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# Effect of Pre-sowing Seed Treatments in *Hypericum perforatum* L: A High Value Medicinal Plant

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**ABSTRACT** *Hypericum perforatum* L. is a high value vulnerable medicinal plant of the Indian Himalayan Region. Seeds of this species showed poor germination. In present study, of the total 15 pre-sowing treatments tried, 13 significantly ($P < 0.05$) stimulated seed-germination, where $GA_3$ (150 μM), $KNO_3$ (150 mM) and $NaHClO_3$ (15 minutes) were found to be most effective. The control showed only 27.8% germination while it increased highest upto 71.1% by $KNO_3$ (150 mM). These treatments were also effective in reducing the time required for germination and mean germination time. In view of the low cost and easy applicability of $KNO_3$ and $NaHClO_3$ compared to the expensive and technically complicated plant growth regulators, these chemicals could be used by poor and unskilled farmers of the Indian Himalayan Region for the *ex-situ* cultivation of *H. perforatum*.

Key words: Chemical treatments, germination. *Hypericum perforatum*, medicinal plant

*Hypericum perforatum* L. (Family Hypericaceae), a high value vulnerable medicinal herb, is native to the Europe, western Asia and North Africa, and is widely distributed throughout the temperate areas of the World. In India, it is distributed in Northwestern Himalaya including Himachal Pradesh [1]. It is commonly known as St. John's Worth and grows mostly in open grassy slopes along with shrubberies at altitudes of 1000-3000 m amsl [2]. The St. John's Worth is very popular medicinal plant today in different countries of the world and is one of the top 15 selling popular herbs in USA and Europe [3].

In the Indian Himalayan Region (IHR), due to over exploitation for pharmaceutical industries *H. perforatum* has depleted from its habitat. Therefore, this species has been categorized as vulnerable for the Himachal Pradesh, Jammu & Kashmir and Uttarakhand [4]. Based on market demand and supply, this species has been identified as potential, commercially viable medicinal plant for *ex-situ* cultivation in the Himachal Pradesh [5].

Although, *H. perforatum* can be propagated by both seeds and vegetative means, but, its seeds generally exhibit very low germination due to dormancy [6]. This is due to the chemical inhibitor (exudate) from the capsule [7]. Moreover, the difficulty in cultivating this plant using vegetative means constrains its production [8]. During 2002-03, some farmers of district Lahaul and Spiti. Himachal Pradesh, India tried to cultivate this species by seeds, where they found no germination (*personal communication*).

The review of literature indicates that information on seed germination in *H. perforatum* is very sparse in the IHR particularly from the Himachal Pradesh [9]. In view of its vulnerable status and economic importance, the study was undertaken to examine the effect of various pre-sowing seed treatments using different concentrations of various chemicals to ascertain the best treatments for breaking dormancy and accelerating the germination rate.

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## MATERIALS AND METHODS

The mature seeds of *Hypericum perforatum* were collected during October 2004 from the Lahaul Valley (altitude, 2600m amsl), district Lahaul & Spiti, Himachal Pradesh. These were dried at room temperature (20±5.0°C) for two weeks and stored in hermetic containers at 4°C in refrigerator until used experimentally. During April 2005, seeds were washed with tap water to remove chemical inhibitors [7] and then soaked in various test solutions viz., gibberellic acid ($GA_3$; 50, 100 & 150 μM); Indole acetic acid (IAA; 50, 100 & 150 μM) and two nitrogenous compounds, thiourea ($CH_4N_2S$: 50, 100 & 150 μM) and potassium nitrate ($KNO_3$; 50, 100 & 150 μM) for 24 hr at 25±2°C in dark. In addition to control (double distilled water), seeds were soaked in Sodium hypochlorite ($NaHClO_3$, 5% available chlorine) solution for 5, 10 and 15 minutes. Subsequently, treated seeds were rinsed thoroughly with double distilled water, and 30 seeds of each treatment in triplicate were placed in Petri dishes (9 cm diameter) lined with moistened filter paper (Qualigens 615 A). The entire set of experiment was placed in BOD at 25°C in alternate light and darkness (16 hr light and 8 hr dark) regimes. The seeds were observed daily and considered to have germinated when radicle was visible (1 mm). The final germination was determined on finding constant readings upto two weeks. In every week, germinated seedlings were transferred to the nursery pots for further development. Mean Germination Time (MGT) was calculated using the equation, $MGT = \Sigma(fx)/\Sigma x$, where $x$ is the number of newly germinated seeds on each day, and $f$ is the number of days after seeds were set to germinate [8].

The data were analyzed statistically using MS Excel 2000. To ascertain the effect of pre-sowing treatments compared to control, analysis of variance was carried out and least significant difference ($P < 0.05$) was estimated [9].

## RESULTS AND DISCUSSION

The statistical analysis of experimental results on seed germination is shown in Table 1. In addition to control, total 15 treatments were tried. Thirteen stimulated seed germinated significantly ($P < 0.05$) over control. The seeds treated with $NaHClO_3$ for 15 minutes germinated earliest i.e., after 6 days of sowing. However, control took 11 days to initiate germination. Further, about 50 per cent germination was achieved within 19 days of sowing under the influence of two best treatments viz., $GA_3$ (150 μM) and $KNO_3$ (100 mM). However, 28 days were taken by control for obtaining the same value. Likewise, these treatments completed germination significantly ($P < 0.05$) earlier (24.0 days and 28.67 days, respectively) than control (89.67 days; Table 1). Earlier studies on this species have suggested positive effect of light in breaking seed dormancy [10, 11]. However, in present case, delayed and low germination in control (27.8%) as compared to that in treatments, revealed that only light condition is not enough to overcome seed dormancy in this species, pre-sowing chemical treatments are necessary to break the dormancy.

Gibberellic acid is known for breaking seed dormancy and promoting germination by increasing the embryo growth potential and/or by reducing the mechanical constraint [12]. In present case, amongst its different concentrations, 150 mM was found most effective ($P < 0.05$) in stimulating germination (70.0%) and reducing MGT (17 days) over control (27.8% and 25 days, respectively).

The IAA is well recognized as germination promoter for many species including medicinal plants [13, 14]. It plays a major role in cell division and differentiation [15]. In the present study, the medium concentration (100 μM) of IAA showed significantly ($P < 0.05$) positive effect on germination and MGT. However, its high and low concentrations were slightly effective.

The stimulatory effects of $KNO_3$ on seed germination in the Himalayan medicinal plants are widely reported [16, 17 & 18]. In the present study, both higher concentrations of $KNO_3$ stimulated germination significantly ($P < 0.05$) over control, whereas, the highest concentration (150 mM) resulted into maximum germination (71.11%) and lowest MGT (19 days). Such stimulatory effect of $KNO_3$ may possibly be through oxidized forms of nitrogen causing a shift in respiratory metabolism to the pentose phosphate pathway [19].

In case of thiourea, its only medium concentration (100 μM) had better germination as

**Table 1. Effect of different pre-sowing chemical treatments on seed germination of *Hypericum perforatum* L.**

| Treatments | Days taken to initiate germination | Days taken for 50% germination | Days taken to complete germination | Mean germination (%) | Mean germination time (days) |
|---|---|---|---|---|---|
| Control | 11.0±5.00 | 27.67±10.12 | 89.67±3.51 | 27.78±5.09 | 24.53±5.27 |
| $GA_3$ (50 µm) | 8.00±2.0 | 24.0±12.29 | 45.33±3.21 | 35.56±1.92* | 26.55±6.62 |
| $GA_3$ (100 µm) | 13.3±4.93 | 25.67±8.33 | 32.0±14.0 | 28.89±3.85* | 24.09±7.09 |
| $GA_3$ (150 µm) | 7.33±0.58 | 18.67±3.21 | 24.0±1.0 | 70.00±3.33* | 17.18±1.88 |
| IAA (50 µm) | 9.00±1.73 | 14.0±2.65 | 63.67±18.0 | 32.22±1.92* | 21.12±2.89 |
| IAA (100 µm) | 8.33±0.58 | 11.33±1.53 | 62.0±12.17 | 42.22±3.85* | 20.47±7.63 |
| IAA 150 µm) | 8.33±1.15 | 16.33±2.52 | 88.0±4.0 | 26.67±3.33ns | 30.27±6.43 |
| $KNO_3$ (50mM) | 9.67±1.15 | 15.67±4.16 | 52.33±23.4 | 27.78±1.92ns | 27.23±4.34 |
| $KNO_3$ (100mM) | 8.67±0.58 | 18.33±3.21 | 39.0±7.0 | 51.11±5.09* | 22.60±2.97 |
| $KNO_3$ (150 mM) | 8.0±1.73 | 19.33±7.51 | 28.67±7.23 | 71.11±5.09* | 19.15±6.62 |
| Thiourea (50mM) | 10.7±0.58 | 19.33±5.86 | 64.33±27.0 | 28.89±1.92* | 27.79±10.92 |
| Thiourea (100mM) | 10.0±0.00 | 32.33±6.66 | 84.0±24.4 | 42.22±12.6* | 39.50±5.96 |
| Thiourea (150mM) | 9.00±1.00 | 14.00±1.00 | 57.67±16.2 | 36.67±10.0* | 21.01±2.06 |
| $NaHClO_3$ (5 minutes) | 6.67±0.58 | 42.33±2.31 | 90.0±11.4 | 34.44±8.39* | 41.47±7.83 |
| $NaHClO_3$ (10 minutes) | 11.3±9.24 | 38.33±3.06 | 54.67±15.0 | 38.89±1.92* | 36.06±2.39 |
| $NaHClO_3$ (15 minutes) | 6.33±0.58 | 31.67±15.70 | 56.33±15.3 | 51.11±1.92* | 29.88±4.32 |
| LSD ($P < 0.05$) | 5.10 | 11.56 | 24.73 | 0.91 | 9.75 |
| F | 1.077ns | 5.233* | 6.288* | 19.30* | 4.492* |

*Significant at $P < 0.05$ level over control; ns: non-significant

compared to control. Both positive [18] and detrimental results [20] of thiourea on seed germination in different plant species are reported.

The sodium hypochlorite is a common surface disinfectant, which has also been used as a means of overcoming seed dormancy by either increasing permeability of seed coats to oxygen through the removal of phenolics [21] or by scarification or other modification of the seed coat [22]. Its treatment upto 30 minutes is recommended for stimulating germination in some medicinal plants [16, 17]. In the present study, all the three soaking periods with $NaHClO_3$ significantly ($P < 0.05$) enhanced seed germination over control. However, the highest germination (51.1%) was achieved by treating seeds for 15 minutes. The same treatment also curtailed days taken to initiate germination (6 days) remarkably over control (11 days). The early germination in treated seeds might be due to altered physiology of embryos, liberating enzymes and helping rapid production of soluble food nutrients, resulting in occurrence of developmental processes more rapidly after sowing [23]. The above treatments would help in the development of early germinating seedlings by providing them a higher competitive ability and then reducing mortality in the field condition.

The results of present study revealed that pre-sowing treatments of $GA_3$ (150 μM) and $KNO_3$ (150 mM) are effective in breaking dormancy, reducing the time required for germination, MGT and increasing total germination in *H. perforatum*. However, $NaHClO_3$ (15 minutes) could be used only for germination stimulation. These results have significance in view of comparing expensive plant growth regulators, the use of $KNO_3$ and $NaHClO_3$ owing to their low cost and easy applicability, would be a beneficial tool to mass multiplication, and can widely be used by poor and unskilled farmers for *ex-situ* cultivation of this species, also, by the academicians interested on the propagation research of this species throughout the IHR.

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