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Standardization of seed germination technique in Khurasani-ajavayan (*Hyoscyamus niger*) seeds

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ABSTRACT

The Khurasani-ajvayan (*Hyoscyamus niger* L) is an important medicinal plant which is having high medicinal value. The germination test was conducted in the laboratory at three different temperatures (20, 25 and 30°C) and substrata (BP, TP and S). The results revealed that species having high percentage of hard seeds ranged from 58.0 -74.0% at 20°C followed by 25°C and 30°C which ranged from 42.0- 55.0% and 44.0 to 47.0%, respectively in all the substrata after 15 days of planting. To overcome this problem of low germination, the seeds were further subjected to various treatments, viz. seed soaking in water (24 and 48 hr), GA_3 (200 and 500ppm), KNO_3 (0.2%) and conc. sulphuric acid (H_2SO_4) 15 seconds to enhance seed germination and tested for seed germination after seed treatment. Among these treatments, GA_3 (200 ppm) recorded the maximum normal seedlings (73.0%) in BP substrata followed by TP (68.0%) at 30°C after 12 days. Hard seeds were also reduced after soaking the seeds in water for 48 hr but it resulted in a subsequent increase in the dead seeds. KNO_3 (0.2%) and GA_3 (500 ppm) seed treatments enhanced the germination up to 50% as compared to control after 13 and 14 days, respectively irrespective of substrata and temperatures. Therefore, it is suggested that the seeds of Khurasani-ajvayan should be tested for germination in BP/TP substrata at 30°C after soaking the seeds in GA_3 (200 ppm) solution for 24hours. The final count for germination should be taken after 12 days.

Key words: Dormancy, Germination, Hard seeds, ISTA, Khurasani-ajvayan, Normal seedlings

Among the medicinal plants, Khurasani-ajavayan (*Hyoscyamus niger* L) is an important medicinal plant which is also known as black henbane or common henbane. Henbane has a strong, unpleasant odour and a bitter taste. Leaves are source of drug, which is used as sedative, narcotic and also in treatment of asthma and whooping cough. Henbane, comprising of the dried leaves and flowering tops of *Hyoscyamus niger* L., commonly called black henbane or common henbane and *H. muticus* L., is an official drug of repute. The herb and seed yield tropane alkaloids (0.3-0.6%), 90% of which are hyoscyamine and hyoscine and the rest are scopolamine, atropine, hyoscipirin, choline, fatty oil, mucilage, albumen and KNO_3. They are used as an anodyne, sedative, tranquillizer, antiseptic, antispasmodic, mydriatic, in asthma and whooping cough. Hyoscine is particularly useful in protection against shocks caused by accidents and loud noises. The commercial henbane in pharmaceutical industry is consisted of foliage and flowering tops of three major temperate species of Hyoscyamus (*H. niger, H. muticus* and *H. albus*) cultivated primarily in Europe and Egypt for production of tropane alkaloids (Pareek *et. al*, 1988). The black henbane or common henbane known, as Khurasani ajvayan is a solanaceous crop having high medicinal values. It is an erect, biennial or annual herb propagated by seeds and vegetative method. Since the seed is not only used for propagation but is also important to cure several severe diseases like gastric or intestinal cramps, diarrhoea, prolapse of rectum, neuralgia, cough, hysteria, skin inflammation and boils, asthma.

The plants, especially the seeds in large doses produce poisonous effects similar to those of datura poisoning, such as dryness of tongue and mouth. Therefore, the crop is having a lot of medicinal properties but it is still to be grown at commercial scale largely because of non-availability of seed standards. Moreover, the crop is also having problem in germination and germination test procedure is not standardized for this crop hence, there is a need to develop and standardize the germination enhancement techniques. Keeping the importance of this

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crop, the present study was undertaken to determine the germination testing procedure with respect to substrata and temperature for seed testing and also to assess the effective seed treatment that would enhance seed germination under laboratory conditions.

MATERIALS AND METHODS

The seeds of Khurasani ajvayan were collected from the Section of Medicinal, Aromatic and Under Utilized plants, Department of Plant Breeding and the study was conducted in the Seed Testing Laboratory, Department of Seed Science & Technology, CCS Haryana Agricultural University, Hisar, India.

Initially, the seeds of Khurasani ajvayan were subjected to germination test in three different substrata, i.e. between the paper (BP), top of paper (TP) and quartz sand (S) at three different temperatures (20, 25 and 30°C) so as to standardize the temperature as well as substrata for seed germination. The seeds of Khurasani ajvayan (Control) showed high percent of hard seeds at the end of the germination test. Fifty seeds in each replication were subjected to tetrazolium (Tz) test to determine the viability. The topographical staining pattern of the embryos (plumule and radical) and cotyledons was studied. Seeds showing a deep red-stained embryo were considered viable. Many pre-sowing treatments are known to improve seed germination in general and hence, different pre sowing treatments were employed to the seeds to enhance the germination.

Further, the seeds were soaked in water (24h, 48h), gibbrellic acid @ 200ppm, 500ppm for 24 hr; KNO_3 (0.2%) for 24 hr and conc. sulphuric acid treatment for 15 seconds in order to increase the normal seedlings/germination percentage**.** All these six seed lots (Treated) with control (Untreated seeds) were further studied in the laboratory for germination testing in three different temperatures and substrata. The number of normal seedlings, abnormal seedlings, hard and dead seeds was counted in all the lots after the end of the germination test. Percentage of normal seedlings at 12 days after planting was considered as the development of these essential structures, which are an indicative of normal seedlings under favourable environmental conditions. Normal seedlings are seedlings having a vigorous primary root or a set of secondary root system, intact hypocotyls and/or epicotyls without damage, at least one attached cotyledon and attached terminal buds. Abnormal seedlings are those having the following defects, no primary root system with weak secondary roots, a lesion in the conducting tissues, more than one cotyledon missing and seedlings with damaged terminal buds (AOSA 1986). Dormant seeds are imbibed seeds which remained firm and apparently viable at the end of the test and were determined by pressing the seeds by the finger (ISTA 1985). All these germination treatment were employed on seed replicating thrice so as to reduce the error at all the three temperatures and substrata. The germination test was conducted for 12-15 days and final count was taken when the maximum normal seedlings have emerged. The factorial experiment in Completely Randomized Design (CRD) was conducted and the statistical software SAS has been used for the analysis.

RESULTS AND DISCUSSION

Results of the present study revealed that the seeds of Khurasani-ajvayan were found to be hard even after 15 days when the final count was taken at the end of germination test. The viability of the seeds was also confirmed from tetrazolium test in the laboratory and 77.0 percent seeds were found viable which showed deep red embryonic axis and cotyledons. The maximum number of hard seeds was recorded at 20°C (74.0%) temperature followed by 25°C (55.0%) and 20°C (47.0%) in BP substrata without any treatment (Control). Minimum number of normal seedlings was found at 20°C temperature which ranged from 5 to 14% (Fig 1). It was also observed that the number of normal seedlings increased at the higher temperature but it was non significant. Therefore, the germination enhancing techniques were applied to convert the hard seeds into normal seedlings. Pre sowing treatments have been shown to enhance seed germination in many species (Kattimani *et al.* 1999, Pandey *et al.* 2000, Joshi and Dhar 2003, Tambat *et al.* 2006).

Among the six treatments, seeds soaked in water (24 hr) did not reduce hard seed significantly whereas number of hard seeds decreased in seeds soaked for 48 hr in water irrespective of temperatures. The number of normal seedlings increased in all the temperatures but high mortality of seed was observed.

The chemical treatments of gibbrellic acid (200 and 500ppm), KNO_3 0.2% and conc. Sulphuric acid (15 seconds) were given to enhance the germination. The results showed that the number of normal seedlings increased in the treatment of GA_3 (200ppm) irrespective of substrata and temperatures. Maximum germination (73.0%) was recorded in BP substrata followed by TP (68.0%) at 30°C. (Fig 2) Gibberellic acid is reported to play important role in improving germination in several plant species (Allen and Meyer 1990, Baskin and Baskin 1990, Choudhary and Gupta 1995, Joshi and Dhar 2003, Tambat *et al.* 2006). The external supply of GA_3 neutralizes inhibitory compounds in seed coat and endosperm and facilitates germination (Evenari 1949, Mary 1972). As the concentration of GA_3 (500ppm) was increased, the number of normal seedlings was decreased but the number of dead seeds increased in all the substrata irrespective of temperatures after 14 days of planting. Germination was reduced as GA_3 concentration increased to 500 ppm, which may possibly due to imbalanced cell activity or toxic effect of GA_3 at higher concentration (Takashashi *et al.* 1991, Deno 1993, Tambat *et al.* 2006). Such results were also noticed by Naidu *et al.* (2000) in *Sapindus trifoliate*, Tigabu and Oden (2001) in *Albizia* sp., Karam and Al-Salem (2001) in *Arbutas andranchne* and Tambat *et al.* (2006) in *Gymnacranthera canarica* Warb. Results also revealed that the number of normal seedlings

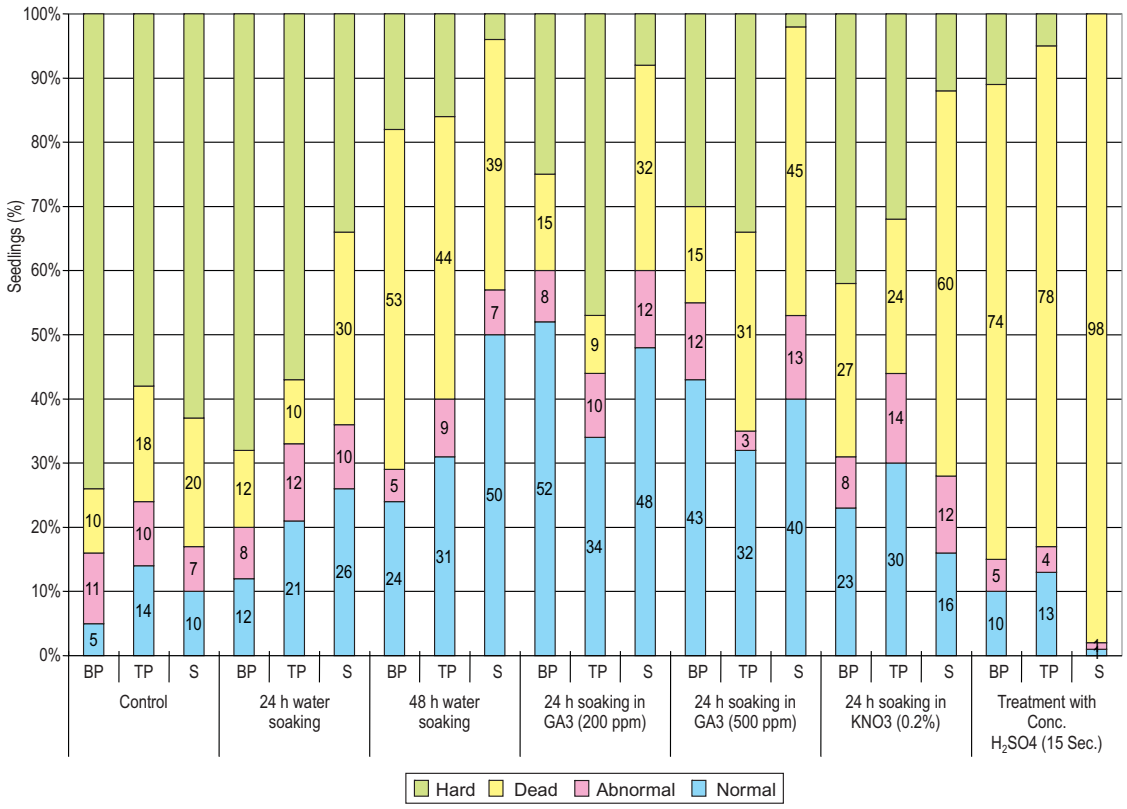
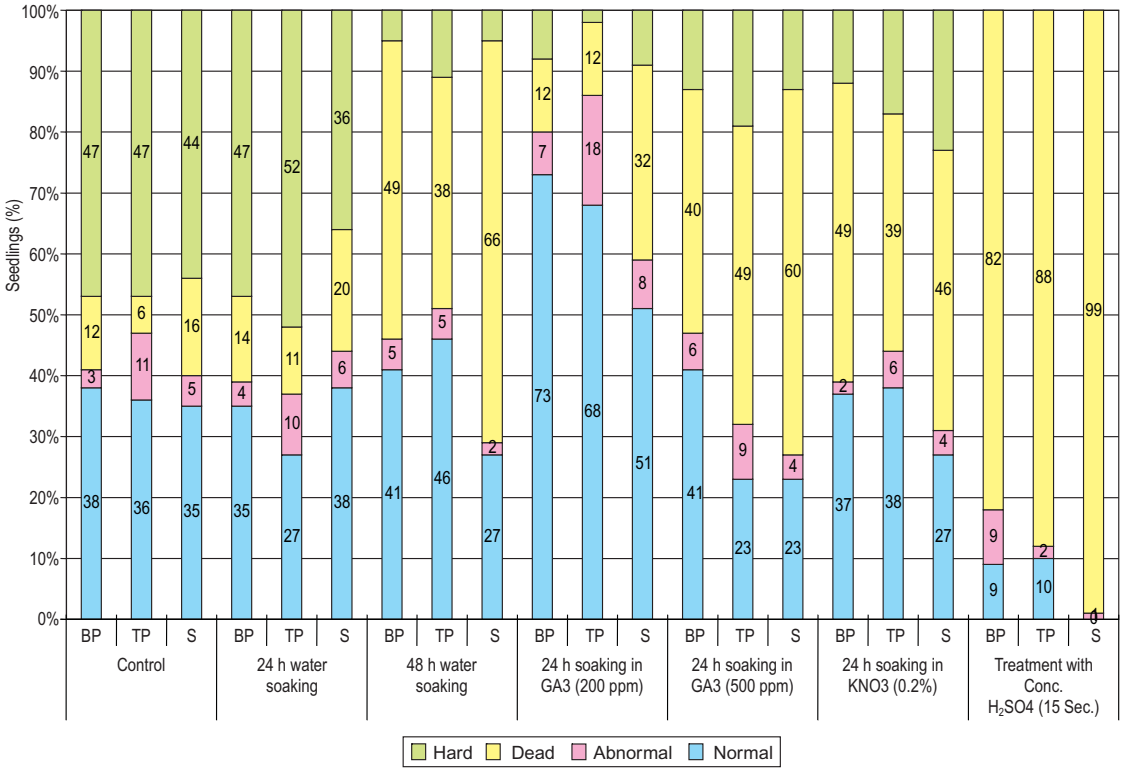


Fig 1 Effect of different treatments on germination (%) in Khurasani-ajvayan seed at 20°C



CD AT α =0.05 BASED ON THE DATA SHOWN IN FIG. 1 TO 2, FOR TREATMENT (TR), SUBSTRATA (S), TEMPERATURE (T), TRXS, SXT, TRXT, TRXSXT IN CORRESPONDING TO NORMAL, ABNORMAL, DEAD AND HARD SEEDS ARE:

NORMAL	0.36, 0.24, 0.24, 0.63, 0.41, 0.63, 1.09;	CV (%) = 2.14
ABNORMAL	0.72, 0.47, 0.47, 1.25, 0.82, 1.25, 2.17;	CV (%) = 11.88
DEAD	0.49, 0.32, 0.32, 0.86, 0.56, 0.86, 1.48;	CV (%) = 2.38
HARD	0.45, 0.30, 0.30, 0.78, 0.51, 0.78, 1.36;	CV (%) = 3.51

Fig 2 Effect of different treatments on germination(%) in Khurasani-ajvayan (niger) seed at 25° C.

was increased with the treatment of KNO_3 (0.2%) as compared to untreated seeds irrespective of substrata and temperatures but the germination was recorded less than 50.0% even after 13 days of planting. It was also observed that the concentrated sulphuric acid treatment resulted in a significant reduction in hard seeds but number of dead seeds was significantly increased in all the substrata and temperatures. Since the seeds are of small size having thin seed coat and hence it resulted in death of embryo.

Results of the present investigation revealed that the maximum germination was observed in the seeds treated with GA_3 (200ppm) in BP substrata (73.00%) followed by TP (68.0%) at 30°C after 12 days. Hence, it was concluded that Khurasani-Ajavayan seeds should be tested in BP/TP substrata at 30°C and the final count should be taken after 12 days of planting.

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