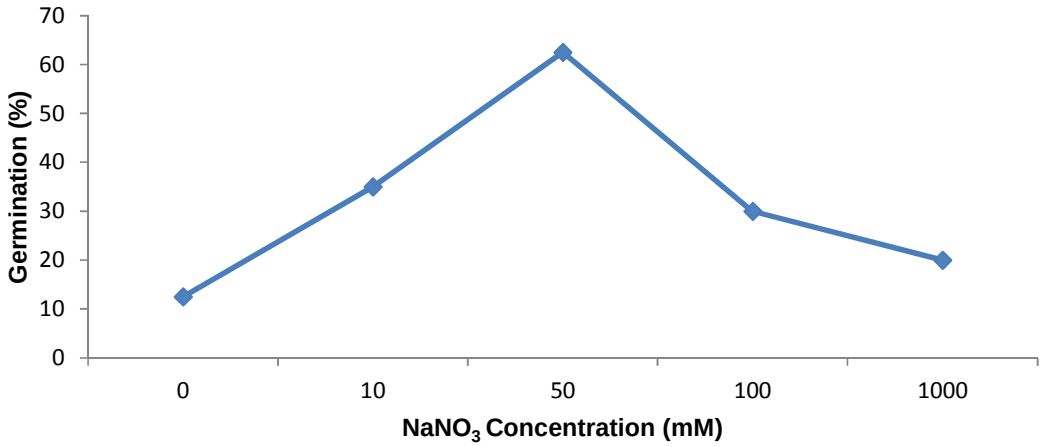

Fig. 4. Germination percentage of *C. verrucosa* seeds subjected different $NaNO_3$ concentration (0, 10, 50, 100 and 1000) treatment

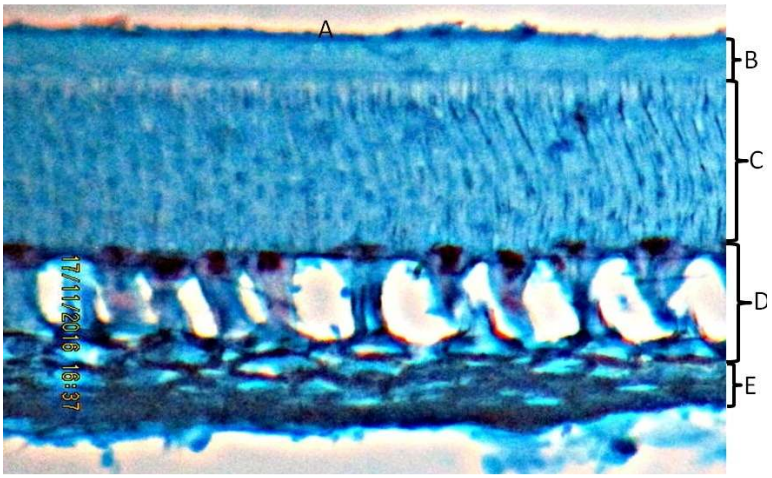

Plate 1. Seed coat anatomy of *Crotalaria verrucosa*
A = Cuticle (4 - 6 µm); B = light line (17 – 26 µm); C = Macrosclereids (107 - 116 µm); D = Osterosclereids (86 – 107 µm) and E = degenerated parenchyma (2 – 3 layers; 9 - 21 µm)

Table 2. The mean germination rate of *C. verrucosa* seeds after $NaNO_3$ treatment

Concentration (mM)	Mean germination
Control (0)	1.25^a
10	3.50^a
50	6.25^b
100	2.00^a
1000	2.50^a
LSD	1.95

Values with the same superscript alphabet are not significantly different

3.5 Seed Coat Anatomy

The seed coat anatomy of *C. verrucosa* revealed the presence of water and gas impermeable tissues consisting of cuticule, macrosclereids, and osteosclereids (Plate 1) which might interfere with the processes like water uptake or regulate gaseous exchange. Consequently, the seed coat may function to regulate germination by offering physical resistance to embryo growth. Seed coat linked dormancy has been observed on some members of the Fabaceae family including species such as *Senna obtusifolia* [13], *Crotalaria senegalensis* [12], *Abrus precatorius* [20]. The seed coat can hinder germination by inhibiting water and gas uptake, and offering mechanical resistance to embryonic development.

4. CONCLUSION

Seed of *C. verrucosa* pretreatment in hot water, sodium nitrate and hydrochloric acid enhanced germination percentage when compared to control. However, maximum germination was achieved with pretreatment in concentrated and 70% sulphuric acid for 20 and 30 minutes respectively (87.5% and 77.5%). The study has shown that *C. verrucosa* exhibits physical or exogenous dormancy which is imposed to a large extent by the seed coat and germination was enhanced by the wear out action of the acid on the seed coat. The avoidance of germination is ecologically advantageous to the plant especially when it grows in harsh climatic conditions. However, this is undesirable when quick and consistent seed germination is required for successful establishment of this economically important plant species. The results demonstrate that this aim can be achieved with chemical scarification of the seeds of *C. verrucosa* using 100% H_2SO_4 for 20 minutes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Asolkar LV, Kakkar KK, Charke OJ. Second supplement to glossary of Indian medicinal plants with active principles part 1(A – K). Council of Scientific and Industrial Research, New Delhi, India; 1992.
2. Narwin K, Mohammad MB, Mohammed SUJ, Avejet R, Radowan A, Nuru I. Antipyretic, antidiabetic, thrombolytic and

CNS depressant potential of ethanol extract of *Crotalaria verrucosa* L. leaves. American Journal of Biomedical Sciences. 2015;7(4):198–204.

3. Yusuf M, Begum, Wahab MA, Chowdhury JU, Begum J. Some tribal medicinal plants of Chittagong Hill Tracts, Bangladesh. Bangladesh Journal of Plant Taxonomy. 2007;14(2):117–128.
4. Kamalakar P, Prabhakar G, Shailaja K. Phytochemical screening and TLC profiling of seeds of *Crotalaria verrucosa* Linn. International Journal of Scientific Research. 2014;3(9):25–30.
5. Prabhakar G, Kamalakar P, Vardhan AT, Shailaja K. *In-vitro* screening of antibacterial activity of seeds of *Crotalaria verrucosa* L. and *Duranta erecta* L. European Journal of Pharmaceutical and Medical Research. 2015;2(4):411–419.
6. Town MH, Thummala C, Ghanta RG. In *vitro* propagation of *Crotalaria verrucosa* L. An important ethnobotanical plant. Journal of Medicinal Plants Research. 2008;2(9): 242–245.
7. Bewley JD. Seed germination and dormancy. The Plant Cell. 1997;9:1055–1066.
8. Nalawadi UG, Bhandary KR, Chandrashekar T. Germination of *Cassia fistula* (Linn.) seeds could be improved by Treatment with sulphuric acid for 20min. Curr. Res. Hortic. Abst. 1977;4(3):42–43.
9. Ramamoorthy K, Rajendran C, Sivasubramanian S. Seed treatment for alleviation of hard seededness in senna (*Cassia angustifolia* L.) Adv. Plant Sci. 2005;18(1):429–430.
10. Al-Menaie HS, Al-Ragam O, Al-Shatti A, Matthew M, Suresh N. The effects of different treatments on seed germination of the *Cassia fistula* L. and *Cassia nodosa* Buch-Ham. ex Roxb. in Kuwait. African Journal of Agricultural Research. 2010; 5(3):230–235.
11. de Morais LF, Almeida JCC, Demincis BB, de Padua FT, Morenz MJF, de Abreu JBR, et al. Methods for breaking seed dormancy of seeds of tropical forage legumes. American Journal of Plant Sciences. 2014; 5:1831–1835.
12. Atif HN, Abdalhalim HE, Faisal EA. Evaluation of different pre-sowing seed treatments to break dormancy of *Crotalaria senegalensis*, a famous rangeland forage in Sudan. Asian Journal of Plant Science and Research. 2015;5(10):16–21.
13. Mensah SI, Ekeke C. Effects of different pretreatments and seed coat on dormancy germination of seeds of *Senna obtusifolia* (L.) H.S. Irwin & Barneby (Fabaceae). International Journal of Biology. 2016;8(2): 77–84.
14. Moise JA, Han S, Gudynaite-Savitch L, Johnson DA, Miki BLA. Seed coats: Structure, development, composition, and biotechnology. *In vitro* Cellular and Developmental Biology – Plant. 2005; 41(5):620–644.
15. Willis CG, Baskin CC, Baskin JM, Auld JR, Venable DL, Cavender-Bares J, et al. The evolution of seed dormancy: Environmental cues, evolutionary hubs, and diversification of the seed plants. New Phytologist. 2014;203:300–309.
16. Levetin E, McMahon K. Plants and Society. 7th Ed. McGrawHill Publishing Company; 2015.
17. Finch-Savage WE, Leubner-Metzger G. Seed dormancy and the control of germination. New Phytologist. 2006;171: 501–523.
18. Footitt S, Douterelo-Soler I, Clay H, Finch-Savage WE. Dormancy cycling in arabidopsis seeds is controlled by seasonally distinct hormone-signaling pathways. Proceedings of the National Academy of Sciences, USA. 2011;108: 20236–20241.
19. Aldrete-Chavez A, Aguilar-Martin L, De La Cruz-Landero N, Guerra-Santos JJ, Brito R, Guevara E, et al. Effects of scarification chemical treatments on the germination of *Crotalaria retusa* seeds. Journal of Biological Sciences. 2010;10(6):541–544.
20. Pallavi HM, Vishwanath K, Harish BS, Prashanth Y, Manjunath T. Seed treatments to break seed dormancy and standardization of viability test procedure in *Abrus precatorius*. Journal of Medicinal Plant Research. 2015;8(4):229–236.
21. Obembe OO, Agboola DA. Seed pretreatments enhance germination in *Occimum gratissimum* (Lameaceae). Life Science Journal. 2008;5(1):87–89.
22. Hayirlioglu-Ayaz S, Beyazoglu O. Seed Anatomy of *Five vicia* L. (Leguminosae) Species. Pakistan Journal of Biological Sciences. 2000;3(9):1440–1442.
23. Ndukwu BC, Okoli BE. Studies on Nigeria *Cucurbita moschata.* Nigeria Journal of Botany. 1992;5:19–26.

24. Metchalfe CR, Chalk L. Anatomy of the Dicotyledons, 2nd Edn. Clarendon Press. Oxford. 1979;1.
25. Abubakar Z, Maimuna A. Effect of hydrochloric acid, mechanical scarification, wet heat treatment on germination of seed of *Parkia biglobosa* African Locust Bean (Daurawa) case study of Gombe Local Government Area. J. Appl. Sci. Environ. Manage. 2013;17(1):119-123.
26. Akobundu IO, Ekelem F, Okoli J. Studies on the growth and development of *Crotolaria verrucisa* L. Biological Agriculture and Horticulture. 1999;17:89-99.
27. Topacoglu O, Sevik H, Guney K, Unal C, Akkuzu E, Sivacioglu A. Effect of rooting hormones on the rooting capability of *Ficus benjamina* L. Cuttings. Šumarski list. 2016a;140(1-2):39-44.
28. Sevik H, Cetin M. Effects of some hormone applications on germination and morphological characters of endangered plant species *Lilium artvinense* L. Onion scales. Bulgarian Chemical Communications. 2016;48(2):256-260.
29. Guney K, Cetin M, Sevik H, Guney KB. Influence of germination percentage and morphological properties of some hormones practice on *Lilium martagon* L. Seeds. Oxidation Communications, 2016a; 39(1-II):466-474.
30. Guney K, Cetin M, Sevik H, Guney KB. Effects of some hormone applications on germination and morphological characters of endangered plant species *Lilium artvinense* L. Seeds, New Challenges in Seed Biology - Basic and Translational Research Driving Seed Technology, Dr. Susana Araújo (Ed.), InTech, 2016b;4:97-112.
31. Sevik H, Cetin M. Effects of water stress on seed germination for select landscape plants. Pol. J. Environ. Stud. 2015;24(2): 689-693.
32. Topacoglu O, Sevik H, Akkuzu E. Effects of water stress on germination of *Pinus nigra* arnold. Seeds, Pak. J. Bot. 2016b; 48(2):447-453.
33. Karaboon S, Ripona S, Thanapompoopong S, Pawelzik E. Breaking dormancy and optimum storage temperature of golden shower (*Cassia fistula*) seeds. Conference on International Agricultural Research for Development, Stuttgart-Honheim; 2005.
34. Babalola SE, Shonubi OO, Olubukanla TO. Seed dormancy in *Cassia fistula* L. population from Nigeria. Journal of American Science. 2014;10(10):85–93.
35. Al-Otaibiand M, Ebid AI. Influence of environmental factors on the germination ecology of *Calligonum comosuma*, medicinal plant from the arid region of Saudi Arabia. International Journal of Plants, Animals and Environmental Sciences. 2015;5:80-86.
36. Shaban M. Effect of water and Temperature on seed germination and emergence as a seed hydrothermal time model. International Journal of Advanced Biological and Biomedical Research. 2013; 1:1688-1691.
37. McDonald M. Physiology of seed germination. Proceedings of the Seed Germination Program. 2000;12-20.
38. Hadas A. Seed preparation; The soil – physical environment of germinated seeds. In Benech- Arnold RL, Sanchez RA. (Eds.), Handbook of Seed Physiology: Application to Agriculture. 2004;3-20.
39. Saberi M, Shahriari A, Tarnian F, Noori S. Comparison the effect of different treatments for breaking seed dormancy of *Citrullus colocynthis.* Journal of Agricultural Science. 2011;3(4):62-67.

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