Evaluation of Chemical Scarification and Priming Treatments to Break Physical Dormancy of *Crotalaria senegalensis* seeds

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***Abstract*—** The present study was conducted to evaluate various chemical scarification methods for breaking physical dormancy of *Crotalaria senegalensis* seeds. The seeds were subjected to the following treatments: (1) soaking in hot distilled water (80°C) for 15 and 30 min, (2) immersion in H_2SO_4 (98%) for 20, 25 and 30 min, (3) immersion in 0.5 %, 1% and 1.5 % KNO_3 and (4) soaking in 50, 100 and 150 µM H_2O_2. All scarification treatments improved the germination capacity of *C. senegalensis* seeds, the highest final germination rate and germinate rate index, were recorded after soaking in 150 µM H_2O_2, followed by soaking intact seeds in H_2SO_4 (98%) for 30 min. and immersion seeds in 1.5 % KNO_3. The results showed that using concentrated H_2SO_4 (98%) for 30 min to break seed physical dormancy of *C. senegalensis* was the second most effective treatments (after 150 µM H_2O_2 treatment) but it is commonly not preferred due to its cost, safety risk and environmental precautions involved. In conclusion, to break both physical and/or physiological dormancy of *C. senegalensis* seeds, soaking in 150 µM H_2O_2 and 1.5 % KNO_3 represent the most recommended treatments.

***Keywords*—** *Chemical Scarification, Crotalaria senegalensis, Germination rate, Hydrogen peroxide, Physical dormancy*.

I. Introduction

ORTHODOX seeds are shed from their mother plants at low water contents and are tolerant to desiccation. On the other hand, recalcitrant seeds are shed at high water contents and are sensitive to desiccation [1]. Most of forage legumes in rangelands and pastures are orthodox seeds. A variety of mechanisms of desiccation tolerance has been suggested to confer protection against the consequence of water loss at different hydration levels, and the effective expression of one or more of these could determine the relative degree of desiccation tolerance [2]. Naturally shed seeds of *Crotalaria senegalensis* are both desiccation tolerance and dormant, requiring warm weather and priming in water or scarification with H_2SO_4 to ensure high levels of germination at 20-25^{o}C [3].

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In Sudan, there are two species, *Crotalaria senegalensis* and *Crotalaria retusa*, locally called "Safari", both grow in areas degraded by erosion on infertile soils and of bad physical properties [3]. The plant is a popular forage shrub due to its high crude protein content (about 17%) and an excellent palatability for grazing animals such as camels, cows and goats in natural ranges of Sudan. *C. senegalensis* (Safari) has an adaptive advantage of having an annual cycle combined with a "seed escape" habit [**3**]. The plant as a self-reseeding legume has developed specific strategies to ensure adaptation and reproduction under harsh climatic conditions [3].

Generally, legumes seeds exhibit hardseededness resulting in dormancy [3] [4]. Several studies have been conducted on legume germination using different seed coat pre-sowing treatments [5] [6] [7]. The function of the seed coat is to protect the embryo and endosperm from desiccation, mechanical injury, unfavorable temperatures and attacks by bacteria, fungi and insects [8]. Similarly to many other taxa of legumes, the seeds of Crotalaria plants remain in a state of physical dormancy until the seed coat is made permeable by some environmental factors in natural conditions [9].

Rapid and uniform field emergence is essential to achieve high yield with respect to quantity and quality of crops [10]. Seed priming currently is widely used to accelerate seed germination rate and improve seedling uniformity in many crops [11]. According to [11], in priming, seeds are exposed to limited water availability under controlled conditions which allows some of physiological processes of germination to occur and also accumulates certain oxidant compounds which activate special enzymes which ultimately break dormancy and accelerate germination speed [11]. It has been known for a long time that an oxidant compound called hydrogen peroxide (H_2O_2) is synthesized at very high rates in the plant cells and involved in virtually all major areas of aerobic biochemistry. Hydrogen peroxide (H_2O_2) is involved in copious quantities by several enzymes (e.g. plasmalemma-bound NADPH-dependent superoxide synthase and other anti-oxidant enzymes. Recently it was reported that exogenously pretreatment with hydrogen peroxide (H_2O_2) leads to breaking seed dormancy and promoted germination of *Zinnia elegans* L. seeds which had hard seed coat [12].

This study aims at evaluating the impact of various chemical treatments on the germination response of *Crotalaria senegalensis* in order to develop an effective method of breaking seed physical dormancy.

II. Materials and Methods

A. Plant materials

Fresh seeds of *Crotalaria senegalensis* seeds were collected at maturity from shrubs growing in Gadarif state in Sudan (rainfall 700 mm per year, mean temperature 25-27^{o}C with a mean winter minimum about 15-2^{o}C and a mean summer maximum about 35^{o}C) in October and November, 2014. The fresh weight of 1000 seeds was 7.0 g and the water content was 4.6% on dry weigh basis, which was estimated after drying the seeds at 70^{o}C for 24h. All seeds were surface sterilized in a solution of 1% sodium hypochlorite (NaOCl) for 3 minutes and then rinsed three times in sterilized water prior to an experimental procedure to prevent fungal contamination.

B. Physical scarification and Priming treatments

Seeds were subjected to different physical scarification and chemical treatments. Physical scarification was carried out by soaking intact seeds in hot distilled water (80°C) for 15 and 30 min. After completion of hot water treatments, seeds were removed from the water and left to cool for 10 min. Chemical scarification (priming) was accomplished by using three different chemical compounds. First, samples of intact seeds were soaked separately in concentrated sulphuric acid (98% H_2SO_4) for 20, 25 and 30 min. Secondly, samples of intact seeds soaked separately in potassium nitrate (KNO_3) at 0.5, 1, 1.5 and 2 % for 24 h. Thirdly, soaking intact seeds in 50, 100 and 150 µM of (H_2O_2) for 30 seconds.

C. Germination assessment

After completion of pre-sowing physical and priming treatments, batches of 20 seeds from each treatment were germinated on moist filter paper in closed Petri dishes (12.5 cm) for 20 days. Germinated seeds were counted every 48 h. All Petri dishes were incubated at 25°C and 16 h photoperiod by a fluorescent light at 40 µmol m^{-2} s^{-1}. According to [9], seeds were considered germinated upon emergence of radicals (length $\geq$ 2 mm).

The following germination parameters were recorded:

1) Final germination percentage (FGP) = (number of number of germinated seeds/number of total seeds) X100

2) Mean time to germination (MTG or G_{50}) was calculated according to the following equation [13].

3) MTG or $G_{50} = \sum Dn / \sum n$

Where,

n = number of seeds which were germinated on day D.

D = the number of days counted from the beginning of germination.

4) Germination rate index (GRI)

According to [14] germination rate index can be calculated as follows:

GRI = [G1/1 + G2/2 + …….+ Gx/x]

Where,

G = the germination on each alternative day after placement.

1, 2, x = the corresponding day of germination

5) Corrected germination rate index (CGRI)

CGRI = (GRI/FGP) X 100 [14]

D. Statistical analysis

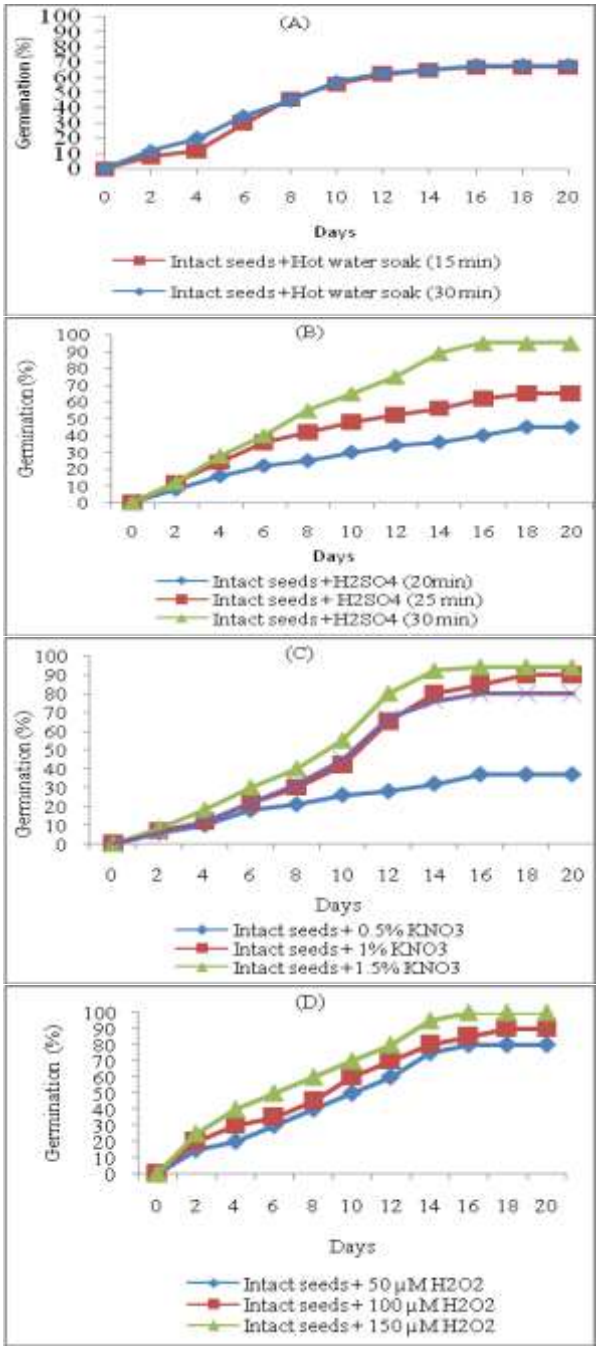
All experiments were arranged in a completely randomized design. There were 11 treatments replicated 4 times, and each replication consisted of 20 seeds. Data were subjected to one way analysis of variance (ANOVA) and mean separation among treatments was carried out by Least Significant Difference (LSD) using SPSS program (version 15). Excel computer software was used for making graphs.

III. Results

The effects of various pre-sowing seed treatments on the time-course changes in germination percentage of *Crotalaria senegalensis* are shown in **(Fig.1)**. In the hot water treatments; germination percentage at both exposure time was identical **(Fig. 1A)**. Intact seeds exposed to hot water for 15 and 30 min hot were capable to break seed dormancy of *C. senegalensis*. On the other hand, in the H_2SO_4 treatments, germination percentage was improved with increasing exposure time of seeds to the acid **(Fig.1B)**. Also, increasing concentration of KNO_3 significantly increased germination percentage of soaked seeds **(Fig. 1C)**. The Results also showed that intact seeds soaked in hydrogen peroxide (150 µM H_2O_2) significantly increased germination rate compared with other treatments **(Fig. 1 D)**.

Effects of pre-sowing treatments on final germination percentage (FGP), germination rate index (GRI), corrected germination rate index (CGRI) and time to 50% of germination (GT_{50}) were shown in **Table 1**. All breaking dormancy treatments significantly ($P < 0.05$) affected germination attributes of *C. senegalensis*. Soaking *C. senegalensis* seeds in hot water significantly ($P < 0.05$) increased FGP **(Table 1).** In this regard, soaking seeds in hot water for 30 minutes recorder higher FGP compared with Hot soak for short time "15min." **(Table 1).** Immersing seeds in H_2SO_4 also significantly ($P < 0.05$) broke the dormancy of *C. senegalensis* seeds at all application times; but exposing seeds to H_2SO_4 for 30 minutes significantly ($P < 0.05$) recorded the highest FGP among the other two H_2SO_4 treatments (**Table 1)**. Interestingly, increasing the time of immersion seeds in concentrated H_2SO_4 significantly increased FGP of *C. senegalensis* seeds (**Table 1).** The results also revealed that soaking the seeds in hot water at both times (15 minutes and 30 minutes caused identical and insignificant

difference in the FGP (**Table 1).**



Moreover soaking seeds in hot water for short period as 15 minutes significantly ($P < 0.05$) recorded higher FGP compared to immersion the seeds in concentrated H_2SO_4 for 20 minutes (**Table 1)**. In KNO_3 treatments using intact seeds of *C. senegalensis* there were significant ($P < 0.05$) increase in the FGP (**Table 1).** The highest FGP was recorded in favor of soaking the seeds in 1.5 % KNO_3 and decreased significantly ($P < 0.05$) by decreasing KNO_3 concentration **(Table 1).**

The germination speed (germination rate index (GRI) and corrected germination rate index (CGRI)) were significantly ($P< 0.05$) affected by all pre-sowing treatments **(Table 1)**. In this regard, soaking the seeds in 150 µM H_2O_2 significantly scored the highest GRI and CGRI among all pre-sowing studied treatments followed by 1.5 % KNO_3 **(Table 1)**. The significant lowest GRI and CGRI were recorded in the immersion treatment in H_2SO_4 for 20 minutes **(Table 1**). The half time of germination (GT_{50}) was also significantly ($P < 0.05$) affected by all studied treatments **(Table 1**). Soaking the seeds in H_2O_2 was significantly ($P < 0.05$) curtailed the GT_{50} **(Table 1**). The highest GT_{50} was recorded by soaking the seeds in 2 % KNO_3 **(Table 1)**. Soaking seeds in hot water for 15 min significantly increased GT_{50} and scored the highest days to reach 50% germination (6.5 d). The Hydrogen peroxide (H_2O_2) treatments significantly increased FGP, GRI and CGRI and decreased GT_{50} **(Table 1**).

TABLE I: EFFECT OF PRE-SOWING TREATMENTS ON FINAL GERMINATION PERCENTAGE (FGP) GERMINATION RATE INDEX (GRI), CORRECTED GERMINATION RATE INDEX (CGRI) AND TIME TAKEN TO REACH 50% OF FINAL GERMINATION PERCENTAGE (GT50) FOR CROTALARIA SENEGALENSIS AFTER 20 DAYS IN CULTURE

Treatments	FGP	GRI	CGRI	GT50
Hot water soak (15 min)	67.3 (55.1)* e	0.20 e	0.36 b	6.5 a
Hot water soak (30 min)	67.5 (55.3) e	0.20 e	0.36 b	5.8 b
H2SO4 (20 min)	45.0 (40.5) g	0.12 f	0.30 c	4.0 cd
H2SO4 (25 min)	65.0 (53.8) f	0.19 e	0.35 b	3.8 d
H2SO4 (30 min)	95.0 (82..5) b	0.32 b	0.39 ab	4.5 c
0.5 % KNO3	37.0 (37..5) h	0.37 a	0.38 a	3.0 e
1 % KNO3	90.0 (72..3) c	0.26 c	0.36 b	5.6 b
1.5 % KNO3	94.0 (80.5) bc	0.31 b	0.39 ab	6.0 ab
50 µM H2O2	80 (62.1) d	0.25 d	0.40 a	2.5 f
100 µM H2O2	90 (72..3) c	0.28 c	0.39 a	2.2f
150 µM H2O2	100 (90)a	0.37 a	0.41a	2.8ef
LSD 0.05	4.2	0.02	0.04	0.6

*Values between two bracts represent arcsine transformation of FGP.

IV. DISCUSSION

The present study evaluates chemical treatments to break physical dormancy of *Crotalaria senegalensis* seeds. The results of the this study revealed that all chemical (priming) and hot water treatments significantly ($P< 0.05$) broke the physical dormancy of *C. senegalensis*. This response provides evidence that the seed coat of the plant is the main inhibitor of germination. In treatments using intact seeds, soaking in hot water increased GP and GRI. Many researchers [15] found that soaking seeds in hot water for specific period break exogenous seed dormancy due to making scratch in hard seed coat which facilitate the imbibition. Moreover this treatment enhance seed germination in many plant species.. This results are in harmony with [16] [17] [18]. The response of H_2SO_4 as a method for breaking seed dormancy in this study was consistent with other studies in different species [19] [20] [21] [22]. Some researchers [23] [24] reported that the seeds

of Crotalaria obtained from a natural environment and the H_2SO_4 scarification treatments simulated pass of the seeds through the digestive tract of animals (birds and rodents), which under natural conditions execute chemical scarification. Although the acid scarification significantly broke the dormancy of crotalaria seeds and enhanced germination , but it is commonly not preferred due to its cost, safety risk and environmental precautions involved, and not reliable or lacking the requisite qualities on seeds of other important plant species [25] [26] [27]. The present study reveals that crotalaria species has dual (physical and physiological) dormancy due to the positive response to chemical scarification treatments such as H_2O_2 and KNO_3. The concentration 1% and 1.5 % of the nitrate treatments in this study enhanced germination compared with the highest concentration 2% of the nitrate in our previous study [3]. These concentrations of nitrate (1 and 1.5%) might simulated the case in the soil after rainfall which dilutes nitrate and make it available for seeds. Supporting evidence was reported by [28]. Nitrate has been stated as being a growth-regulating substance in some plant species such as Salvia [3].

The results of this study also revealed the importance of H_2O_2 for breaking the physical dormancy of *C. senegalensis* and the concentration (150 µM) of this oxidant was the best treatment to break dormancy and accelerate germination of *C. senegalensis* seeds. In accord with this finding, some authors have also observed increased germination of other species by soaking seeds in H_2O_2[29]. Previously, a researcher in his recent paper [29] showed that O_2 and H_2O uptake is substantially increased in H_2O_2 soak treatment for Pseudotsuga *menziesii* seeds than in the water control, suggesting an enhanced conversion rate of reserve lipids to carbohydrates and, consequently, increased synthesis of cellular components.

V. Conclusion

The present study reveals that crotalaria exhibit physical or exogenous dormancy and is entirely imposed by the hard seed coat. The integument is able to withstand unfavorable conditions such as heat, teeth of dispersing agent and mechanical damage prevailing in the natural habitat. This avoidance of germination is ecologically advantageous to the plant grows in harsh climatic conditions , in that seeds accumulate in the soil to increase the chance that some of these seeds will germinate and create new population to maintain the species. But this is limiting when quick and consistent seed germination is desirable for successful establishment of economically important forage plant species. Our results demonstrate that mechanical scarification and soaking in water for 24 h , soaking in 1.5 % KNO_3 solution break dormancy and promote germination of *Crotalaria senegalensis*. On the other hand, this study clearly found the positive effect of the oxidant agent H2O2 in breaking dormancy of *C. senegalensis*. Moreover, our results has established a successful methodology for overcoming seed dormancy and optimizing seed germination of *Crotalaria senegalensis* in order to satisfy the demand for fresh materials as a forage in rangeland and pastures.

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References

[1] S. Q. Song, P. Berjak , N. Pammenter, (2004). Desiccation sensitivity of *Trichilia dregeana* Axes and antioxidant Role of ascorbic acid. Acta Botanica Sinica, 46(7), p.p. 803-810.

[2] S.Q. Song, C. L. Long and S.H. Yin and Q.Y. Lan (2003). Desiccation behavior of seeds and their molecular mechanisms. Acta Botanica Yunnan, 25, p.p. 465-479. (in Chinese with English Abstract).

[3] A.H. Naim, A.H. El Hadi and F.E. Ahmed (2015). 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, 5 (10), pp. 16-21.

[4] P. Ortega Baes, M.L. De Viana and S. Sühring. (January 2002). Germination on Prosopis ferox seeds: effects of mechanical, chemical and biological scarificators. Journal of Arid Environments [Online]. 50 (1). pp.185-189.Available: http://dx.doi.org/10.1006/jare.2001.0859

[5] C. Patanè and F. Gresta (October 2006). Germination of Astragalus hamosus and Medicago orbicularis as affected by seed-coat dormancy breaking techniques. Journal of Arid Environments [Online]. 67 (1). pp.165-173.Available: http://dx.doi.org/10.1016/j.jaridenv.2006.02.001

[6] I.S. Travlos, G. Economou and A.I. Karamanos. (February 2007). Germination and emergence of the hard seed coated Tylosema esculentum (Burch) A. Schreib in response to different pre-sowing seed treatments. Journal of Arid Environments [Online]. 68 (3). pp. 501-507. Available: http://dx.doi.org/10.1016/j.jaridenv.2006.07.001

[7] J.H. Ko, S.H. Yang, and Han, K.H. (August 2006). Upregulation of an Arabidopsis RING-H2 gene, XERICO, confers drought tolerance through increased abscisic acid biosynthesis. Plant Journal [Online]. 47 (3) .pp.343-355. Available: http://dx.doi.org/10.1111/j.1365-313X.2006.02782.x

[8] H.J. Ness and K. Bressmer (March 2005). A biotic influences on the behaviour of rodents, ants and plants affect an ant-seed mutualism. Ecoscience [Online]. 12 (1) .pp. 76 - 81. Available: http://dx.doi.org/10.2980/i1195-6860-12-1-76.1

[9] R.K.Sharma, S. Sharma (2010). Effect of storage and cold-stratification on seed physiological aspects of Bunium persicum: A threatened medicinal herb of Trans- Himalaya. International Journal of Botany [Online]. 6 (2). Pp. 151 – 156. Available: http://dx.doi.org/10.3923/ijb.2010.151.156

[10] K.D. Subedi and B.L. Ma (2005). Seed priming does not improve corn yieldin a humid temperate environment. Agronomy Journal , 97, pp. 211-218.

[11] P. Halmer (2003). Methods to improve seed performance. In: R.L. Benech-Arnold and R.A. Sanchez (eds). Seed Physiology, Applications to agriculture. Food Product Press, New York.

[12] K. Ogawa, and M. Iwabuchi, (2001). A mechanism for Promoting the Germination of *Zinnia elegans* Seeds by Hydrogen Peroxide. Plant Cell Physiol,42, 286–29. http://dx.doi.org/10.1093/pcp/pce032

[13] P. Moradi Dezfuli, F. Sharif-zadeh and M. Jan Mohammadi (2008). Influence of Priming Techniques on Seed Germination Behavior of Maize Inbred Lines (*Zea mays* L.). ARPN Journal of Agricultural and Biological Sciences, 3,22-25.

[14] H.A. Esechie (April 1994). Interaction of salinity and temperature on the germination of sorghum. Journal of Agronomy and Crop Sciences [Online]. 172 (3). pp. 194 – 199. Available: http://dx.doi.org/10.1111/j.1439-037X.1994.tb00166.x

[15] C.A. Previero, L. Martins, R.H.A. Fonseca, and D. Groth (1996). Effect of storage of guinea grass (Panicum maximum Jacq.) on treatment to break dormancy. Revista Brasileira de Sementes 18.pp. 143-148. http://dx.doi.org/10.17801/0101-3122/rbs.v18n1p143-148

[16] R. Lindig-Cisneros and S. Lara-Cabera (March 2004). Effect of scarification and growing media on seed germination of Crotalaria pumila (Ort.). Seed Science and Technology 32 (1).pp. 231-234.Available:

[17] T.N. Kaye and K. Kuykendall (January 2001) . Effects of scarification and cold stratification on seed germination of lupines sulphuerus spp. Kincaidil. Seed Science and Technology 29.pp. 663-668. Available:

http://www.researchgate.net/profile/Thomas_Kaye2/publication/260942702

[18] L. A. Hermansen, M.L. Duryea and T. L. White (2000). Viability in seed coat dormancy in Dimorphandra mollis. Seed Science and Technology 28 (3). Pp. 567-580. Available: http://cat.inist.fr/?aModele=afficheN&cpsidt=883886

[19] N. Righini, J.C. Serio-Silva, V. Rico-Gray and R. Martinez-Mota (June 2004). Effect of different primate species on germination of Ficus (Urostigma) seeds. Zoology and Biology 23.pp.: 273-278.Available: http://onlinelibrary.wiley.com/doi/10.1002/zoo.20008/abstract

[20] E. Ates (2011). Influence of some hardseededness-breaking treatments on germination in Persian clover (trifolium resupinatum ssp. typicum Fiori et Paol.) seeds. Romanian Agricultural Research.28.pp. 229–236. Available: http://www.incda-fundulea.ro/rar.htm

[21] W.E. Finch-Savage and Leubner-Metzger, G. (July 2006). Seed dormancy and the control of germination. New Phytologist. 171 (3) .pp. 501-523. Available: http://dx.doi.org/10.1111/j.1469-8137.2006.01787.x

[22] J.M. Baskin, N. Xiaoying and C.C. Baskin (December 1998). A comparative study of seed dormancy and germination in an annual and a perennial species of Senna (Fabaceae). Seed Science Research 8 (4). pp. 501-512. Available: http://dx.doi.org/10.1017/S0960258500004475 http://dx.doi.org/10.15258/sst.2004.32.1.25

http://dx.doi.org/10.1016/j.jaridenv.2005.06.009

[23] A. Alderete-Chavez, L. Aguilar-Marín and N. de la cruz-Landero (2010). Effects of scarification chemical treatments on the germination of Crotalaria retusa L. seeds. Journal of Biological Sciences 10 (6).pp. 541–544. Available: http://dx.doi.org/10.3923/jbs.2010.541.544

[24] A. Kak, L. Chitra Devi, V. Gupta and N. Singh (2007). Response of seed treatments on seed germination in wild crotalaria species. International Conference on Indigenous Vegetables and Legumes. Prospectus for Fighting, Poverty and Malnutrition. ISHS Acta Horticulture 752. http://dx.doi.org/10.17660/ActaHortic.2007.752.42

[25] E. Yücel (2000). Effects of different salt (NaCl), nitrate (KNO3) and sulphuric acid (H2S04) concentrations on the germination of some salvia species seeds. Seed Science and Technology 28 (3):853-860. Available: http://www.cabdirect.org/abstracts/20013009239.html;jsessionid=0ADF469FD3AF84F7B508A919F63DC228

[26] P. C. Bethke, I. G.L. Libourel and R.J. Jones (2006). Nitric oxide reduces seed dormancy in Arabidopsis. Journal of Experimental Botany 57 (3).pp. 517 -526. Available: http://dx.doi.org/10.1093/jxb/erj060

[27] D. W. Chuanren, L. Bochu, C. Wanqian, L. J. Jing and Z. Huan (september 2004). Effect of physical factors to improve the germination rate of Echinacea angustifolia seeds. Colloids Surf B: Biointerfaces 37(3-4): 101-105. Available: http://dx.doi.org/10.1016/j.colsurfb.2004.07.003

[28] A. Tigabu and P.C. Oden (2001). Effect of scarification, gibberellic acid and temperature on seed germination of two multipurpose Albizia species from Ethiopia. Seed Science Research 29 (1): 11-20. Available: http://cat.inist.fr/?aModele=afficheN&cpsidt=986031

[29] G . Sarath, R . Mitchell, G . Hou, L.M. Baird (2007) Reactive oxygen species, ABA and nitric oxide.interactions on the germination of warm season C4-grasses. Planta 226:697-708.