International Journal of Nursing, Midwife and Health Related Cases

Vol.4, No.2, pp.1-10, March 2016

Published by European Centre for Research Training and Development UK (www.eajournals.org)

STUDIES ON METHODS OF BREAKING SEED DORMANCY AND GERMINATION ENHANCEMENT IN *SENNA ALATA* (L.) ROXB., A PLANT WITH GREAT MEDICINAL VALUE

Sunday Arowosegbe

Department of Plant Science, Faculty of Science, Ekiti State University, PMB 5363, Ado-Ekiti, Nigeria.

ABSTRACT: *Senna alata is a plant with great medicinal value that belongs to Fabaceae family and grows as wild perennial shrubby species. Seeds of this species possess seed coat-induced dormancy. The seeds were subjected to different treatment methods and durations of exposure to break the dormancy and enhance germination. Treatments includes scarification with 60, 80 and 100% H_2SO_4 and HCL for 2, 4 and 6 min; exposure to 60, 80 and 100 ^{0}C dry (oven) and wet (hot water) heat for 2, 4 and 6 min; and soaking in water for 12, 24, 36, 48, 60, 72, 84 and 96 h. The experimental results revealed that seeds scarified with 100% H_2SO_4 for 4 and 6 min were the most effective treatments for enhancing seed germination (both gave 100.00% germination), followed by 100 ^{0}C wet heat for 6 min (77.50%) and 80% H_2SO_4 for 6 min (70.00%). Other treatments were less effective. The treatments that gave significantly higher germination percentages also produced low MGT (1.80, 1.73, 2.71 and 1.51 days, respectively) and increased GI (90.25, 92.67. 63.00 and 65.00, respectively) without having any significant negative effects on the radicle length of the seedlings. These are desirable for field establishment and production of uniform plant population of S. alata*

KEYWORDS: Seed Dormancy, Medicinal Plants, Scarification, Germination

INTRODUCTION

The importance of medicinal plants is no longer limited to the people to whom traditional medicine serves as the only opportunity for their primary health care, but also to those who use pharmaceutical products derived from plants (Thirupathi *et al*., 2012). Natural products derived from plants are being tested to discover new drugs with new mode of pharmaceutical action (Ogunjobi and Abiola, 2013). Higher plants are known for their capacity to produce a large number of secondary metabolites (Castello *et al*., 2002) that have been implicated for various therapeutic properties.

Senna alata (L.) Roxb. is one of such plant species with great medicinal value. The plant belongs to the family Fabaceae and subfamily Caesaipinioideae. It is an erect, perennial shrubby legume that grows to about 183 cm in height. The plant has dark green and pinnately compound leaves, each with 16-28 obviate leaflets measuring 5-10 cm and alternating bilateral pattern. Leaves are usually obviated in shape and are evergreen (Chatterjee *et al*., 2012). Flowers are beautiful and in columns looking like yellow candle sticks, hence the common name candle stick or candle bush (Gbadamosi and Shaibu, 2013). According to Chatterjee *et al*. (2012), the flowers are actually hypogynous but appear to be perigynous because of the hypanthium, a floral structure consisting of bases of sepals, petals and stamens fused together (a distinguishing characteristic of the family Fabaceae). The flowers later develop into pods of 5-17 cm long containing 50-60 flattened seeds that are triangularly shaped. The pods are

ISSN 2053-4108(Print), ISSN 2053-4116(Online)

usually soft and green while young but eventually harden and turn dark brown or nearly black as they mature.

The fresh leaf extract of *S. alata* is universally used as a remedy for parasitic skin diseases (Oliver-Bever, 1986).The plant is also popular for its laxative or purgative and wound healing properties and for the treatment of abscesses (Ogunti and Elujobi, 1993; Monkheang *et al*., 2011). Validation of the medicinal properties of *S. alata* revealed that the plant has biological activities such as antibacterial, antifungal, diuretic analgesic and choleretic (Palanichamy and Nagarajan, 1990; Reezal *et al*., 2002; Ogunjobi and Abiola, 2013). Unfortunately, the population of many medicinal plants including *S. alata* is reducing rapidly on daily basis. This reduction is largely due to the changing environmental conditions over the past decades and the excessive harvesting of the wild plants from their natural habitat. To mitigate this undesirable trend, it is necessary to propagate and domesticate such plants in order to enhance greater productivity, higher population and availability.

According to Gupta and Bandopadhyay (2013), seeds are excellent dispersal unit and means of propagating higher plants which have emerged in the course of plant evolution. However, seeds of *S. alata* are difficult to germinate due to dormancy. A viable seed (or other germination unit) is said to be dormant when it does not have capacity to germinate in a specified period of time under normal physical environmental factors that otherwise is favourable to its germination (Aghilan *et al*., 2014). Baskin and Baskin (2001) had reported the different types of seed dormancy. The seed dormancy exhibited by *S. alata* is not strange because, Zare *et al*. (2011) reported specifically that the seeds of many medicinal plant species have dormancy issues. According to these authors, the seeds germinate within the native environment but fail to show good germination under laboratory conditions or under cultivation. Bryant (1996), also stated that seeds of most cultivated plant species lose their dormancy before or shortly after being separated from the parent plant, whereas seeds of most wild shrubs have long period of seed dormancy.

In order to domesticate and cultivate any plant species, information is needed on the seed germination and how to overcome the problem of dormancy of such plant species. The Association of Official Seed Analysts (AOSA) and the International Seed Testing Association (ISTA) have reported different ways for breaking seed dormancy and stimulating germination (Aghilian *et al*., 2014). The objective of this work therefore, was to find method(s) to break seed dormancy in *S. alata* for achieving rapid, uniform and higher germination which will lead to higher plant stand establishment.

MATERIALS AND METHODS

Seed collection

Mature pods of *S. alata* were collected from the parent plant in January, 2015 from its natural habitat in Ado-Ekiti (7^{o} 29^{1}N, 5^{o} 13^{1}E). Seeds were removed manually from the mature and dry pods and the good ones selected were kept in paper envelopes for the germination study. The weight of 1000 seeds was 33 g

ISSN 2053-4108(Print), ISSN 2053-4116(Online)

Seed Sterilization

All the *Senna alata* seeds (2,800) collected for these experiments were surface-sterilized by soaking in 70% alcohol for 1 min and were immediately soaked in 2.5% Sodium hypochlorite (NaOCl) for 3 min according to the method of Ashtari *et al*. (2013). The seeds were thoroughly rinsed in several rounds of distilled water before applying the various dormancy-breaking treatments

Dormancy-breaking Treatments

Before germination experiments, the seeds were subjected to the following pre-sowing treatments;

Scarification with Acids (H_2SO_4 and HCL)

Seeds (50) were soaked separately in different concentrations (60, 80 and 100%) of both Tetraoxosulphate (vi) acid (H_2SO_4) and Hydrochloric acid (HCL) for 2, 4 and 6 min each. The seeds were carefully removed from the Petri dishes according to the time required for soaking. They were then placed immediately under a cool running water to terminate the chemical reactions and to remove every trace of acid from the seeds. Untreated seeds were used as control.

Exposing the seeds to Wet heat (Hot water) and Dry heat (Oven)

Fifty seeds each of S. alata were separately soaked in 60, 80 and 100 ^oC of distilled water for 2, 4 and 6 min. The same numbers of seeds were placed in sterilized non-disposable (Pyrex) Peti dishes and kept in the oven at 60, 80 and 100 ^oC for 2, 4 and 6 min each before they were removed. Untreated seeds were used as control.

Soaking in water

Fifty seeds each of S. alata were separately soaked in distilled water in beakers for 12, 24, 36, 48, 60, 72, 84 and 96 h. Untreated seeds were used as control.

Germination assay

Ten (10) Seeds from each of the seed lots in the treatments described above were placed in 9 cm sterilized Petri dishes lined with double-layered Whatman No.1 filter papers. The papers were moistened with 6 mL distilled water and covered-up with their respective covers (Arowosegbe and Afolayan, 2013).

The experiments were set up in a Randomized Complete Block Design with four replicates each. Untreated seeds were used as control. All the Petri dishes were placed on the germination table in the laboratory at room temperature (35± 2 ^oC). Seed germination was counted and recorded daily until no further germination occurred. A seed was considered to have germinated when the tip of the radicle had grown free from the seed coat to 2 mm. Radicle length (cm) were taken 7 days after germination.

Major Calculations.

The germination percentage (%) was determined using the relation;

ISSN 2053-4108(Print), ISSN 2053-4116(Online)

Germination % = $\frac{G}{N}$ X 100

Where G = Total number of seeds that germinated.

N = Total number of seeds in the Petri dish.

The mean germination time (MGT) was determined as described by Orchard (1977) as follows;

$$MGT = \frac{\Sigma f.x}{\Sigma f}$$

Where f = Number of seeds that germinated on day x

The germination index (GI) was calculated according to the Benech Arnold *et al*. (1991) equation;

$$GI = (10\ X\ n1) + (9\ X\ n2) + (8\ X\ n3) \ldots\ldots + (1\ X\ n10)$$

Where; $n1, n2, n3 \ldots\ldots n10$ = Number of germinated seeds on first, second, third and subsequent days until 10th day; 10, 9…..and 1 are weights given to the number of germinated seeds on the first, second and subsequent days, respectively.

Statistical Analysis

The data were analysed using one-way Analysis of Variance (ANOVA) and the means were separated at P < 0.05 using Duncan's Multiple Range Test (DMRT). All statistical analyses were done using SAS software, 1999 version.

RESULTS

Table 1: .Effect of seed scarification with acids (H_2SO_4 and HCL) on breaking dormancy and germination enhancement in seeds of *S. alata*

Dormancy breaking treatments	Germination (%)	MGT (days)	GI	Radicle length (cm)
Control	17.50^{ef}	5.01^{abcd}	8.50^{ef}	7.44^{bcde}
H_2SO_4 (60%) for 2 min	10.00^{f}	2.50^{de}	8.50^{ef}	7.02^{def}
H_2SO_4 (60%) for 4 min	20.00^{ef}	3.67^{bcde}	15.50^{ef}	7.87^{bcde}
H_2SO_4 (60%) for 6 min	17.50^{ef}	2.83^{de}	14.50^{ef}	8.62^{abc}
H_2SO_4 (80%) for 2 min	20.00^{ef}	2.50^{de}	17.00^{e}	7.86^{bcde}
H_2SO_4 (80%) for 4 min	42.50^{d}	2.10^{de}	37.75^{d}	8.08^{bcde}
H_2SO_4 (80%) for 6 min	70.00^{b}	1.51^{e}	65.00^{b}	7.87^{bcde}
H_2SO_4 (100%) for 2 min	57.50^{c}	2.44^{de}	51.00^{c}	7.95^{bcde}
H_2SO_4 (100%) for 4 min	100.00^{a}	1.80^{e}	90.25^{a}	7.10^{cdef}
H_2SO_4 (100%) for 6 min	100.00^{a}	1.73^{e}	92.67^{a}	7.66^{bcde}
HCL (60%) for 2 min	15.00^{ef}	5.63^{abcd}	8.50^{ef}	9.02^{ab}
HCL (60%) for 4 min	20.00^{ef}	8.38^{a}	4.75^{ef}	9.85^{a}
HCL (60%) for 6 min	15.00^{ef}	5.5^{abcd}	8.25^{ef}	8.60^{abcd}

ISSN 2053-4108(Print), ISSN 2053-4116(Online)

HCL (80%) for 2 min	25.00^{e}	6.42^{abc}	12.00^{ef}	6.98^{ef}
HCL (80%) for 4 min	10.00^{f}	5.00^{abcd}	6.00^{ef}	8.36^{abcde}
HCL (80%) for 6 min	15.00^{ef}	3.13^{cde}	12.00^{ef}	8.88^{ab}
HCL (100%) for 2 min	15.00^{ef}	3.75^{bcde}	11.75^{ef}	8.96^{ab}
HCL (100%) for 4 min	15.00^{ef}	4.14^{bcd}	10.50^{ef}	6.79^{f}
HCL (100%) for 6 min	10.00^{f}	7.00^{ab}	4.00^{f}	5.86^{f}

Values with the same letter(s) within the column are not significantly difference at P<0.05 by Duncan's Multiple Range Test (DMRT).

Table 2: Effect of dry heat (oven) and wet heat (hot water) on breaking dormancy and germination enhancement in seeds of *S. alata*

Dormancy breaking treatments	Germination (%)	MGT (days)	GI	Radicle length (cm)
Control	15.00^{d}	5.10^{ab}	8.50^{d}	7.40^{bcde}
Dry heat (60 ^{0}C) for 2 min	15.00^{d}	7.13^{ab}	6.50^{d}	6.21^{efghi}
Dry heat (60 ^{0}C) for 4 min	12.50^{d}	7.63^{a}	5.00^{d}	5.67^{ghi}
Dry heat (60 ^{0}C) for 6 min	17.50^{d}	7.13^{ab}	5.00^{d}	5.90^{fghi}
Dry heat (80 ^{0}C) for 2 min	15.50^{d}	5.92^{ab}	12.25^{d}	8.01^{bcd}
Dry heat (80 ^{0}C) for 4 min	12.50^{d}	7.63^{a}	5.00^{d}	7.50^{bcde}
Dry heat (80 ^{0}C) for 6 min	15.00^{d}	7.92^{a}	6.25^{d}	6.85^{defgh}
Dry heat (100 ^{0}C) for 2 min	10.00^{d}	6.75^{ab}	4.25^{d}	5.52^{hi}
Dry heat (100 ^{0}C) for 4 min	10.00^{d}	7.75^{a}	3.25^{d}	5.08^{i}
Dry heat (100 ^{0}C) for 6 min	10.00^{d}	6.75^{ab}	4.25^{d}	4.73^{i}
Wet heat (60 ^{0}C) for 2 min	12.50^{d}	5.30^{ab}	9.25^{d}	8.47^{abc}
Wet heat (60 ^{0}C) for 4 min	12.50^{d}	7.00^{ab}	5.25^{d}	5.69^{ghi}
Wet heat (60 ^{0}C) for 6 min	20.00^{d}	3.87^{bc}	11.75^{d}	7.47^{bcde}
Wet heat (80 ^{0}C) for 2 min	15.00^{d}	4.88^{abc}	9.75^{d}	9.65^{a}
Wet heat (80 ^{0}C) for 4 min	17.50^{d}	5.54^{abc}	10.75^{d}	7.13^{cdefg}
Wet heat (80 ^{0}C) for 6 min	47.50^{c}	4.20^{abc}	33.00^{bc}	7.32^{bcdef}
Wet heat (100 ^{0}C) for 2 min	45.00c	4.15^{abc}	30.75^{c}	7.90^{bcd}
Wet heat (100 ^{0}C) for 4 min	60.00b	3.77^{bc}	44.25^{b}	8.75^{ab}
Wet heat (100 ^{0}C) for 6 min	77.50a	2.71^{c}	63.00^{a}	7.53^{bcde}

Values with the same letter(s) within the column are not significantly difference at P<0.05 by Duncan's Multiple Range Test (DMRT).

Table 3: Effect of soaking in water on breaking dormancy and germination enhancement in seeds of *S. alata*

Dormancy breaking treatments	Germination (%)	MGT (days)	GI	Radicle length (cm)
Control	15.00^{a}	5.10^{bc}	8.50^{a}	7.44^{bcd}
Soaking for 12 h	12.50^{a}	9.25^{a}	2.50^{a}	7.68b^{cd}
Soaking for 24 h	12.50^{a}	5.38^{bc}	7.00^{a}	9.10^{ab}
Soaking for 36 h	12.50^{a}	7.88^{ab}	4.00^{a}	6.57^{d}
Soaking for 48 h	12.50^{a}	7.63^{ab}	4.75^{a}	7.49^{cd}
Soaking for 60 h	17.50^{a}	4.17^{c}	10.25^{a}	8.41^{abc}
Soaking for 72 h	12.50^{a}	7.50^{ab}	5.00^{a}	7.20^{cd}
Soaking for 84 h	12.50^{a}	7.88^{ab}	4.25^{a}	9.32^{a}
Soaking for 96 h	10.00^{a}	7.50^{ab}	3.50^{a}	6.70^{d}

ISSN 2053-4108(Print), ISSN 2053-4116(Online)

Values with the same letter(s) within the column are not significantly difference at P<0.05 by Duncan's Multiple Range Test (DMRT).

Effects of scarification with acids (H_2SO_4 and HCL)

Effects of acids (H_2SO_4 and HCL) on seed germination percentage, Mean germination time (MGT), germination index (GI) and seedlings' radicle length of *S. alata* differed significantly (P< 0.05) among the various treatments (Table1**).** From the results obtained, Seeds scarified with HCL at different concentrations and duration produced low germination percentages that were not significantly different from the control. However, Seeds treated with concentrated (100%) H_2SO_4 for 4 and 6 min significantly enhanced best germination percentage (100%). This was followed by 80% H_2SO_4 for 6 min, 100% H_2SO_4 for 4 min and 80% H_2SO_4 for 4 min with germination of 70.00, 57.50 and 42.50%, respectively. A closer look at the results of this study (Table 1) also revealed that minimum MGT (1.51, 1.73 and 1.80 days) was detected in *S. alata* seeds treated with 80, 100 and 100% H_2SO_4 for 6, 4 and 6 min, respectively. All were statistically the same but significantly different from the control (5.01 days). Moreover, maximum GI (92.67, 90.25 and 65.00) was observed in seed lots treated with 100, 100 and 80% H_2SO_4 for 6, 4 and 6 min, respectively. These were significantly (P < 0.05) higher than the control (8.50). The GI value for seeds treated with 100% H_2SO_4 for 2 min was also significantly higher (51.00) than the control. Interestingly, soaking *S. alata* seeds in H_2SO_4 seemed not to have any significant effect on the radicle length of the seedlings, while longer radicle length (9.85 cm) was recorded in seed lot treated with 60% HCL for 4 min (but with low germination percentage), However, significantly lower radicle length was found in seeds treated with 100% HCL for 6 and 4 min (5.86 and 6.79 cm, respectively) when compared with the control.

Effects of dry heat (ovum) and wet heat (hot water)

Table 2 shows the effect of dry heat (oven) and wet heat (hot water) treatments on breaking the dormancy and germination of *S. alata* seeds. From these results, exposing the seeds to dry heat irrespective of temperature or duration of exposure had no significant effect on the germination percentage. However, soaking the seeds in 100 ^{0}C wet heat for 6 min produced 75.50 % germination which was significantly higher than the untreated seeds (15.00%). This was followed by seeds treated with 100 ^{0}C wet heat for 4 and 2 min; and 80% wet heat for 6 min with germination of 60.00, 45.00 and 47.00%, respectively. Moreover, a desirable minimum MGT (2.71 days) was observed in seed lot subjected to 100 ^{0}C wet heat for 6 min which was significantly different from the control (5.10 days). Meanwhile, higher GI was also obtained for *S. alata* seeds treated with 100 ^{0}C wet heat for 6, 4 and 2 min, and seeds treated with 80 ^{0}C wet heat for 6 min (63.00, 44.25, 30.75 and 33.00, respectively). The longest radicle length (9.65 cm) was recorded in seedlings of seeds soaked in 80 ^{0}C hot water for 2 min (but with very low germination percentage), while shorter radicle length were recorded in seedlings of seed lots exposed to dry heat at 100 ^{0}C for 2, 4 and 6 min. The values raged from 4.73 to 5.52 cm. However, soaking *S. alata* seeds in 100 ^{0}C wet heat irrespective of the duration had no significant effect on the radicle length of the seedlings when compared with the control. This was also the same for seeds soaked in 80 ^{0}C wet heat for 6 min with no significant effect on the radicle length.

ISSN 2053-4108(Print), ISSN 2053-4116(Online)

Effects of soaking in water

Seed germination of *S. alata* was not significantly affected by soaking in water irrespective of the duration of soaking (Table 3). This also reflected in the GI, where no significant effect was recorded when compared with the control. Moreover, soaking seeds in water seemed not to have any effect on the MGT except in seeds soaked for 12 h (9.25). Longer radicle length (9.32 cm) was observed in seedlings of seeds soaked in water for 84 h when compared with the control.

DISCUSSION

Seeds of members of Fabaceae family are known to exhibit dormancy due to hard seed coats that are impermeable to water and oxygen (Ali *et al*., 2011; Pallavi *et al*., 2014). According to Baskin and Baskin (2001), seed coats can also impose dormancy because they may contain growth inhibitors or may prevent the leaching of inhibitors from the embryo. It is interesting to note that the results of different treatments in this present study confirm that *S. alata* seeds truly exhibit dormancy due to hard seed coat. Generally, the germination percentage obtained in the untreated seeds was found to be very low. This further corroborates the report of Rao *et al*. (2006) that unlike cultivated species, seeds of wild plant species are generally dormant.

As observed in these present studies, breaking the seed dormancy of *S. alata* by scarification with H_2SO_4 resulted in a significant ($P < 0.05$) increase in the germination percentage (42.50 to 100.00 %) when compared with the control. The increase was found to be dependent on the concentration of the acid and the duration of treatment. The highest germination percentage was found in the seed lots soaked in 100% H_2SO_4 for 4 and 6 min, where 100% germination were both recorded. However, soaking the seeds longer than 6 minutes might lead to a decline in the germination percentage. Previous work on *Parkia biglobosa* (Aliero, 2004), *Enterolobium contortisiliquum* (Malavasi and Malavasi, 2004), *Rhynchosia capitata* (Ali *et al*., 2011) also showed that soaking of seeds of these plants in H_2SO_4 can break dormancy and increase germination percentage. The mechanism involved in the possible seed germination influenced by H_2SO_4 could be due to the capacity of the acid to break the seed coat (Ali *et al*., 2011) by softening it thereby allowing water imbibition and oxygen penetration into the seed. According to Nadjafi *et al*. (2006), scarification of seed coat with acids such as H_2SO_4 usually leads to elimination of exogenous dormancy. However, the case was not the same for Scarification with HCL in this study, where soaking of *S. alata* seeds in HCL irrespective of its concentration or duration of treatment resulted in very low germination percentages ($\leq$ 20.00%).

Soaking *S. alata* seeds in wet heat for 6 min also brought about significant increase in the germination percentage (45.00 to77.50%), with soaking in 100 ^{0}C for 6 min having the higher germination percentage (77.50%). Whereas, seeds exposure to dry heat irrespective of temperature and duration of exposure did not improve the seed germination.This implied that it was not the high temperature of the wet heat (hot water) that brought about the breaking of dormancy in *S. alata* seeds, but the softening of the seed coat by the hot water. Although dormancy in *Azadiracta indica* (Owonubi *et al*., 2005) and *Adansonia digitata* (Ibrahim and Otegbeye, 2004) seeds were successfully broken by soaking in water, no appreciable improvement was recorded in *S. alata* seeds soaked in ordinary water irrespective of the time

ISSN 2053-4108(Print), ISSN 2053-4116(Online)

of soaking. This is in similar to the findings of Zare *et al*. (2011) who reported that soaking in water was not effective in breaking the seed dormancy of *Ferula assa foetida.*

According to Timson (1965), the final germination percentage of seeds alone is not enough for reporting results of any germination assay since it does not indicate the rapidity of germination but only its final extent. It is therefore necessary to use the final germination percentage with the mean germination time (MGT) and the germination index (GI) to reflect the percentage and speed of germination separately and in combination. The significant decrease in MGT and the corresponding increase in the GI recorded in *S. alata* seeds scarified with 80% H_2SO_4 for 6 min and 100% H_2SO_4 for 4 and 6 min, and soaking in wet heat (100 ^{0}C) for 6 min are good indications that H_2SO_4 at higher concentrations and wet heat (100 ^{0}C) for 6 min did not only induce higher germination percentages, but also increase the speed of germination. The implication of this is that these treatments were capable of breaking the hard seed coat of *S. alata* seed and also induce uniform germination within a short time. It is also interesting to note that all the treatments that gave significantly higher germination percentage, desirable low MGT and increased GI did not have any negative effects on the radicle length of the seedling as observed in these studies.

CONCLUSION

The results of these studies have demonstrated that seeds of *S. alata* like some other wild medicinal plants, exhibits dormancy imposed by hard seed coat. Softening the seed coat by scarifying with concentrated H_2SO_4 and soaking in wet heat (hot water) significantly increased seed germination percentage, reduced the MGT and increased the GI without having any effects on the radicle length of the seedling. All these are desirable for the field establishment and production of uniform plant population.

REFERENCES

Aghilian,S., Khajeh-Hosseini, M and Anvarkhah, S. (2014). Evaluation of seed dormancy in forty medicinal plant species. *International Journal of Agriculture and Crop Species*. 7(10): 760-768

Ali, H.H., Tanveer, A., Nadeem, M.A. and Asghar, H.N. (2011). Methods to break seed dormancy of *Rhynochosia capitata.*, A summer annual weed. *Chilean Journal of Agricultural Reserarch*. 71(3): 483-487.

Aliero, B.I. (2004). Effects of sulphuric acid, mechanical scarification and wet heat treatments on germination of seeds of African locust bean tree. *Parkia biglobosa*. *African journal of Biotechnology* 3: 179-181

Arowosegbe, S. and Afolayan, A.J. (2013). Germination and growth inhibitory effects of aqueous extracts of different parts of *Bidens pilosa* Var, Radiata on some vegetable crops. *Bulletin of Pure and Applied Science* Vol. 32 B- Botany (No.1):11-20.

Ashtari, R., Heidari, M., Omidi, M. and Zare, A.R. (2013). Seed germination and dormancy breaking techniques for *Ducrosia anethifolia* (DC.). *Trakia Journal of Science* 11(1): 82-87

Baskin, C.C. and Baskin, J.M. (1998). *Seeds: Ecology, biogeography and evolution of dormancy and germination*. Academic Press, New York, USA. P. 5-26.

ISSN 2053-4108(Print), ISSN 2053-4116(Online)

Benech Arnold, R., Fenner, M. and Edwards, P. (1991). Changes in germinability, ABA content and ABA embryonic sensitivity in developing seeds of *Sorghum bicolor* (L.) Moench. induced by water stress during grain filling. *New Phytologist*. 118: 339-347.

Bewley, J.D. (1997). Seed germination and dormancy. *Plant Cell*. 9(7): 1055-1066.

Bryant, G.A. (2005). *Seed Physiology*. Edward Arnord, London. 1: 54-60.

Castello, M.C., Phatak, A., Chandra, N. and Sharon, M. (2002). Antimicrobial activity of crude extracts from plant parts and corresponding calli of *Bixa orellana* L. *Indian Journal of Experimental Biology*. 40(12): 1378-1381.

Chatterjee, S., Chatterjee, S and Dutta, S. (2012). An overview on the ethnophytopathological studies of *Cassia alata*- an important medicinal plant and the effect of VAM on its growth and productivity. *International Journal of Research in Botany.* 2(4):13-19.

Gbadamosi, A.E. and Shaibu, B. (2013). Influence of the phytohormones on the in-vitro regeneration in *Senna alata* (L.). *Academia Journal of Biotechnology* 1(3): 041-045

Gupta, S. and Bandopadhyay, A. (2013). Overcoming seed dormancy of *Acalypha indica* L. (Euphorbiaceae): an important medicinal plant. *Indian Journal of Plant Science*. 2(1): 72-79.

Ibrahim, A. and Otegbeye, G.O. (2004). Methods of achieving optimum germination in *Adansonia digitata*. *Bowen Journal of Agriculture*. 1(1): 54-58.

Malavasi, U.C. and Malavasi, M. (2004). Dormancy breaking and germination of *Enterolobium contortisiliquum* (Vell.) Morong seed. *Brazilian Archives of Biology and Technology*. 47:851-854.

Monkheang, P., Sudmoon, R., Tanee, T., Noikotr, K., Bletter, N. and Chaveerach, A. (2011). Species diversity, usages, molecular markers and barcode of medicinal *Senna species* (Fabaceae, Caesalpinioideae). *Thailand Journal of Medicinal Plants Research.* 5(26): 6173-6181

Nair, R.M., Craig, A.D., Rowe., T.D., Biggins, S.R. and Hunt, C.H. (2004). Genetic variability and heritability estimates for hardseededness and flowering in Bslansa clover (*Trifolium michelianum* Savi) population. *Eughytica*. 138(3): 192-203.

Nadjafi, F., Bannayana M., Tabrizia, L. and Rastgoo, M. (2006). Seed germination and dormancy breaking techniques for *Ferula gummosa* and *Teucrium polium*. *Journal of Arid Environment*. 64:542-547.

Ogunjobi, A.A. and Abiola, M.A. (2013). Antimicrobial activity of *Senna alata* and *Phyllanthus amarus*. Global *Journal of Pharmacology*. 7(2): 198-202.

Ogunti, E.O. and Elujobi, A.A. (1993). Laxative activity of *Cassia alata*. *Filoterapia.* 64(5): 437- 439.

Oliver-Bever, B. (1986). *Medicinal plants in tropical West Africa*. Cambridge University press, London. Pp. 134

Orchard, T. (1977). Estimating the parameters of plant seedling emergence. *Seed Science and Technology*. 5: 61-69.

Owonubi, J.J., Otegbeye, G.O. and Nwokkedi, C. (2005). Development of Pre-germination technique for *Azadiracta indica*: Preliminary investigation In: *Sustainable forest management in Nigeria: Lessons and prospect*. Annual Forestry Association Conference held in Kaduna, Nigeria, 7- 11th Nov.2005. pp. 29-38.

Palanichamy, S. and Nagarajan, S. (1990). Antifungal activity of *Cassia alata* leaf extract. *Journal of Ethnopharmacology*. 29(3): 337-340

Pallavi, H.M., Vishwanath, K., Harish, B.S., Prashanth, Y. and Manjunath T. (2014). Seed treatments to break seed dormancy and standardization of viability test procedure in *Abrus precatorious*. *Journal of Medicinal Plant Research.* 8(4): 229-236.

ISSN 2053-4108(Print), ISSN 2053-4116(Online)

Rao, N.K., Hanson J., Dulloo, M.E., Ghoch, K., Nowel, D. and Larinde, M. (2006). *Manuel de manipulation des semences dans les banques de genes.* Manuel pour les banques de genes no. 8. Biodiversity International Rome, Italy 165 p

Reezal, I., Somchit, M.N. and Rahim, M.A. (2002). *In-vitro antifungal properties of Cassia alata (GELENGGANG BESAR).* Proceedings of the Regional Symposium on Environment and Natural Resources. April 10-11, Hotel Renaissance Kuala Lumpur, Malaysia. Pp 654-659

SAS. (1999). Proprietary software release 8.2 SAS Institute Inc. NC, USA.

Thirupathi, M., Kiran Kumar, M., Sriniva, D., Rajendar, K. and Jaganmohan Raddy, K. (2012). A study on seed germination of *Cassia alata* Linn., an antiallergenic plant. *Journal of Phytology*. 4(5): 13-15

Timson, J. (1965). New method of recording germination data. *Nature*. 207: 216-217.

Zare, A.R., Solouki, M., Omidi, M., Irvani, N., Oladzad, A., Basabad, A. and Mahdi Nezad, N. (2011). Effect of various treatments on seed germination and dormancy breaking in *Ferula assa faetida* L. (Asafetida)., a threatened medicinal herb. *Trakia Journal of Sciences*. 9(2): 57-61.

ISSN 2053-4108(Print), ISSN 2053-4116(Online)