AUSTRALIAN JOURNAL OF BASIC AND APPLIED SCIENCES

ISSN:1991-8178 EISSN: 2309-8414
Journal home page: www.ajbasweb.com

Pre-germination treatments and their relation with *Aegiphila sellowiana* seeds germination

[1]Paulo do Nascimento, [2]Bruno Oliveira Lafetá, [3]Jéssica Cristina Barbosa Ferreira

[1]Instituto Federal de Educação, Ciência e Tecnologia de Minas Gerais/Tecnologia em Silvicultura, Primeiro de Junho Avenue, n° 1043 – Center – 39705-000 São João Evangelista, Minas Gerais – Brazil.
[2]Instituto Federal de Educação, Ciência e Tecnologia de Minas Gerais/Tecnologia em Silvicultura, Primeiro de Junho Avenue, n° 1043 – Center – 39705-000 São João Evangelista, Minas Gerais – Brazil.
[3]Universidade de Brasília – Forestry Department/ Masters degree in Forestry Sciences. UnB. Asa Norte – 70910-900 Brasília, Distrito Federal, Brazil.

Address For Correspondence:
Jéssica Ferreira. Universidade de Brasília. Departamento de Engenharia Florestal. Faculdade de Tecnologia. 70.910-900. Brasília. Brasil.

ARTICLE INFO

Article history:
Received 18 January 2017
Accepted 28 March 2017
Available online 15 April 2017

Keywords:
Cattle manure, Dormancy interruption, Subtract, Tamanqueira Tree

ABSTRACT

Aegiphila sellowiana is a pioneer useful to restore locations affected by human action, because it grows fast and produces fruits for birds' consumption. Due its low germination rates, BOD experiments were conducted and in a greenhouse, using seeds deriving from red fruits in an entirely random design, aiming to maximize species germination rates and include it in the damaged areas restoration programs. Seeds immersion in sodium hypochlorite 2% during two minutes was efficient to allow germination in laboratory. Quality seedlings formation is obtained in the cattle manure subtract, which enabled higher growth rates.

INTRODUCTION

Tropical forests number have been decreasing progressively, meanwhile, the damaged areas number have been increasing. Considering the need to replace the native vegetation and/or restore it, it is important to understand reproductive biology aiming to restore forests rationally.

Among the factors needed to be understood, seeds dormancy affects seeding production directly (Ipef, 1997). Mechanic and chemical scarification, soaking and high temperature treatments under humid and dry conditions aim to overcome seed dormancy and obtain a fast and uniform germination.

Temperature variations affect germination percentage, speed and uniformity (Carvalho and Nakagawa, 2000). Usually, pioneer species require temperature rotation to overcome dormancy (Brancalion *et al.*, 2010).

The subtract influences seeding capacity viability maintenance (Brasil, 2009), and, according to Wagner Júnior *et al*. (2006) its basic functions are plant support nutrient supply, water and oxygen.

Aegiphila sellowiana Cham belongs to the Lamiaceae family is a pioneer tree (Leonhardt *et al*., 2008), rustic, heliophytic, quick growth and very common in the Atlantic Forest (Lorenzi, 2008). This species' seed is exalbuminous (Barroso *et al*., 1999), stenospermic, oblong and the integument is hard but brittle (Biruel, 2006). It had potential to restore damaged areas, but its seeding percentage and speed are low, it emerges between 50 and 100 days (Lorenzi, 2008).

This research aimed to characterize the this species and obtain *A. sellowiana* soaking seeds curve, deriving from red fruits; to evaluate pre-germination treatments during seeding; to verify the presence of germination

To Cite This Article: Paulo do Nascimento, Bruno Oliveira Lafetá, Jéssica Cristina Barbosa Ferreira., Pre-germination treatments and their relation with Aegiphila sellowiana seeds germination. ***Aust. J. Basic & Appl. Sci.,*** *11(5): 69-79, 2017*

inhibitors in the seed-vessel and/or in the seed; to evaluate how storage time in room temperature affects germination and determine the best subtract type for seedlings growth in greenhouse.

MATERIALS AND METHODS

The experiments were conducted in a greenhouse and Seed Technology Laboratory of the Federal Institute of Education, Science and Technology Minas Gerais, Campus São João Evangelista (IFMG-SJE), located at Vale do Rio Doce, under the coordinates 18º32'15" South and 42º46'00" West, its average height is 680m (São João Evangelista, 2014). The climate is defined as Cwa, sub-tropical with drought period, the winter, and a rainy season, the summer (Köppen, 1948).

A. sellowiana fruits were collected at the frontier area between São João Evangelista and Paulistas, in Minas Gerais, Brazil, at the MG 117 highway's margins, and at the José Procópio de Oliveira Street, in São João Evangelista. After the harvest, red fruits were extracted manually of the inflorescences, and then, they were packed in paper bags and in allocated in plastic trays. Infected fruits by larvae were discarded.

For this research experiments only red fruits seeds were used, since Orange fruits are physiologically immature (Nascimento, 2014), the seeds were collected on February, 2, 2013. One day after harvesting, the immersion test was conducted using distilled water. There were four replicates of 50 seeds of *A. sellowiana*, derived from red fruits. These seeds were immersed in water for three hours during the first 12 hours and thereafter every 12 hours until the process has reached 108 hours. In order to obtain immersion curve species, seeds were weighed using a balance accurate to 0.001 mg.

Seeds characterization was performed by determining how much a thousand seeds weight (a total of eight sub-samples groups containing 100 seeds each); number of seeds by kilogram and humidity levels using the 105 ± 3 °C greenhouse method during 24 hours, according to the Regras de Análise de Sementes (Brasil, 2009).

On February 23 of 2013, an experiment was performed in which seeds were submitted to pre-germination treatments, described on Table 1.

Table 1: Pre-germination treatments in *Aegiphila sellowiana* seeds, derived from red fruits and put to germinate in BOD, under alternate temperatures of 20/30°C.

Nº	Treatments
T1	Control treatment
T2	Immersion in NaClO 2% during 2 minutes
T3	Immersion in H_2O, 50°C during 5 minutes + NaClO 2% during 2 minutes
T4	Immersion in H_2O, 80°C during 5 minutes + NaClO 2% during 2 minutes
T5	Immersion in H_2O, 80°C during 5 minutes + immersion H_2O, 50°C/5 min.+ NaClO 2%/2min
T6	Scarification with a nº 120m sandpaper + NaClO 2% during 2min
T7	Immersion in HCl PA during 2 minutes
T8	Immersion in HCl PA during 4 minutes

The seed were distributed on two sheets of paper germitest moistened with distilled water, transparent gerboxes (measuring 11.5 x 11.5 x 3.5 cm) and placed in germination type Biochemical Oxygen Demand (BOD) at temperatures alternating (20 / 30ºC) for 8 and 16 hours, respectively.

The germination process was evaluated during 45 days. Seeds with a primary root measuring 2 millimeters were considered germinated (Borghetti, 2004).

In another experiment, seeds were add in a kraft paper bag and stored in the laboratory for 90 days at room temperature during until 90 days under room temperature. From time to time, part of those seeds were submitted to the treatments listed on Table 2, and put to germinate in BOD, as described, previously.

Table 2: Pre-germination treatments applied to *Aegiphila sellowiana* seeds, coming from red fruits, submitted to different storage times under room temperature and germinated in BOD.

Nº	Treatments
T1	Control treatment (without storage and without 2% sodium hypochlorite during 2 min)
T2	Seeds without storage+ 2% sodium hypochlorite during 2 min
T3	Seeds storage during 10 days + 2% sodium hypochlorite during 2 min
T4	Seeds storage during 30 days + 2% sodium hypochlorite during 2 min
T5	Seeds storage during 60 days + 2% sodium hypochlorite during 2 min
T6	Seeds storage during 90 days + 2% sodium hypochlorite during 2 min

For each treatment, except the Control treatment, seeds were immersed in a 2% sodium hypochlorite solute during two minutes before germination.

Another experiment conducted by BOD under alternated temperatures of 20/30 ºC, using the dry heat, previously, in which seeds were drought in a greenhouse with forced air, in 70ºC, during 48 hours, except the Control Treatment, as shown on Table 3.

Table 3: Pre-germination treatments in *Aegiphila sellowiana* seeds, coming from red fruits, submitted to dry heat and germinated by BOD.

Nº	Treatments
T1	Control treatment (without storage and without 2% sodium hypochlorite during 2 min)
T2	Seeds storage during 10 days + immersion in 2% NaClO during 2 min
T3	Seeds storage during 10 days + immersion in 2% NaClO during 4 min
T4	Seeds storage during 10 days + 120 sandpaper + immersion in 2% NaClO during 2 min
T5	Seeds storage during 10 days under 15 °C + immersion in 2% NaClO during 2 min
T6	Seeds storage during 10 days under 15 °C + immersion in 2% NaClO during 4 min
T7	Seeds storage during 20 days + immersion in 2% NaClO during 2 min
T8	Seeds storage during 20 days + immersion in 2% NaClO during 2 min

After the results of the experiments that demonstrated the existence of some kind of dormancy in *A. sellowiana* seeds, a bioassay was performed using lettuce seeds, aiming to identify any kind of germination inhibitor in the studied species' seed-vessel.

The bioassay was performed using seeds and fruits extracts in two dilutions; such activity was conducted by BOD, at the Forest Seeds Laboratory of Department of Forestry of the Federal University of Viçosa (DEF/UFV). Treatments are shown in Table 4.

Table 4: Treatments used in the bioassay using *Aegiphila sellowiana* seeds and fruits extracts applied in *Lactuca sativa* seeds put to germinated by BOD.

Nº	Treatments
T1	Control treatment
T2	Fruit extract, 1:10 dilution
T3	Fruit extract, 1:100 dilution
T4	Seed Extract, 1:10 dilution
T5	Seed Extract, 1:100 dilution

The extracts were obtained according to the methodology described by Coutinho and Hashimoto (1971) and Jackson and Willerseem (1976), cited Borges *et al*. (1993). Fruits and seeds were dried in an oven at 80 ° C for 24 hours and allocated in tightly closed containers. After that, they were ground into crucibles separately for Macera them. Five grams of the mash was transferred to a round-bottomed flask were added 50 ml of 80% ethanol, and then the system was kept to stand for 5 minutes. Then the flask with the extracts was subjected to a temperature of 70 ° C for 90 minutes. The extract was filtered through filter paper and placed in a vacuum evaporator at a temperature 45 ° C to remove ethanol. Finally, the crude extract obtained water volume was completed with distilled water to 10 ml. Solutions at dilutions 1:10 and 1: 100 were prepared from the extracts and then continued bioassay germitest wetting the paper with extracts prepared. Control treatment the germitest role of bioassay was moistened with the extracts obtained. Lettuce seeds were germinated arranged on germitest paper in Petri dishes and maintained in BOD at a temperature of 25 ° C for 24 hours photoperiod. Germination was assessed on the fifth day after sowing and the results expressed as a percentage of germination.

After completion of the previous experiments, especially the first and the last, conducted an experiment to produce seedlings in nurseries in order to detect the substrate providing the best emergency percentage (% E), the best East Emergency Time (EET) seeds, but the best Quality Index of Dickson (QID) of seedlings of *A. sellowiana*. For this, the two best treatments for germination in laboratory conditions (BOD) were repeated in nursery conditions, as shown in Table 5.

Table 5: Experiment performed on July/2013, in a greenhouse, using seeds from *Aegiphila sellowiana* red fruits in different subtracts.

Nº	Treatments
T1	Control treatment (seeds without storage and distilled water)
T2	Seeds storage during 50 days in room temperature + immersion in 2% NaClO during 2 min.
T3	Seeds storage during 50 days in room temperature + immersion in H_2O in 50°C temperature during 5 min + immersion in 2% NaClO during 2 min.

This experiment was conducted between April 21 and June 29 of 2013, using seeds from red fruits, using three subtracts: Izo Flok GR 2/3 expanded vermiculite (modular), granulometry from 0,9 to 4 mm, density from 120 to 125 kg/m³; cattle manure and Forest Basaplant commercial subtract for native species. The manure as sifted using a 4mm sieve to equalize it.

The experiments were performed in DIC in 50 seeds using four repetitions. When necessary data expressed as percentages were transformed into "arc sin ($\sqrt{x}$ / 100)" or "$\sqrt{x}$" in order to obey normal Lilliefors and homogeneity of variances seconds Cochran (Banzatto and Konkra, 2006). A variance analysis and Tuckey test with a 5,0% significance level were performed. The Statistica 7.0 software (Statsoft, 2007) was used for the statistical analysis.

RESULTS AND DISCUSSION

Results of characterization of *A. sellowiana* seeds are shown in Table 6.

Table 6: *Aegiphila sellowiana* humidity levels, thousand seeds weight and number of seeds by kilogram.

Humidity levels in humidity base (%)	1.000 seeds weight (g)	Number of seeds by kilogram
14,7	33,7	29.694

Humidity levels (14.7%) of seeds of *A. sellowiana* differ water content of the same kind (8.97%), found by Biruel (2006) the classified as orthodox. The weighted mass of 1,000 seeds was equal to 33,7g. These data differ from Biruel (2006) that was 27,9g as the mass of 1000 seeds (17.2% difference), and showed different patterns of weight in accordance with environmental variations and species location, as seen in the studies this author, which occurred in Analândia - São Paulo, Brazil and in this survey, conducted in St. John the Evangelist - Minas Gerais Brazil.

An amount of 29.694 seeds by kilogram was obtained. Biruel (2006) found 36.842 seeds by kilogram. As Biruel (2006) used seeds from orange fruits, which are in the process of maturation and accumulation of reserves, the interruption of these processes, post-harvest, may have been responsible for values, since this favors the presence of smaller seeds and lower weight in work samples. However, it is necessary to emphasize that biometric data obtained by Biruel (2006) attributes a 16% weight variation on *A. sellowiana* seeds, which might justify seeds amount by kilogram number difference when compared to data obtained in São João Evangelista. Besides, inter-population variables might contribute for understanding such difference for 1.000 seeds weight.

The curve imbibition seeds in distilled water lying in Figure 1, where it appears that the initial weight of the seed became 1.74 g before starting the imbibition process (time zero) to 1.86 g after last reading (108h after the start).

Quick water absorption was verified only during the beginning and second reading (3h later); after that, the absorption was slow and gradual, until the seventh reading, 36 h later. From the eighth reading (48h) until the last one (108h) water absorption was established. According to Carvalho and Nakagawa (2012), seeds first germination phase presents a quick water absorption and on cotyledon seeds, water content varies from 35 to 40%. It did not occur on the *A. sellowiana* seeds that took around 36h during the first soaking phase (Figure 1). Besides, before the soaking, seeds' water content presented 14,7% water content average, after 36h this number added 6,9% to seeds mass, showing that said content was 20,2% after the soaking.

Biruel (2006) studied seeds hydration during 13 days, when protruding of the roots occurred. The soaking curve presented by said author did not have a three-phase soaking pattern, although the germination occurred on the 13th day.

Caesalpinia pyramidalis Tull seeds were adjusted to the three-phase model, with the first phase completed in 24 hours and third phase started after 51h of soaking. In 54 h of soaking there was 10% of seedlings with protruding roots and a 76% seeds' weight increasing (Dantas *et al*., 2008).

From the seventh determination (Figure 1) seeds, probably on phase II of soaking, almost did not absolve water until the thirteenth reading and the last one (108h later). It shows that *A. sellowiana* seeds do not enter on the third phase of soaking, which indicates some kind of difficulty to absorb more water of some blocking on the embryo, such as a physiological dormancy. Biruel (2006) explained that the slow absorption might be due the presence of sclereids on seeds' foreheads anatomic cuts, which makes the integumentary rigid and waterproof them; however, did not suggest any integumentary dormancy for said seeds. Ferreira *et al.* (2009) leached *A. sellowiana* seeds, within or without integument, using running water during different periods, adding alternate temperatures (30/35ºC) and white lights, with a 12h photoperiod and detected germination 9 hours after the lixiviate process, only in seeds without integument. Since seeds with integuments did not germinate, the authors concluded that the species dormancy is integumentary.

Tukey test results for the pre-germination treatments of the first experiment are found on Table 7.

Table 7: Germination (G), Average Germination Time (AGT), Germination Speed Index (GSI) of *Aegiphila sellowiana* seeds, from red fruits, under distinct pre-germination treatments.

Treatments	G (%)		AGT (day)		GSI	
T1	15,0	bcd	14,3	a	0,6	abcd
T2	80,5	a	24,7	a	1,9	a
T3	56,0	ab	25,8	a	1,3	ab
T4	0,0	d	0,0	b	0,0	d
T5	40,5	abc	21,5	a	0,7	abcd
T6	44,0	ab	28,5	a	0,9	abc
T7	14,5	bcd	24,0	a	0,3	bcd
T8	3,0	cd	16,3	a	0,1	cd

Averages followed by the same letter do not differ according to the Tukey Test ($p > 0,05$)

T1- Control treatment. T2- 2% sodium hypochlorite/ 2 minutes. T3 – immersion in H_2O in a 50ºC temperature/ 5 minutes. T4 - immersion in H_2O in a 80ºC temperature/ 5 minutes. T5 - immersion in H_2O in a 80ºC temperature/ 5 minutes + immersion in H_2O in a 50ºC temperature/ 5 minutes. T6 – Scarification using sandpaper. T7 – immersion in HCl PA/2 minutes and T8 – immersion in HCl PA/4 minutes.

Control Treatment the seeds of the control (T1) showed only 15% germination, signaling some difficulty to carry out this process. The lack of germination in seeds dipped in H 2 O at 80 ° C / 5min. (T4) indicates that this temperature agrees embryo structures probably killing the seeds or in some other triggering a secondary dormancy, since soaking in H2 O at 80 ° C / 5 min. followed by immersion in H2 O at 50 ° C / 5 min still enabled 40.5% germination. For Carvalho & Nakagawa (2012, p. 172) "the environmental factors that, more often, it turns induce secondary dormancy have been high temperatures and low relative humidity of the air, especially when associated". Soaking the seeds in water at 80 ° C for 10 and 30 seconds was effective in breaking dormancy of seeds P*iptadenia moniliformis* (Ferreira *et al*., 2014).

Seeds immersion in sodium hypochlorite at 2% during two minutes (T2) enabled 80,5% germination, as seen on Table 7. In *A. sellowiana* seeds insoluble fibers in neutral detergent represent 53,66% of the diaspore components. Such fibers, part of the cell wall, are constituted of cellulose, hemicellulose, lignin and protein (Biruel, 2006). Therefore, hypochlorite activity might weaken the tegument and enable water input in the seed, causing its germination. Acids or bases are used to cause cracks at the seed tegument, and necessary for the permanence period of such substances, since they cannot invade the tegument, under consequence of peeling it and being attacked by fungus or even damaging its embryonic axis, which compromises seeds' strength and usefulness (Perez, 2004).

Seeds previous immersion in sodium hypochlorite in different proportions and periods, associated, or not with, to other treatments was, also, relevant to germinate *Albizia lebbeck* seeds (Benedito *et al*., 2009) and decreasing fungus emergence (Smiderle and Schwengber, 2011), it happened to *Coffea arábica* seeds; IAC 44 Red Catuí (Lima *et al*., 2012) and *Bowdichia virgilioides* seeds.

Immerging *A. sellowiana* seeds in H_2O at 50ºC during 5 minutes (T3) enabled the second higher germination level (56,0%) and sandpaper scarification (T6) enabled 44,0% of germination. *A. sellowiana* seeds leached and lixiviated under running water during 24 hours presented a 44% and 43% germination rating, respectively, both statistically equivalent to the control that turned the germination possible (Biruel, 2006). However, considering seedlings production for commercial proposes, treatments germination rates are still low. Scarification using sandpaper was efficient to overcome seed dormancy of *Myracrodruon urundeuva* seeds (Pacheco *et al*., 2006) dormancy and *Bauhinia variegata* var. candida (Lopes *et al*., 2007), as well.

Scarification using hydrochloric acid at 2% during two or four minutes (T7 and T8) enabled *A. sellowiana* seed low germination rates, 14,5% and 3,0%, respectively. Treatment using this acid at 36% p.a during 15 minutes enabled a 16% germination rate of *Capsicum baccatum* var. *praetermissum* seeds. Such result was superior to the one obtained in the Control treatment, which did not germinate, however, was statically inferior to the 36,7% rate, obtained with seeds immerged in HNO_3 at 67% p.a. during 10 minutes (Athanázio *et al*., 2012). Hydrochloric acid at 71% p.a. in different concentrations has not overcome the numbness of *Zeyheria montana* Mart seeds. (Dousseau *et al*., 2007).

In general, treatments used in this research, show non Table 7, did not interfere at the AGT, since the answer pattern was statistically equivalent in all germination treatments; since the germination speed index (GSI) was in absolute numbers higher in T2 and T3.

Stoking seeds in room temperature, indicated, through variance analysis, significant differences according to the F test ($p < 0,05$) in treatment levels for all attributes evaluated and in accordance to that, the Tukey Test was performed, as seen on Table 8.

Table 8: Germination percentage (G). Average Germination Time (AGT) and Germination Speed Index (GSI) for *Aegiphila sellowiana* seeds from red fruits, stocked in room temperature.

Treatments	G (%)		AGT (dias)		GSI	
T1	15,00	c	14,3	b	0,6	b
T2	80,50	a	24,7	a	1,9	a
T3	45,50	b	23,9	a	1,1	ab
T4	37,00	bc	22,0	a	1,0	b
T5	14,50	c	25,4	a	0,3	b
T6	11,50	c	18,7	ab	0,3	b

Averages followed by the same letter are not different according to the Tukey Test ($p > 0,05$).
T1- control treatment (seeds without storing nor sodium hypochlorite (NaClO) at 2%/2 min.)
T2- Seeds without storing + NaClO at 2%/2 min.
T3- Seeds stored during 10 days in room temperature NaClO at 2%/2 min.
T4- Seeds stored during 30 days in room temperature NaClO at 2%/2 min.
T5- Seeds stored during 60 days in room temperature NaClO at 2%/2 min.
T6- Seeds stored during 90 days in room temperature NaClO at 2%/2 min.

The highest average germination (80.5%) was obtained in seeds without storage (T2) treated with sodium hypochlorite at 2% for two minutes before the germination test (Table 8), as in the previous experiment. The germination percentage was decreased along with the GSI with stoking period, as seen on treatments from T3 to T6, whose seeds were storage during periods varying from 10 to 90 days. That data signalize the occurrence of

some kind of deterioration in seeds that were stocked for a certain period, slowing the germination process down.

Based on humidity percentage (9,3%) at the post-harvest immediate and also due the fact that seeds keep being useful until 90 days of stocking, Biruel (2006) characterized the seeds as orthodox. In another study from the same author, aged seedlings emergence percentage increased, said seeds were aged for 6h and 12h at 45ºC and 100% UR and the educed emergency percentage for aged seeds for longer periods (24 and 48 h) indicating reduction the viability and vigor with the storage time.

In relation to AGT, in general, the T2 to T6 treatments had AGT greater than the average obtained at T1 (control) confirming some deterioration in seeds, with the storage time. Orthodox seeds of *Caesalpinia echinata* stored at room temperature can lose viability in less than three months; already under 7 ° C (tolerate desiccation to 7.6% water) seeds remained viable up to 18 months, with germination over 80% (Barbedo *et al*., 2002).

Variance analysis showed significant differences according to the F test ($p < 0,05$) in treatments level regarding percentage, for average time and for the germination speed index in treatments that used dry heat, previously. Considering that, the Tukey test was performed, as seen on Table 9.

The higher germination ratings occurred on the T3, T7 and T8 treatments. It was verified low germination rates on the Control (15%), which did not receive sodium hypochlorite (Table 9). On previous experiments it was stated this substance efficiency for germinating *A. sellowiana* seeds.

The effect of low temperature was negative in T5 and T6 treatments and may have canceled the effect of the sodium hypochlorite treatments such as the average germination was statistically equal to the average obtained for the T1 treatment. These results indicate that the seeds of this kind can be intermediate or recalcitrant behavior in relation to storage.

Table 9: Tukey Test for Germination percentace (G), Average Germination Time (AGT) and Germination Speed Index (GSI) for *Aegiphila sellowiana* seeds from red fruits, exposed do dry heat at 70ºC during 48 hours.

Treatments	G (%)		AGT (days)		GSI	
T1	15,0	c	14,3	b	0,6	abc
T2	24,5	bc	21,2	a	0,6	abc
T3	42,5	abc	23,5	a	1,0	abc
T4	17,0	bc	21,8	a	0,4	bc
T5	13,5	c	27,1	a	0,3	c
T6	13,0	c	26,0	a	0,3	c
T7	58,5	a	23,0	a	1,5	a
T8	48,0	ab	22,8	a	1,2	ab

Averages followed by the same later do not differ according to the Tukey Test ($p > 0,05$).
T1- Control (seeds without storage and without sodium hypochlorite)
T2- Seeds stored for 10 days in room temperature + NaClO at 2% during 2 min
T3- Seeds stored for 10 days in room temperature + NaClO at 2% during 4 min.
T4- Seeds stored for 10 days in room temperature + sandpaper number 120 + NaClO at 2% during 2 min
T5- Seeds stored for 10 days at 15ºC + NaClO at 2% during 2 min
T6- Seeds stored for 10 days at 15ºC + NaClO at 2% during 4 min.
T7- Seeds stored for 20 days s in room temperature + NaClO at 2% during 2 min
T8- Seeds stored for 20 days in room temperature + NaClO at 2% during 4 min.

Furthermore, when comparing the overall results of germination in the experimental treatments described in Table 08, with the dried seeds in an oven at 70 °C for 48 h (Table 9) and the results of the first experiment in BOD (Table 7), with no dry heat seeds without low temperatures and without storage, there is greater germination experiment in the treatment of T2 in Table 7, signaling intolerance seeds of *A. sellowiana* storage to dry at low temperatures..

These results indicate be the seeds of this species can be intermediate or recalcitrant regarding the behavior in storage.

There were significant diferences among the bioessay treatments by the F test ($p < 0,05$) and because of this was held Tukey's test (Figure 2).

The negative effect of the extract of *A. sellowiana* seed germination of lettuce seeds did not occur in the treatment T5 (1: 100 dilution), which showed 90% germination (Figure 2). As the obtained extract of the fruits in the same dilution (T3) reduced the germination percentage to 58%, there is probably a greater concentration or range of germination inhibitors in the fruit. Since there was no germination at 1:10 dilution (T2 and T4), there should be inhibitors in the seeds and fruits of this species. For Baskin and Baskin (2004), the presence of inhibitors or the absence of germination promoters are common forms of dormancy.

The bioassay is a common instrument to detect allelochemicals and diagnose its effects in a certain species. The hydroalcoholic extracts HA1:1 and HA1:2 *Cymbopogon citratus* inhibited germination, the shoot height and radicle growth of *Bidens pilosa* and *Lactuca sativa*; all dry extracts reduced the% F, IVG and growth of lettuce. None of the extracts had influenced on *B. pilosa* initial growth (Lousada *et al*., 2012).

In the experiment conducted in greenhouse, the emergence percentage of *A. sellowiana* seedlings was higher in the T3 treatment (76.3%) and was greater in AET T1 and T2, showing the efficiency of the pool water

at 50 ° C and hypochlorite 2% sodium / 2 min in increased seedling emergence, as well as reducing the number of days for this emergence (Table 10).

Table 10: Emergence percentage (E), Average Emergency Time (AET) and Dickson Quality Index (DQI) for *Aegiphila sellowiana* seeds, germinated in a greenhouse under different treatments.

Treatments	E (%)	AET (days)	DQI
T1	5,67 c	51,85 a	0,04 a
T2	65,33 b	47,53 ab	0,02 b
T3	76,33 a	45,03 b	0,01 b

Averages followed by the same letter do not differ according to the Tukey Test (p > 0,05).
T1 – Control Treatment (without storing nor NaClO at 2% during 2 min).
T2 – Seeds stored for 50 days at room temperature (rt) + NaClO at 2% during 2 min.
T3 - Seeds stored for 50 days (rt) + immersion in H_2O at 50°C during 5min + NaClO at 2% /2 min.

On the T1 treatment there were a higher DQI average, however, the number of emerged plants was lower and its emergence period was higher (Table 10), which increases seedlings period of permanence at the greenhouse and in consequence increases production costs. According to Rego *et al*. (2009), germination in a smaller period is beneficial, because, the faster the process, the lower seeds exposure to adverse environmental conditions, increasing the possibilities to establish quality seedlings.

Erythrina falcata overcome dormancy after immersion in water at 80ºC, followed by resting it on the same water, off heating, during 24 hours, or in a 25ºC temperature during 48 hours (Fowler and Bianchetti, 2000). Water at 97ºC overcome *Acacia mearnsii* wild seeds dormancy (Roversi *et al*., 2002).

Biruel (2006) found higher *A. sellowiana* seeds emergence rates (53%) in cerrado soil, whether using sandpaper scarification or not. In the agricultural subtract with leached seeds under running water during 24 hours the emergence rate was 50%. It is evident that the first procedure is lengthy and the second demands a lot of water, both proceedings encumber costs of producing seedlings in nurseries. Therefore, seed storage for 50 days at room temperature and then immerge them in H_2O at 50ºC during 5 minutes and add NaClO 2% during two minutes is a sustainable proceeding and must be adopted for producing seedlings in this species.

Substrates tanned bovine manure and commercial Basaplant Forest enabled the highest average percentage of emergence of seedlings of *A. sellowiana* regarding vermiculite (Table 11).

Table 11: Emergence percentage (%E) and Dickson Quality Index (DQI) for *Aegiphila sellowiana* seeds from red fruits, germinated in the nursery, on the vermiculite subtracts, commercial and cattle manure.

Subtracts	E (%)	DQI
Vermiculite	41,00 b	0,04 a
Commercial	52,33 a	0,02 b
Cattle Manure	54,00 a	0,01 b

Averages followed by the same letter do not differ according to the Tukey Test (p > 0,05).).
T1 – Control (without storage and without NaClO 2% for 2 min).
T2 – Seeds stored during 50 days in room temperature (ta) + NaClO at 2% during 2 min.
T3 - Seeds stored during 50 days (ta) + immersion in H_2O at 50°C during 5min + NaClO at 2% /2 min.

Even with the IDQ being higher on the vermiculite, such subtract presented the lower seedlings emergence rate (41%) and must be deprecated regarding the others for *A. sellowiana* seedlings formation. The vermiculite is used to germinate forestry species (Martins *et al*., 2009; Pacheco *et al*., 2006; Silva *et al*., 2002). Oliveira *et al*., (2012) concluded that the vermiculite substract has a good potential to germinate *Anadenanthera colubrina*. To Medeiros and Abreu (2005) no special treatment for breaking dormancy in *A. sellowiana*, which must be placed to germinate vermiculite. These results contrast with those obtained in this work, in which vermiculite was the worst substrate for seedling emergence.Said results contrast with the ones obtained in this research, in which vermiculite was the worst subtract for seedlings emergence.

Crescentia cujete seeds presented the best germinate performance (72% for germination and GSI = 2,16) when exposed to alternate temperatures 20/30ºC and combined with vermiculite subtracts (Azevedo *et al*., 2010). 62% of *Caesalpinia pyramidalis* seeds germination under alternated temperatures at 20/30ºC and 60% of the germination at 20/35ºC, using the vermiculite subtract, was attributed to low density and good water absorption capacity in said subtract (Lima *et al*., 2011).

DQI for calculating the average dry weight of shoot and root seedlings are important parameters (Guedes *et al*., 2010). These authors observed higher average dry matter in vermiculite in relation to commercial plantmaxon *Amburana cearenses* (Allemão) A. C. Smith seedlings. Regarding cattle manure and commercial subtracts there was not any significant difference in DQI averages. On the experiment conducted at IFMG-SJE, DQI data was higher on the vermiculite, in which was possible to detect a good shoot and root development in *A. sellowiana* plants. However, since the vermiculite presented an inferior emergence (Table 11) and its costs is elevated, it is suggested to use cattle manure to produce seedlings of this species.

Fruit (like seeds) Red and scarified *A. sellowiana*, allowed a maximum of 42.3% of emergency, the tanned bovine manure substrate (Nascimento, 2014). In the current experiment, using the seeds from red fruits

emergency reached 54.0% over the same substrate (Table 11). Furthermore, independent of the substrate, the emergence was 76.3% (Table 10). These results justify the withdrawal pericarp and the formation of this species seedlings from the seeds and not the fruit themselves. Considering the results of the bioassay (Figure 2), it is assumed that the withdrawal of the fruit pulp removes some of the inhibitors and favors a better relationship between promoters and inhibitors, which enables a higher emergence, the plants formed from seeds.

According to IBF (2014) for the formation of *A. sellowiana* seedlings are needed three fruits or seeds per hole, with no need for pre-germination treatments; germination rate is low and the mean emergence time is 60 days. In this work the emergency median time was 45 days and the emergence was 76%, using seed subjected to a pretreatment relatively easy to be operated in nurseries. It is suggested, then soaking the seeds in H_2O at 50 °C for five minutes and then in 2% NaClO for two minutes to form seedling quality in this species.

Subtracts chemical, physical and biological characteristics are essential for plants rood and vegetable development (Cunha *et al*., 2006; Fonseca, 2001), said characteristics, demanded by the species, and economical aspects are relevant for subtracts choice (Gomes and Silva, 2004). The subtract must guarantee a quality seedling development, must have good water containing capacity and drainage and must not have toxic substances (Alfenas *et al*., 2004; Cunha *et al*., 2006). In addition, the substrate should allow adequate growth to the plant and the material used in its composition must be abundant in the region and low cost. As commercial substrates are purchased at high costs to define the best substrate for the formation of *A. sellowiana* seedlings lies with the tanned manure bovine, which must have provided at least in part, the chemical, physical and biological development seedlings; besides being quite common in the region of São João Evangelista region.

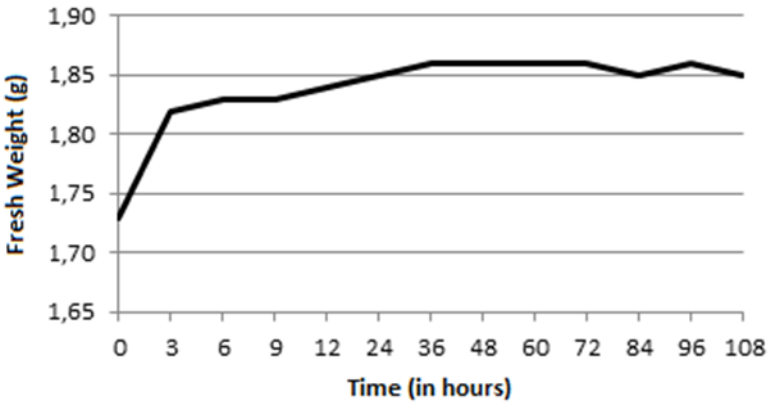

Fig. 1: Aegiphila sellowiana seeds soaking curve in distilled water.

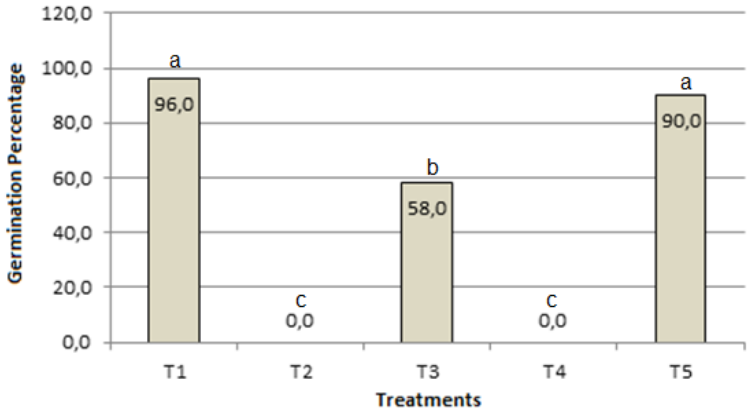

Fig. 2: Lettuce seeds germination in humid subtracts with diluted extracts (1:10 and 1:100) from A. sellowiana red fruits and seeds from red fruits.

Averages followed by the same letter on the histograms do not differ according to the Tukey Test ($p > 0,05$).

T1 = Control (distilled water)
T2 = Fruit extract, 1:10 dilution.
T3 = Fruit extract, 1:100 dilution
T4 = Seed extract, 1:10 dilution.
T5 = Seed extract, 1:100 dilution.

Conclusion:

The immersion of seeds in *A. sellowiana* sodium hypochlorite at 2% for two minutes was efficient to guarantee the germination under laboratory conditions and immersion in water at 50 °C for five minutes, followed by immersion in sodium hypochlorite 2 % for two minutes was the treatment that made possible the largest emergency seeds of this species under nursery conditions.

There germination inhibitors in the pericarp and the seed itself *A. sellowiana*, since the diluted extracts of fruits and seeds of the species inhibited the germination of lettuce seeds in a bioassay.

The species' seeds are damaged with a long stoking period in room temperature, reducing the rates and elevating germination average period.

In order to produce *Aegiphyla sellowiana* seedlings, it is recommended to use seeds from red fruits, immerged in sodium hypochlorite at 2% during two minutes, followed by immersion in water at 50ºC during five minutes, using cattle manure in platic tubes measuring 17 x 10 cm.

ACKNOWLEDGEMENTS

The authors want to thank the CNPq, for scientific initiation scholarship granted, to the team of the Laboratory of Seeds of UFV and of IFMG by providing infrastructure needed to perform the research.

REFERENCES

Alfenas, A.C., E.A.V. Zauza, R.G. Mafia and T.F. Assis, 2004. *Clonagem e doença de eucalipto*. Viçosa, MG: UFV, pp: 104.

Athanázio, J.C., R.J. Kosteski, J. Castelo, R. dos and P.R.F. Pereira, 2012. *Germinação de sementes de pimenta cumari (Capsicum baccatum var. praetermissum*). Disponível em: <<http://www.abhorticultura.com.br/biblioteca/arquivos/Download/Biblioteca/45_0391.pdf>>. Acesso em: 27/10/2013.

Azevedo, C.F., R.L.A. Bruno, E.P. Gonçalves and Z.G.M. Quirino, 2010. Germinação de sementes de cabaça em diferentes substratos e temperaturas. *Revista Brasileira de Ciências Agrárias*, 5(3): 354-357.

Banzatto, D.A. and S.N. Kronka, 2006. *Experimentação Agrícola*, 4 ed. Jaboticabal: Funep, pp: 237.

Barbedo, C.J., D.A.C. Bilia and R.C.L. Figueiredo-Ribeiro, 2002. Tolerância à dessecação e armazenamento de sementes de *Caesalpinia echinata* Lam. (pau-brasil), espécie da Mata Atlântica. *Revista Brasileira de Botânica*, 25(4): 431-439.

Barroso, G.M., M.P. Morim, A.L. Peixoto and C.L.F. Ichaso, 1999. *Frutos e sementes: morfologia aplicada à sistemática de dicotiledôneas*. Viçosa, MG: UFV, pp: 443.

Baskin, J.M. and C.C. Baskin, 2004. A classification system for seed dormancy. Seed Science Research, 14: 1-16.

Benedito, C.P., M.C.C. Ribeiro, M.K.T. Oliveira, I.P. Guimarães and G.S. Rodrigues, O. De, 2009. Influência da cor e métodos de superação de dormência em sementes de albizia. *Caatinga*, 22(2): 121-124.

Biruel, R.P., 2006. *Caracterização e germinação de sementes de Aegiphyla sellowiana CHAM*. Tese de doutorado. São Carlos, SP: Universidade Federal de São Carlos.

Borges, E.E.L., E.S. Lopes and G.F. Silva, 1993. Avaliação de substâncias alelopáticas em vegetação de uma floresta secundária. 1 - Árvores. Revista Árvore, 17(1): 69-84.

Borghetti, F., 2004. Dormência embrionária, pp. 109-124 in: Ferreira, A.G.; Borghetti, F. (Eds) *Germinação: do básico ao aplicado*. Porto Alegre, Artmed.

Brancalion, P.H.S., A.D.L.C. Novembre and R.R. Rodrigues, 2010. Temperatura ótima de germinação de sementes de espécies arbóreas brasileiras. Revista Brasileira de Sementes, 32(4): 15-21.

Brasil., 2009. Ministério da Agricultura, Pecuária e Abastecimento. *Regras para análises de sementes*. Brasília: MAPA/ACS, pp: 399.

Carvalho, N.M. and J. Nakagawa, 2000. *Sementes: ciência, tecnologia e produção*. 4.ed. Jaboticabal: UNESP, pp: 588.

Carvalho, N.M. and J. Nakagawa, 2012. *Sementes: ciência, tecnologia e produção*. 5.ed. Jaboticabal: FUNEP, pp: 590.

Coutinho, L.M. and F. Hashimoto, 1971. Sobre o efeito inibitório da germinação de sementes produzido por folhas de *Calea cuneifolia* DC. Ciência e Cultura, 23(6): 759-764.

Cunha, A. de M., G. de M. Cunha, R. de A. Sarmento, G. de M. Cunha and J.F.T. Amaral, 2006. Efeito de diferentes substratos sobre o desenvolvimento de mudas de Acacia sp. Revista Árvore, 30(2): 207-214.

Dantas, B.F., J. de S. Correia, L.B. Marinho and C.A. Aragão, 2008. Alterações bioquímicas durante a embebição de sementes de baraúna (*Schinopsis brasiliensis* Engl.). Revista Brasileira de Sementes, 30(2): 214-219.

Dousseau, S., A.A. Alvarenga, E.M. Castro, L.O. Arantes and F.C. Nery, 2007. Superação de dormência em sementes de *Zeyheria montana* Mart. Ciência e Agrotecnologia, 31(6): 1744-1748.

Ferreira, A.R., G.V. Pinto and H.R. Ferreira, 2009. Superação de dormência em sementes de *Aegiphila sellowiana*: (tamanqueiro). Trabalho de Conclusão de Curso (Curso Superior de Tecnologia em Silvicultura). Instituto Federal de Educação, Ciência e Tecnologia de Minas Gerais – Campus São João Evangelista, pp: 34.

Ferreira, M.S., V.P. Matos, R.B. Silva, H.H.D. Santos and L.H.M. Sena, 2014. Thermal scarification to overcome *Piptadenia moniliformis* seeds dormancy. Revista Brasileira de Ciências Agrárias, 9(1): 79-83. DOI:10.5039/agraria.v9i1a2488.

Fonseca, T.G., 2001. *Produção de mudas de hortaliças em substratos de diferentes composições com adição de CO_2 na água de irrigação*. Dissertação (Mestrado em Agronomia) - Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, SP, pp: 72.

Fowler, J.A.P. and A. Bianchetti, 2000. *Dormência em sementes florestais*. Colombo: Embrapa Florestas, Documentos, pp: 40.

Gomes, J.M. and A.R. Silva, 2004. Os substratos e sua influência na qualidade de mudas, pp. 190-225 in: Barbosa, J.G., Martinez, H.E.P., Pedrosa, M.W. and Sediyama, M.A.N. (Eds) *Nutrição e adubação de plantas cultivadas em substratos*. Viçosa, UFV.

Guedes, R.S., E.U. Alves, E.P. Gonçalves, J.M. Braga Júnior, J.S. Viana and P.N.Q. Colares, 2010. Substratos e temperaturas para testes de germinação e vigor de sementes de *Amburana cearensis* (Allemão) A. C. Smith. Revista Árvore, 34(1): 57-64.

IBF - Instituto Brasileiro de Florestas. Tamanqueiro: sementes. 2014. Disponível em: <http://ibflorestas.org.br/loja/tamanqueiro-semente.html >. Acesso em: 20/09/2014.

IPEF. Métodos de quebra de dormência de sementes. 1997. Disponível em: <http://www.ipef.br/tecsementes/dormencia.asp>. Acesso em: 20/09/2014.

Jackson, J.R. and R.W. Willemseem, 1976. Allelopathy in the first stage of secondary sucession on the piedmont of New Jeressey. American Journal of Botany, 63(7): 1015-1023.

Köppen, W., 1948. *Climatologia*, pp.553-557. México: Fundo de Cultura Económica.

Leonhardt, C., O.L. Bueno, A.C. Calil, A. Busnello and R. Rosa, 2008. Morfologia e desenvolvimento de plântulas de 29 espécies arbóreas nativas da área da Bacia Hidrográfica do Guaíba, Rio Grande do Sul, Brasil. Iheringia, série botânica, 63(1): 5-14.

Lima, C.R., M.V. Pacheco, R.L.A. Bruno, C. Ferrari, J.M. Braga Júnior and A.K.D. Bezerra, 2011. Temperaturas e substratos na germinação de sementes de *Caesalpinia pyramidalis* Tul. Revista Brasileira de Sementes, 33(2): 216-222.

Lima, J.S., E.F. Araújo, R.F. Araújo, L.A.S. Dias, D.C.F.S. Dias and F.C. Rena, 2012. Uso da reidratação e do hipoclorito de sódio para acelerar a emergência de plântulas de cafeeiro. Revista Brasileira de Sementes, 34(2): 323-333.

Lopes, J.C., L.G. Barbosa, M.T. Capucho, 2007. Germinação de sementes de Bauhinia spp. Revista Floresta, 37(2): 265-274.

Lorenzi, H., 2008. *Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil*. 5. ed. Nova Odessa: Instituto Plantarum.

Lousada, L.L., G.C.S. Lemos, S.P. Freitas, R.F. Daher and B.S. Esteves, 2012. Bioatividade de extratos hidroalcoólicos de *Cymbopogon citratus* (DC.) Stapf. sobre picão-preto (*Bidens pilosa* L.) e alface (*Lactuca sativa* L.). *Revista Brasileira de Plantas Medicinais*, 14(2): 282-286.

Martins, C.C., M.L.A. Bovi and S.H. Spiering, 2009. Umedecimento do substrato na emergência e vigor de plântulas de pupunheira. Revista Brasileira de Fruticultura, 31(1): 224-230.

Medeiros, A.C. de S. and D.C.A. de. Abreu, 2005. Instruções para testes de germinação de sementes florestais nativas da Mata Atlântica. Colombo: Embrapa Florestas, Comunicado técnico 151, pp: 5.

Oliveira, K.S. de, K. Oliveira and M.A.I. Aloufa, 2012. Influência de substratos na germinação de sementes de *Anadenanthera colubrina* (Vell.) Brenan em condições de casa de vegetação. Revista Árvore, 36(6): 1073-1078.

Pacheco, M.V., V.P.M atos, R.L.C. Ferreira, A.L.P. Feliciano and K.M.S. Pinto, 2006. Efeito de temperatura e substrato na germinação de sementes de *Myracrodruon urundeuva* Fr. All. (Anacardiaceae). Revista Árvore, 30(3): 359-367.

Perez, S.C.J.G.A., 2004. Envoltórios, pp.125-134 in Ferreira, A.G., Borghetti, F. (Eds) *Germinação: do básico ao aplicado*. Porto Alegre, Artmed.

Rego, S.S., A.C. Nogueira, Y.S. Kuniyoshi and Á.F. SANTOS, dos., 2009. Germinação de sementes de *Blepharocalyx salicifolius* (H.B.K.) Berg. em diferentes substratos e condições de temperaturas, luz e umidade. Revista Brasileira de Sementes, 31(2): 212-220.

Roversi, T., V.L. Mattei, P. Silveira Júnior and G.L. Falck, 2002. Superação da dormência em sementes de acácia negra (*Acacia mearnsii* Willd.). Revista Brasileira de Agrociência, 8(2): 161-163.

São João Evangelista. Informações sobre o município. Disponível em: <http://www.sje.mg.gov.br/index.php?option=com_content&view=article&id=26&Itemid=40>. Acesso em: 25/05/2014.

Silva, L.M.M., T.J.D. Rodrigues and E.B. Aguiar, 2002. Efeito da luz e a temperatura na germinação de sementes de aroeira (*Myracrodruon urundeuva*). Revista Árvore, 26(6): 691-697.

Smiderle, O.J. and L.A.M. Schwengber, 2011. Superação da dormência em sementes de paricarana (*Bowdichia virgilioides* kunth.). Revista Brasileira de Sementes, 33(3): 407-414.

Statsoft, INC. Statistica (data analysis software system), version 7. (2007) Disponível em: <<http://www.statsoft.com>> Acesso em 25/05/2014.

Wagner Junior, A., R.S. Alexandre, J.R. da S. Negreiros, L.D. Pimentel, J.O. Costa e Silva and C.H. Bruckner, 2006. Influência do substrato na germinação e desenvolvimento inicial de plantas de maracujazeiro amarelo (*Passiflora edulis* Sims f. *flavicarpa* Deg). *Ciência e* Agrotecnologia, 30(4): 643-647.