INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE
ISSN: 2067-533X Volume 11, Issue 3, July-September 2020: 799-806 www.ijcs.ro

IMPACT OF TEMPERATURE AND GROWTH REGULATORS ON GERMINATION OF SOME MEDICINAL PLANTS

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Abstract

Three medicinal plant species viz., Saussurea costus (kuth), Withania somnifera (Ashwagandha) and Ocimum sanctum (Tulsi) were selected with an objective to observe the germination behavior under different conditions. During the experiments, different treatments were given to the seeds and seeds were tested at different temperature condition. All the experimental tested attributes of seeds viz. seed germination, onset of germination, completion of germination and speed of germination were showed best results in seed germinator at 25°C temperature with water pre soaked for 24 hours and in seeds treated with 5% KNO_3 for 24 hours just before showing in contrast to other conditions and treatments. Seeds of S. costus, W. somnifera and O. sanctum treated with KNO_3 (5%) showed 46, 90 and 94 percent germination in seed germinator at 25°C temperature. Maximum seeds of all the three experimental species treated with 5% KNO_3 were maximum germinated within 7-12 days after sowing and started germination within 1 to 3 days after sowing at 25°C temperature condition.

Keywords: *Medicinal plants; Germination; Temperature; Growth regulator*

Introduction

India is the country having rich biodiversity and the great diversity region Himalaya is an example of it. The Himalaya covers plants as well as animals, in case of plants 18% of the Indian sub continent, more than 50% of Indian forest, and having 40% of India's endemic species [1] . Biological diversity in the world we can say they are boon of god for the local peoples.

So many plants species are used in various purpose like making medicine, shelter and food. Around 1748 species of medicinal and aromatic plants (MAPs) of Indian Himalayan region used in formulation of different medicine [2]. Medicinal plants cultivation attracted farmers for their socioeconomic upliftment because demands of medicinal plants are increasing day by day along with decreasing availability in natural conditions. Since till today mostly these plants harvested unscientifically from their natural habitat, may be serious implications to the survival of such valuable plants.

The Uttarakhand state is well known for rich biological diversity having around 964 species of 158 families known for medicinal value and received a significant attention since long back in curing various diseases [3-5]. In conservation point of view medicinal and aromatic plants are in high priority as their high pharmaceutical value, MAPs trade will play a significant role in future. It is also necessary to ensure the long term availability of such

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valuable plants by applying adequate scientific policy. Cultivation is an only option to reduce the pressure from natural population.

Now a day's people are shifting from modern medicines to the traditional system of medicines, such as AYUSH. Medicinal plants are important for health care of human beings as in ancient time Indians used only the traditional system to cure the diseases. During the Second World War, due to acute scarcity of drugs led to cultivation of medicinally important plant species, it was the first step of Indian's towards the cultivation of medicinal plants [6] .

Seed are the basic input and only the quality or vigour seeds may provide the quality planting material for cultivation. To evaluate the planting value of seeds, to minimize the risk of failure in planting low vigour seeds, testing of seed is an essential step. Among the different tests for determination of seed quality, germination test is very important to check the reproducibility, reliability and uniformity of seeds. Among the large number of medicinal plants that are still to be exploited, there are some important species that are commercially as well as economically valuable but have problem of seed germination, storage, testing etc. So *Saussurea costus, Withania somnifera* and *Ocimum sanctum* species were selected for the study to observe the germinability of seeds.

Saussurea costus (Falc.) *Lipschitz, syn Saussurea lappa c.b. Clark*e, is commonly known as costus and kuth in Hindi. Due to remarkable biological activity of S. costus and its constituents it will have an appropriate place in various systems of medicines all over the globe. The genus Saussurea dc. of the family Asteraceae comprises about 300 species in the world of which about 61 species exist in India. Total of 62 sp. were recorded from the IHR, of them 37 sp. were native to the Himalayan region, 8 were endemic and 21 were near endemic to the IHR [7] .

Withania somnifera (l.) dunal, (solanaceae), also known as Ashwagandha in Hindi and popularly known as Winter cherry or Indian Ginseng. It is an important herbal medicine that comes from the Ayurvedic tradition. Ashwagandha roots are used in many formulations prescribed in variety of manifestation viz. musculoskeletal conditions. It is a general tonic for overall health [8] .

Ocimum sanctum (Lamiaceae) has a holistic approach to health and disease that focused on preserving and promoting good health and preventing disease through healthy lifestyle practices. These practices include consumption of fresh, minimally processed foods, the use of rasayanas (formulas) that eradicate ageing and disease. Ocimum is commonly known as tulsi. Tulsi is an aromatic shrub in the basil family *Lamiaceae* (tribe ocimeae) that is throughout to have originated in north central India and now grows native throughout the eastern world tropics [9] .

Within ayurveda, tulsi is known as "the incomparable one", "mother medicine of nature" and "the queen of herbs", and is revered as an "elixir of life" that is without equal for both its medicinal and spiritual properties [10]. Tulsi is also credited with giving luster to the complexion, sweetness to the voice and fostering beauty, intelligence, stamina and a calm emotional disposition [10-13] .

Keeping in the view the critically endangered status and huge medicinal potential of the species, the present investigation was undertaken to standardize some basic techniques of seed germination to overcome seed dormancy and to enhance seed germination that will finally help in effective conservation and utilization of the species.

Material and Methods

The Experiment was carried out to observe the germination behavior of selected plant species viz. *S. costus, W. somnifera* and *O. sanctum*. The seeds of selected species were collected from Herbal Research and Development Institute Gopeshwar Chamoli Uttarakhand, India and brought to the laboratory at Deptt. of Botany Govt. P. G. College Gopeshwar,

Chamoli, Uttarakhand India. The seeds were cleaned and dried properly and stored in air tight containers. The seeds were divided into different lots and used as per requirement of the experiment.

Moisture content of seeds was determined by hot air oven method, in which whole seeds were dried in ventilated electric oven at 105°C for 15 hours. To observe the moisture content, 50 seeds of each species were taken.

Seed germination studies were conducted to observe the effect of temperature, growth regulators (One lot was treated as control, while 2nd, 3rd, 4th and 5th lots were treated with GA_3 50ppm, GA_3 100ppm, 5% and 10% of KNO_3 respectively and lots were treated with) and presoaking of seeds in H_2O, on the germination of selected species. All the seeds were treated for 24 hours just before sowing. To find out the effect of temperature on germination, mature seeds were kept for germination at 20°C±6°C (room condition), 25°C±4°C and 35°C±4°C (in seed germinator). Each treatment was comprised in triplicate and each replicate was of 25 seeds. Filter papers were moistened regularly with distilled water during the experiment. The observations were made regularly from sowing to completion of germination. For this purpose, the seeds were placed between Whatman no.1 filter papers in petri-plates, in triplicate. Radicle emergence was noted as a seed germinated.

To see the effect of presoaking, seeds of selected plant species were divided into two lots, one was untreated (control), and second was soaked in H_2O for 24 hours just before sowing (presoaking treatment), and observed up to the completion of experiment.

Moisture Content calculated by using below mentioned formula:

$$Moisture\ Content\ (\%) = Fresh\ Wt.\text{-}\ Dry\ Wt./Fresh\ Wt. \times 100$$

Speed of germination was calculated using a formula that uses the daily number of germinations and the number of days, as follows:

$$p_1/d_1 + p_2/d_2 + p_3/d3 + \ldots + p_i/d_i,$$

where, p_1, p_2, p_3 ... p_i are no. of seeds germinated until the nth day and d_1, d_2, d_3 ... d_i are the days when the seeds are germinated to the nth days respectively.

Results and Discussion

Now-a-days medicinal crops are gaining much importance and are exploited from wild for their valuable disease curing properties without any side effects. So the cultivation of medicinal crops needs attention to meet the demand and to conserve their germplasm, at their natural habitat.

For the successful cultivation of a crop, complete package of practice is needed. As the propagation of *Saussurea costus*, *Ocimum sanctum* and *Withania somnifera* are through seeds, therefore an attempt has been made to investigate seeds germination ability and the speed of germination behavior of these selected medicinal crops.

Various government and NGO initiated medicinal plants cultivation in different parts of the world. Now in In India farmers and some industries focused on farming of MAPs to get a regular income and continuous supply of raw materials respectively [14-16] .

Moisture Content Analysis

The fresh wt of seeds of *S. costus, W. somnifera* and *O. sanctum* was 265.75, 10.50 and 9.25mg respectively. Dry wt. of seeds of *Saussurea costus* was 213.50mg, followed by *Withania somnifera* (9.00mg) and *Ocimum sanctum* (7.50mg). The data of dry wt, fresh wt and moisture content are presented in figure 1 and table 1.

Maximum moisture content approximately 19.7% was observed in seeds of *S. costus* while lowest moisture content was in *W. somnifera* seeds i.e. 14.26%. *O. sanctum* seed showed 18.92% moisture content. Moisture content was calculated on fresh weight basis.

Table 1. Fresh wt., Dry wt. and moisture content of selected species

Name of species	Fresh wt. (mg)	Dry wt. (mg)	Moisture content (%)
Saussurea costus	265.75	213.5	19.7 ±3.5
Withania somnifera	10.5	9	14.28 ±2.4
Ocimum sanctum	9.25	7.5	18.92 ±3.6

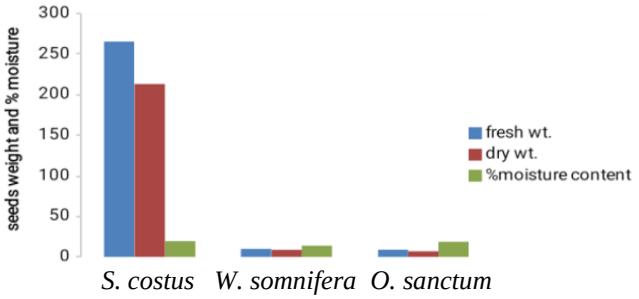

Fig. 1. Fresh weight, Dry weight and Moisture content of selected species

Germination Analysis

Effect of different temperature conditions viz. room condition (20±6°C), at 25°C±4°C and 35°C±4°C on seeds germination were observed on all the three experimental plant species. Overall, the germination percent, speed of germination, onset of germination and completion of germination in all the experimental species showed better result at 25°C in contrast to room condition and 35°C. In case of *S. costus*, 17, 16 and 13 percent of germination was observed in room condition, at 25°C and at 35°C respectively. Speed of germination also followed same trend as percent germination. Highest (3.88) speed of germination was observed at 20°C (room condition), followed by 3.00 speed of germination at 25°C and lowest (2.00) speed of germination was observed at 35°C temperature condition. Seeds started germinate just after 13, 5 and 10 days of sowing in room conditions, at 25°C and 35°C temperature conditions respectively. Maximum germination 17%,16% and 13% was observed just after 22 days in room condition, after 8 days at 25°C and after 10 days at 35°C conditions respectively. The poor performance of Saussurea seeds regarding germination may be due to low quality like immature and empty seeds. The observation on effect of different temperatures is presented in figure 2 and table 2.

In case of *Withania somnifera*, maximum 64% germination was observed at 25°C with 4 days required for onset and 12 days for completion of germination. This was followed by seeds, which were kept in room condition for germination and showed 32% germination, took 5 days to start and 15 days to complete germination and showed 7.8 speed of germination.

Different experimental attributes were found lowest at 35°C in contrast to 25°C and room conditions. As the seeds of *W. somnifera* showed 30% germination required 6 days for onset and 16 days for completion of germination and showed 7 speed of germination. The investigation on different temperatures is presented in table 2 and figure 2.

Table 2. Effect of Temperature on Germination

Species	Different Temperature Conditions											
	Room temperature				25°C				35°C			
	A	B	C	D	A	B	C	D	A	B	C	D
S. costus	13	22	17	3.88	5	8	16	3.0	10	20	13	2
W. somnifera	5	15	32	7.81	4	12	64	19.1	6	16	30	7
O. sanctum	5	17	50	25.2	2	10	66	57.3	3	20	45	21

A = Days required for onset of germination (DROG),
B = Days required for completion of germination (DRCG),
C = % germination (PG), D = Speed of germination (SG)

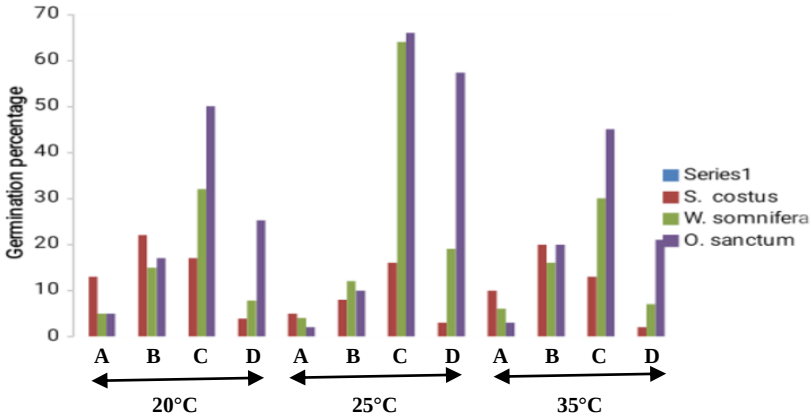

Fig. 2. Effect of temprature on germination percentage

Some preliminary observations on seed germination of W. somnifera have been reported in seed lots collected from different eco geographical zones [17, 18]. In field conditions, seed germination was found poor and mortality rate of seedlings were high [19].

At 25°C, highest 66% germination was observed in *Ocimum sanctum* at 25°C with only 2 days required for onset and 10 days required for completion of germination and 57.30 speed of germination. In room conditions *O. sanctum* showed lowest (45%) germination in contrast to other experimental conditions, with days required for onset 3 and 20 days required for completion of germination and showed 21 speed of germination (table 2 and figure 2).

Water soaked seeds of *S. costus*, *W. somnifera* and *O. sanctum* showed respectively 30%, 74% and 86% germination at 25ºC condition. Days required for onset of germination were 4, 3 and 1 in S. costus, *W. somnifera* and *O. sanctum* respectively. For completion of germination S. costus tooks 8 days, *W. somnifera* tooks 10 days and *O. sanctum* tooks 8 days. Seeds of *S. costus*, *W. somnifera* and *O. sanctum* showed respectively 3.29, 18.47, 89.36 speed of germination (figure 3 and table 3).

Table 3. Effect of Presoaking on Germination of Selected Species

Species	Control				Water soaked for 24 hrs.			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**
S. costus	5	8	16	0.7	4	8	30	3.3
W. somnifera	4	12	64	26.8	3	10	74	18.5
O. sanctum	2	10	66	4.8	1	8	86	89.4

A = Days required for onset of germination (DROG), B = Days required for completion of germination (DRCG), C = % germination (PG), D = Speed of germination (SG)

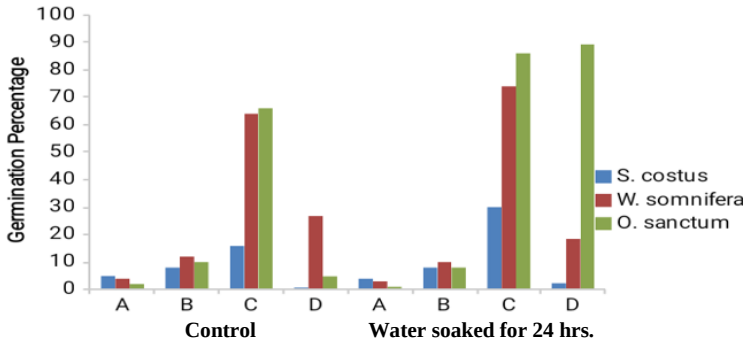

Fig. 3. Effect of water presoaking on germination percentage

Seed germination is one of the most important processes in the plant's natural life cycle. In seeds to initiate the process of germination water must imbibe under favourable environmental conditions [20]. Several factors such as temperature, oxygen levels, water availability and environmental conditions influences germination process. Seeds of different species have different temperature requirements. The most adequate temperature for germination is generally the one at which maximum seeds germinated within minimum time. Optimum temperature for germination test of *Catharanthus roseus* was 20 to 25°C while 25°C temperature was optimum for conducting germination test in *Plantago ovate* [21 and 22]. The optimum temperature for germination of *Solanum nigrum* was 20/30°C [23].

To improve germination, seeds of all the three experimental species were treated for 24 hours with different concentration of GA_3 (100 and 50ppm) and KNO_3 (5 and 10%). In respect of all growth regulators used for experiment, all the three experimental species showed best result in seeds treated with 5% KNO_3. Seeds of *S. costus, W. somnifera* and *O. sanctum*, treated with KNO_3 (5%) showed 46, 90 and 94% germination, tooks 3, 3 and 1 days for onset of germination, tooks 7, 12 and 10 days for completion of germination and showed 6.16, 18.4 and 89.36 speed of germination respectively (table 4 and fig 4). Seeds treated with $GA_3$100 ppm showed 44, 82 and 90% germination in seeds of *S. costus, W. somnifera* and *O. sanctum* respectively. GA_3 50ppm treated seeds showed little bit high (almost same) as compared with GA_3 100ppm treated seeds. While the seeds of *S. coustus* and *W. somnifera* treated with10% of KNO_3 also showed almost similar results like $GA_3$100 and 50ppm treated seeds. But the seeds of *O. sanctum* treated with10% of KNO_3 showed around 20% reduction in germination in comparison to GA_3 treated seeds. Positive impacts of GA_3 observed on seed germination of high altitude medicinal plants [24] .

Table 4. Effect of Growth Hormones on Germination of Selected Species

Species	**Hormonal concentration**															
	GA_3 50ppm				**GA_3 100ppm**				**KNO_3 5%**				**KNO_3 10%**			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
S. costus	5	8	42	3.2	5	8	44	4.65	3	7	46	6.2	4	7	44	5.2
W. somnifera	4	12	84	19.4	4	12	82	19.4	3	12	90	19.3	4	12	82	11.2
O. sanctum	1	7	92	92.3	1	7	90	84.9	1	10	94	61.2	2	12	72	11.3

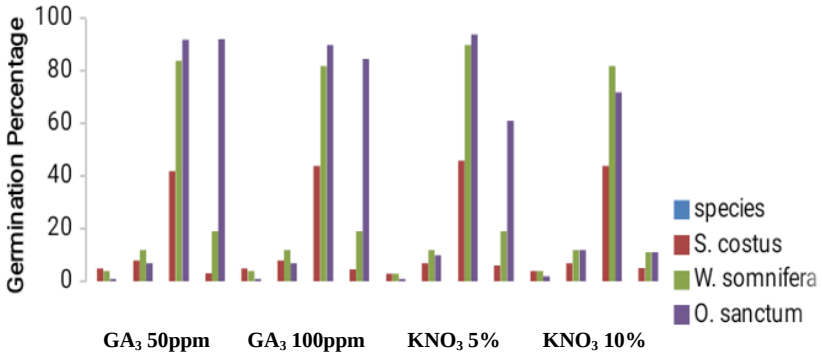

Fig. 4. Effect of Growth Hormones on Germination Percentage

GA_3 (5-40ppm) was found very effective to overcome the seed dormancy problem and most effective dose was 20ppm, KCl, KNO_3 and H_2O_2 salts also found to be useful at 1-10%, concentrations (most while effective doses were 5 and 10%) [25]. These increases seed germination capacity, germination velocity index and seedling vigour index in *Ocimum sps*.

The effect of different chemicals like GA_3, KH_2PO_4 and thiourea on seed germination of *Withania somnifera* was studied and found highest 65.3% germination in seeds treated with GA_3 (250 ppm) in contrast to other chemicals [26] .

Soaking of seeds in water just before sowing showed enhancement of germination in all the experimental species. Water soaked seeds of all the experimental species showed significant enhancement in germination in contrast to control conditions at 25°C temperature condition.

Conclusion

Three medicinal plant species viz., *Saussurea costus* (kuth), *Withania somnifera* (Ashwagandha), *Ocimum sanctum* (Tulsi) were selected with an objective to observe the germination behavior under different conditions. During the experiments, different treatments were given to the seeds and seeds were tested at different temperature condition. The results of the present investigation are summarized below.

All the experimental tested attributes of seeds viz. seed germination, onset of germination, completion of germination and speed of germination were showed best results in seed germinator at 25°C temperature with water pre soaked for 24 hours and in seeds treated with 5% KNO_3 for 24 hours just before showing.

Seeds of *S, costus, W. somnifera* and *O. sanctum* treated with KNO_3 (5%) showed 46, 90 and 94 percent germination in seed germinator at 25°C temperature. Maximum seeds of all the three experimental species treated with KNO_3 (5%) were germinated within 7-12 days after sowing and started germination within 1 to 3 days after sowing at 25°C temperature condition in seed germinator.

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Received: August 24, 2019
Accepted: August 26, 2020