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Effect of various pre-treatments for breaking the dormancy of *Nigella sativa* Linn.

Mudasir Qadir and Fatima Khan*

Department of Botany Govt. College of Science and Commerce Benazeer, Bhopal-462003 (India)

Abstract

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

The highest germination percentage for *N. sativa* seeds (93%) was achieved with pre-soaking treatment for 6 hours. It was followed by 84% with IAA. The seeds kept as control germinated to the tune of 59%. The scarified and GA_3 treated seeds exhibited 55% germination each, whereas thiourea, H_2SO_4 and hot water treated seeds showed 45% germination each. The stratified seeds, those subjected to alternate high and low temperature, KNO_3, mechanically injured and those subjected to electric current, coumarin and brassinolide showed 46%, 42%, 33%, 51% , 31%, 31% and 75% germination respectively. Thus, for achieving higher germination percentage of *N. sativa*, pre-soaking of the seeds for 6 hours is the best option.

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.

It is commonly known as black cumin or *Kalonji*. It is a member of the family Ranunculaceae. The seeds are attributed with a number of medicinal properties. The seeds as well as its oil is used against a variety of ailments. According to the Prophet Muhammad (S A W), this black seed can cure every ailment except death. It is an annual flowering plant, native to south and south west Asia. It grows to 20-30 cm tall, with finely divided, linear

Present Address: Govt. College Nasrullah Ganj (M.P.)

leaves. The flowers are delicate and usually coloured pale blue and white with five to ten petals. The fruit is a large and inflated capsule composed of three to seven united follicles, each containing numerous seeds. The seed is used as a spice. The seeds are frequently referred to as black cumin. Its seed are used in Bosnia and particularly its capital Sarajevo to flavour pastries (Bosnian: somun) often baked on muslim religious holidays. It has a pungent bitter taste and smell. It is used primarily in confectionery and liquors. Peshawari naan is as a rule, topped with Kalonji seeds. It is also used in American string cheese called Majdouleh or Majdouli in the Middle East. Recent researchers have shown that this seed has antidiabetic, anticarcinogenic and carminative properties as well as an excellent remedy for the treatment of leucoderma.

In the present study, seeds of this plant were tested for their germination potential and shortening of dormancy period. Initial studies exhibited that there was only 59% germination till 17th day of sowing recorded under untreated seeds. Therefore, it was thought imperative to undertake this investigation to find out the 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 influences 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_3SO_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.

Davis, (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[16]. Effects of light, temperature and their interaction on the germination of seeds was investigated by Toole[27].

Hayes & Klein[12] investigated special quality influence of light during development of *Arabidopsis thaliana* plants in regulating seed germination. Bewley and Black[3] studied the physiology and biochemistry of seeds. Isoenzymes of sugar phosphate metabolism in endosperm of germinating castor beans were studied by Nishimura[21]. Seed germination and dormancy have been studied by Bewley[2]. Improvement of seed germination in *Asparagus racemosus* has been reported by Gupta,[11] *et al.,*

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[22]. Siva[25] *et al.,* have studied the enhanced seed germination of *Psoralea corylifolia* L. by heat treatment. Musara,[19] *et al.,* 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,[4] *et al.,* have reported seed dormancy QTL identification across a *Sorghum bicolor* segregating population. Dave,[6] *et al.,* 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 and Shinde[7]. 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[8] *et al.* Transcriptome analysis of seed dormancy after rinsing and chilling in ornamental peaches (*Prunus persica*) has been investigated by

Table-1. Showing the effect of various treatments on the germination percentage of *Nigella sativa.*

D A S→ Treatment ↓	3rd day	5th day	7th day	9th day	11th day	13th day	15th day	17th Day
Control	0	19	20	51	58	59	59	59
Hot water	11	21	29	30	36	44	44	45
Scarification	10	16	24	44	45	51	55	55
Stratification	0	11	21	26	31	43	45	46
Alt. high & low temp.	0	16	25	25	36	42	42	42
KNO_3	4	14	19	25	26	31	33	33
Thiourea	10	10	19	28	37	44	44	45
Kinetin	16	24	36	51	59	64	66	66
GA_3	8	26	30	34	42	51	53	55
H_2SO_2	11	19	25	31	37	44	45	45
Presoaking	9	31	42	69	82	91	93	93
Coumarin	0	5	14	20	27	31	31	31
Electric current	0	4	16	21	27	31	31	31
Brassinolide	11	19	27	46	61	74	75	75
Mechanical injury	9	24	36	36	45	50	50	51
IAA	25	38	53	66	74	82	84	84

Kanjana[13] *et al.*

Effect of different pretreatments and seed coat on dormancy and germination of seeds of *Senna obustifolia* has been studied by Mensah and Ekeke[17]. Mishra[18] has investigated the effect of temperature and light on the seed germination of *Sida cordifolia*. Redwood, *et al.,*[23] 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[26].

Effect of various dormancy breaking treatments on seed germination, seedling growth and seed vigour of medicinal plants has investigated by Warghat, *et al.,*[28]. Zohra, *et al.,*[29] 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.,*[20].

Healthy seeds of *Nigella sativa* 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 highest germination percentage for *N. sativa* seeds (93%) was achieved with pre-soaking treatment for 6 hours. It was followed by 84% with IAA. The seeds kept as control germinated to the tune of 59%. The scarified and GA_3 treated seeds exhibited 55% germination each, whereas thiourea, H_2SO_4 and hot water treated seeds showed 45% germination each. The stratified seeds, those subjected to alternate high and low temperature, KNO_3, mechanically injured and those subjected to electric current, coumarin and brassinolide showed 46%, 42%, 33%, 51%, 31%, 31% and 75% germination respectively (table-1).

In nature, germination percentage of this plant species is not very high. Only 59% of the seeds kept as control could germinate after 17th day of sowing. However, presoaking of the seeds for 6 hours resulted in 93% germination in the same time period followed by 84% and 75% respectively in the seeds treated with IAA and brassinolide respectively.

On the 3rd day, the germination percentage was 0, 9, 25 and 11 respectively in the seeds kept as control, presoaked, treated with IAA and brassinolide. As pointed out earlier, presoaked seeds of *Andrographis paniculata* exhibit a higher germination percentage[15]. Thus, for achieving higher germination percentage of *N. sativa* seeds, presoaking is the best option for this extraordinarily important medicinal plant, other options are IAA and brassinolide treatment of the seeds, which are expensive.

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