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## Mid storage invigouration – An attempt to extend seed vigour and viability in soybean [*Glycine max* (L.) Merrill]

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### Abstract

A storage experiment was carried out to extend the longevity of soybean seeds through mid storage seed invigouration technique by following moist sand conditioning-soaking-drying method in five months old seeds in the Department of Seed Science and Technology, UAS, Raichur, Karnataka, India. The experiment consisted of nine treatments using different salts and botanicals viz., T<sub>1</sub>: control; T<sub>2</sub>: Water, T<sub>3</sub>: NaCl (10<sup>-3</sup>M), T<sub>4</sub>: KI (10<sup>-3</sup> M), T<sub>5</sub>: Na<sub>2</sub>HPO<sub>4</sub> (10<sup>-3</sup> M), T<sub>6</sub>: Iodine (0.1%), T<sub>7</sub>: CaCl<sub>2</sub>.2H<sub>2</sub>O (1%), T<sub>8</sub>: pongamia leaf extract (1%) and T<sub>9</sub>: custard apple leaf extract (1%). From the experiment it was found that various salts and botanicals effectively reduced the physiological deterioration during subsequent storage period under ambient environmental conditions compared to control. Among the treatments, Iodine (0.1 %), water and CaCl<sub>2</sub>.2H<sub>2</sub>O (1%) shown great promise in extending the storage potential of soybean seeds by an extra one month by registering higher germination values (74.63, 73.13 and 72.88 %, respectively) compared to control (67.38 %), possibly by acting as a free radical scavenger that prevent propagation of radical during peroxidation of lipids. Since the availability of water to the farmers is more economical, it can also be used in extending the storage potential for another one month instead of iodine and CaCl<sub>2</sub>.2H<sub>2</sub>O.

**Keywords:** Soybean, invigouration, iodine, water, storability and alpha amylase

### Introduction

Soybean [*Glycine max* (L.) Merrill] is one of the most important protein and oil rich crop grown throughout the world. Despite its high yielding potential, the yield per unit area of soybean is low in India (Mewael *et al.*, 2010) [13]. Poor germination and low seed viability are the serious problems limiting the productivity of soybean. Availability and accessibility of good quality seeds is the key for successful agriculture in increasing production of any crop (Satish and Bhaskaran, 2013) [18]. However, use of seeds of low physiological quality is a common practice under tropical and subtropical conditions, leading to inadequate plant population in the field (Mewael *et al.*, 2010) [13].

Some protective measures need to be taken to improve the germination and field emergence of this particular crop leading to adequate plant stand. Such measures comprise either pre-sowing hardening practised just before planting or mid storage hydration – dehydration treatment in which seeds are treated 3 – 5 months after storage (mid way between harvest and next sowing time). These mid-storage seed invigouration treatments are physiological treatments that imply an improvement in physiological status of seed, thereby achieving improved germinability, greater storability and better performance than the untreated seeds (Basu, 1994) [6]. In several crops (rice, wheat, tomato and sunflower), mid storage invigouration treatment with or without chemicals has been found successful in improving vigour, viability and productivity (Mitra and Basu, 1979; Basu and Pal, 1980) [14, 4].

The germination of seeds when reduced marginally below minimum seed certification standards (70 %) before sowing time, so if any measures to increase the viability would be of great importance. To improve the germination of unutilized stock and as well as to prolong their storability mid-storage invigouration treatments are highly warranted (Renugadevi *et al.*, 2006) [16]. These treatments would invigorate the low vigour seeds to achieve better emergence and establishment. Considering the above facts in the present investigation an attempt was made to extend the seed vigour and viability of soybean seeds through various mid storage corrections in five months old seeds.

## Materials and methods

The experiment was conducted during the year 2015-16 in the Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur, Karnataka, India. It consisted of 18 treatments laid out in CRD (completely randomised design) two factors in four replications with two varieties as main factor and nine mid storage correction treatments as sub factors. The seeds of the two varieties (JS-335 and DSB-21) were procured from ARS-Bidar in the month of November 2014 which were packed and stored under ambient environmental conditions and after five months (April) mid storage invigoration treatments was performed as per the procedure suggested by Renugadevi *et al.* (2006) [16] with little modification. Here, the sterilized air-dry sand was pre-moistened to 60 per cent with water and then five months old seeds were thoroughly mixed with moist sand in 3:1 ratio (3 parts of sand and 1 part of seed) and kept for four hours for slow absorption of moisture to avoid imbibition injury and the moistened seeds were soaked in water for two hours followed by drying back to original moisture content (9 %) under shade. Similarly, seeds were invigorated with various salts and botanicals by soaking the seeds in respective concentrations of the solutions for two hours (seed to solution ratio of 1:0.3) after moist sand conditioning for four hours. Then the seeds were dried back to original moisture content under shade.

The seeds imposed with different invigoration treatments were subjected for germination test in four replicates of 100 seeds each following between paper method in walk-in seed germinator maintained at  $25 \pm 2$  °C temperature and 90 per cent RH for 8 days. Similarly, the electrical conductivity of seed leachate was carried out according to ISTA (2013). While, the seedling vigour index was computed by adopting the formula suggested by Abdul-Baki and Anderson (1973) [1]. Whereas, the dehydrogenase enzyme activity was determined as per the procedure described by Kittock and Law (1968) [11], alpha amylase by Simpsons and Naylor (1962) [19] and mean germination time (MGT) as per Azimi (2013) [3]. The experimental data thus obtained were statically analysed by the procedure prescribed by Sundararaj *et al.* (1972).

## Results and discussion

Mid storage seed invigoration of two varieties had a significant effect on seed germination, mean germination time, seedling vigour index, electrical conductivity, dehydrogenase activity and alpha amylase activity.

Between the varieties, immediately after mid storage invigoration, V<sub>2</sub> (DSB-21) recorded significantly higher seed germination (84.47 %), seedling vigour index (3620), dehydrogenase activity (0.982) and alpha amylase activity (23.8 mm) than the variety V<sub>1</sub> (JS-335) which recorded (82.58 %, 3061, 0.932 and 23.0 mm, respectively). While, V<sub>2</sub> recorded significantly lower mean germination time (2.09) and electrical conductivity (0.405 dSm<sup>-1</sup>) compared to V<sub>1</sub> (2.14 and 0.464, respectively). However, V<sub>2</sub> was able to maintain higher seed germination (71.53 %), seedling vigour index (2477), dehydrogenase activity (0.686) and alpha amylase activity (17.2 mm) and significantly lower mean germination time (2.24) and electrical conductivity (0.442 dSm<sup>-1</sup>) than V<sub>1</sub> even at the end of fourth months of storage period (Table 1, 2 and 3). It is noted that, during the entire physiological and biochemical test, the variety DSB-21 with the advancement of storage period after invigoration was comparatively a good storer than JS-335 which is evident

from significantly higher values for germination, vigour indices and enzymes activities.

This may be attributed to existence of variation in variety for maintaining viability during storage. Deterioration in seed quality as evident with decreased seedling length, dry matter production and vigour index with the passage of time during storage has been reported by Singh *et al.* (1972). Vijay *et al.* (2010) [22] also noticed varied response for seed storability between varieties of soybean wherein, JS-335 was superior to that of Kalitur in maintaining higher vigour parameters upon subjecting natural ageing conditions. The variation within the varieties may be because of varied response to loss of seed membrane integrity due to accumulation of free radicals upon lipid peroxidation during storage which in turn might have reduced the vigour and viability of the stored seeds (McDonald, 1999) [12].

The results revealed that, all the mid storage invigoration treatments improved the germination markedly ranging from 82.50 to 86.75 per cent over control (82.13 %). However, among the treatments, mid storage invigoration with iodine (0.1 %) gave significantly higher germination (86.75 %) followed by CaCl<sub>2</sub>.2H<sub>2</sub>O (1 %) with 84.75 per cent seed germination in the initial month after mid storage seed invigoration (Table 1) compared to control (82.13 %) *i.e.*, 5.6 and 3.2 per cent increase over control due to invigoration with iodine (0.1 %) and CaCl<sub>2</sub>.2H<sub>2</sub>O (1 %), respectively in the initial month after mid storage invigoration (Fig. 1). At the end of storage period, iodine (0.1 %), water hydration and CaCl<sub>2</sub>.2H<sub>2</sub>O (1 %) were able to register 10.8, 8.5 and 8.1 per cent increased seed germination over control. McDonald (1999) [12] claimed that the improvement in germination is influenced by pre-soaking of seeds due to activation of repair mechanisms and metabolic process which occur during water absorption. Better performance exhibited by iodine might be due to its involvement in repair and control mechanism of membrane damage incurred during post harvest storage which is evident from low electrical conductivity across the storage period *i.e.*, in initial, two and four months after storage. Further, iodine rapidly passes into the vapour phase and is also characterised by its great affinity for double bonds of unsaturated lipid constituents of the cell (Gopa and Mukherjee., 1984) [8].

Among the mid storage invigoration treatments, T<sub>6</sub> was significantly superior in maintaining lower mean germination time (1.94) compared to control (2.71) and all other treatments immediately after mid storage invigoration (Table 1). While, the mean germination time went on increasing irrespective of the treatment with the advancement of the storage period to four months. During storage, T<sub>6</sub> was able to maintain significantly lower mean germination time (1.99 and 2.07) followed by T<sub>2</sub> (2.05 and 2.13) and T<sub>7</sub> (2.05 and 2.13) respectively at two and four months after storage.

Similarly, T<sub>6</sub> recorded significantly higher seedling vigour index (3826) compared to all other treatments and control (3090) at initial month of storage (Table 2). The next best treatments were T<sub>7</sub> (3609) and T<sub>2</sub> (3571). Wherein, the seedling vigour index decreased significantly with an increase in the storage period up to four months. Still, T<sub>6</sub> was able to maintain higher seedling vigour index (3649, and 2768) compared to all other treatments including control (2554 and 1924) respectively at two and four months after storage. However, T<sub>6</sub> was followed by T<sub>7</sub> (3433 and 2594) and T<sub>2</sub> (3396 and 2575).

Significantly low mean germination time noticed due to seed invigoration with iodine (0.1%) might be attributed to

hydration and dehydration of seeds during invigoration; which has accelerated the germination process (Airin and Khosro, 2013) [2]. Besides, seed invigoration also permits early DNA replication, increased RNA production and protein synthesis, increased enzyme activity, greater ATP availability, faster embryo growth and efficient repair of deteriorated parts of seed (Mewael *et al.*, 2010) [13]. All these activities might have initiated quicker radical protrusion through seed coat and have accelerated the process of germination and other parameters by shortening the germination time (Elouaer and Hannachi, 2012) [7]. Thus, there was increased seedling vigour indices which was evident from higher values obtained in the invigorated seeds irrespective of the salts and botanicals used and in particular iodine (0.1 %) registered significantly higher seedling vigour index at the end of storage period. Similar results were also reported by Jamadar and Deshpande (2014) [10] in pigeon pea.

As far as electrical conductivity of seed leachate is concerned (Table 2), iodine (0.1 %) was significantly superior in maintaining low electrical conductivity ( $0.406 \text{ dSm}^{-1}$ ) from that of other treatments and control ( $0.486 \text{ dSm}^{-1}$ ). It was followed by T<sub>7</sub> ( $0.419 \text{ dSm}^{-1}$ ) and T<sub>2</sub> ( $0.420 \text{ dSm}^{-1}$ ) in the initial month of storage. Irrespective of the treatments, the electrical conductivity went on increasing with an increase in the storage period up to four months from mid storage invigoration. However, T<sub>6</sub> was able to maintain significantly lower electrical conductivity ( $0.406$  and  $0.473 \text{ dSm}^{-1}$ ) compared to control ( $0.486$  and  $0.542 \text{ dSm}^{-1}$ ) respectively at two and four months after storage. The beneficial effect can, perhaps, be explained in terms of the great affinity of iodine for the carbon-carbon double bonds of unsaturated lipids. In fact, Basu and Rudrapal (1980) [5] have suggested that iodine may react with carbon-carbon double bonds of polyunsaturated fatty acids, hence stabilizing them and making them less susceptible to further oxidation. On the other hand, CaCl<sub>2</sub> and other salts might have a role in maintenance of cell membrane integrity which rendered less susceptible to peroxidative and free radical reactions (Ravi *et al.*, 2007) [15]. Hence, the deterioration of invigorated seeds was less compared to untreated seeds.

Mid storage invigoration treatment with iodine (0.1%) recorded (Table 3) significantly higher OD values for dehydrogenase activity (1.118) compared to other treatments and control (0.589). Even at the end of storage (four months),

T<sub>6</sub> was still able to maintain higher dehydrogenase enzyme activity (1.109) compared to control (0.556). However, T<sub>6</sub> was on par with T<sub>2</sub> in second months after storage (0.987). In the same line Iodine (0.1 %) recorded significantly higher amylase activity (26.8 mm) compared to all other treatments and control (17.8 mm). Wherein, T<sub>6</sub> was on par with T<sub>7</sub> (26.1 mm) in the initial month of storage. With the advancement of storage period to four months, amylase activity decreased irrespective of the treatment imposed. However, Iodine (0.1 %) was able to maintain higher amylase activity throughout the storage period (24.4 and 19.1 mm) compared to control (17.3 and 14.5 mm) at two and four months of storage, respectively (Table 3).

The primary effect of seed invigoration is attributed to certain enzymatic activities that take place in seed, while it is being held in moist condition. At four months of storage, significantly higher dehydrogenase and alpha amylase activity was recorded by invigorating the seeds with iodine (0.1 %) which was on par with water and CaCl<sub>2</sub>.2H<sub>2</sub>O (1 %). Lower lipid peroxidation and lower free fatty acid formation in treated seeds particularly with iodine over untreated suggest that invigoration treatment may act at least partly in scavenging free radical formed during dry storage (McDonald, 1999) [12]. Saha *et al.* (1990) [17] showed that priming caused increased amylase and dehydrogenase activity in aged soybean seed compared to un-primed seeds by lowering lipid peroxidation. The invigoration treatment may act as a preventive measure against ageing.

### Conclusion

Mid storage seed invigoration improved all the seed quality parameters. Among the different salts and botanicals used for invigoration, iodine (0.1 %), water and CaCl<sub>2</sub>.2H<sub>2</sub>O (1%) showed best results in extending the storability of soybean seeds to extra one month compared to control. Since the availability of water to the farmers is more economical, it can also be used in extending the storage potential for another one month instead of iodine and CaCl<sub>2</sub>.2H<sub>2</sub>O.

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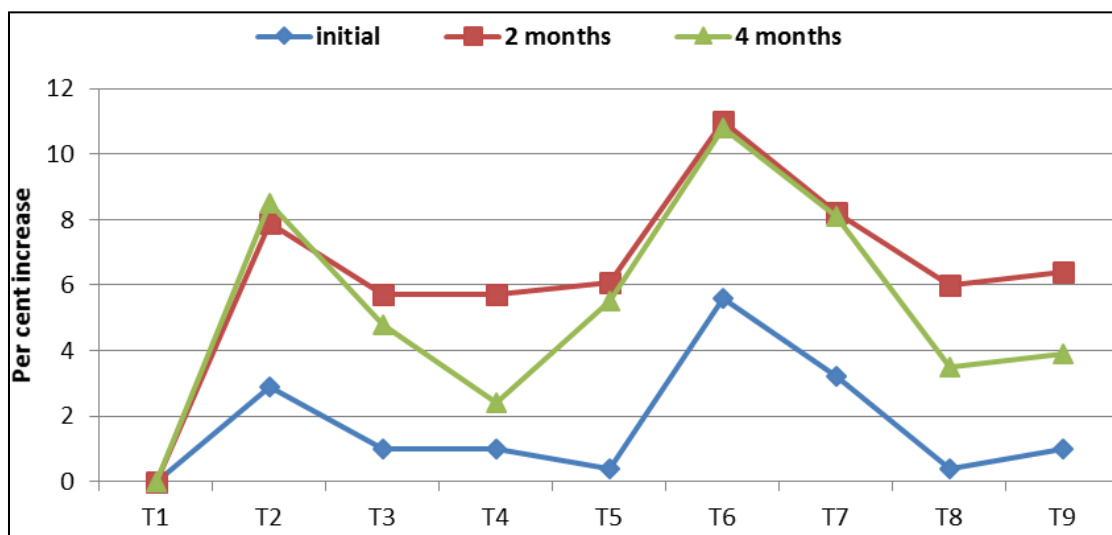


Fig 1: Germination per cent increase over control

**Table 1:** Influence of mid storage invigoration on seed germination (%) and mean germination time of soybean varieties

Varieties Treatments	Months after storage																	
	Seed germination (%)									Mean germination time								
	Initial			2			4			Initial			2			4		
V <sub>1</sub>	V <sub>2</sub>	Mean	V <sub>1</sub>	V <sub>2</sub>	Mean	V <sub>1</sub>	V <sub>2</sub>	Mean	V <sub>1</sub>	V <sub>2</sub>	Mean	V <sub>1</sub>	V <sub>2</sub>	Mean	V <sub>1</sub>	V <sub>2</sub>	Mean	
T <sub>1</sub>	81.00	83.25	82.13	75.75	76.50	76.13	66.75	68.00	67.38	2.72	2.69	2.71	2.92	2.84	2.88	3.11	2.92	3.01
T <sub>2</sub>	82.75	86.25	84.50	80.50	83.75	82.13	71.75	74.50	73.13	2.03	1.96	2.00	2.09	2.02	2.05	2.15	2.10	2.13
T <sub>3</sub>	82.25	83.50	82.88	80.00	81.00	80.50	70.75	70.50	70.63	2.07	1.98	2.03	2.13	2.04	2.09	2.19	2.12	2.16
T <sub>4</sub>	81.75	84.00	82.88	79.50	81.50	80.50	68.75	69.25	69.00	2.13	2.10	2.11	2.19	2.16	2.17	2.25	2.24	2.25
T <sub>5</sub>	81.50	83.50	82.50	79.75	82.50	81.13	71.00	71.25	71.13	2.11	2.03	2.07	2.17	2.09	2.13	2.23	2.17	2.20
T <sub>6</sub>	86.50	87.00	86.75	84.25	84.75	84.50	72.75	76.50	74.63	1.97	1.90	1.94	2.03	1.96	1.99	2.09	2.04	2.07
T <sub>7</sub>	84.50	85.00	84.75	82.25	82.50	82.38	71.75	74.00	72.88	2.00	1.99	2.00	2.06	2.05	2.05	2.12	2.13	2.13
T <sub>8</sub>	81.25	83.75	82.50	79.50	82.00	80.75	69.75	69.75	69.75	2.12	2.09	2.11	2.18	2.16	2.17	2.24	2.24	2.24
T <sub>9</sub>	81.75	84.00	82.88	79.75	82.25	81.00	70.00	70.00	70.00	2.11	2.08	2.09	2.16	2.14	2.15	2.23	2.22	2.22
Mean	82.58	84.47		80.14	81.86		70.36	71.53		2.14	2.09		2.21	2.16		2.29	2.24	
Factors	S.Em±	CD at 1%		S.Em±	CD at 1%		S.Em±	CD at 1%		S.Em±	CD at 1%		S.Em±	CD at 1%		S.Em±	CD at 1%	
V	0.27	0.77		0.33	0.95		0.36	1.02		0.01	0.02		0.01	0.02		0.01	0.02	
T	0.57	1.63		0.71	2.01		0.76	2.17		0.02	0.04		0.02	0.04		0.02	0.05	
V x T	0.81	2.61		1.00	3.07		1.08	3.18		0.02	0.06		0.02	0.06		0.02	0.07	

Legend  
**V<sub>1</sub>**- JS-335  
**V<sub>2</sub>**- DSB-21  
**T<sub>1</sub>**- Control  
**T<sub>2</sub>**- Water  
**T<sub>3</sub>**- NaCl (10<sup>-3</sup>M)  
**T<sub>4</sub>**- KI (10<sup>-3</sup>M)  
**T<sub>5</sub>**- Na<sub>2</sub>HPO<sub>4</sub> (10<sup>-3</sup>M)  
**T<sub>6</sub>**- Iodine (0.1%)  
**T<sub>7</sub>**- CaCl<sub>2</sub>.2H<sub>2</sub>O (1%)  
**T<sub>8</sub>**- Pongamia leaf extract (1%)  
**T<sub>9</sub>**- Custard apple leaf extract (1%)

**Table 2:** Influence of mid storage invigoration on seedling vigour index and electrical conductivity of soybean varieties

Varieties Treatments	Months after storage																	
	Seedling vigour index									Electrical conductivity (dSm <sup>-1</sup> )								
	Initial			2			4			Initial			2			4		
V <sub>1</sub>	V <sub>2</sub>	Mean	V <sub>1</sub>	V <sub>2</sub>	Mean	V <sub>1</sub>	V <sub>2</sub>	Mean	V <sub>1</sub>	V <sub>2</sub>	Mean	V <sub>1</sub>	V <sub>2</sub>	Mean	V <sub>1</sub>	V <sub>2</sub>	Mean	
T <sub>1</sub>	2829	3351	3090	2324	2785	2554	1807	2040	1924	0.507	0.466	0.486	0.496	0.476	0.486	0.610	0.474	0.542
T <sub>2</sub>	3240	3903	3571	3078	3714	3396	2378	2772	2575	0.454	0.387	0.420	0.449	0.376	0.412	0.534	0.425	0.479
T <sub>3</sub>	3004	3592	3298	2846	3410	3128	2157	2468	2312	0.460	0.391	0.425	0.460	0.389	0.424	0.545	0.438	0.491
T <sub>4</sub>	2883	3438	3160	2728	3260	2994	2011	2278	2144	0.471	0.423	0.447	0.491	0.417	0.454	0.576	0.466	0.521
T <sub>5</sub>	2930	3487	3209	2795	3368	3081	2128	2403	2265	0.461	0.396	0.429	0.461	0.392	0.427	0.546	0.441	0.494
T <sub>6</sub>	3622	4030	3826	3450	3848	3649	2610	2926	2768	0.433	0.379	0.406	0.446	0.367	0.406	0.531	0.416	0.473
T <sub>7</sub>	3470	3748	3609	3302	3563	3433	2517	2671	2594	0.449	0.389	0.419	0.450	0.387	0.418	0.535	0.436	0.485
T <sub>8</sub>	2766	3519	3142	2633	3369	3001	1956	2369	2163	0.470	0.414	0.442	0.475	0.394	0.434	0.560	0.443	0.501
T <sub>9</sub>	2802	3510	3156	2660	3360	3010	1980	2364	2172	0.468	0.397	0.433	0.461	0.392	0.427	0.546	0.441	0.494
Mean	3061	3620		2868	3409		2171	2477		0.464	0.405		0.465	0.399		0.554	0.442	
Factors	S.Em±	CD at 1%		S.Em±	CD at 1%		S.Em±	CD at 1%		S.Em±	CD at 1%		S.Em±	CD at 1%		S.Em±	CD at 1%	
V	18	52		20	57		16	47		0.002	0.006		0.002	0.005		0.001	0.004	
T	39	111		42	121		35	99		0.003	0.009		0.003	0.008		0.003	0.008	
V x T	55	157		60	170		49	148		0.004	0.011		0.004	0.012		0.004	0.011	

Legend  
**V<sub>1</sub>**- JS-335  
**V<sub>2</sub>**- DSB-21  
**T<sub>1</sub>**- Control  
**T<sub>2</sub>**- Water  
**T<sub>3</sub>**- NaCl (10<sup>-3</sup>M)  
**T<sub>4</sub>**- KI (10<sup>-3</sup>M)  
**T<sub>5</sub>**- Na<sub>2</sub>HPO<sub>4</sub> (10<sup>-3</sup>M)  
**T<sub>6</sub>**- Iodine (0.1%)  
**T<sub>7</sub>**- CaCl<sub>2</sub>.2H<sub>2</sub>O (1%)  
**T<sub>8</sub>**- Pongamia leaf extract (1%)  
**T<sub>9</sub>**- Custard apple leaf extract (1%)

**Table 3:** Influence of mid storage invigoration on dehydrogenase activity and alpha amylase activity of soybean varieties

Varieties Treatments	Months after storage																	
	Dehydrogenase activity (OD value)									Alpha amylase (mm)								
	Initial			2			4			Initial			2			4		
	V <sub>1</sub>	V <sub>2</sub>	Mean	V <sub>1</sub>	V <sub>2</sub>	Mean	V <sub>1</sub>	V <sub>2</sub>	Mean	V <sub>1</sub>	V <sub>2</sub>	Mean	V <sub>1</sub>	V <sub>2</sub>	Mean	V <sub>1</sub>	V <sub>2</sub>	Mean
T <sub>1</sub>	0.608	0.570	0.589	0.581	0.532	0.556	0.577	0.556	0.567	17.0	18.5	17.8	16.0	18.5	17.3	14.0	15.0	14.5
T <sub>2</sub>	1.034	1.177	1.106	0.949	1.103	1.026	0.738	0.809	0.773	24.3	26.3	25.3	22.3	23.5	22.9	16.8	20.8	18.8
T <sub>3</sub>	0.940	1.025	0.982	0.855	0.950	0.903	0.644	0.681	0.663	24.3	24.8	24.5	22.3	22.0	22.1	16.8	16.8	16.8
T <sub>4</sub>	0.897	0.895	0.896	0.812	0.821	0.816	0.601	0.628	0.614	20.8	20.8	20.8	18.8	18.0	18.4	15.3	16.0	15.6
T <sub>5</sub>	0.901	1.003	0.952	0.816	0.928	0.872	0.605	0.659	0.632	22.8	25.0	23.9	20.8	22.3	21.5	15.8	16.5	16.1
T <sub>6</sub>	1.137	1.240	1.188	1.052	1.166	1.109	0.780	0.872	0.826	26.3	27.3	26.8	24.3	24.5	24.4	18.0	20.3	19.1
T <sub>7</sub>	1.100	1.027	1.064	1.020	0.953	0.987	0.757	0.729	0.743	26.0	26.3	26.1	24.0	23.5	23.8	17.5	19.8	18.6
T <sub>8</sub>	0.884	0.948	0.916	0.798	0.873	0.836	0.597	0.633	0.615	22.8	22.0	22.4	20.8	19.3	20.0	15.3	14.5	14.9
T <sub>9</sub>	0.888	0.954	0.921	0.803	0.879	0.841	0.589	0.610	0.600	23.3	23.0	23.1	21.3	20.3	20.8	16.0	15.0	15.5
Mean	0.932	0.982		0.854	0.912		0.654	0.686		23.0	23.8		21.1	21.3		16.1	17.2	
Factors	S.Em±	CD at 1%		S.Em±	CD at 1%		S.Em±	CD at 1%		S.Em±	CD at 1%		S.Em±	CD at 1%		S.Em±	CD at 1%	
V	0.008	0.023		0.013	0.037		0.008	0.023		0.2	0.6		0.3	NS		0.2	0.6	
T	0.017	0.048		0.028	0.078		0.017	0.048		0.4	1.2		0.6	1.8		0.4	1.2	
V x T	0.024	0.068		0.039	0.108		0.024	0.068		0.6	1.9		0.9	2.7		0.6	1.7	

Legend  
**V<sub>1</sub>**- JS-335  
**V<sub>2</sub>**- DSB-21  
**T<sub>1</sub>** - Control  
**T<sub>2</sub>** - Water  
**T<sub>3</sub>** - NaCl (10<sup>-3</sup>M)  
**T<sub>4</sub>** - KI (10<sup>-3</sup>M)  
**T<sub>5</sub>** - Na<sub>2</sub>HPO<sub>4</sub> (10<sup>-3</sup>M)  
**T<sub>6</sub>** - Iodine (0.1%)  
**T<sub>7</sub>** - CaCl<sub>2</sub>.2H<sub>2</sub>O (1%)  
**T<sub>8</sub>** - Pongamia leaf extract (1%)  
**T<sub>9</sub>** - Custard apple leaf extract (1%)

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