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# Effect of various pre-treatments for breaking the dormancy of *Indigofera linifolia* Retz.

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**Abstract**

In the present investigation, seeds of *Indigofera linifolia* Retz. were subjected to various treatments to achieve early germination by breaking dormancy.

The seeds of this plant species were subjected to various physical and chemical treatments but the highest germination percentage (77%) was achieved for the seeds exposed to alternating high and low temperature. Whereas, the germination percentage was 71 and 65 under the influence of IAA and brassinolide respectively till 17$^{th}$ day of sowing. So, the best method for breaking its dormancy was found to be treatment with alternating high and low temperature.

The seeds of some plants easily germinate after sowing in nature but the seeds of a number of plants do not germinate easily and exhibit dormancy for varying period of time. The dormancy may be due to internal factors or may be due to external factors. Certain plants may immediately germinate after the harvest, it can be best exemplified by the seeds of *Pisum sativum*, which sometimes germinate in the fruit itself which is still on the plant, a phenomenon known as vivipary. However, sometimes the dormancy period is very prolonged and can take months together for germination. This is true for the seeds of *Malus domestica* which has a hard seed coat and *Entada gigas* which has a very thick seed coat and do not germinate easily.

*Indigofera linifolia* Retz. is an erect woody perennial or annual herb of the family Fabaceae with pink flowers and spherical single seeded legumes. It is known by the name *Pandarphali*. It has a great medicinal importance. The seeds are rich in potassium, calcium, $M_n$ and copper. In times of famine, the seeds can be ground and used as a food source. Its seeds are highly dormant.

So, in the present study, seeds of this plant were tested for their germination potential and shortening of dormancy period. There was only 23% germination till 17$^{th}$ day of sowing recorded under untreated seeds. Therefore, it was thought imperative to undertake this investigation to find out the

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substance that can break the dormancy of this plant. The seeds were subjected to various treatments which are mentioned in table 1.

According to Berlyn (1972), germination is a sequential series of morphogenetic events that result in the transformation of an embryo into a seedling. The seeds of every plant have the capability to germinate but their germination is affected due to some factors, such as seed coat, hard seed coat, rudiment embryo, over-ripening, presence of plant growth inhibitors, due to absence of water, oxygen and due to unfavourable conditions. Dormancy of seeds is due to external factors or due to internal factors. When it is caused due to internal factors, it is called as true dormancy or innate dormancy or primary dormancy. And when it is caused due to external factors, it is called as imposed dormancy or quiescent dormancy or secondary dormancy. Both of these primary and secondary dormancy inûuences are mutually dependent and can not be singled out. True dormant seeds do not germinate even if they are provided with suitable environmental factors. Secondary dormant seeds may germinate immediately after shed off. After some storage, they fail to germinate and thus exhibit secondary dormancy. Some seeds such as *Brassica alba, Ambrosia tripolia* and *Xanthium pennsylvanicum* exhibit secondary dormancy. Secondary dormancy is opposite to after ripening. Presence of high carbondioxide concentration, absence of light and very high or low temperature induce the secondary dormancy.

A number of techniques are available for breaking the dormancy of seeds, such as; scarification, exposure to light, alternating high & low temperatures, stratification, impaction, pressure, electric current, pretreatment with coumarins, kinetin, $GA_3$, $H_2SO_4$, thiourea, $KNO_3$ and hot water.

Studies on germination and dormancy of seeds have been carried out by various workers on different types of species. These include; the studies of Shul, (1911) on the oxygen minimum and the germination of *Xanthium* seeds. A detailed account of seed dormancy mechanics was given by Crocker, (1916). Influence of low temperature in improving germination percentage was found out by Conville, (1920). Similarly, alternating temperatures to break the dormancy was used by Harrington, (1923). Morinaga, (1926) has studied the germination of seeds under water.

Davies, (1928) used high pressure to achieve higher seed germination. Denny & Stanton, (1928) suggested chemical treatments for breaking the seed dormancy. Joseph, (1929) investigated the germination and vitality of birch seeds. Barton, (1930) investigated on coniferous seeds. In 1936, Crocker investigated the effect of visible spectrum upon the germination of seeds and fruits. In 1938, Crocker also gave an account of life-span of seeds.

Chouard[5] has investigated vernalization and its relation to dormancy. Experimental induction of dormancy in *Betula pubescens* was investigated by Eagles & Wareing[9]. Evanari[10] has studied the physiology of seed dormancy, after ripening and germination. Ribosome and enzyme changes during maturation and germination of the castor bean seeds was investigated by Marre[15]. Effects

of light, temperature and their interaction on the germination of seeds was investigated by Toole[26].

Hayes & Klein[12] investigated special quality influence of light during development of *Arabidopsis thaliana* plants in regulating seed germination. Bewley and Black,[2] studied the physiology and biochemistry of seeds. Isoenzymes of sugar phosphate metabolism in endosperm of germinating castor beans were studied by Nishimura[20]. Seed germination and dormancy have been studied by Bewley, (1997). Improvement of seed germination in *Asparagus racemosus* has been reported by Gupta, *et al.,*[2].

Effect of pre-sowing treatment on seed germination of *Babchi* (*Psoralea corylifolia*) and Senna (*Cassia angustifolia*) in nursery has been reported by Koppad and Umarbhadsha[14]. Seed germination behaviour of *Asparagus racemosus* (*Shatavari*) under *in-vivo* and *in-vitro* conditions has been investigated by Raghav and Kasera[21]. Siva, *et al.,*[24] have studied the enhanced seed germination of *Psoralea corylifolia* L. by heat treatment. Musara, *et al.,*[18] have investigated the evaluation of different seed dormancy breaking techniques on Okra (*Abelmoschus esculentus* L.) seed germination. Asha and Illa[1], have studied the effect of seed direction and growth media on *in vitro* seed germination and seedling establishment of *Pterocarpus marsupium*.

Cantoro, *et al.,*[4] have reported seed dormancy QTL identification across a *Sorghum bicolor* segregating population. Dave, *et al.,*[6] have investigated the regulation of *Arabidopsis thaliana* seed dormancy and germination by 12-oxo-phytodienoic acid. *Entada phaseoloids* seed dormancy and germination: implications for conservation and restoration has been reported by Deepa, C. and Shinde, N. W. (2016). The effect of the use of temperature on the breakage of dormancy and the subsequent performance of rice (*Oryza* spp.) has been investigated by Doku, *et al.,*[8]. Transcriptome analysis of seed dormancy after rinsing and chilling in ornamental peaches (*Prunus persica*) has been investigated by Kanjana, *et al.,*[13].

Effect of different pretreatments and seed coat on dormancy and germination of seeds of *Senna obustifolia* has been studied by Mensah and Ekeke[16]. Mishra[17], has investigated the effect of temperature and light on the seed germination of *Sida cordifolia*. Redwood, *et al.,*[22] have reported seed longevity and dormancy state in a disturbance-dependent forest herb, Ageratina. Germination pretreatments to break hard-seed dormancy in *Astragalus cicer* L. has been studied by Statwick[25].

Effect of various dormancy breaking treatments on seed germination, seedling growth and seed vigour of medicinal plants has investigated by Warghat, A. R. *et al.,* (2016). Zohra, *et al.,*[28] have reported the effect of salicylic acid on germination of *Ocimum gratissimum* seeds induced into dormancy by chlormequat. The release of dormancy, a wake-up call for seeds to germinate has reported by Nee, *et al.,*[19].

Healthy seeds of *Indigofera linifolia* were collected from the seed market (Bhopal).

The seeds were washed with running tap water three to four times and once surface sterilized with 0.1% $H_gCL_2$ solution for 5 minutes to remove the surface adhering microbes. After surface sterilization, the seeds were again washed with double distilled water. Uniform sized seeds were then transferred to sterilized Petri Plates provided with filter paper pads.

Three replicates of treated and control seeds were kept for germination studies. The filter paper pads were moistened as and when needed. The emergence of radical was taken as germination.

The germination percentage was 5, 15, 8, 7, 31, 9, 11, 10, 8, 13, 16, 8, 12, 0, 16 and 24 respectively in the seeds kept as control, those treated with hot water, subjected to scarification, stratification, alternating high and low temperature, $KNO_3$, thiourea, kinetin, $GA_3$, $H_2SO_4$, pre-soaking, electric current, mechanical injury, coumarin, brassinolide and IAA on the 3rd day after sowing. The germination percentage was 18, 35, 17, 13, 61, 23, 29, 20, 19, 34, 37, 18, 22, 24, 46 and 48

Table-1. Showing the effect of various treatments on the germination percentage of *Indigofera linifolia*.

| D A S → Treatment ↓ | 3rd day | 5th day | 7th day | 9th day | 11th day | 13th day | 15th day | 17th Day |
|---|---|---|---|---|---|---|---|---|
| Control | 5 | 5 | 11 | 18 | 20 | 23 | 23 | 23 |
| Hot water | 15 | 18 | 27 | 35 | 35 | 48 | 49 | 49 |
| Scarification | 8 | 12 | 12 | 17 | 22 | 31 | 31 | 31 |
| Stratification | 7 | 9 | 13 | 13 | 17 | 26 | 28 | 28 |
| Alt. high & low temp. | 31 | 46 | 55 | 61 | 65 | 75 | 77 | 77 |
| $KNO_3$ | 9 | 13 | 18 | 23 | 28 | 34 | 34 | 34 |
| Thiourea | 17 | 11 | 25 | 29 | 33 | 33 | 33 | 33 |
| Kinetin | 10 | 13 | 15 | 20 | 29 | 32 | 32 | 32 |
| $GA_3$ | 8 | 12 | 12 | 19 | 26 | 26 | 27 | 27 |
| $H_2SO_4$ | 13 | 18 | 27 | 34 | 34 | 42 | 42 | 42 |
| Presoaking | 16 | 20 | 29 | 37 | 42 | 53 | 53 | 54 |
| Coumarin | 0 | 9 | 15 | 24 | 31 | 36 | 36 | 36 |
| Electric current | 8 | 12 | 12 | 18 | 25 | 31 | 31 | 31 |
| Brassinolide | 16 | 28 | 37 | 46 | 51 | 63 | 65 | 65 |
| Mechanical injury | 12 | 18 | 22 | 22 | 28 | 36 | 36 | 27 |
| IAA | 24 | 25 | 41 | 48 | 57 | 69 | 71 | 71 |

respectively in the seeds kept as control, those treated with hot water, scarification, stratification, alternating high and low temperature, $KNO_3$, thiourea, kinetin, $GA_3$, $H_2SO_4$, pre-soaking, electric current, mechanical injury, coumarin, brassinolide and IAA on the 9th day from the date of sowing. Incidently, there was no improvement in germination percentage after 17th day from the date of sowing. On 17th day from the date of sowing, highest germination percentage was recorded for the seeds subjected to alternating high and low temperature, under which as high as 77% seeds germinated followed by 71% germination in the seeds which were subjected to IAA treatment. Brassinolide treatment resulted in 65% germination. Under other treatments, the germination percentage was 49, 31, 28, 34, 33, 32, 27, 42, 54, 31, 37 and 36 respectively in the seeds treated with hot water, scarification, stratification, $KNO_3$, thiourea, kinetin, $GA_3$, $H_2SO_4$, presoaking, electric current, mechanical injury and coumarin. There was only 23% germination during this time period in the seeds kept as control thus alternating high and low temperature proved to be useful for the induction of germination of *I.linifolia* seeds (table 1).

The behaviour of all the dormant seeds taken in the present investigation was not uniform against a particular dormancy breaking technique. For instance, the highest germination percentage (77%) for the seeds of *I. linifolia* was achieved for the seeds exposed to alternating high and low temperature. Whereas, the germination percentage was 71 and 65 under the influence of IAA and brassinolide respectively. Initially, on the 3rd day, the germination percentage was 5, 31, 24 and 16 respectively in the seeds kept as control, subjected to alternate high and low temperature treatment, IAA and brassinolide treatment. Even after 17 days of sowing, only 23% seeds of *I. linifolia* kept as control could germinate. So the best method for breaking its dormancy was found to be treatment with alternating high and low temperature (table 1).

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