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In Situ Germination and Seedling Characteristics of Sweet Wormwood (*Artemisia annua* L.)

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Abstract: ***An experiment was conducted in the Botanical Garden of the Department of Biological Sciences, Isa Kaita College of Education, Dutsin-ma, Katsina State, Nigeria; to determine the most effective treatment for rapid germination of Artemisia annua. The seeds were subjected to physical treatment by soaking in warm water and incubated at 20˚C, 40˚C and 60˚C for 1, 3 and 5 minutes respectively, while cold water treatment at room temperature was done by soaking seeds for 2, 4 and 6 hours respectively. Chemical treatment was done by soaking seeds in 10%, 20% and 30% methanol concentration for 1, 2 and 3 minutes respectively. Experiments were performed based on a completely randomized design with three replicates. Results of Analysis of Variance (ANOVA) indicated significant difference ($p \leq 0.05$) between the treatments with respect to days to germinate, % germination, Number of leaves, fresh plant weight, dry weight, plant height and vigor. Highest germination percent (45.87%) was observed in cold water treatment for 4 hours. Using cold 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. Therefore, this treatment is recommended for A. annua and related member of the family. Hence, Wide range of chemical (methanol) concentration level should be used in order to have a close comparison between the various concentrations.***

Keywords: *Artemisia*, In Situ, Germination, Seedling Growth, Methanol

1. Introduction

Sweet Wormwood (*Artemisia annua* L) is a tree plant belonging to the family Asteraceae, (Compositae). The plant is characterized by extreme bitterness in its part [1]. Its cultivation has expanded from its Centre of origin (China) to Nigeria in response to the call by the World Health Organization (WHO) for the use of Artemisinin-Combination Therapies (ACT) for treating malaria fever [2]; [3]. Artesunate, Artemether and Artemisinin are the three common derivatives found in *Artemisia annua* [4]. 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 [5]. 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 [6]; [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]; [9].

However, 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.

This research was carried out 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

The experiment was conducted in the botanical garden of the Department of Biological Sciences Isa Kaita College of Education, Dutsin-ma Katsina State, Nigeria.

2.2 Materials

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

2.3 Experimental Design

The experiment was conducted using a completely randomized design (CRD) with three replications.

2.4 Soil Preparation

The soil of experiment was sandy loamy soil with neutral pH. The organic carbon content of the soil is 0.2990(w/w). The soil had 127kgha-1 available nitrogen, 24kgha-1 available phosphorus and 11.9kgha-1 available potassium.

2.5 Treatments

The seeds were subjected to the following treatments:

2.5.1 Chemical Treatment

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

2.5.2 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.

2.5.3 Cold Water Treatment
Seeds were washed and soaked in cold water at room temperature for 2 hours, 4 hours, & 6 hours, before sowing.

2.6 Sowing

The *Artemisia* seeds were sown in polythene bags containing sandy loamy soil and monitored for germination. Transparent polythene material was used to cover the seeds (Muhammad *et al.,* 2014). This is to help maintain adequate moisture, temperature and humidity levels in the soil, which are essential for Artemisia seed germination.

2.7 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.

2.8 Parameters Studied

a) Days to germination
b) % Germination
c) Vigor
d) Number of leaves
e) Fresh plant weight
f) Dry weight
g) Plant height

2.9 Observation and Data collection

Leaves germination was observed Two days after sowing, % germination, vigor, number of leaves, Fresh plant weight, Dry weight, and Plant heights is recorded and continue at a week interval for eight weeks.

a) Days to germination was determined by number of days to germinate per treatment after sowing
b) % regeneration was calculated according to Wiese and Binning (1987) where Gr = (number germinating since n-1)/ n. Where: Gr = germination (regeneration) rate; n = the days of incubation.
c) Vigor was determined based on morphological appearance, seedling emergence and early percentage germination adopting the procedure of (Gibson, 1980). A scale of 1-5 was used where 1= very high vigor and 5=very low vigor.
d) Fresh plant weight was obtained by using an electronic weighing scale.
e) Dry plant weight Samples was obtained after shade drying for two weeks. Dry plant weight was measured using an electronic weighing scale.
f) Plant height was determined by spreading a thread against the length of the plantlet after which it was placed on a measuring tape to measure its height.

2.9.1 Data Analysis
The data generated from this work was analyzed using analysis of variance (ANOVA), SAS (2002) statistical package, where significant. Least significant difference (LSD) was used to separate the 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).

Figure 1: Seeds sawn and covered with transparent polythene

Hypogeal type of germination was observed to occur 2 - 4 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).

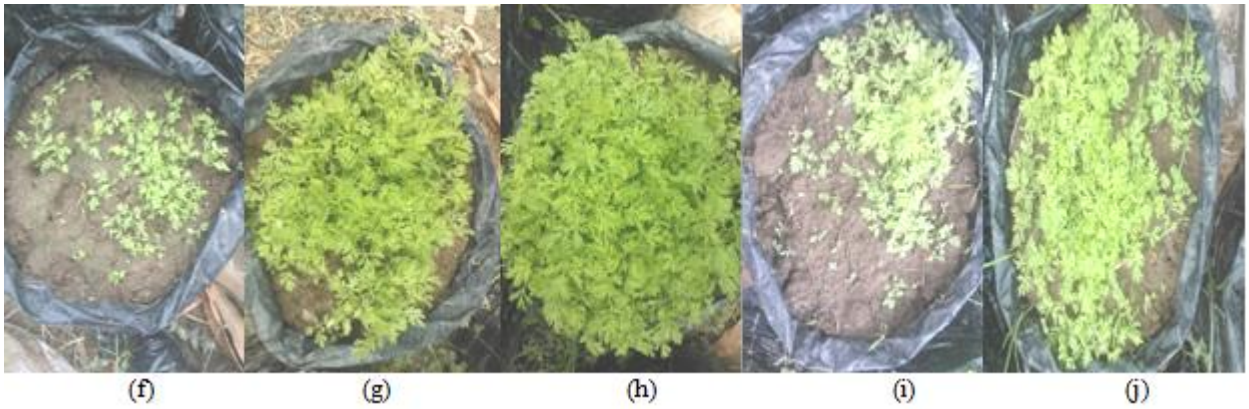

Figure 2: Artemisia annua seeds subjected to different treatments

a) 10%/1minute methanol concentration,
b) 20%/2minute methanol concentration,
c) 30%/3minute methanol concentration,
d) warm water treatment at 20˚C/1 minute,
e) warm water treatment at 40˚C/3 minutes,
f) warm water treatment at 60˚C/5 minutes,
g) cold water treatment for 2 hours,
h) cold water treatment for 4 hours,
i) cold water treatment for 6 hours,
j) control.

Results obtained from this study showed significant difference between the treatments with respect to germination, shoot length and plant weight.

3.1 Days for Germination

In chemical treatment**,** seeds treated with 10%/1minute and 20%/2 minutes methanol concentration germinated in three days after sowing, this is followed by 30% concentration which germinated in four days after sowing (Table 1).

Warm water treatment at 40˚C/3 minutes had the least days to germination i.e. it germinate in 3 days after sowing, Warm water treatment at 20˚C and 60˚C for 1 minute and 5 minutes respectively germinate exactly 4 days after sowing (Table 1).

In cold water treatment, seeds that were washed and soaked in cold water for 2 hours and 4 hours germinated in 3 days after sowing, and then followed by 6 hours which germinated in 4 days after sowing (Table 1). Similarly, the controls germinate in exactly 4 days after sowing (Table 1).

3.1.1 Germination Percentage

Chemical treatments show that 10% methanol concentrations have the highest percentage germination as compared to the control. This is followed by 20% concentration, and lastly 30% methanol concentration (Table 1).

Similarly in warm water treatments, Warm water treatment and incubated at 60˚C for 5 minutes had the least percentage germination. There is no significant difference between 20˚C for 1 minute and 40˚C for 3 minutes as compared to the control (p=0.05) (Table 1).

In cold water treatment, seeds that were washed and soaked in cold water for 4 hours had the highest percentage germination, followed by 2 hours as compared to the control (Table 1). Seeds that were washed and soaked in cold water for 6 hours had the least percentage germination (Table 1).

Table 1: Effects of Methanolic, warm and cold water treatments on *in situ* germination of *A. annua*

Treatment	*GMP (%)*	*DG*	*VG*
Methanol			
10%/1min	35.70^{bb}	3^{a}	1.67^{bb}
20%/2min	24.17^{cc}	3^{a}	2.67^{cc}
30%/3min	9.73^{f}	4^{b}	4.00^{d}
Warm Water			
20˚C/1min	25.40^{cc}	3^{a}	2.67^{cc}
40˚C/3min	23.27^{dc}	3^{a}	3.00^{cc}
60˚C/5min	13.33^{ff}	4^{b}	4.00^{d}
Cold Water			
2 hours	33.83^{b}	3^{a}	1.67^{bb}
4 hours	45.87^{a}	3^{a}	1.00^{aa}
6 hours	17.60^{de}	4^{b}	4.00^{d}
CONTROL	26.90^{cc}	4^{b}	3.00^{cc}
LSD	5.74	0.76	0.62

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

Keys: GMP Germination percentage, DG Days to Germinate, VG Vigor.

3.2 Average Fresh Plant Weight

Chemical treatment shows that 10%/1 minute gives the highest Average plant weight compare to control, followed by 20%/2 minutes methanol concentration, then lastly 30% concentration which give the least average fresh plant weight (Table 2).

In warm water treatment, Warm water treatment at 60˚C/5minutes had the least plant weight, followed by 40˚C/3minutes, and lastly 20˚C/1minute which had the highest plant weight of 2.41 gram (Table 2).

In cold water treatment, seeds that were washed and soaked in cold water for 4 hours had the highest average fresh plant weight, followed by 2 hours as in compared to the control. And lastly 6 hours which gave the least average fresh plant weight (Table 2).

3.3 Average Dry Plant Weight

This also shows that in chemical treatment, 10%/1minute methanol concentration gives the highest average dry plant weight, followed by the control, then 20%/2minute and lastly 30%/3minute concentration (Table 2).

In warm water treatment, Warm water treatment at 60 ˚C/ 5 minutes had the least dry plant weight, followed by 40˚C/3 minutes, and lastly 20˚C/1 minute which had the highest dry plant weight (Table 2).

In cold water treatment, seeds that were washed and soaked in cold water for 4 hours had the highest dry plant weight, followed by 2 hours as in compared to the control and lastly 6 hours (Table 2).

3.4 Average Plant height

Chemical treatment shows that methanol concentration of 10%/1minutes has the highest shoot length compare to the control, followed by 20%/2minutes and lastly 30%/3minutes which had the least average plant height (Table 2).

In warm water treatment, Warm water treatment at 60˚C/5 minutes had the least average plant height, followed by 40˚C/3 minutes, and lastly 20˚C/1 minute which had the highest average plant height (Table 2).

In cold water treatment, seeds that were washed and soaked in cold water for 4 hours had the highest plant height, followed by 2 hours and lastly 6 hours (Table 2).

3.5 Average Number of Leaves:

Chemical treatment shows that methanol concentration of 10%/1minutes has highest average number of plantlet, followed by 20%/2minutes concentration as in compared to the control, then by 30%/3minutes methanol concentration which gave the least average number of leaves (Table 2).

In warm water treatment, Warm water treatment at 60˚C/5 minutes had the least average number of leaves, followed by 20˚C and 40˚C for 3 minutes and 5 minutes respectively which had the same average number of leaves (Table 2).

In cold water treatment, seeds that were washed and soaked in cold water for 4 hours had the highest average number of leaves, followed by 2 hours and lastly 6 hours (Table 2). The control has the average number of leaves of 28 (Table 2).

3.6 Average Plant Vigor

Chemical treatment shows that methanol concentration of 10%/1minute gives the highest strength than the control followed by 20%/2 minutes and then lastly 30%/3 minutes concentration (Table 1).

So also in warm water treatment, Warm water treatment at 60˚C/5 minutes had the least strength than that of 20˚C and 40˚C for 1minute and 3 minutes respectively which have the same strength (Table 2).

In cold water treatment it shows that seeds that were washed and soaked in cold water for 4 hours had the highest strength than that of 2 hours, followed by the control and lastly 6 hours (Table 2).

Table 2: Effects of methanolic, warm and cold water treatments on seedling growth of *A. annua*

TREATMENT	*NL*	*FPW(g)*	*DPW(g)*	*PH(cm)*	*VG*
Methanol					
10%/1min	38.67^{a}	3.71^{bb}	1.66^{a}	26.51^{a}	1.00^{aa}
20%/2min	28.67^{cc}	1.85^{dc}	0.50^{cd}	17.42^{de}	2.67^{cc}
30%/3min	22.00^{dd}	1.33^{e}	0.39^{d}	13.00^{g}	4.00^{c}
Warm Water					
20˚C/1min	27.67^{cd}	2.41^{cc}	0.99^{bb}	18.79^{dc}	2.67^{cc}
40˚C/3min	27.33^{cd}	2.14^{da}	0.75^{cb}	17.36^{e}	2.67^{cc}
60˚C/5min	23.00^{c}	1.53^{d}	0.51^{cc}	14.68^{f}	4.00^{c}
Cold Water					
2 hours	30.67^{bb}	3.41^{b}	1.12^{bb}	19.01^{dc}	1.67^{bb}
4 hours	40.33^{aa}	5.26^{a}	2.01^{aa}	23.48^{b}	1.00^{aa}
6 hours	22.00^{dd}	1.45^{d}	0.88^{cb}	15.17^{ff}	4.00^{c}
CONTROL	28.33^{cc}	2.30^{cc}	1.03^{bb}	19.70^{cc}	2.67^{cc}
LSD	5.78	0.77	0.43	1.64	0.7

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

Keys: NL Number of Leaves, FPW Fresh Plant Weight, DPW Dry Plant Weight, PH Plant Height, VG Vigor.

4. Discussion

Germination is a vital phenomenon during the life cycle of a plant [9]. 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 [10].

All warm water treatments showed a significant decrease in the growth of *A. annua* compared to the control. This is contrary to the findings of [11] who reported that seeds of *A. annua* treated with warm water showed significant increase in growth compared to the control.

Cold water treatment for 4 and 2 hours gave the best performance in all the parameters studied compared to the control. Similar result was obtained by [12] who studied the effect of hot and cold water (at room temperature) pretreatment on the emergence of the *Acacia senegal* seeds.

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 [13]. It might also be due to the exposure of the shoot tip to light which enabled it to photosynthesize thereby straightening the epicotyls. All treated seeds were covered with transparent polythene thereby exposing them to light which is an important regulatory environmental signal that triggers germination [14]; [11]. And they responded favorably [15]; [11]. This is

contrary to the findings of [16] who reported that seeds of *A. annua* germinated after exposure to dark. Germination of seeds of *A. annua*, commenced 2 - 4 days after sowing and 96% of the seeds responded to treatment. This is contrary to the findings of [17] 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 dark green, tripinnate, aromatic, deeply dissected leaves ranging from 3.0 to 8.5 cm in length observed, is similar to the findings of [1];[11]. The leaflets are linear and have dentate margin with netlike venation. The germinating seeds of *A. annua* exhibited a taproot system. A taproot system is one in which the primary root becomes the main root of the plant with minimal branching consisting of secondary smaller lateral roots [18]. Generally, plants with taproots system are deep rooted in comparison with those having fibrous type. The tap root system enables the plant to anchor better to the soil and obtain water from deeper source [19].

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 [20].

5. Conclusion

In conclusion, cold water treatment for 4 hours, 2 hours and 10% methanol concentration for 1 minute increased *A. annua* growth compared to the control. All methanol concentrations show significant effect on the *in situ* germination and seedling growth of *A. annua*. Warm water and cold water treatments also show significant effect on the *in situ* germination and seedling growth of *A. annua*. However there is significant difference between warm water and cold water treatment in all the parameters studied. Based on the results obtained it has been found that cold water treatment for 4 hours had the best performance on the growth parameters (Days to germination**,** % Germination**,** Vigor**,** Number of leaves**,** Fresh plant weight**,** Dry weight**,** Plant height). Using cold 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*.

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References

[1] J. Ferreira, J. Janick, "Annual Wormwood (*Artemisia annua* L.)", 2009. Retrieved from www.hort.purdue.Edu/newcrop/cropfactsheets/Artemisia.pdf

[2] J.F.S. Ferreira, J.C. Laughlin, N. Delabays, P.M.D. Magalhaes, "Cultivation and genetics of *Artemisia annua* for increased production of the Anti-malarial Artemisinin". *Plant Genetic Resources*. (3), pp. 206–229, 2005.

[3] E.A. Brisibe, "Challenges and opportunities in the local production of artemisinin-based therapies against malaria in Nigeria". *Journal of Pharmaceuticals Science*. (8) 49-59, 2006.

[4] S.M. Tahir, S. Usman, M.D. Katung, M.F. Ishiyaku, "In vitro regeneration of *Artemisia annua* (wormwood) using seed explants". *International Journal of Biotechnology*. 2 (1), 171-181, 2013.

[5] N.B. Paniego, A.M. Giulietti, "*Artemisia annua* L.: Dedifferentiated and Differentiated Cultures". *Plant Cell Tissue and Organ Culture*, (36), 163-168, 1994. http://dx.doi.org/10.1007/BF00037715

[6] M. Jensen, E.N. Eriksen, "Development of Primary dormancy in Seeds of *Prunus avium* during Maturation". *Seed Science and Technology*, 29, 307-320, 2001.

[7] A.R. Zare, M. Solouki, M. Omidi, N. Irvani, A.A. Oladzad, N. Mahdi, "Effect of Various Treatments on Seed Germination and Dormancy Breaking in *Ferula assa foetida* L. (Asafetida), a Threatened Medicinal Herb". *Trakia Journal of Sciences*, (9), 57-61, 2011.

[8] F. Al-Quadan, A. Ibrahim, F.M.R. Al-Charchafchi, "Effect of Chlorogenic and Caffeic Acids on Activities and Isoenzymes of G6PDH and 6PGDH of *Artemisia Herba Alba* Seeds Germinated for One and Three Days in Light", Pakistan, 2008.

[9] L.D. Geraldine, A.D. Lisa, "Water Potential and Ionic Effects on Germination and Seedling Growth of Two Cold Desert Shrubs". *American Journal of Botany*, (86), pp. 1146-1153, 1999. http://dx.doi.org/10.2307/2656978

[10] G. Nicolas, "The Biology of Seeds Recent Research Advances: Proceedings of the Seventh International Workshop on Seeds, Salamanca, Spain 2002". CABI Pub. Page, Wallingford, 2003.

[11] T.S. Muhammad, I.S. Usman, M.D. Katung, M.F. Ishiyaku, "*In Situ* Germination and Early Seedling Growth of Wormwood (*Artemisia annua* L.)". *American Journal of Plant Sciences*, (5), 1694-1701, 2014. http://dx.doi.org/10.4236/ajps.2014.511184

[12] A. Usman, O.A. Sotannde, Y.P. Mbaya, Y. Musa, "Effects of hot and cold water pretreatments on emergence of *Acacia senegal* seeds in the nursery". *Journal of research in forestry, wild life and environment*. 2(2), pp. 384-391, 2010.

[13] E.A. Van Geldre, E. Vergauwe E.E. Van, "State of the art of the production of the antimalarial compound artemisinin in plants". *Plant Molecular Biology*. (33), pp. 199–209, 1997.

[14] C.C. Basking, J.M. Basking, "Dormancy Types and Dormancy-Breaking and Germination Requirements in Seeds of Halophytes". In: Khan, M.A. and Ungar, I.A., Eds., *Biology of Salt Tolerant Plants*, University of Karachi, Karachi, pp. 23-30, 1995.

[15] H.M. Modares, "Loss of Dormancy and Raising of 8 Slat Desert Species and Their Manner of Establishment in the Site, a Research Plan Report". Research Institute of Forests and Rangelands, Tehran, 2003.

[16] Z.O. Jamaleddine, P. Lyam, O. Fajimi, A. Giwa, A. Aina, E.F. Lawyer, A.U. Okere, W.T. Odofin, "In *Vitro* Growth Response of *Artemisia annua* Seeds to Different Concentrations of Plant Growth Regulators". *African Journal of Biotechnology*, (10), pp. 17841-17844, 2011. http://dx.doi.org/10.5897/AJB10.1289

[17] A. Mannan, T.N. Syed, M.A. Yameen, N. Ullah, T. Ismail, I. Hussain, B. Mirza, "Effect of Growth Regulators on *In Vitro* Germination of *Artemisia absinthium*". *Scientific Research and Essays*, (**7**), pp. 1501-1507, 2012. http://dx.doi.org/10.5897/SRE11.1894
[18] B. Linda, L.R. Berg, "Introductory Botany plants, people and the environment". Cengage learning. pp. 112, 2007. ISBN 978-0-534-4669-5
[19] W.H.O. "World Malaria Report 2011 fact sheet". Global Malaria Programme. December, 2011.
[20] F. Micheal, "Seed Ecology". Chapman and Hall, 2-6 Boundary Row, London, pp. 208, 1993.

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