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## Studies on duration of seed dormancy and effect of various dormancy breaking treatments on seed quality of spinach (*Spinacia oleracea* L.)

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**Abstract**

Dormancy is a form of developmental arrest and is a mechanism by which seeds maintain their viability in unfavourable conditions. The laboratory experiments were carried at Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences Raichur, to know the dormancy duration and effect of dormancy breaking treatments on seed quality of spinach. The experiment was laid down in Completely Randomized Design (CRD) with three replications. The first experiment was conducted to assess the duration of dormancy in spinach. The dormancy duration of 35 days was observed with 65.5 per cent of germination. The second experiment consisted of 21 dormancy breaking treatments, among them thiourea (1%) recorded significantly highest seed quality parameters viz., normal seedlings (88.25%), speed of germination (26.95), root length (7.33 cm), shoot length (8.22 cm), seedling dry weight (11.56 mg), seedling vigour index (1372) and lowest electrical conductivity (0.219 dSm<sup>-1</sup>) compared to control.

**Keywords:** Spinach, dormancy duration, thiourea, seed quality parameters

**Introduction**

The true seed in *Spinacia oleracea* L. lies within dead maternal perianth tissue and is made up of dense layers of sclerenchyma cells. The upper part of the pericarp contains an ovary cap, which is termed the operculum. The bottom part of the pericarp has a basal pore, this is essentially a pole like pericarp structure consisting of loose cells. Both the operculum and the basal pore have been proposed as major pathways for water and oxygen to access the germinating embryo in *Spinacia oleracea* L. Fruit (Perry and Harrison, 1974) [26]. The spinach fruit as consisting of several dry fruits forming a cluster as a result of fusion of flower parts because of this phenomenon, unprocessed or natural fruit may contain a number of true seeds, most often two or three (Byford, 1963) [6]. Therefore, these fruits are known as "multigerm", i.e. more than one seedling may emerge from each fruit.

*Spinacia oleracea* L. fruits have however been historically reported as possessing coat imposed dormancy. A number of factors may be involved i.e. physical restriction of radicle protrusion and restriction of gas exchange between the embryo and the environment by the operculum, the presence of a mucilaginous layer that also prevents access of oxygen to the embryo and the presence of germination inhibitors in the pericarp (Heydecker *et al.*, 1971; Taylor, *et al.*, 2003) [15, 35]. This dormancy has caused germination problems both in the laboratory and in the field (Habib, 2010; Tekrony and Hardin, 1968) [13, 36].

Seed germination and subsequent seedling growth define crucial steps for entry into the plant life cycle and proper seed germination is a basic pre-requisite for getting a better crop yield. An important problem encountered in the cultivation of spinach is the poor germination of the seeds when planting is done in extremely warm temperatures, which may delay or inhibit seed germination in the field, reduce uniformity total stand establishment and ultimately reduces the yield. Keeping all these points in view, the present investigation entitled "Studies on duration of seed dormancy and effect of dormancy breaking treatments on seed quality of spinach."

**Materials and Methods**

The research studies were carried out in the laboratory of Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur. Geographically, the station is situated in the North-Eastern dry zone (Zone-2) of Karnataka State at 16° 15' North latitude and 77° 20' East longitude and at an altitude of 389 meter above mean sea level. Fresh seeds of spinach variety "Annapoorna" were obtained from University of Horticultural Sciences, Bagalkot.

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The Annapoorna is an improved spinach variety with test weight of 10 - 12 g which matures in 150- 180 days. Spinach crop can be grown on a wide range of soils provided that soils are sufficiently fertilized and well drained.

#### Experiment I: Study the duration of seed dormancy

Germination was recorded at weekly intervals from harvest up to the stage where the germination reached to Minimum Seed Certification Standards (60%).

#### Experiment II: Effect of dormancy breaking methods on seed quality

The experiment was laid out in CRD (Completely Randomized Design) with three replication. The experiment consisted of 21 treatments which were used for breaking dormancy in spinach seeds various physiological and biochemical parameters were recorded as follows.

#### Observations recorded

##### Normal seedlings (%)

Germination test was conducted using eight replicates of 50 seeds each in pleated paper towels where seeds were placed in between the pleats of germination paper and incubated in the walk-in seed germination room at  $25 \pm 2^\circ\text{C}$  temperature and  $90 \pm 5$  per cent RH. Seedling evaluation was done when seedlings have reached a stage with all the essential structures were fully expressed. Sufficient time was given for the seeds to germinate and produce all essential structures showing potentiality to develop into normal plant under favourable conditions. Such seedlings were considered as normal seedlings and counted to compute the germination percentage. The number of normal seedlings in each replication was counted at the end of 14th day and the germination percentage was calculated and was expressed in percentage (ISTA, 2013).

$$\text{Normal seedlings \%} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

##### Abnormal seedlings (%)

From the germination test, those seedlings which did not show the capacity of continued development into normal plants under favourable conditions of water supply, temperature and light were counted and expressed in percentage.

$$\text{Abnormal seedlings (\%)} = \frac{\text{No. of Abnormal seedlings}}{\text{Total number of seeds}} \times 100$$

##### Fresh un-germinated seeds (%)

From the germination test, those seeds which at the end of the test absorbed water but did not show germination under favourable conditions of water supply, temperature and light were counted and expressed in percentage.

$$\text{Fresh un-germinated seeds (\%)} = \frac{\text{No. of fresh un-germinated seeds}}{\text{Total number of seeds}} \times 100$$

##### Hard seeds (%)

From the germination test, those seeds which at the end of the test neither absorbed water nor showed germination under favourable conditions of water supply, temperature and light were counted and expressed in percentage.

$$\text{Hard seeds (\%)} = \frac{\text{No. of hard seeds}}{\text{Total number of seeds}} \times 100$$

##### Dead seeds (%)

From the germination test, those seeds which at the end of the test period, were neither hard nor fresh and have not produced the seedlings are counted and expressed in percentage.

$$\text{Dead seeds (\%)} = \frac{\text{No. of dead seeds}}{\text{Total number of seeds}} \times 100$$

##### Speed of germination

Seeds were germinated on paper medium with eight replications of 50 seeds each and the daily germination counts were taken up to final count (14 days). The speed of germination was calculated by using the formula given by Maguire (1962) [21].

Where,

$$\text{Speed of Germination} = \frac{G_1}{D_1} + \frac{G_2}{D_2} + \dots + \frac{G_n}{D_n}$$

$G_1, G_2, \dots, G_n$  are the number of seeds germinated on  $D_1, D_2, \dots, D_n$  day

##### Root length (cm)

From the germination test, ten normal seedlings were selected randomly in each treatment from each replication on 14<sup>th</sup> day. The root length was measured from the tip of the primary root to hypocotyl and mean root length was expressed in centimeter.

##### Shoot length (cm)

From the germination test, ten normal seedlings were selected randomly in each treatment from each replication on 14th day. The shoot length was measured from the base of the primary leaf to hypocotyl and mean shoot length was expressed in centimeter.

##### Seedling dry weight (mg)

From the germination test the same ten seedlings used for measuring the root and shoot length were kept in a butter paper packet and dried in hot air oven maintained at  $70^\circ \pm 2^\circ\text{C}$  for 24 hours. Then the seedlings were cooled in a desiccator for 30 minutes and the weight of the dry seedlings was recorded using electronic balance and was expressed in milligrams (mg)/ 10 seedlings.

##### Seedling vigour index (SVI)

The seedling vigour index was calculated by using the formula suggested by Abdul-Baki and Anderson (1973) [12].

Seedling vigour index = Germination (%) x Total seedling length (cm)

##### Electrical conductivity ( $\text{dSm}^{-1}$ )

Five grams of seeds in four replications were soaked in acetone for half a minute and thoroughly washed in distilled water three times. Then, the seeds were soaked in 25 ml distilled water and kept in an incubator maintained at  $25^\circ\text{C} \pm 1^\circ\text{C}$  for 12 hours. The seed leachate was collected and the volume was made up to 25 ml by adding distilled water. The electrical conductivity of the seed leachate was measured in the digital conductivity bridge (ELICO) with a cell constant 1.0 and the mean values were expressed in deci simons per meter ( $\text{dSm}^{-1}$ ) (Milosevic *et al.*, 2010) [24].

## Procedure for the preparation of the dormancy breaking treatments

### Procedure for preparation of KNO<sub>3</sub>

KNO<sub>3</sub> solution of 0.5 percent and 1.0 percent were prepared by dissolving 0.5 g and 1.0 g of KNO<sub>3</sub> in 100 ml distilled water respectively (Plate 1) and seeds were soaked in respective KNO<sub>3</sub> solutions separately for 24 hour.

### Procedure for preparation of GA<sub>3</sub>

GA<sub>3</sub> solution of 100 ppm and 150 ppm were prepared by dissolving 10 mg and 15 mg of GA<sub>3</sub> in 100 ml distilled water respectively (Plate 1) and seeds were soaked in respective GA<sub>3</sub> concentration solutions separately for 24 hours.

### Procedure for preparation of thiourea

Thiourea solution of 0.5 percent and 1.0 percent were prepared by dissolving 0.5 g and 1.0 g of Thiourea in 100 ml distilled water respectively (Plate 1) and seeds were soaked in respective thiourea solutions separately for 24 hours.

### Procedure for preparation of ethrel

Ethrel solution of 25 ppm and 50 ppm were prepared by dissolving 2.5 µl and 5 µl of Ethrel in 100 ml distilled water respectively (Plate 1) and seeds were soaked in the ethrel solutions of respective concentration separately for 24 hours.

### Water

Spinach seeds were soaked in water for 12, 24 and 36 hours.

### Hot Water treatment

Spinach seeds were soaked in hot water for 5, 10 and 15 minutes at 50 °C.

### Heat treatment

Spinach seeds were exposed to heat in hot air oven for 1, 2 and 3 days at 50 °C. These seeds were used for testing seed quality parameters.

### Sun drying

Spinach seeds were exposed to sunlight for 24, 48 and 72 hours. These seeds were used for testing seed quality parameters.

**Note:** The treated seeds were surface dried and tested for germination and the germination per cent expressed based on normal seedlings on 14<sup>th</sup> day of testing for spinach.

## Results and Discussion

The ability of the seeds to delay their germination until the time and place are right is an important survival mechanism in plants. Seed dormancy may be a complex and puzzling challenge to the seed analyst and seed researcher but it is the method through which plants are able to survive and adapt to their environment. Seed dormancy is a state in which seeds fail to germinate even under favourable condition of moisture, temperature and oxygen for germination (Wareing, 1963) [40]. The main disadvantage of seed dormancy is that, they cannot be used immediately after harvest for seed purpose. Dormancy in spinach is imposed by certain physical and physiological factors. The nature of these germination blocks, their mode of action and processes regulating the release of dormancy are not fully understood. Agro-climatic conditions under which the mother plant is grown and harvested may influence the state of dormancy of the seeds. The intensity of dormancy depends on many factors, including the species and

variety, year, place and time of harvest and the stage of development of seeds (Lenoir, 1983 and Come *et al.*, 1984) [19, 8].

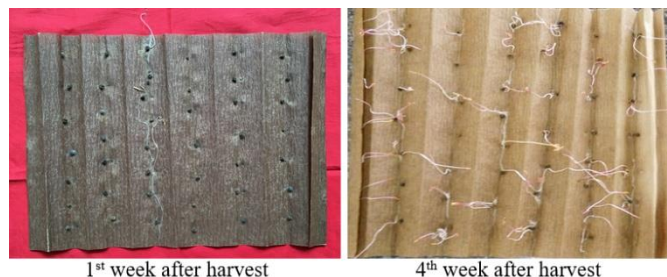
## Experiment I: Study the duration of seed dormancy

The information on dormancy duration in spinach is very important when grown as a pure crop. Seed dormancy variation in one way is a desirable trait for cultivation during *kharif* season when harvest coincides with rain. But, in another way it is disadvantageous for *rabi* sowing.

The variety 'Annapoorna' showed dormancy for 4 weeks (35 days) with an initial germination of 4.50 per cent and dormancy was released after 35 days where the germination percentage recorded was 65.50 per cent (Table 1) which was above the Minimum Seed Certification Standards (60%) (fig. 1a & 1b). Similar findings were reported by the Vanden Born (1993) [37] in coriander seed that exhibits complete or near complete dormancy in freshly harvested seeds and dormancy was broken after three to six weeks. Bosland and Votava, (2000) [5] observed that the freshly harvested seeds of wild capsicum species exhibited dormancy for 40-45 days after harvest. Randle and Homna, (1981) [30] where in chilli seeds required an after ripening period of 45 days to release dormancy. The results are in agreement with Shivhare *et al.* (1995) [33] in chilli, Philpot (1976) [27] in sugar beet, Randle and Homna (1982) in capsicum and Vinodkumar (1998) [39] in chilli.

**Table 1:** Duration of seed dormancy in spinach seeds after harvest

Weeks	Per cent seed germination (weeks after harvest)
Immediately after harvest (0-7 days)	4.50
1 (7-14 days)	14.75
2 (14-21 days)	27.50
3 (21-28 days)	53.75
4 (28-35 days)	65.50



**Fig 1a & 1b:** Duration of seed dormancy weeks after harvest

## Experiment II: Effect of dormancy breaking methods on seed quality

The seed production depends on seedling emergence and uniform plant stand establishment in spinach as influenced by the presence of seed dormancy.

The seeds were subjected to various pre-treatments to break dormancy which showed significant differences on seed physiological parameters of spinach, while low seed physiological parameters were observed in untreated seeds, indicating pre-treatments are required to overcome the dormancy so as to improve the seedling emergence and uniform plant stand.

In the present investigation, the normal seedling germination percentage was improved above MSCSs (60%) when seeds were treated with thiourea (1%) (88.25%) (Fig. 2a& 2b) followed by thiourea at 0.5 per cent (85.75%) and GA<sub>3</sub> at 150 ppm (83.75%),

**Table 2:** Effect of dormancy breaking methods on normal seedlings (%), abnormal seedlings (%), fresh un-germinated seeds (%), hard seeds (%) and dead seeds (%) of spinach

Treatments	Normal seedlings (%)	Abnormal seedlings (%)	Fresh un-germinated seeds (%)	Hard seeds (%)	Dead seeds (%)
T <sub>1</sub> - Control	48.00	2.00	4.00	34.00	12.00
T <sub>2</sub> - KNO <sub>3</sub> 0.5%	77.50	9.50	3.50	4.75	4.75
T <sub>3</sub> - KNO <sub>3</sub> 1%	80.75	7.25	3.25	4.75	4.00
T <sub>4</sub> - GA <sub>3</sub> 100 ppm	81.25	6.25	4.00	5.25	3.25
T <sub>5</sub> - GA <sub>3</sub> 150 ppm	83.75	5.00	3.00	3.25	5.00
T <sub>6</sub> - Thiourea 0.5%	85.75	6.00	3.00	3.25	2.00
T <sub>7</sub> - Thiourea 1%	88.25	5.00	3.00	1.75	2.00
T <sub>8</sub> - Ethrel 25 ppm	75.25	10.50	2.75	7.50	4.00
T <sub>9</sub> - Ethrel 50 ppm	77.75	9.50	2.25	5.50	5.00
T <sub>10</sub> - Water soaking 12 h	67.00	6.00	5.50	18.00	3.50
T <sub>11</sub> - Water soaking 24 h	73.00	10.75	6.50	5.00	4.75
T <sub>12</sub> - Water soaking 36 h	65.25	19.25	4.50	3.75	7.25
T <sub>13</sub> - Hot Water 5 min 50 °C	61.75	8.00	3.50	21.50	5.25
T <sub>14</sub> - Hot Water 10 min 50 °C	71.25	14.75	3.75	3.50	6.75
T <sub>15</sub> - Hot Water 15 min 50 °C	61.25	18.75	6.00	1.25	12.75
T <sub>16</sub> - Heat treatment 1 day 50 °C	72.75	13.50	5.00	4.75	4.00
T <sub>17</sub> - Heat treatment 2 day 50 °C	67.25	19.00	6.25	3.25	4.25
T <sub>18</sub> - Heat treatment 3 day 50 °C	58.50	22.00	2.50	2.50	14.50
T <sub>19</sub> - Sun drying 24 h	64.50	6.75	8.50	15.75	4.50
T <sub>20</sub> - Sun drying 48 h	68.75	12.00	5.00	11.00	3.25
T <sub>21</sub> - Sun drying 72 h	62.50	21.50	4.00	3.50	8.50
MEAN	71.10	11.10	4.27	7.79	5.58
S.Em±	0.783	0.271	0.204	0.227	0.207
CD @ 1%	2.218	0.768	0.578	0.643	0.585

**Table 3:** Effect of dormancy breaking treatments on physiological parametrs of spinach

Treatments	Speed of germination	Root length (cm)	Shoot length (cm)	Seedling dry weight (mg)	Seedling vigour index	Electrical conductivity (dSm <sup>-1</sup> )
T <sub>1</sub> - Control	3.55	4.05	5.57	5.29	462	0.574
T <sub>2</sub> - KNO <sub>3</sub> 0.5%	15.06	5.58	7.27	7.78	996	0.381
T <sub>3</sub> - KNO <sub>3</sub> 1%	18.36	6.08	7.60	8.64	1105	0.422
T <sub>4</sub> - GA <sub>3</sub> 100 ppm	19.32	6.08	7.85	9.56	1132	0.336
T <sub>5</sub> - GA <sub>3</sub> 150 ppm	22.20	6.20	7.85	9.74	1177	0.263
T <sub>6</sub> - Thiourea 0.5%	25.48	7.16	8.14	11.21	1312	0.238
T <sub>7</sub> - Thiourea 1%	26.95	7.33	8.22	11.56	1372	0.219
T <sub>8</sub> - Ethrel 25 ppm	13.89	5.64	7.57	7.70	994	0.440
T <sub>9</sub> - Ethrel 50 ppm	15.98	5.78	7.52	8.11	1034	0.415
T <sub>10</sub> - Water soaking 12 h	10.55	4.99	7.31	7.06	824	0.364
T <sub>11</sub> - Water soaking 24 h	12.83	5.44	7.42	7.32	939	0.419
T <sub>12</sub> - Water soaking 36 h	8.74	4.76	7.07	7.06	772	0.484
T <sub>13</sub> - Hot Water 5 min 50 °C	8.93	4.42	6.00	6.05	643	0.478
T <sub>14</sub> - Hot Water 10 min 50 °C	11.30	5.12	7.51	7.30	900	0.515
T <sub>15</sub> - Hot Water 15 min 50 °C	7.80	4.76	6.37	6.41	682	0.749
T <sub>16</sub> - Heat treatment 1 day 50 °C	11.98	5.22	7.44	7.30	921	0.295
T <sub>17</sub> - Heat treatment 2 day 50 °C	10.96	5.16	7.45	7.15	848	0.330
T <sub>18</sub> - Heat treatment 3 day 50 °C	7.45	4.53	5.99	5.89	615	0.589
T <sub>19</sub> - Sun drying 24h	7.97	4.79	6.97	7.00	759	0.564
T <sub>20</sub> - Sun drying 48h	8.86	4.92	7.27	7.07	838	0.575
T <sub>21</sub> - Sun drying 72h	7.17	4.65	6.95	6.98	725	0.574
MEAN	13.11	5.36	7.21	7.72	907	0.437
S.Em±	0.089	0.084	0.067	0.074	13.00	0.007
CD @ 1%	0.252	0.239	0.019	0.210	36.910	0.019

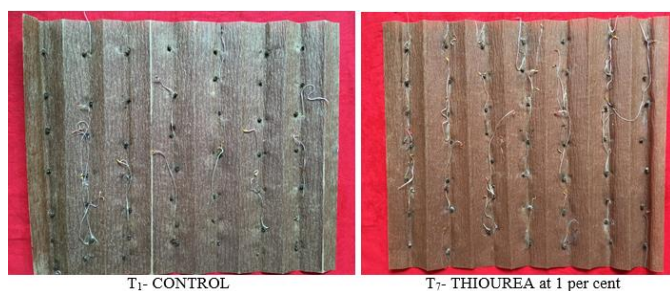
while control recorded significantly least germination of 48 per cent. The increased normal seedling percentage in thiourea treated seeds might reduce the preventive effect on seed coat and increase cytokinin activity to overcome inhibition that leads to stimulate the seed germination (Cetinbas *et al.*, 2006) [7]. Byford (1963) [6] demonstrated the role of thiourea as a light substitute to enhance the germination. However significantly maximum speed of germination (26.90) was observed in the seeds treated with thiourea (1%) followed by thiourea (0.5%) (25.48) and

significantly lowest speed of germination was recorded in control (3.55). Enhanced speed of germination may be due to enhanced metabolic activity (Airin and Khosro, 2013) [4]. Similarly the results were also obtained by Germchi *et al.* (2010) [12] in potato, Hassan *et al.* (2007) [14] in lettuce, Rahman *et al.* (2002) [28] in spinach and Shobha (2016) [34] in ashgourd.

The differential response of spinach to chemical and physical treatment in breaking seed dormancy suggests that the mechanism of seed dormancy is not only due to exogenous



barrier imposed by pericarp seed coat complex but may also be due to the presence of germination inhibitors in the seed coat, especially ABA, the amount of which vary from cultivar to cultivar leading to differential response (Patil and Zode, 1990) [25].



**Fig 2a & 2b:** Effect of dormancy breaking treatments on normal seedlings (%)

Further, the freshly harvested seeds treated with thiourea (1%) also recorded significantly highest root length (7.33 cm), shoot length (8.22 cm) and seedling dry weight (11.56 mg), compared to control (4.05 cm, 5.57 cm and 5.29 mg, respectively). Increase in seedling dry weight may be attributed to increased seedling length and dry matter. The enhancement in root length and shoot length may be due to enhanced metabolic and enzyme activity (Airin and Khosro, 2013) [4]. The seedling vigour index was significantly highest in thiourea (1%) (1372) followed by thiourea (0.5%) (1312) treated seeds and significantly lowest was recorded in control (462) (Table 2). The increase in seedling vigour index might be due to increase in seedling length, germination and dry weight. The results are in conformity with the findings of Venkatasubramaniam and Umarani, (2007) [38] in tomato; Bijanzadeh *et al.* (2010) [4] in rapeseed; Divya, (2013) [9] in chilli; Rahman, (2014) [29] in ash gourd and Saleem *et al.* (2014) [32] in bitter gourd.

In the present investigation the significantly minimum percentage of abnormal seedlings were observed in control (2.00%), whereas heat treatment for 3 days at 50 °C (22.0%) treatment significantly resulted in maximum number of abnormal seedlings due to exposure of seed to heat for longer period which may damage the embryo and seed coat resulting into more leachates by increasing cracks in the seed coat or reducing the peroxidase activity in the seed covering structures thereby promoting the degradation and evaporation of short chain saturated fatty acids (SCSFAs) from the dormant seeds thereby increasing the abnormal seedlings. Similar results was observed by Farhodi *et al.* (2007) [10] in potato seed. Significantly highest fresh un-germinated seeds were observed in sun drying for 24 hour (8.50%), whereas significantly minimum number of fresh un-germinated seeds were observed in ethrel 50 ppm due to softening effect on seed coat, denaturation of inhibitors and enhanced after-ripening process. This might be associated with the washing away of the inhibitor, ABA, and further during the process, the porosity of the seed coat might increase which leads to increased number of fresh un-germinated seeds (Maiti *et al.*, 2005) [22]. Similar findings were reported by Cetinbas and Koyuncu, (2006) [7] and Mani *et al.* (2013) [23] in potato cultivars.

However, significantly highest percentage of hard seeds (34.00%) were observed in control due to hard seed coat of spinach seed. However significantly lowest percentage of hard seeds were observed in hot water treatment for 15 minutes at 50 °C (1.25%) due to softening of seed coat

(Farooq *et al.*, 2007) [11]. Similarly the seeds exposed to heat treatment for 3 days at 50 °C recorded more number of dead seeds (14.50%), whereas significantly minimum dead seeds per cent were observed in the thiourea at 1 and 0.5 per cent (2.00%). The more number of dead seeds was observed in heat treatment for 3 days at 50 °C may be due to presence of dead embryo and endosperm due to excessive heat exposure. The results are in conformity with the Janaiah *et al.* (2006) [17] and Abdul *et al.* (2012) [1] in bittergourd.

Among all the treatments significantly highest electrical conductivity ( $\text{dSm}^{-1}$ ) was recorded in hot water treatment for 15 minutes at 50 °C (0.749) followed by heat treatment for 3 days at 50 °C (0.589  $\text{dSm}^{-1}$ ). This increased electrical conductivity might be due to soaking of seeds for longer period of time at high temperature which causes the damage to the seed coat resulting in more leachates from the seed. Significantly lowest value were observed in thiourea at 1 per cent (0.219  $\text{dSm}^{-1}$ ) and it was on par with thiourea at 0.5 per cent (0.238  $\text{dSm}^{-1}$ ) (Table 3). These results are in conformity with the findings of Jin *et al.* (2006) [18] they noticed the higher degrees of seed coat damage caused by hot water treatment in Chinese cabbage. Similar results were also reported by Liela *et al.* (2005) [20] in fenugreek and Reshma *et al.* (2009) [31] in *Desmanthus virgatus*.

## Conclusion

- The seed dormancy in Annapoorna variety was released 35 days after harvest with 65.50 per cent germination which is above the Minimum Seed Certification Standards (60%).
- Among the dormancy breaking treatments thiourea at 1 per cent is found to be effective in overcoming dormancy.

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