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Effect of Temperature and Plant Growth Regulators on Seed Germination Response of *Oroxylum indicum*-A High Value Threatened Medicinal Plant of Sikkim Himalaya

Research Article

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Abstract

Oroxylum indicum is an integral component of Indian Ayurvedic medicine system. Owing to depletion of its natural populations, the plant is facing threats of extinction. Different plant growth regulators (viz. Benzyl amino purine, Indole-3-acetic acid, Gibberellic acid) and temperatures (cold and hot water) were used to improve seed germination in *O. indicum*. Cold water treatment had significantly improved the seed germination as compared to control, hot water treatment and tested plant growth regulators. Cold water at 4 °C for 24h was found to be optimum, which resulted in to more than 80% seed germination response. Here, it is the first time we have described the seed germination requirement of *Oroxylum indicum*, using growth hormones and temperatures. The study suggested that the seed germination and seedling growth of *Oroxylum* can be improved by using simple and economic method.

Keywords: Conservation; *Oroxylum indicum*; Seed Germination; Stress

Abbreviation

BAP-Benzyl amino purine; IAA-Indole-3-Acetic Acid; GA_3 -Gibberellic acid

Introduction

Oroxylum indicum (L), commonly known as Sonapatha, Shyonaka or tatola, is an important medicinal plant of the family Bignoniaceae. This high value multipurpose tree is reported to be vulnerable in Sikkim Himalaya [1]. Different parts of the plant, particularly leaves, bark and root have been extensively used in Indian System of Medicine [2]. Its root is an important constituent of Dashmula which is a compound decoction of 10 roots [3]. Plant is also included in famous tonic formulations, such as Chyawanprash. *O. indicum* is reported to have anticancer, antioxidant, hepatoprotective and immunomodulaory properties [4-6]. In addition, *Oroxylum* has antibacterial, analgesic and gastro-protective properties [7, 8]. These properties are mainly due the presence of diverse range of phytocompounds in the plant. Major medicinally important bioactive compounds present in this species are baicalein, oroxylin A, chrysin and its derivatives [9,10].

At present, the entire commercial requirement of *O. indicum* is met solely from the wild natural populations. The species is generally propagated by seeds but its germination rate and viability is very low due to seed abortion [11,12]. Destructive and non-sustainable collection methods coupled with low regeneration and habitat destruction have posed serious threats to the survival and availability of *O. indicum* [13]. To keep pace with the growing demand of this tree, its propagation by conventional and biotechnological method

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is highly warranted. In the present study, to obtain rapid, uniform and high seed germination response of *O. indicum,* different pre-sowing treatments were performed. Specifically, we studied the pre-treatment effect of temperature (hot and cold water) and plant growth regulators on *Oroxylum* seed germination response and established a suitable method to overcome its low and erratic germination rate.

Materials and Methods

Collection of material

Mature fruits of *O. indicum* were collected in October 2013 from a mother plant growing in natural habitat in Asam-Lingzay, East Sikkim (ca. 1000 m a.s.l.). The fruits were washed thoroughly under running tap water to remove the surface contaminants. After proper drying at room temperature, the fruits were split open to collect seeds.

Seed viability test

To ensure that the seeds used for the experiment were viable and of high quality; viability test using the tetrazolium chloride technique was performed. Three replicates of 24 seeds each were used. Using the procedure described by Peters [14], the seeds were imbibed for 24 h in water, cut along the margin without damaging the embryo and soaked in colourless 0.1% solution of 2,3,5-triphenyltetrazolium chloride (TTC) solution for 24 h at 25 °C in the dark. The seeds were removed from TTC solution and washed with distilled water, and viewed under a light microscope to observe the stained embryos. Whole embryos of viable seeds appeared bright red in colour. Seed viability was observed immediately at the time of collection and after 3 and 6 months of storage at normal room temperature.

Effect of seed wing and sulphuric acid (H_2SO_4)

To investigate the effect of wing on seed germination, experiments were conducted using seeds with wings and without wings. To overcome seed epicarp dormancy if any, seeds were given pre-sowing H_2SO_4 treatments. Seeds were immersed in 96% sulphuric acid for 30 sec, and then rinsed thoroughly in running water for 45 min.

Effects of plant growth regulators

The effects of three plant growth regulators viz. Gibberellic acid (GA_3), Benzylamino purine (BAP) and Indole 3-acetic acid (IAA) at 50, 100, 150 and 200 µM concentrations were tested for *O. indicum* seed germination in a controlled environment system. Twenty four seeds of uniform size were immersed in different concentrations of three plant growth regulators for 24 h, distilled water used for control treatment. Seeds were then rinsed with distilled water and placed in Petri dishes on filter paper. Petri dishes were watered with distilled water, as needed, to ensure adequate moisture for seed germination. The incubation temperature was 25 °C. All the seeds were checked daily for germination during 30 days.

Temperature treatments

Based on literature, the following pre-sowing temperature treatments were used to establish methods for overcoming seed dormancy: cool water (soaking in water at 4 °C for 6, 12, 24 and 48h), hot water (soaking in boiling water for 30, 60, 90 and 120 s). All the results were compared with control.

Seed germination conditions

Each germination treatment, including control, was performed with three replications. In each replicate, 24 seeds were used, giving a total of 72 seeds in each pre-sowing treatment. The Petri dishes were placed in a seed germination chamber at 25± 2 °C, with alternating light (14/10 h photoperiod). The seeds were kept moist (using DDW) and checked every day. Visible protrusion of the radical was the criterion to score seed germination. Final observation on the percentage germination, percentage relative germination, percentage dormancy, percentage relative dormancy and seedling morphology were taken after 30 day.

Statistical analysis

Results were analyzed using analysis of variance (ANOVA) and significance was determined at $p < 0.05$. The data was analyzed statistically using SPSS (version 16) software and significant differences among the mean values were assessed on the basis of the Duncan's multiple range test. In graph, data represented as mean ± standard deviation. The following parameters were determined:

Germination = number of germinating seeds/ number of seeds initiated × 100

Relative germination = number of germinating seeds/number of viable seeds initiated × 100

Dormancy = number of ungerminated seeds but viable seeds/ number of seeds initiated × 100

Relative dormancy = number of ungerminated seeds but viable seeds/number of viable seeds initiated × 100

Mean germination time (MGT), mean germination rate (MGR), uncertainty of germination and synchrony of germination were calculated by following Ranal et al. [15].

Results

Seed viability

Seed viability significantly decreased with duration. At the time of seed collection 83.33% seeds were viable. After 3 months of storage at room temperature, seed viability was decreased to 62.5% ($p < 0.05$), and after 6 months storage it plunged to 41.67%.

Effect of seed wing and sulphuric acid (H_2SO_4)

In the present study, to break the physical dormancy of seed wing and epicarp, *Oroxylum* seeds were subjected to dewinging and H_2SO_4 treatments. H_2SO_4 have had a corrosive physical action on *Oroxylum* seeds as its seed coat is very thin. Dewinging also showed poor results. The seeds with wings germinate earlier than the dewinged seeds. Moreover, in comparison to dewinged seeds, seedlings developed from winged seeds were healthy and fast growing (**Figure 1a-d**).

Effect of plant growth regulators

In the present study, tested plant growth regulators have influenced the seeds germination and radical length to different extent. Among the tested growth regulators, BAP was found to be highly effective to induce seed germination. At 50 µM concentration

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of BAP, only 50% seeds were germinated, whereas 100-200 µM concentrations of BAP helped >70% seed germination. There was no significant ($p< 0.05$) difference in the percent germination from 100-200 µM BAP treatments, but BAP significantly influenced the development of radical length with increasing concentration (**Figure 2a; Figure 3**). At 200 µM concentration of BAP, 76.4% seeds were germinated having rudimentary roots.

Effect of GA_3 treatments was not found to be significant over control (**Figure 4**). At lower concentrations (50 and 100 µM) of GA_3, significant reduction in germination percentage was observed. Nevertheless at higher GA_3 concentrations (150 and 200 µM), germination percentage had increased but germinated seedlings were found to be abnormal with long hypocotyls. Moreover, GA_3 treatment had caused vitrification of seedlings (**Figure 2b**.). Again, GA_3 treatment was inhibitory to root development. Effect of IAA was found to be completely inhibitory.

Results of growth regulators pre-treatments not only showed differences in the germination percentage, but also in the mean germination time, mean germination rate, uncertainty of germination, synchrony of germination and size of the seedlings (**Figure 2; Table 1**). In comparison to control and BAP, GA_3 pre-treatments resulted in earlier and faster emergence (**Table 1**). Moreover, seedlings from GA_3 pretreatments were bigger than seedlings from BAP treatments. Hypocotyl length had increased with the concentration of GA_3. At 150 µM concentrations, the hypocotyl length was 1.92 cm in GA_3 treated seeds which are much longer than the 0.79 cm length from BAP.

Effect of temperature

Among hot and cold water pretreatments, cold water pretreatments significantly improved the seed germination response. Soaking *Oroxylum* seeds in water at 4 °C for 12, 24 and 48h resulted in a significantly ($p< 0.05$) higher germination percentage than 6h pre-treatment and control (**Figure 5**). Results indicate that cold water pre-treatments significantly reduced the mean germination time and increased the mean germination rate. The minimum mean germination time was recorded in seeds subjected to 24h cold water pre-treatment which is significantly different than 6, 12 and 48 h treatments (**Table 1**). Cold pre-treatments produced seedlings of significantly higher vegetative growth vigour than those of other treatments and control (**Figure 2c & d**).

Figure 1: (A) An open pod of *Oroxylum indicum* showing numerous seeds; (B) Seeds of *Oroxylum indicum* (C&D) One-week old germinated dewinged and winged seeds of *Oroxylum indicum* (arrow marked).

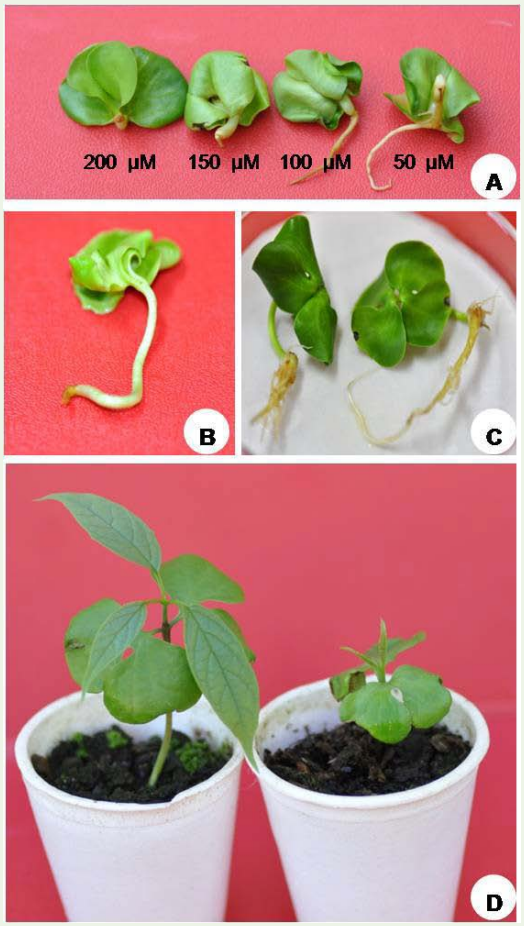

Figure 2:
(A) Three-week old BAP treated *Oroxylum indicum* seedlings showing decrease in radicle length with increase in BAP concentration.
(B) Three-week old GA_3 treated *Oroxylum indicum* seedling having long hypocotyl and rudimentary root.
(C) Three-week old cold water treated *Oroxylum indicum* seedlings having green leaves and well developed roots and root hair.
(D) Cold pretreated (left) and control (right) *Oroxylum indicum* plants after 9 weeks

In comparison to cold water pre-treatment, the effect of hot water on the seeds of *Oroxylum* gave adverse result. This shows that hot water pre-treatment is not an appropriate pre-treatment technology in the seeds of *Oroxylum*.

Discussion

The propagation of plants by seeds is easy, fast and most preferred method of plant conservation because it preserves genetic variations. However, seeds of many plant species specifically trees exhibit dormancy and fail to germinate even in favourable conditions. Depending on the plant species and type of dormancy, various methods like scarification, pre-treatment with growth regulators and

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Table 1: Effect of BAP, GA_3 and cold water pre-treatments on mean germination time, mean germination rate, uncertainty and synchrony of *Oroxylum indicum*.

Treatment	Mean Germination Time	Mean Germination Rate	Uncertainty	Synchrony
Control	9.45^{a}	0.10^{h}	2.47^{ab}	0.11^{d}
GA_3 (µM)				
50	6.88^{c}	0.15^{e}	2.22^{bcd}	0.14^{cd}
100	6.27^{d}	0.16^{cd}	1.37^{f}	0.21^{a}
150	5.41^{f}	0.19^{a}	1.80^{e}	0.21^{a}
200	5.68^{ef}	0.18^{ab}	2.18^{cd}	0.19^{abc}
BAP (µM)				
50	8.99^{a}	0.11^{gh}	2.65^{a}	0.10^{d}
100	7.94^{b}	0.13^{f}	2.37^{bcd}	0.16^{abc}
150	8.16^{b}	0.12^{fg}	2.44^{abc}	0.15^{bcd}
200	8.06^{b}	0.12^{f}	2.34^{bcd}	0.18^{abc}
Cold (4°C)				
6h	6.40^{d}	0.15^{de}	2.27^{bcd}	0.17^{abc}
12h	6.38^{d}	0.16^{de}	2.15^{d}	0.17^{abc}
24h	5.90^{de}	0.17^{bc}	2.25^{bcd}	0.17^{abc}
48h	6.32^{d}	0.16^{cd}	2.08^{d}	0.19^{ab}

Data are mean value (n =3). Values in a column followed by different letters are significantly (p, 0.05) different according to Duncan's test.

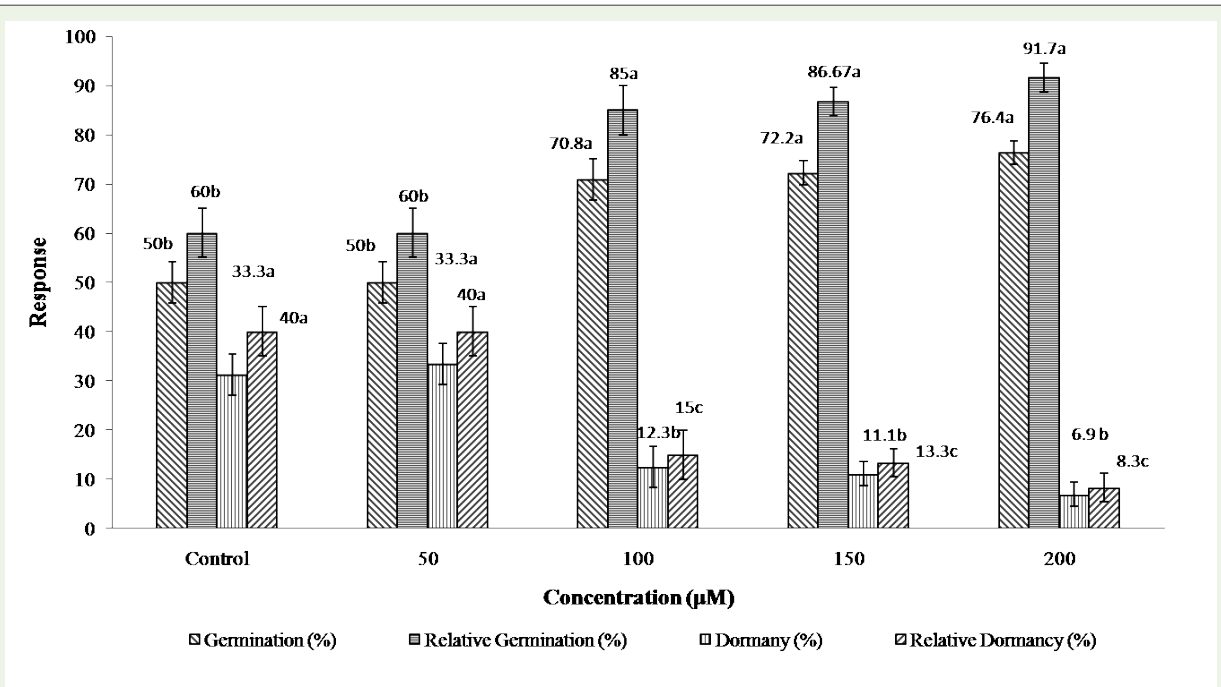

Figure 3: Effect of BAP treatment on seed germination response of Oroxylum indicum. Data are means (n =3) ± standard deviation (n = 3). Values in a column followed by different letters are significantly (p, 0.05) different according to Duncan's test.

temperature shocks are used to break dormancy [16]. In the present study, to break *Oroxylum* seed dormancy, different germination experiments were carried out to investigate the effect of plant growth regulators (viz. GA_3, BAP and IAA), scarification and temperature (cold and hot water) on germination response of *Oroxylum* seeds. Despite of being a high value plant, so far, there is not much documentation on *Oroxylum* seed germination; therefore, much more insights are needed on this aspect for large scale propagation of this tree.

Obtaining commercially useful seedlings of economically important *Oroxylum* tree is hindered by innumerable factors

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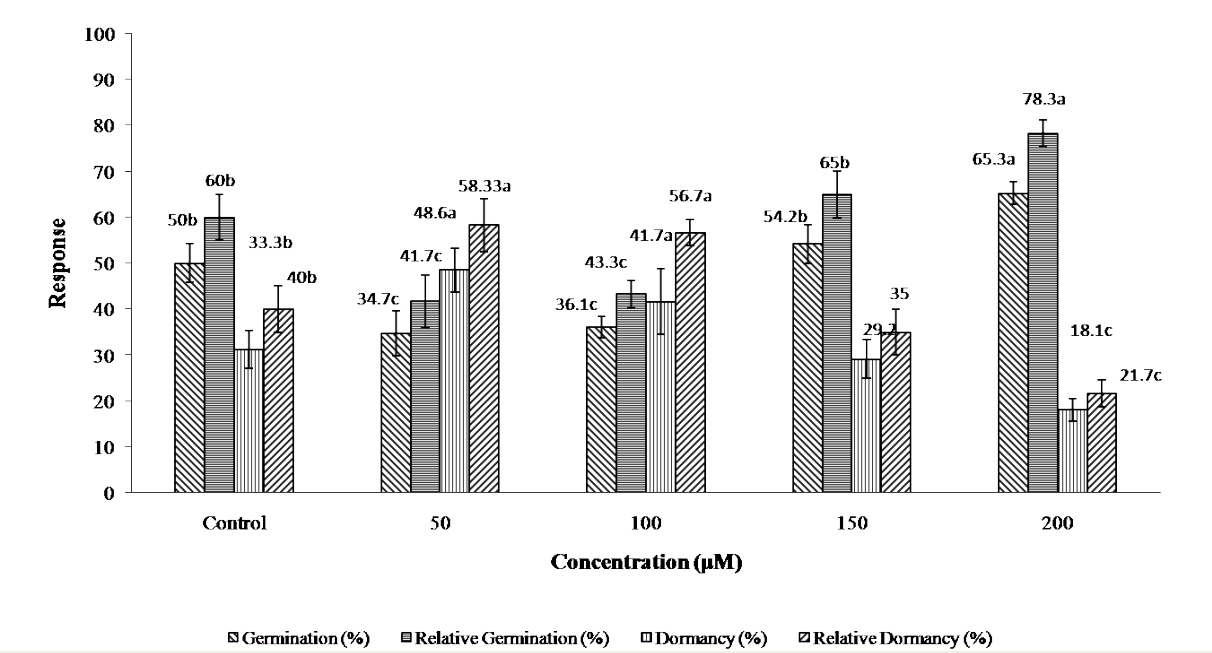

Figure 4: Effect of GA_3 treatment on seed germination response of *Oroxylum indicum*. Data are means (n =3) ± standard deviation (n = 3). Values in a column followed by different letters are significantly (p, 0.05) different according to Duncan's test.

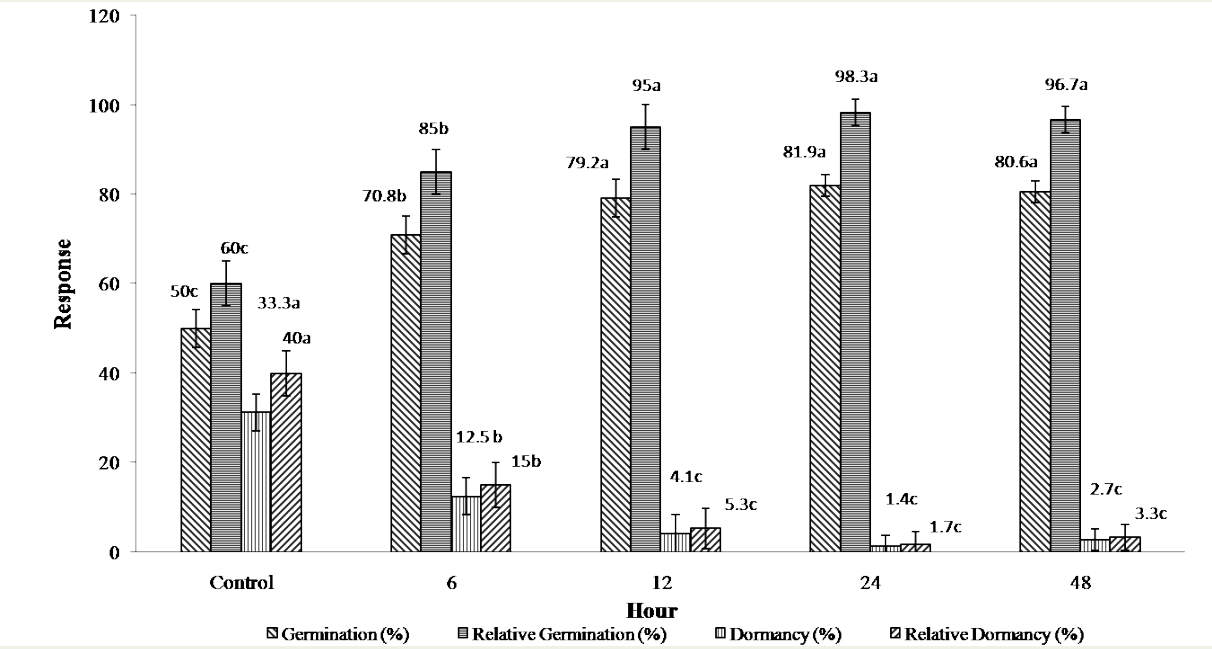

Figure 5: Effect of cold treatment on seed germination response of *Oroxylum indicum*. Data are means (n =3) ± standard deviation (n = 3). Values in a column followed by different letters are significantly (p, 0.05) different according to Duncan's test.

including uneven germination, reducing seed longevity and hence viability [11,12]. The results of viability test using TTC showed that the seed viability decreased from 83.33 to 41.67 % after six months of storage at room temperature. Loss of moisture content and very low reserve of nutrients are most probably the main reasons of *Oroxylum* seed deterioration during storage conditions. Similar studies were carried on viability testing of the seeds using TTC in *Magnolia officinalis* and *Panax notoginseng* [17]. Viability study using TTC is very important in seed trade, crop production and also in germplasm conservation and management.

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Scarification increases the seed germination rate of a number of plant species [18,19] indicating that seed outer covering impermeability to water and/or gases may be involved in the seed dormancy. However, in the present study, scarification viz. dewinging and H_2SO_4 showed negative effect. Dewinged seeds though germinated but their germination success was very low. Moreover, seedlings developed from dewinged seeds showed stunted growth. Seeds treated with H_2SO_4 did not show any response. This might be due to the presence of thin seed coat in *Oroxylum* as a consequence of which acid have ruptured vital parts of the embryo.

Plant seed germination is controlled by specific endogenous growth promoting and inhibiting compounds [20] and there is a strong correlation among applied hormones, hormone concentration, specific developmental stage and metabolic activities [21]. Plant growth regulators in minute quantities are known to enhance the germination percentage and reduce the germination time. In the present study, auxin (IAA), cytokinin (BAP) and gibberelic acid (GA_3) were tested at different concentrations to improve the seed germination response of *Oroxylum*. In comparison to control, BAP and GA_3 improved seed germination at optimum concentrations. However, IAA effect was found to be inhibitory. Pre-sowing seed treatments with growth substances such as plant growth regulators have been found to improve the seedling growth of many other species [22-26].

The effect of cold pre-treatment on seed dormancy breaking has also been confirmed in some other plants such as: *Capparis ovate* [27], *Ferula ovina* [28] and *Pinus roxburghii* [29]. It has been reported that the promontory effect of cold treatment on seed germination might be due to increase in the level of the organic phosphates like fructose 2,6-biphosphate [30], ATP [31] and nucleotides [32]. Several scientists have confirmed this elucidation.

Conclusion

The results suggest that the seeds of *Oroxylum indicum* could be germinated without pre-treatment, however, to attain higher germination percentage and reduced dormancy period, the seeds should be pre-treated with cold water. Cold water treatment at 4 °C for 24h seems to be most effective method and could be easily adopted by the potential farmers for economic cultivation of this species.

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