



## SEED DORMANCY/GERMINATION STATUS OF FRESHLY HARVESTED SEEDS OF PUSILLA (*ELSHOLTZIA ERIOSTACHYA*); A MEDICINAL PLANT SPECIES OF HIMACHAL PRADESH

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### ABSTRACT

*Pusilla (Elsholtzia eriostachya)* is an important medicinal plant species of Himachal Pradesh. Besides having medicinal value this plant species is also aromatic in nature. The above said plant species is collected from Lahaul and Spiti region of Himachal Pradesh. The seeds of this plant species exhibited deep dormancy. In the present study we will discuss the germination of freshly harvested seeds which will indicate the dormancy status of this medicinal plant.

**KEYWORDS :** Seed, Germination, Medicinal Herb, Aromatic

### INTRODUCTION:

The present study is focussed on dormancy/ germination of freshly harvested seeds of *Pusilla (Elsholtzia eriostachya)* belongs to family Lamiaceae (Kachroo, 1977), which is an aromatic and medicinal herb. The height of this plant is about 15-37 cm and bears purple coloured flowers. The seeds of this plant are collected from Lahaul and Spiti region of Himachal Pradesh. The entire region of this district is dry with annual rain fall of about 17 cm (Bajpai, 1987; Kapadia, 1996). This tribal region is rich in ethnobotanical and cultural heritage. The region has a number of medicinal plants scattered throughout the area of study, among which "Pusilla" is one of the important medicinal plant species. Different species of this plant has been used in north east Asia as an ingredient of folk medicines for treating cough, headache and inflammation (Kim et al., 2003).

### MATERIALS AND METHODS:

**Seed collection:** The seeds were collected from the wild populations and separated manually. The seeds were air dried and stored in plastic air tight jars at room temperature for subsequent studies. Seeds of this medicinal plant species were collected from pattan valley of Lahaul and Spiti district, Himachal Pradesh, during August-September.

**Seed germination assays:** The seeds selected for uniformity were surface sterilized and soaked in distilled water for 24 h at  $25 \pm 2^\circ\text{C}$ . The seeds were transferred to Petri dishes lined with three layers of filter papers moistened with distilled water and allowed to germinate in the seed germinator at  $25 \pm 2^\circ\text{C}$  under continuous illumination provided by the fluorescent white light (PAR:  $40 \text{ mol m}^{-2} \text{ sec}^{-1}$ ). Emergence of 2-5 mm radicle was taken as seed germination (ISTA, 1966). Seed germination percentage was recorded at periodic intervals until the final count.

### Physico-chemical and hormonal treatments for dormancy removal

**Stratification:** The surface sterilized seeds soaked in distilled water for 24 h, were subjected to low temperature ( $2-4^\circ\text{C}$ ) treatment in a refrigerator for variable periods.

**Scarification with sulphuric acid ( $\text{H}_2\text{SO}_4$ ):** Seeds were treated with concentrated  $\text{H}_2\text{SO}_4$  (50%) for 3 min. This duration was decided on the basis of certain preliminary experiments. Thereafter, the seeds were washed thoroughly under tap water and soaked in distilled water for 24 h and then transferred to germination conditions as described above.

**Sodium hypochlorite (SHC):** The seeds were first treated with sodium hypochlorite for 3 min. Thereafter, they were washed thoroughly with tap water and transferred to moist substratum for germination.

**Sodium nitroprusside (SNP):** The seeds were surface

sterilized with mercuric chloride and then they were soaked in 1 and 10 mM sol. of SNP for 24 h. Thereafter, they were transferred to the moist substratum for germination. For moistening the substratum, distilled water was used.

**Gibberellic acid ( $\text{GA}_3$ ):** The surface sterilized seeds were kept in aqueous solution of gibberellic acid ( $\text{GA}_3$ , 0.1 and 1.0 mM) for 24 h. Thereafter, the seeds were transferred to moist substratum for germination. For moistening the substratum, distilled water was used.

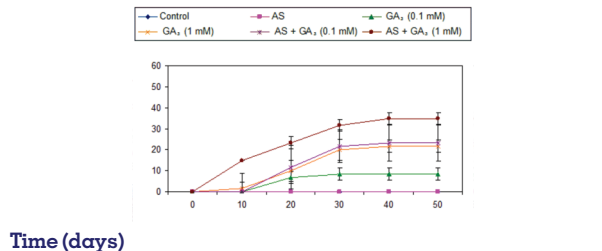
**Combined treatment with  $\text{H}_2\text{SO}_4$  (AS) and  $\text{GA}_3$ :** The seeds were first treated with  $\text{H}_2\text{SO}_4$  (50%) for 3 min. Thereafter, they were washed thoroughly with tap water and soaked in aqueous solution of  $\text{GA}_3$  (0.1 and 1.0 mM) for 24 h. The seeds were then transferred to moist substratum for germination. For moistening the substratum, distilled water was used.

**Combined treatment of leaching (L) and acid scarification (AS) with  $\text{GA}_3$ :** Seeds were surface sterilized with mercuric chloride (0.1%) and then subjected to continuous leaching for 7 days under running tap water. Thereafter, they were scarified with sulphuric acid (3 min.) and soaked in  $\text{GA}_3$  for 24 h. The seeds were then shifted to petri plates for germination.

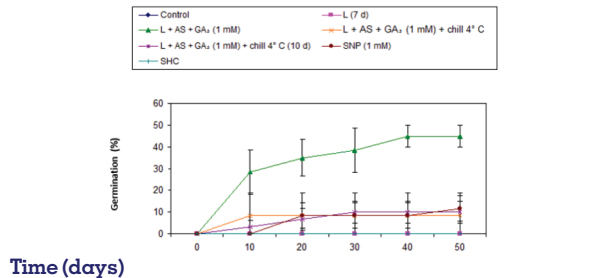
### RESULTS AND DISCUSSION:

Freshly harvested seeds of *Pusilla (E. eriostachya)* exhibited deep dormancy as was apparent from no germination in control for at least 60 d, when seeds were subjected to optimum germination conditions. Various pre-treatments ( $\text{GA}_3$ , SNP, acid scarification (AS), leaching (L), chilling and the stated combined treatments) applied to break the dormancy were effective to varying degrees. Among all the treatments,  $\text{GA}_3$  (1 mM) was found to be most effective in causing the dormancy removal. It was of particular interest to examine whether the magnitude of  $\text{GA}_3$  effect would be further enhanced through application of certain specific physical treatments of seeds prior to  $\text{GA}_3$  treatments. In this regard, besides acid scarification, that was routinely tested for all other species, seed leaching under continuously flowing water and low temperature treatments were considered. The effect of  $\text{GA}_3$  was further enhanced when  $\text{GA}_3$  was applied to acid scarified seeds. Maximum germination was observed with  $\text{GA}_3$  applied to acid scarified seeds after 7 d leaching (L) under running water. Thus, in freshly harvested seeds, 45, 35 and 21% germination with an MGT of 18, 26 and 20 d, with L + AS +  $\text{GA}_3$  (1 mM), AS +  $\text{GA}_3$  (1 mM) and  $\text{GA}_3$  (1 mM) treatment, respectively was observed as compared to no germination in control (Fig. 1, 2). The whole plant contains an essential oil consisting of *Elsholtzia* ketones, thymol, monoterpenes (Bestmann et al., 1995), sesquiterpenes, a trans-bergamotene, rosefuran epoxide (Melkani et al., 1994). The essential oil of *Elsholtzia* plants exhibited antibacterial and antifungal properties (Bestmann et al., 1997). Essential oils extracted from *E. splendens* possess good antibacterial as well as anti-

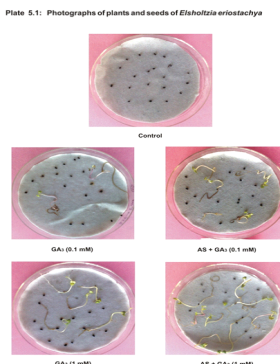
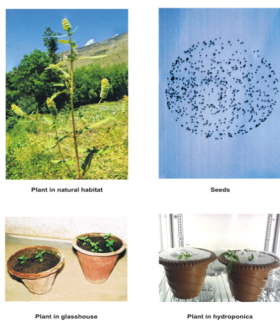
inflammatory activities against acne-inducing bacteria, whereas inducing no cytotoxicity with human cell lines (Kim et al., 2008). *E. splendens* also possesses antioxidant effect (Jeong et al., 2005). Besides being medicinal and aromatic, *Elsholtzia* sps. has a great potential for phytoremediation (Yang et al., 2002; Jing et al., 2004). *E. splendens* is a Cu tolerant plant. Cu is accumulated in vascular tissues of stem and petiole (Shi et al., 2004). *E. haichonvensis* seedlings were able to tolerate upto 250 mole Cu. Phytoavailability of free Cu was higher at higher pH (Jing et al., 2004). Very little work has been done on seed germination or growth of *E. eriostachya*.



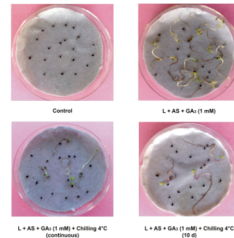
**Fig. 1: Time-course of germination of freshly harvested seeds of *Elsholtzia eriostachya* as affected by acid (H<sub>2</sub>SO<sub>4</sub>) scarification (AS)/GA<sub>3</sub> treatment. Data are average of 3 replicates each ± s.d.**



**Fig. 2 Time-course of germination of seeds of *Elsholtzia eriostachya* as affected by GA<sub>3</sub>, leaching, SNP, SHC and chilling (4°C) applied individually or in desired combinations. Data are average of 3 replicates each ± s.d.**



**Plate 5.2: Photographs showing the effect of GA<sub>3</sub> applied alone or following acid (H<sub>2</sub>SO<sub>4</sub>) scarification (AS) of seeds on germination of freshly harvested seeds of *Elsholtzia eriostachya***



**Plate 5.3: Photographs showing the effect of the combined treatments of leaching, acid scarification, chilling and GA<sub>3</sub> on germination of freshly harvested seeds of *Elsholtzia***

**REFERENCES**

1. Bajpai S.C. 1987. Lahaul- Spiti, a forbidden land in Himalayas. Indus Pub. Co. (New Delhi).
2. Bestmann H.J., Rauscher J., Vostrowsky O., Pant A.K., Mathela C.S. and Singh A.K. 1995. Terepenoids from *Elsholtzia* species. J. Essen. Oil Res. 1(1): 85-87.
3. Bestmann H.J., Rauscher J., Vostrowsky O., Pant A.K. and Mathela C.S. 1997. The volatile constituents of *Elsholtzia flava*. Planta Medica. 63(1): 88-90.
4. International Seed Testing Association. 1966. Proc. Inter. Seed. Testing Assoc. 31: 1-152.
5. Jeong J.H., Sohn H.O., Shin H.J., Hyun H.C., Lee D.W. and Lim H.B. 2005. Biological activities of flavor components extracted from *Elsholtzia ciliata* and *Elsholtzia splendens*. J. Kor. Soc. Tobacco Sci. 27: 19-30.
6. Jing S., Jie Z.F., Ming L.Y., McGrath S.P. and Hao Z. 2004. Copper uptake by *Elsholtzia splendens* and *Silene vulgaris* and assessment of copper phyto availability in contaminated soil. Environ. Pollu. 128 (3): 307-315.
7. Kachroo P., Sapru B.L., Ph.D., Uppendra D. 1977. Flora of Ladhakh. Shiva Printers Onkar Road Dehra Dun. pp. 65.
8. Kapadia H. 1996. Spiti: adventures in the trans- Himalaya. Indus Pub. Co. New Delhi, India.
9. Kim D.W., Son D.W., Chang H.W., Bae K., Kang S.S. and Kim H.P. 2003. Anti-inflammatory activity of *Elsholtzia splendens*. Arch. Pharm. Res. 26(3): 232-236.
10. Kim S.S., Jeong H.O., Jeong S.P., Tai H.O., Pil Y.Y., Kim S.C., Lee H.N. and Chang G.H. 2008. Chemical composition and biological activities of *Elsholtzia splendens* essential oil. J. Appl Biol. Chem. 51(2): 69-72.
11. Melkani A. B., Beauchamp P.S., Dev V., Whalen C., Mathela C.S. and Dev. V. 1994. Rosefuran epoxide from *Elsholtzia densa* Benth. J. Essen. Oil Res. 6(5): 475-479.
12. Shi J.Y., Chen Y.X., Huang Y.Y. and He W. 2004. SRXRF micropoobe as a technique for studying elements distribution in *Elsholtzia splendens*. Micron. 35 (7): 557-564.
13. Yang M.J., Yang X.E. and Romheld V. 2002. Growth and nutrient composition of *Elsholtzia splendens* Nakai under Copper toxicity. J. Plant Nutr. 25(7): 1359-1375.