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Storage-dependent Detection of Germination Inhibitors in Seeds of *Aconitum heterophyllum* Wall ex Royle from Kinnaur (Himahcal Pradesh)

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ABSTRACT: Remarkably, the dormancy status could vary even within a species, depending upon the factors such as differences between individuals, location, climatic conditions as well as nature and duration of seed storage after collection. Apparently, seed dormancy is of immense significance in ensuring the survival of plant species during adverse conditions. However, it hampers the seed germination that turns out to be a disadvantage for the seed based propagation/cultivation of plant species. The full realization of the potential of seeds in achieving plant regeneration/cultivation of a plant species depends to a great extent on the understanding of the basic mechanism(s) of dormancy. In the present study, the presence of germination/chemical inhibitors in the dormant seeds of important plant species namely, *Aconitum heterophyllum* 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 seed leachate of freshly harvested, 1, 2 and 3 year stored seeds of *A. heterophyllum*, a 45, 36, 25 and 23% inhibition of *B. juncea* seed germination was evident. Similar observations were obtained with seed extract with some quantitative differences. The findings reveal the presence of chemical inhibitors involved in seed dormancy regulation of concerned plant species. Remarkably, there appeared a loss of inhibitors with storage of seeds as revealed by the reduction in the degree of inhibition of *B. juncea* seeds.

Keywords: *Aconitum heterophyllum; Brassica juncea;* Chemical inhibitors; Seed dormancy and Storage.

INTRODUCTION: The medicinal plants are currently receiving unprecedented attention all over the world due to the reports of their erosion and resurgence of interest in herbal medicine. According to a World Health Organization (WHO) estimate, approximately 80% of the more than 4000 million inhabitants of the world rely on traditional system of medicine, mainly in the developing countries, for primary health care needs (Farnsworth et al., 1995). Besides, in recent years, the herbal medicine has also become acceptable in the developed countries. Many herbal extracts are being used as prescription drugs and nautraceuticals/health food in these countries. More than a decade ago, the market for herbal preparations was estimated to be at about US $ 80-250 billion in USA and Europe (Brower, 1998). As also indicated in the ancient literature, several age-related diseases like liver disorders, asthma, arthritis etc. could be effectively cured by herbal medicine. There are apparent limitations in modern medicine for the cure/management of such diseases (Kamboj, 2000).

The Indian Himalayan Region (IHR) has been reported to support over 1748 (32.2% of India) plant species of known medicinal value. Most of the high altitude medicinal plants of Himalaya are habitat specific (Dhar et al., 2000). H.P. in IHR is particularly significant in the context of medicinal and aromatic plant wealth and the associated indigenous knowledge (Chauhan and Khosla, 1988; Anonymous, 1997). To date, approximately 3,300 species have been recorded from H.P. Of these, 150 species are attributed with diverse medicinal uses. About 100 plants are of confirmed medicinal value. Furthermore, about 40 plant products of medicinal value are regularly supplied by the state. H.P. is associated with the development of Ayurveda; this ancient science of medicine is believed to have its origin in Himachal Pradesh in about 80 B.C. Further, it is believed that Punarvasu Atreya, a great scholar of Ayurveda, lived in Chandra-Bhaga river catchment in Lahaul and Spiti district (H.P.). It is revealed from the survey of literature and the outcome of a CAMP (Conservation Assessment and Management Prioritization)

workshop held at the Himalayan Forest Research Institute (HFRI), Shimla (2003) for assessment of medicinal plants of Jammu and Kashmir, Himachal Pradesh and Uttaranchal that several medicinal plants are at varying levels of threat status in the areas considered. In H.P., there are 12 (18%) critically endangered, 21 (31%) endangered, 27 (40%) vulnerable, 2 (3%) near threatened, 3 (4%) least concern and 3 (4%) data deficient plants (Ved et al., 2003). In view of the risk of extinction, several of these threatened medicinal and aromatic plant species have been included in the negative list of export by the Govt. of India.

MATERIAL AND METHODS:

The area of study: Kinnaur, the north eastern frontier of Himachal Pradesh, is a region of high mountain ranges, enclosing the narrow valley of Satluj and its tributaries (Figure 1). It is situated between 31º 50' 30" and 32º 05´ 15" north latitude and between 77º 45' 30" and 79º 00' 35" east longitude. It is bounded on the East by the Ngari region of Western Tibet; the district is separated from Tibet by the Zanskar mountains. The Dhaula Dhar range of mountains forms its Southern boundary and its separates Uttar-Kashi district of Uttarakhand and Rohru tehsil of Shimla district from it. Srikhand Dhar separates the district from Kullu and Rampur region in the West. In the North, Kinnaur district is separated by the rivers Spiti and Parechu near international boundary with Tibet (Aswal and Mehrotra, 1994).

Figure 1: Glimpse of the study area Kinnaur (Himachal Pradesh).

Kinnaur is particularly significant so far as the medicinal plant wealth is concerned. It is of interest that the medicinal plant populations from high altitudes generally yield markedly superior active principles as compared to their lower altitude counterparts (Sharma et al., 2000). As a consequence to multiple factors, several high value medicinal plant species of the region are getting depleted at an alarming rate. Such factors, among others, include overuse of the plants, collection by unskilled workers, degradation of plant habitats by soil erosion, landslides, grazing and lack of cultivation of medicinal plants.

The plant species under study is critically endangered as an indiscriminate and unscientific extraction of tubers of *A. heterophyllum* in large quantities has reduced it towards rarity (IUCN, 1993; Nautiyal et al., 2002; Ved et al., 2003). Vegetative propagation of *A. heterophyllum* is slow as well as time consuming to achieve a large scale production of propagules. Likewise, the propagation through seeds is seriously limited with seed dormancy. Moreover, seed germination in many medicinal plants belonging to the family Ranunculaceae has been reported to be restricted by different kinds of environmental factors or due to underdeveloped embryo which lead to morphological and/or physiological dormancy (Baskin and Baskin, 2004). However, in the present study, detection of germination inhibitors, if any, in the dormant seeds is of significance in developing the dormancy removal measures.

Seed source: Seeds of the concerned medicinal plant species were collected from Kinnaur district (Himachal Pradesh), during August-October 2007 and same duration of subsequent years. The seeds were collected from wild population. 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 (room temperature) until they were used in described studies. The seeds were subjected to various analyses following harvest and subsequently at regular intervals during a storage period of three years. In order to understand certain specific mechanism(s) involved in seed dormancy/germination of the stated medicinal plants, seeds of *Brassica juncea* cv. Jai Kisan procured from the seed production unit, Indian Agriculture Research Institute, New Delhi were used.

Detection of germination inhibitors based on phytometer test: In order to determine the presence of germination inhibitors, if any, in the dormant seeds of the species under consideration, two methods were employed (Sharma et al., 1986). In these methods the

effect of leachate and seed extract of dormant seeds on the germination of seeds of a phytometer species namely, *Brassica juncea* was monitored. The seed extract of different plant species was prepared by homogenising a certain number of seeds (variable in a species specific manner) in 60 ml H_2O which was used as a source of inhibitor(s), if any. Alternatively, the seeds (number as above) were soaked in distilled water (60 ml) for 24 h. Thereafter, the external medium was used as seed leachate. The seeds of *Brassica juncea* were used as a phytometer. The surface sterilized seeds of *B. juncea* were soaked in leachate or extract, prepared from the dormant seeds as described, for 24 h. In case of control, the seeds were soaked in distilled water for 24 h. Thereafter, the seeds were transferred to moist (H_2O) substratum for germination. Germination of seeds of the phytometer species was recorded at regular intervals. The degree of inhibition reflected the relative presence of seed germination inhibitors.

All the experiments in the present study were carried out in triplicate. Data are presented as arithmetic means and standard deviation.

RESULTS AND DISCUSSIONS:

***Aconitum heterophyllum* Wall ex Royle:** *A. heterophyllum* belonging to family Ranunculaceae is a perennial, erect stemmed herbaceous plant with biennial, paired and tuberous roots. Leaves are more or less heteromorphous with varying shape and size: the lowest (oldest) leaf has long petiole (upto 13 cm); blade orbicular-cordate or ovate-cordate in outline with a usually narrow sinus (1-1.5 cm deep); 5-lobed to middle, amplexicaul. Inflorescence is a slender racemose, leafy panicle, crispopubescent; sepals bluish or violet (rarely whitish), obliquely erect and shortly beaked (18-20 cm high and 8-9 cm wide). Carpels 5, elliptic-oblong, straight (16-18 cm long). Fruits follicles, 16-18 mm long.

Salient features of seeds: The seeds of *A. heterophyllum* are pyramidal; 3-4 mm long, blackish brown with angles more or less winged. The average seed weight of air-dried and H_2O-soaked (24 h) seeds was 5 and 11 mg, respectively. Thus, a 120% increase in seed mass was observed after 24 h soaking of seeds in distilled water (Plate 1).

Evaluation of germination inhibitors in seeds:

Effect of seed leachate or extract of *A. heterophyllum* on seed germination of *Brassica juncea*: The aqueous leachate or extract prepared from freshly harvested as well as from differentially stored seeds of *A. heterophyllum* inhibited the germination of *B. juncea* (used as a phytometer) seeds. Furthermore, the degree of inhibition due to leachate/extract observed in case of freshly harvested seeds were maximum and declined with the storage of seeds. Thus, with *A. heterophyllum* seed leachate, 45% inhibition of *B. juncea* seed germination was observed after 5 d incubation. The magnitude of this inhibition was reduced to 36, 25 and 23% in case of leachate prepared from 1, 2 and 3-year stored seeds, respectively. Similar pattern of inhibition was observed with seed extract with some quantitative variations (Figure 2).

Plate 1: Seeds of *A. heterophyllum* collected from wild habitat.

The present study was considered for the determination the chemical inhibitors in the dormant seeds of *A. heterophyllum* 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. The nature of chemical inhibitors was not elucidated. Abscissic acid (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).

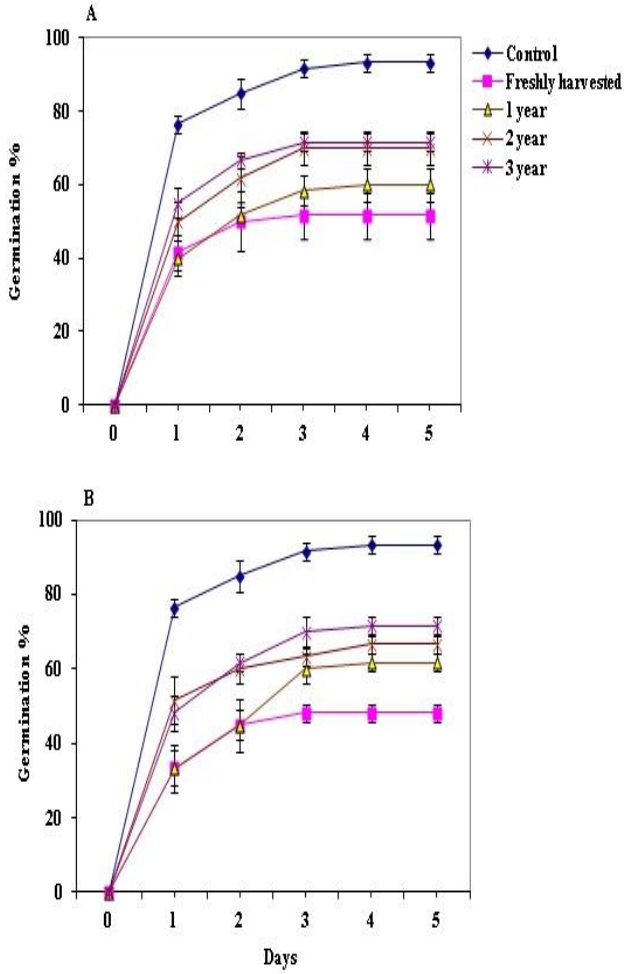

Figure 2: Effect of aqueous seed leachate (A) and extract (B) of *A. heterophyllum* on seed germination of *Brassica juncea*. Data are arithmetic means of 3 replicates ± S.D.

Besides, they could also be phenolic compounds that have been classified as natural growth inhibitors (Kafeli and Kadyrov, 1971). 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. However, the nature of inhibitors present/involved in seed dormancy of *A. heterophyllum* could not be revealed in the present study. The data suggest the presence of some of germination/chemical inhibitors that retard the seed germination performance in the plant species considered. These might help identify the factors for dormancy removal in the seeds of *A. heterophyllum* so that optimum germination, and in turn plant regeneration could be achieved.

CONCLUSION: The findings from the present study suggest that the dormant seeds of *A. heterophyllum* suppressed the germination of *Brassica juncea* (used as phytometer) seeds. This could be taken to be due to some unidentified chemicals inhibitors in the seeds of *A. heterophyllum.*

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