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Seed physiological studies on *Pleurospermum candollii,* an high altitude medicinal herb from cold desert area of Pattan valley, Lahaul and Spiti in Western Himalayas

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ABSTRACT: Himachal Pradesh lies in the Indian Himalayan Region (IHR), is one of the richest reservoirs of biological diversity in the world, having a rich wealth of medicinal and aromatic plant wealth. The *Pleurospermum candollii,* is very important and rare medicinal herb found at very high altitude of 4500m amsl. The various species of Pleurospermum possess medicinal and antioxidant properties. Moreover, some species of Pleurospermum are also used in perfumery. *Pleurospermum candollii* is used in the treatment of dysentery, dyspepsia, renal pain, stomachache and flatulence. The seeds of *Pleurospermum candollii* were deep dormant and have very poor germination (7%), besides having high viability status of 98%. In the present paper seed storage studies on dormancy status and germination status is discussed. The effect of various germination inhibitor tests on germination of various phytometer species is also studied. The implications of the present research will be very productive and helpful in the seed based conservation of the various medicinal plant species in which the germination is poor and have dormancy.

Keywords: medicinal plants, seed storage, viability, germination and dormancy

INTRODUCTION

Indian Himalayan Region (IHR) is one of the richest reservoirs of biological diversity in the world and extends from Jammu and Kashmir in the north to Arunachal Pradesh in the east, covering a geographical area of 500,000 sq. km and a flora of 18440 species [8 & 10]. Himachal Pradesh (H.P.), in the IHR, is very rich in terms of medicinal and aromatic plant wealth is concerned. *Pleurospermum candollii* Benth. Ex Clarke is an important medicinal herb belonging to the family Apiaceae. The seeds of *Pleurospermum candollii* were collected from Lahaul and Spiti, one of the district of H.P., also known as cold desert[6]. Traditionally this medicinal herb is used by tribals of the Pin Valley National Park, Himachal Pradesh, India, for the treatment of dysentery [9]. Other species of *Pleurospermum* also possess medicinal properties. *P. kamatschaticum* exhibited antioxidant activity [1]. Antioxidant properties are due to the presence of high amount of flavonoids and total phenolic compounds in *P. kamatschaticum* [4]. Antioxidant and vitamins present in *P. kamatschaticum* have preventive effects on degenerative diseases which have been associated with free radical mediated events. The Furanocoumarins derivatives from *P. rivalorum* exhibited inhibitory effects on human drug metabolizing enzyme cytochrome P. 450 3 A (CYP 3A) [7]. Besides being used as medicine, some species of *Pleurospermum* are also used for economic purposes. Another high altitude Himalayan herb, *P. densiflorum* is used in perfumery. A new ester, octacosanyl hexadeconoate was isolated from the aerial parts of *P. densiflorum* and furanocourmarins, psoralen and bergaptenand beta sitosterol were also identified. *P. wrightianum* contained octadencenoic acid and octatetraene [5].

MATERIAL AND METHODS

Seed Collection: The seeds of *Pleurospermum candollii* were collected from wild habitat at an altitude of 4500m amsl in the month of August.

Photograph of the plant *Pleurospermum candollii* in natural habitat

Viability Test: Seed viability was determined, using a biochemical test "Topographical Tetrazolium Test". In this reduction process, the living cells are made visible by the reduction of an indicator. The indicator used is a colourless solution of 2,3,5-triphenyl tetrazolium chloride. The seeds were surface sterilized with 0.1% aqueous solution of mercuric chloride for 3 min. Thereafter, they were washed thoroughly under tap water and soaked in distilled water for 24 h at 25±2^{o}C. Thereafter, the seeds were cut off 1/3rd at the broad end opposite the radicle in order to expose the embryos. Then the seeds were soaked in 0.1% aqueous solution of TTC at 25± 2^{o}C in dark. After 24 h, qualitative viability was determined by counting the coloured embryos. Seeds having a completely stained embryo were considered viable. The experiment was done in triplicate taking 30 seeds of each species studied. The measurements were taken as percent viability.

Seed germination assays: The seeds selected for uniformity were surface sterilized and soaked in distilled water for 24 h at 25±2^{o}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±2^{o}C under continuous illumination provided by the fluorescent white light (PAR: 40 µmol m^{-2} 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. The latter varied in a species- specific manner.

Determination of mean germination time (MGT): The seed germination data were also presented as mean germination time (MGT). MGT was calculated as follows (Hartmann et al., 1989).
MGT = Σ (nd)/N Where,
n = number of seeds germinated after each incubation period in days, d
N = total number of seeds emerged at the end of the test

Physico-chemical and hormonal treatments for dormancy removal: The dormant seeds were subjected to the following Physico-chemical and hormonal treatments for achieving the removal of dormancy and improving seed germination

Stratification: The surface sterilized seeds soaked in distilled water for 24 h, were subjected to low temperature (2- 4^{o}C) treatment in a refrigerator for variable periods (one to two months) for different species. Thereafter, the seeds were subjected to germination conditions as described earlier.

Scarification with sulphuric acid (H_2SO_4): Seeds were treated with concentrated H_2SO_4 (50%) for 2 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.

Potassium nitrate (KNO_3): The surface sterilized seeds were soaked in 0.1% aqueous solution of KNO_3 for 24 h. Thereafter, these were transferred to moist substratum for germination. For moistening the substratum, distilled water was used.

Sodium hypochlorite (SHC): The seeds were first treated with sodium hypochlorite for 3-4 min. depending upon the seed. 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 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 (GA_3): The surface sterilized seeds were kept in aqueous solution of gibberellic acid (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 H_2SO_4 (AS) and GA_3: The seeds of all plants species were first treated with H_2SO_4 (50%) for 2-3 min. Thereafter, they were washed thoroughly with tap water and soaked in aqueous solution of 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.

RESULTS AND DISCUSSION

A) Seed viability: Seed viability of *P. candollii* was determined shortly after collection/harvesting and at regular intervals during 24-month storage period by using 2, 3, 5-triphenyl tetrazolium chloride (TTC) test. Upon 24 h incubation with 0.1% TTC solution, the freshly harvested seeds of *P. candollii* exhibited 98% viability. The viability status remained unchanged for a short period and thereafter, the viability of seeds declined gradually. Thus, at 0, 6, 12, 18 and 24 months storage, 98, 94, 84, 80 and 70% viability, respectively was observed (Fig. 1).

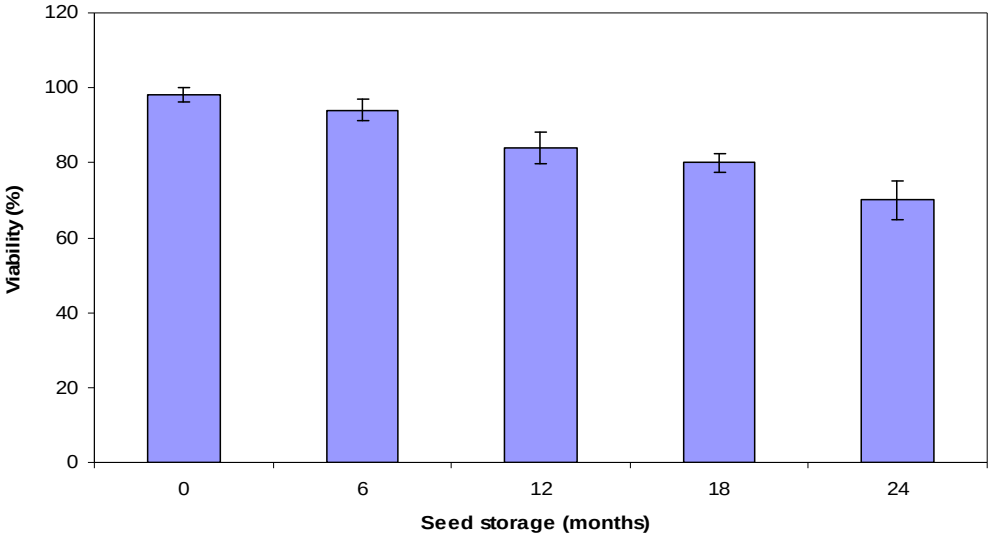

Figure 1: Storage-dependent changes in qualitative seed viability of *Pleurospermum candollii*. Data are average of 3 measurements each ± s.d.

B) Seed dormancy/germination in *Pleurospermum candollii*: The seeds of *P. candollii* were deep dormant as was evident with no germination in control for 70 d. The seeds remained dormant

throughout the storage period of 6-month. Various treatments were applied to alleviate dormancy. Among all the physico-chemical (SNP, SHC, AS, KNO_3 and chilling) and hormonal (GA_3) treatments applied, GA_3, SHC and chilling were found to be effective to a limited extent in alleviation of dormancy. A 7, 7, 4 and 2% germination was achieved by GA_3 (1 mM), chilling 4°C (continuous), sodium hypochlorite and GA_3 (0.1 mM) treatments with an MGT of 40, 50, 59 and 70 d, respectively. All other treatments namely, acid scarification + GA_3 (0.1 and 1 mM), SNP (10 mM), KNO_3 (0.1%), acid scarification and chilling 4°C (30 d), were found to be ineffective in alleviating dormancy (Fig. 2, 3; Table 1). The order of effectiveness of various seed pretreatments was in following order: GA_3 (1 mM) > chilling 4°C (continuous) > sodium hypochlorite > GA_3 (0.1 mM).

Table 1: Storage-dependent changes in mean germination time (MGT) of seeds of *Pleurospermum candollii* as affected by different physico-chemical and hormonal treatments. Data are arithmetic means of 3 replicates each ± s.d.

Mean Germination Time (MGT), days (d)		
Treatments	**Seed storage (months)**	
	0 month	**6 months**
Control	0.00	0.00
AS	0.00	0.00
GA_3 (0.1 mM)	70.00	0.00
GA_3 (1 mM)	40.00	0.00
AS + GA_3 (0.1 mM)	0.00	0.00
AS + GA_3 (1 mM)	0.00	0.00
KNO_3 (0.1%)	0.00	0.00
SNP (10 mM)	0.00	0.00
SHC	58.55	0.00
Chilling 4°C (continuous)	50.00	0.00
Chilling 4°C (30 d)	0.00	0.00

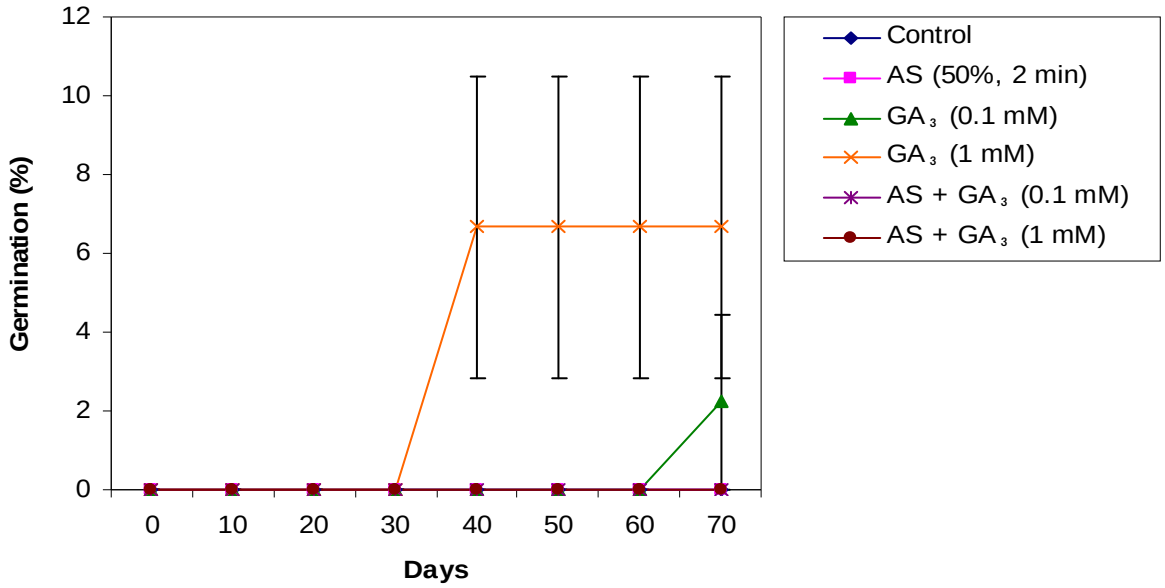

Figure 2: Time-course of germination of 6-month stored seeds of *Pleurospermum candollii* as affected by different physico-chemical and hormonal treatments. Data are average of 3 replicates each ± s.d.

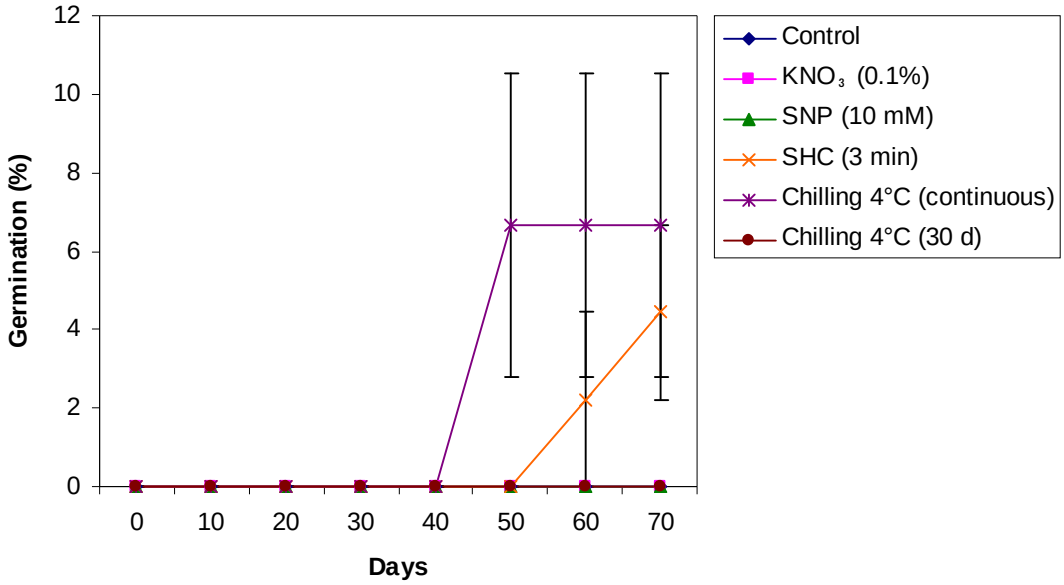

Fig.3: Time-course of germination of 6-month stored seeds of *Pleurospermum candollii* as affected by different physico-chemical and hormonal treatments. Data are average of 3 replicates each ± s.d.

C) Detection of germination inhibitors: The presence of chemical inhibitors in seeds is one of the major causes of seed dormancy. Often the depth of dormancy is found to be correlated, with the level of inhibitors. In order to examine whether the dormancy in seeds is due to the presence of chemical inhibitors, effect of seed extract on TTC reduction by excised embryo axes of *Brassica juncea* and *Vigna radiata* was determined. The seed leachate of *Brassica juncea* and *Vigna radiata* was found to be

inhibitory. The seed homogenate has no effect on seed germination of *Brassica juncea* seeds while in case of *Vigna radiata* was found to be promotory (Fig 4, 5).

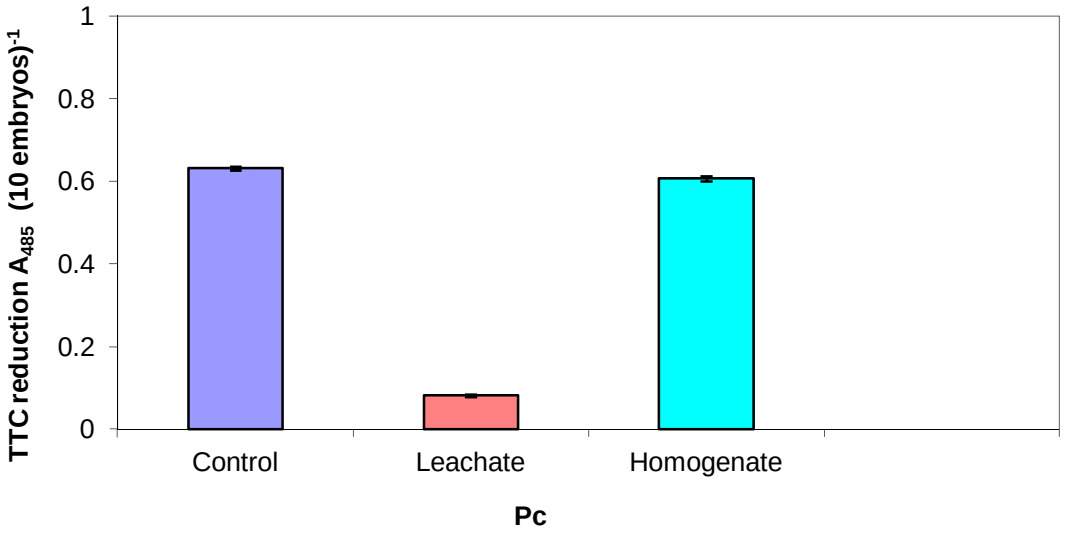

Fig. 4: Effect of aqueous seed leachate and homogenate of 6-month stored seeds of *Pleurospermum candollii (Pc)* on on TTC reduction by excised embryo axes of *Brassica juncea*. Data are average of 3 replicates each ± s.d.

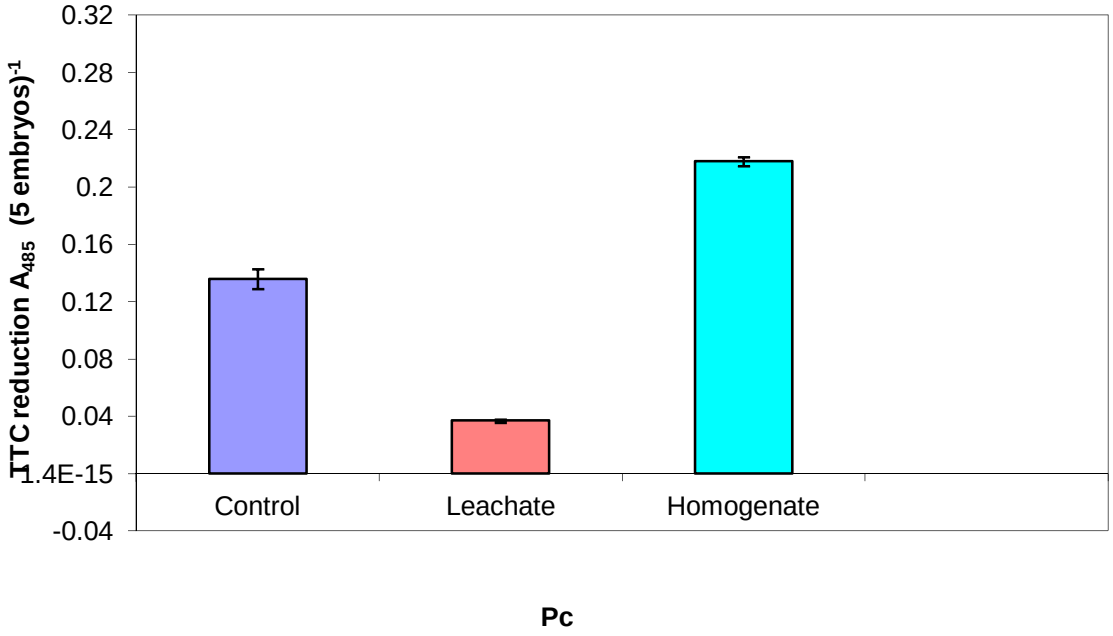

Fig. 5: Effect of aqueous seed leachate and homogenate of 6-month stored seeds of *Pleurospermum candollii (Pc)* on on TTC reduction by excised embryo axes of *Vigna radiata*. Data are average of 3 replicates each ± s.d.

CONCLUSION

P. candollii is an important medicinal and aromatic plant belonging to family Apiaceae. The plant is commonly known as ban and used by tribals of the Pin Valley National Park, Himachal Pradesh, India, for the treatment of dysentery (Srivastava and Sekar, 2004). Antioxidant and vitamins present in *Pleurospermum* sps. are also used in perfumery.

The seeds (freshly harvested) of *P. candollii* showed high (98%) viability status and were deep dormant as was apparent from no germination in control seeds. Very poor germination; only 7% germination could be achieved through chilling and GA_3 pre-treatments. The viability status declined gradually with the storage period of 2 years. The dormancy in *P. candollii* seems to be due to the immaturity of embryos which is alleviated by chilling and GA_3 pre-treatments. Thus, seeds of *P. candollii* possess morphophysiological dormancy (MPD). The phytometer tests indicated the presence of germination inhibitors in the dormant seeds of *P. candollii*. The present study is of considerable significance as the plant species chosen for the study is lesser known but of high medicinal value and have greater potential to be explored.

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