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In Situ Germination and Early Seedling Growth of Wormwood (*Artemisia annua* L.)

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Abstract

Experiments were conducted on the farm of Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria, during the 2012 hot season and 2013 cold season to determine the most effective treatment for rapid germination of *Artemisia annua* by subjecting the seeds to physical treatment by soaking in cold and warm water for 2, 4 & 6 hours and 1, 2 & 3 minutes, chemical treatment by soaking in 10%, 20% & 30% Sulphuric Acid (H_2SO_4) for 1, 2 & 3 minutes respectively and hormone treatment (GA3) by soaking in 100 pp, 200 pp, 300 pp & 500 pp for 6, 12 & 24 hours. Results of Analysis of Variance (ANOVA) indicated no significant difference between the seasons with respect to germination, shoot and root lengths ($p \geq 0.05$). However, warm water treatment at 60˚C for 2 minutes during the rainy season trial had the least days to germination. Similarly, warm water treatments in both rain and irrigation trials at 40˚C for 3 minutes and 60˚C for 3 minutes produced the best vigor. Highest germination percent (96%) was observed during the rainy season trial with 10% Sulphuric acid treatment. Using warm water is a simple and affordable treatment especially to local farmers which will give the best result in the germination and seedling production of *A. annua*. Early March is recommended as the ideal planting period so that seedlings are transplanted on the field at the onset of rainfall.

Keywords

Artemisia, *In Situ*, Germination, Seedling, Growth

1. Introduction

Artemisia annua L. (Wormwood) belongs to the tribe Anthemideae of the Asteroideae, a subfamily of the Asteraceae (Compositae) [1]. Its cultivation has expanded from its centre of origin (China) to Nigeria in response to

the call by the World Health Organization for the use of Artemisinin-Combination Therapies (ACT) for treating malaria fever. Likewise its effectiveness has been demonstrated in the treatment of skin diseases and it has also been shown to be an effective non-selective herbicide such as glyphosate [2] [3].

Members of some plant families exhibit erratic germination due to seed dormancy [4]. They readily germinate within the native environment, but fail to show good germination under alien condition depending on the plant species and type of dormancy, several methods are used to break dormancy in order to induce germination [4]-[7].

Artemisia seeds were observed to undergo chemical dormancy due to the Presence of some chemical compounds (such as Phenolics) on the surface. This was linked with seeds germination inhibition and dormancy of the plant. Phenolics accumulation played a protective role in strengthening the plant cell walls during growth by polymerization into lignin [8]. In Nigeria, productive *Artemisia* seed is expensive and not readily available. Successful and quantitative production of biomass is an important step towards maximizing Artemisinin content in *Artemisia* for the treatment of malaria fever. However, this has been hindered by several biotic and abiotic factors such as the pest, diseases and climatic constraints. Consequently, this has led to poor performance, hence decrease in yields. Similarly, there is inadequate agro-technological information regarding the ideal planting dates, seed density, harvesting system, post-harvesting and optimum fertilizer application rates required for higher yields. There is therefore, the need to determine the most effective treatment for the germination of *Artemisia* seeds as a commercially viable means to production of Artemisinin.

2. Materials and Methods

2.1. Study Area

Two separate experiments were conducted during the 2012 & 2013 hot and cold seasons respectively on farm of the Institute for Agricultural Research (IAR) Ahmadu Bello University, Zaria, latitude 11˚11'N and 07˚38'E, altitude 670 m above sea level, 640 km from the Atlantic shores of Nigeria in the south.

2.2. Materials

Fresh and healthy seeds of Chinyong variety of Artemisia *annua* were sourced from the Artemisia Programme Unit in the Institute for Agricultural Research (IAR) Ahmadu Bello University, Zaria for the experiment.

2.3. Treatments

The seeds were subjected to the following treatments:

1) Chemical Treatment

Seeds were soaked into 10%, 20% & 30% Sulphuric acid (H_2SO_4) for 1, 2 and 3 minutes respectively with a control (0%).

2) Hormone Treatment

The seeds were soaked in three different GA3 concentrations (100 ppm, 300 ppm, & 500 ppm) with a control (0%) for 6 hrs, 12 hrs & 24 hours respectively.

3) Warm Water Treatment

Seeds were soaked in water bath and incubated under 20˚C, 40˚C and 60˚C for 1 minute, 3 minutes and 5 minutes respectively.

4) Cold Water Treatment

Seeds were washed and soaked in cold water for 2 hrs, 4 hrs & 6 hrs before planting.

2.4. Sowing

The *Artemisia* seeds were sown in polythene bags containing sterilized river soil. [9]. Under the pre-nursery stage the seeds were sown in polythene bags containing sterilized river sand and monitored for germination. Transparent polythene material was used to cover the seeds. This is to help maintain adequate moisture, temperature and humidity levels in the soil, which are essential for Artemisia seed germination (**Figure 1**). Two weeks after germination, the tender seedlings were transferred to the nursery stage into a mixture of 50% each of river sand and cow dung to facilitate drainage and enhance their growth.

The germination percent was calculated according to [10] where Gr = (number germinating since n − 1)/n.

Where: Gr = germination rate; n = the days of incubation.

Seedling vigor was determined by field rating based on morphological appearance, seedling emergence and early percentage germination adopting the procedure of [11]. A scale of 1 - 5 was used where 1 = very high vigor and 5 = very low vigor.

1) Watering

While waiting for the Artemisia seeds to germinate and depending upon the humidity in the area, the soil was kept moist and damp by regular watering.

2) Preparation of Seedlings

Sixty days after germination, the plants were hardened gradually by exposing them to sunlight while reducing watering, prior to transplanting.

3) Parameters Studied

The following parameters were studied:

Days to germination;

Percentage germination;

Seedling vigor;

Seedling height;

Root length.

2.5. Data Analysis

The data generated from this work was analyzed using analysis of variance (ANOVA), SAS (2002) statistical package. Least significant difference (LSD) was also used to compare treatment means ($p < 0.05$).

3. Results

Covering the seeds with transparent polythene was observed to maintained suitable temperature and moisture condition thereby enhancing the germination of *A. annua*. These were seen to be very vital in the germination of this plant (**Figure 1**). During the 2012 study period, an average temperature of 37˚C, a humidity of 9% and

Figure 1. Seeds sawn and covered with transparent polythene.

sunshine duration of 7 hours were recorded. However, 33˚C temperature, 22% humidity and 8 hours sunshine duration were recorded for 2013 experiment. Hypogeal type of germination was observed to occur 2 - 3 days after sowing (**Figure 1**). Leaves were found to be aromatic, deeply dissected and range from 3.0 to 8.5 cm in length (**Figure 2**). Transferring the seedlings singly into bags containing a mixture of river sand and cow dung in the ratio of 50:50 was also observed to further facilitate the growth and development of *A. annua* (**Figure 2**). Results obtained from this study showed no significant difference between the seasons with respect to germination, shoot and root lengths. However, warm water treatment at 60˚C for 2 minutes during the rainy season trial had the least days to germination (**Table 1**). Similarly, warm water treatments in both rain and irrigation trials at 40˚C for 3 minutes and 60˚C for 3 minutes produced the best vigor (**Table 2**). Highest germination percent (96%) was observed during the rainy season trial with 10% Sulphuric acid treatment (**Table 1**). Fourteen days after germination, the young seedlings were transfrered to the nursery stage (**Figure 3(b)**).

4. Discussion

Germination is a vital phenomenon during the life cycle of a plant [12]. The early germination observed may be attributed to the covering with polythene material which helped in maintaining adequate moisture, warm and humidity levels in the soil, which are essential for Artemisia seed germination. Dormancy of some seeds was reported to be inhibited when soil temperatures are too warm. They therefore germinate only at high temperatures [13]. Similarly, germination depends on weakening of the seed coat by heating hence providing the optimum temperature suitable for influencing the rate of enzyme-controlled reactions [14] [15]. The chemical dormancy reported in *Artemisia* seeds due to the accumulation of some chemical compounds such as Phenolics on the surface, played a protective role in strengthening the plant cell walls during growth by polymerization into lignin. This physiologically contributes to the maintenance of dormancy by impeding water and gas to and from the embryo, chemically by inhibiting germination and mechanically by restricting the growth of the embryo [8] [15] [16]. This eventually serves as a barrier that restrict water uptake by the impermeable outer part of the epidermal layer of malphigian cells hence restraining expansion of the radicle and manifestation of germination [17].

The aromatic, deeply dissected leaves ranging from 3.0 to 8.5 cm in length observed, is similar to the findings of [18]. The germinating seeds of *A. annua* exhibited a hypogeal type of germination by having the Cotyledon remaining below the soil surface. A seed was considered germinated when the tip of the radicle had grown free of the seed coat emerging through the outer covering [10] [19]. Exposure of the shoot tip to light enabled it to photosynthesize thereby straightening the epicotyls [20] [21].

All treated seeds were covered with transparent polythene thereby exposing them to light which is an important regulatory environmental signal that triggers germination [22] and they responded favorably [23]. This is contrary to the findings of [24] who reported that seeds of *A. annua* germinated after exposure to dark.

(a) (b)

Figure 2. Artemisia seedlings at pre-nursery stage.

Table 1. Effect of treatments on germination during raining season trial.

Treatment	Days to Germination	Vigor	Germination %	Shoot Length	Root Length
GA3					
100 pp/6hrs	3.67 hij	2.00 ef	73.67 d	1.98 abcdef	0.48 f
100 pp/12hrs	2.67 jkl	1.33 f	63.50 h	2.48 f	0.53 f
100 pp/24hrs	3.33 hijk	1.33 f	81.00 b	2.67 abcdef	0.48 abcde
300 pp/6hrs	3.67 hij	2.33 de	57.50 k	2.62 abcde	0.53 abcde
300 pp/12hrs	3.67 hij	3.33 bc	49.67 no	2.51abc	0.58 abcde
300 pp/24hrs	3.67 hij	4.33 a	26.83 u	2.11 bcdef	0.58 abcde
500 pp/6hrs	5.00 efg	3.33 bc	37.83 r	2.57 def	0.58 abcde
500 pp/12hrs	5.00 efg	3.33 bc	48.83 o	2.43 abcdef	0.60 abcde
500 pp/24hrs	0.00 m	0.00 g	0.00 w	0.00 abcdef	0.60 abcde
Cold Water					
2 hours	2.33 kl	4.33 a	58.83 j	2.32 abcdef	0.55 abcde
4 hours	4.00 ghi	3.67abc	45.33 p	2.99 a	0.60 abcde
6 hours	3.33 hijk	4.33 a	42.37 q	2.31 abcdef	0.61 abcde
Sulphuric Acid					
10%/1min	4.67 ghi	1.67 ef	95.50 a	2.36 abcdef	0.64 abcdd
10%/2mins	4.00 ghi	2.33 de	70.17 e	2.22 abcdef	0.61 abc
10%/3mins	2.33 kl	3.00 cd	51.00 m	2.74 bcdef	0.62 abcd
20%/1min	3.67 hij	2.33 de	58.33 jk	2.46 abcd	0.61 abc
20%/2mins	8.67 a	3.33 bc	50.67 nm	2.83 abcdef	0.62 abc
20%/3mins	6.00 de	4.00 ab	25.67 u	2.25 abc	0.61 abc
30%/1min	6.33 cd	4.33 a	21.00 v	2.57 bcdef	0.56 abc
30%/2mins	2.33 kl	1.67 ef	65.67gf	2.05 abcdef	0.55 abcde
30%/3mins	5.33 def	3.33 bc	41.17 q	2.20 ef	0.55 abcde
Warm Water					
20˚C/1min	8.00 ab	4.33 a	21.67 v	2.87 bcdef	0.52 abcde
20˚C/2mins	2.67 jkl	2.33 de	55.83 l	2.41 abcdef	0.56 abcdef
20˚C/3mins.	7.33 fgh	3.67 abc	30.83 t	2.86 ab	0.58 abcde
40˚C/1min	4.33 ghf	3.67ab	35.50 s	2.52 abc	0.60 abcde
40˚C/2mins	2.33 kl	2.33 de	60.83 i	2.65 abcdef	0.55 abcd
40˚C/3mins	3.00 ijkl	1.33 f	66.17 gf	2.46 abcde	0.60 abcde
60˚C/1min	2.67 jkl	1.67 ef	65.17 g	2.59 abcdef	0.61 abcde
60˚C/2mins	2.00 l	2.00 f	66.67 f	2.62 abcdef	0.68 abcd
60˚C/3mins	2.67 jkl	1.33 f	72.67 d	2.54 abcdef	0.65 a
Control	3.67 hij	2.33 de	75.50 c	2.53 abcdef	0.45 ab

Means within a column followed by the same letter are not significantly different (p = 0.05).

Table 2. Effect of treatments on germination during the Irrigation trial.

Treatment	Days to Germination	Vigor	Germination %	Shoot Length	Root Length
GA3					
100 pp/6hrs	3.33 klm	2.33 def	61.17 h	1.90 abcdefg	0.38 b
100 pp/12hrs	3.00 nml	2.33 def	78.50 d	1.64 abcdefg	0.41 b
100 pp/24hrs	4.67 ghi	3.00 h	60.50 k	2.10 abcdefg	0.48 ab
300 pp/6hrs	6.00 fe	4.33 a	52.00 k	0.56 abcdefg	0.50 c
300 pp/12hrs	6.33 de	4.33 a	46.17 m	1.78 g	0.48 b
300 pp/24hrs	5.33 fg	3.33abcd	50.83 kl	1.67 abcdefg	0.51 b
500 pp/6hrs	5.00 gh	2.67 cdef	55.00 j	1.93 abcdef	0.51 b
500 pp/12hrs	7.00 cd	2.67 cdef	84.67 b	1.67 h	0.53 b
500 pp/24hrs	6.00 ef	1.67 fg	27.17 q	1.71 abcdefg	0.52 b
Cold Water					
2 hours	4.33 hij	2.00 efg	75.67 e	2.21 efg	0.55 ab
4 hours	3.33 klm	2.33 def	90.83 a	1.97 abcdefg	0.55 b
6 hours	5.33 fg	2.67 cdef	60.83 h	1.86 abcdefg	0.56 b
Sulphuric Acid					
10%/1min	4.67 ghi	2.33 def	84.00 b	1.79 abcdefg	0.58 b
10%/2mins	7.00 cd	2.67 cdef	80.83 c	2.00 bcdef	0.58 b
10%/3mins	2.67 nm	2.33 def`	61.17 h	1.91 a	0.58 b
20%/1min	4.33 hij	4.33 a	50.00 l	1.83 bcdefg	0.59 b
20%/2mins	2.67 nm	3.67 abc	31.33 p	2.26 abcdefg	0.57 ab
20%/3mins	13.00 a	3.67 abc	30.83 p	1.98 cdefg	0.58 b
30%/1min	13.00 a	3.33abcd	35.33 o	1.79 abcde	0.55 b
30%/2mins	3.33 klm	4.00 ab	51.17 kl	1.94 bcdefg	0.55 b
30%/3mins	7.33 c	4.33 a	46.17 m	1.86 abcd	0.55 a
Warm Water					
20˚C/1min	13.00 a	4.33 a	32.17 p	2.87 bcdefg	0.52 a
20˚C/2mins	3.67 jkl	4.00 ab	54.50 j	2.16 abcdefg	0.56 ab
20˚C/3mins	10.67 b	3.33abcd	55.67 j	2.11 gf	0.58 ab
40˚C/1min	7.33 c	4.00 ab	38.83 n	1.82 efg	0.60 b
40˚C/2mins	3.33 klm	3.33abcd	58.50 i	1.98 ab	0.56 b
40˚C/3mins	4.33 hij	2.67 cdef	60.67 h	2.04 abcdefg	0.60 b
60˚C/1min	3.33 klm	2.67 cdef	68.33 g	1.97 abcdefg	0.61 b
60˚C/2mins	2.33 n	1.67 fg	71.50 f	2.16 abc	0.68 ab
60˚C/3mins	2.67 nm	1.00 g	72.50 f	2.04 abcdefg	0.65 b
Control	4.00 ijk	3.00 bcde	50.83 kl	1.85 abcdefg	0.46 b

Means within a column followed by the same letter are not significantly different (p = 0.05).

(a) (b)

Figure 3. Artemisia seedlings at the nursery stage.

Germination of seeds of *A. annua*, commenced 2 - 3 days after sowing and 96% of the seeds responded to treatment. This is contrary to the findings of [25] and other workers who observed that seeds of *A. annua* and *A. absinthium* germinated in 6 - 7 days as obtained when grown under field conditions.

The insignificant height observed with the Artemisia young seedlings may be attributed to lack of ample reserved nutrients such as Carbohydrate, lipid and protein to enable the seedlings achieve critical size advantage [26].

5. Conclusion

Using warm water is a simple and affordable treatment especially to local farmers which will give the best result in the germination and seedling production of *A. annua*. In Nigeria, early March is recommended as the ideal planting period so that seedlings are transplanted on the field at the onset of rainfall.

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