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DETECTION OF SEED GERMINATION INHIBITORS IN DORMANT SEEDS OF *ANGELICA GLAUCA* EDGEW. AND *POLYGONATUM VERTICILLATUM* (L.) ALL. FROM KINNAUR (HIMACHAL PRADESH)

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Although seed dormancy is advantageous in an ecological context, it hampers the plant regeneration. Seed dormancy could inter alia be ascribed to an array of germination inhibiting substances present in the embryo or other seed parts. In the present study, the presence of germination inhibitors in the dormant seeds of two medicinally important plant species namely, *Angelica glauca* and *Polygonatum verticillatum* from Kinnaur (Himachal Pradesh) has been determined by monitoring the effect of leachate and extract of dormant seeds on germination of seeds of a phytometer species namely, *Brassica juncea.* Due to the treatment with the extract of freshly harvested, 1, 2 and 3 year stored seeds of *A. glauca*, a 39, 46, 32 and 25% inhibition of *B. juncea* seed germination was evident. Similar observations were obtained with seed leachate with some quantitative differences. A comparable suppression of *B. juncea* seed germination was also observed due to the extract/leachate of *P. verticillatum* seeds. The findings reveal the presence of inhibitors involved in seed dormancy regulation of concerned species. Interestingly, there appeared a loss of inhibitors with storage of seeds as revealed by the reduction in the degree of inhibition of *B. juncea* seeds.
Key words: *Angelica glauca, Brassica juncea,* Germination inhibitors, *Polygonatum verticillatum, S*eed dormancy.

The medicinal plants possess some active principles that could be used for therapeutic purpose. They naturally synthesize and accumulate some secondary metabolites e.g. alkaloids, glycosides, tannins, volatile oils, certain minerals and vitamins (Johnson and William 2002). The medicinal plants are currently receiving tremendous global attention due to their erosion and resurgence of interest in herbal medicine. This is primarily because of the fewer side effects and also due to the fact that the herbal preparations have better compatibility with the human body (Trivedi 2006). Himachal Pradesh in Indian Himalayan Region (IHR) is a rich repository of many a medicinally important plant species. The medicinal plant populations from high altitudes generally yield superior active principles as compared to their lower altitude counterparts (Sharma *et al.* 2000). Owing to multiple factors such as overuse of the plants, collection by unskilled workers, degradation of plant habitats, several high value medicinal plant species of the region are getting depleted at an alarming rate. In the prevailing circumstances, the affected plant species are placed at diverse threat status ranging from low risk, near threatened to critically endangered (Uniyal *et al.*, 2002, Ved *et al*., 1998, 2003, Kala 2003, Badola and Pal 2003). Therefore, it is of utmost significance to develop and implement appropriate measures aimed at restoration and regeneration of medicinal plant wealth. Among others, *ex-situ* cultivation seems to be an effective strategy that would lower the pressure on natural populations. Out of the common means of plant propagation, the seed based regeneration appears most convenient. However, this is hampered by seed dormancy (Bewley and Black 1982) that is otherwise advantageous in the ecological context. Dormancy is a block to the completion of germination of an intact viable seed under favourable conditions (Hillhorst 1995, Bewley 1997, Li and Foley 1997). It is often encountered in the seeds of temperate zone plants. Embryo dormancy refers to the failure of viable, mature embryo to germinate even when it has been isolated from the seed. This could be ascribed to an array of germination inhibiting substances present in the embryo. Some of the naturally occurring inhibitors are aldehydes, alkaloids, ammonia releasing substances, essential oils, various organic acids, phenols, unsaturated lactones and cyanide releasing complexes (Bewley and Black 1982). The alleviation of this type of dormancy relies on the loss of inhibitors e.g. through leaching, chilling (Villiers and Wareing 1965, Hamilton and Carpenter 1976). The GA application also relieves this type of dormancy as the seed germination promoter to

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inhibitor ratio could be favourably altered. In the present study, the seed germination inhibitors have been monitored by assessing the impact of extract and leachate from dormant seeds of *Angelica glauca* and *P. verticillatum,* two important medicinal plant species from Kinnaur (H. P.). *A. glauca* Edgew, endemic to the Indian Himalaya (Badola and Aitken, 2003), is an endangered medicinal herb (Ved *et al.* 2003). Its natural populations in H. P. are very low, which may be, among other reasons, due to poor seed germination. Seed dormancy and irregular patterns of seed germination in *A. glauca* have been suggested (Butola and Badola 2004). *P. verticillatum,* commonly known as salam misri, is used in various Ayurvedic formulations. *P. verticillatum* is categorized as vulnerable in Himachal Pradesh (Ved *et al*.003). Detection of germination inhibitors, if any, in the dormant seeds is of significance in developing the dormancy removal measures.

MATERIALS AND METHODS

Seed source:

Seeds of *Angelica glauca* Edgew. and *Polygonatum verticillatum* (L.) All. were collected from from wild populations in Nichar (2110 m amsl) in Kinnaur district (Himachal Pradesh) during August-October 2007 and same duration of subsequent years. The seeds were separated manually from the fruits, air-dried for about a fortnight at room temperature and stored in polyethylene jars under ambient conditions until they were used. The seeds were subjected to detection of inhibitors following harvest and subsequently at regular intervals during a storage period of three years. The seeds of *Brassica juncea* cv. Jai Kisan were procured from Indian Agriculture Research Institute, New Delhi.

Detection of germination inhibitors based on phytometer test:

The presence of germination inhibitors, if any, in the dormant seeds was determined by monitoring the effect of leachate and seed extract of dormant seeds on the germination of seeds *Brassica juncea*, employed as a phytometer species (Sharma et al., 1986). The seed extract was prepared by homogenising the seeds (number variable in a species specific manner) in 60 ml distilled H_2O which was used as a source of inhibitor(s), if any. Alternatively, the seeds were soaked in 60 ml distilled H_2O for 24 h. Thereafter, the external medium was used as seed leachate. The surface sterilized seeds of *B. juncea*, used as a phytometer, were soaked in leachate or extract, prepared from the dormant seeds. In case of control, the seeds were soaked in distilled water for 24 h. Thereafter, the seeds were transferred to Petri dishes lined with moist (H_2O) filter papers for germination. Germination of *B. juncea* seeds was recorded at regular intervals. The degree of inhibition reflected the relative presence of seed germination inhibitors. The experiments were done in triplicate and repeated once. The data are presented as arithmetic means and standard deviation

RESULTS

The seeds of *A. glauca* and *P. verticillatum* did not germinate when subjected to favourable conditions for 20 d and 25 d, respectively. Thus they exhibited a high degree of dormancy (data not shown). Therefore, the seed germination inhibitors, if any, were determined.

Effect of seed leachate or extract of *A. glauca* on seed germination of *Brassica juncea*

The aqueous leachate or extract prepared from freshly harvested as well as differentially stored seeds of *A. glauca* suppressed the germination of seeds of *B. juncea*. The degree of suppression of *B. juncea* seed germination by the leachate of freshly harvested seeds, observed initially (41%) i.e. after 1 d incubation was marginally reduced to 32% after 5 d of incubation (Fig. 1). Also, the magnitude of inhibition of *B. juncea* seed germination due to the seed leachate of *A. glauca* consistently declined as the storage of *A. glauca* progressed. Thus, 32, 30, 23 and 25% inhibition of *B. juncea* seed germination was observed with the leachate obtained from freshly harvested, 1, 2 and 3-year stored seeds

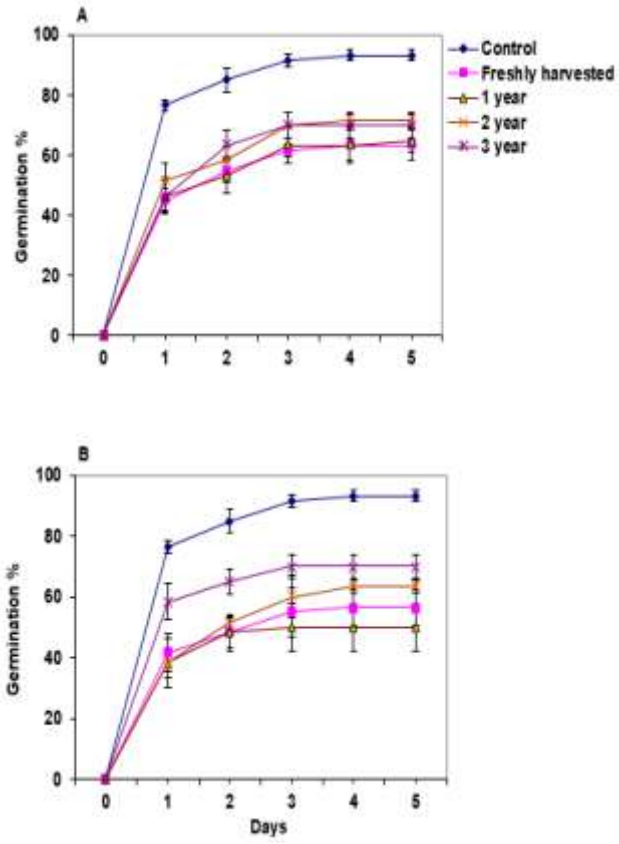

Figure 1. Effect of aqueous seed leachate (A) and extract (B) of *A. glauca* on seed germination of *Brassica juncea*. (Data are arithmetic means of 3 replicates ± S.D.)

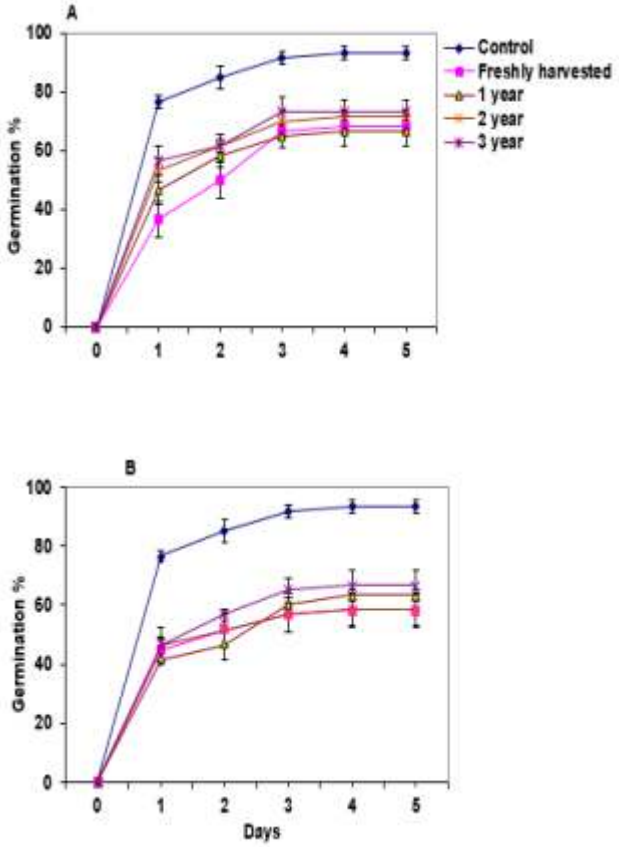

Figure 2. Effect of aqueous seed leachate (A) and extract (B) of *P. verticillatum* on seed germination of *Brassica juncea*. (Data are arithmetic means of 3 replicates ± S.D.)

of *A. glauca*, respectively (Fig. 1 A). Similar observations were obtained with the seed extract of *A. glauca* with some quantitative differences. For example, 39, 46, 32 and 25% inhibition of *B. juncea* seed germination was evident with the extract of freshly harvested, 1, 2 and 3 year stored seeds of *A. glauca*, respectively (Fig. 1 B).

Effect of seed leachate or extract of *P. verticillatum* on seed germination of *Brassica juncea*

As in case of *A. glauca*, the aqueous leachate or extract of freshly harvested and differentially stored seeds of *P. verticillatum* seeds suppressed the germination of seeds of *B. juncea*. The degree of suppression gradually declined with the passage of incubation period. Thus, the leachate of *P. verticillatum* seeds suppressed the germination of *B. juncea* seeds by 52, 41 and 27% after 1, 2 and 3 d incubation, respectively (Fig. 2 A). Also, the leachates from stored seeds were responsible for relatively lesser suppression of *B. juncea* seed germination. Thus, with seed leachate, 27, 29, 23 and 21% inhibition of *B. juncea* seed germination was observed in freshly harvested, 1, 2 and 3-year stored seeds of *P. verticillatum*, respectively. In contrast, 38, 32, 38 and 29% inhibition was observed with seed extract in above stated storage periods, respectively (Fig. 2 B). Other features of seed extract (*P. verticillatum)* induced changes in *B. juncea* seed germination were comparable to those observed in case of seed leachates with some quantitative variations.

DISCUSSION

The aim of present study was to determine the chemical inhibitors in the dormant seeds of *A. glauca* and *P. verticillatum* by monitoring the seed germination response of *B. juncea*,

employed as a phytometer. *B. juncea* seeds were completely non-dormant and exhibited high germination rates in control. The leachate as well as the extract from dormant seeds of concerned medicinal plant species suppressed the seed germination of *B. juncea.* In a straightforward manner this could be ascribed to the presence of certain specific chemical inhibitors in the leachate or extract. Apparently, they were water soluble. We did not determine the chemical nature of these inhibitors. ABA could be an important factor as its role in the regulation of seed dormancy and germination is well established (Mc Court 1999, Koornneef *et al*. 2002). Besides, they could also be phenolic compounds that have been classified as natural growth inhibitors (Kafeli and Kadyrov 1971). The involvement of phenolics in seed dormancy regulation has been suggested (Bewley and Black, 1982; Sharma et al. 1988). Other germination inhibitors include aldehyde, alkaloids, ammonia releasing substances, essential oils, organic acids, phenols and cyanide releasing complexes. Interestingly, the magnitude of seed germination inhibition imposed initially (1 d) by the leachate/extract was reduced subsequently. It seems that the inhibitors were metabolized by the germinating *B. juncea* seeds. Likewise, the findings involving differentially stored seeds revealed the loss of inhibitors with storage. The similarity between the effects of leachate and extract suggest the water solubility of the chemical inhibitors which were able to leak from the seeds upon imbibition. However, the nature of inhibitors present/involved in seed dormancy of *A. glauca* could not be revealed in the present study. The data suggest the presence of some of chemical inhibitors that hampers the seed germination performance in the plant species considered. These might help identify the factors for dormancy removal in the seeds of *A. glauca* and *P. verticillatum* so that optimum germination, and in turn plant regeneration could be achieved.

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