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Effect of seed treatments and storage containers on seed quality parameters of stored blackgram var. ADT 3

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Abstract
Laboratory experiment were conducted to evaluate the efficacy of comprehensive seed treatments of black gram seed during storage in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2010 - 2011. The seeds were treated with Polymer @ 3ml kg^{-1} of seed, Polymer @ 3ml kg^{-1} of seed + Imidacloprid @ 2ml kg^{-1} of seed + Carbendazim @ 2g kg^{-1} of seed, Polymer @ 3ml kg^{-1} of seed + Halogen mixture @ 3g kg^{-1} of seed (chlorine based) ($CaCl_2$ + $CaCO_3$ in 1:1 ratio), rhizome powders of turmeric (*Curcuma longa*) and vasambu (*Acorus calamus*) @ 3g kg^{-1} of seed along with control. After treatment, seeds were stored in cloth bag and polythene bag container for evaluation of seedling quality and biochemical parameters where data was analysed by factorial experiment laid out in completely randomized design. The results revealed that, seed treated with Polymer @ 3ml kg^{-1} of seed + Imidacloprid @ 2ml kg^{-1} of seed + Carbendazim @ 2g kg^{-1} of seed recorded highest germination percentage (82), root length (15.9 cm), shoot length (25.4 cm), drymatter production (254 mg / 10 seedlings) and vigour index (3699) over all other seed treatments after storage period of nine months in polythene bag without loss in vigour and viability of seeds.

Keywords: Blackgram seed, Comprehensive seed treatment, Storage container

Introduction
Seed being a biological entity, deterioration is inevitable, irreversible and inexorable. Loss of vigour and viability is hereditary in nature and senescence is common for the entire living organism. Seed deterioration is a phenomenon, which begins immediately after attaining physiological maturity even on the mother plant itself (Helmer *et al.*, 1962) [16]. One of the most important basic needs for higher productivity is quality seed characterized by higher viability and vigour (Yaklich *et al*., 1979) [60]. These two characters cannot be maintained in storage especially in legumes, because they rapidly deteriorate under ambient storage condition. Pulses are mesobiotic in storage behaviour, which can only be stored upto a storage period of three years (Ewart, 1908) [12] and their higher lipoprotein content which gain moisture on storage undergo rapid lipid peroxidation (Agrawal and Dadlani, 1995) [2] that leads to degradation of seed quality characters and finally ends with death of seed. During storage, qualitative and quantitative losses upto 8.3 per cent have been reported in India (Anon, 1978) [6]. The quality of seed in storage is determined both by intrinsic and extrinsic factors. Among the diversified factors, pre storage treatment (Kalyan and Dadhich, 1999) [24] play a vital role in extending the storability of seeds to longer duration without appreciable loss in vigour and viability would be heeled for farmers and seed producers. Hence, studies were made to evaluate the pre storage seed treatment for seed quality parameters in stored black gram seeds.

Materials and methods
Genetically pure, freshly harvested breeder seeds of blackgram var.ADT 3 obtained from Agricultural Research Station, Bhavanisagar served as the base material for the study. The experiments were carried out in the Department of Seed Science and Technology, TNAU, Coimbatore during 2010-2011. The seeds were pre-cleaned and dried to eight per cent moisture content and were imposed with various pre-storage seed treatments viz., Polymer @ 3ml kg^{-1} of seed, Polymer @ 3ml kg^{-1} of seed + Imidacloprid @ 2ml kg^{-1} of seed + Carbendazim @ 2g kg^{-1} of seed, Polymer @ 3ml kg^{-1} of seed + Halogen mixture @

3g kg^{-1} of seed (chlorine based) ($CaCl_2$ + $CaCO_3$ in 1:1 ratio), rhizome powders of turmeric (Curcuma longa) and vasambu (*Acorus calamus*) @ 3g kg^{-1} of seed along with control. The treated seeds were kept in cloth bag and polythene bag stored under ambient conditions for a period of nine months and evaluated the seed quality and biochemical parameters.

Moisture content (%)

Five gram of seeds in triplicate were taken separately in a pre-weighed (M_1) moisture estimation bottle and the sample weights along with the bottle were recorded (M_2). The bottles were kept in a hot air oven maintained at 105±2ºC for 6 h. Then the bottles were taken out and cooled in a desiccator with calcium carbonate for 30 minutes. The weight of bottle along with dried seeds were recorded (M_3) individually. The moisture content was calculated on wet weight basis adopting the following formula and expressed as percentage (ISTA, 1999) [18].

$$\text{Moisture content (\%)} = \frac{M_2\text{-}M_3}{M_2\text{-}M_1} \times 100$$

Germination (%)

Germination test, in quadruplicate of 100 seeds, each with four sub replicates of 25 seeds were carried out in roll towel in a germination room maintained at temperature of 25 ± 1º C and RH of 96 ± 2% with diffused light. Final count based on normal seedlings was recorded on seventh day and the mean recorded as germination in percentage (ISTA, 1999) [18].

Root length (cm)

After the germination period of seven days, ten normal seedlings were selected at random in each of the replication, and were measured for root length, from the collar region to the tip of primary root using measuring scale. The mean expressed as root length in centimetre.

Shoot length (cm)

Seedlings used for measuring root length were also used for measuring shoot length. The length between the collar regions to tip of the primary leaf (Plumule) was measured and the mean expressed as shoot length in centimetre.

Dry matter production (mg/ 10 seedlings)

Seedlings used for growth measurement were dried in a hot air oven maintained at 85±2°C for 24 h and cooled in a desiccator for 30 min. and weighed in an electronic balance and the mean expressed as dry matter production per 10 seedlings in milligram.

Vigour index

Vigour index (VI) was calculated by using the formula suggested by Abdul-Baki and Anderson, 1973 [5] and the mean expressed in whole number.

VI = Germination (%) x [root length (cm) + shoot length (cm)].

Electrical conductivity (dSm^{-1})

Four replicates of fifty seeds from each treatment were taken at random, prewashed with deionised water to remove the adhering seed coating materials and soaked in 50 ml of deionized water for 6 hours at room temperature. The seed leachate was decanted and electrical conductivity was measured in digital conductivity bridge with a cell constant of one. The electrical conductivity values were expressed in decisiemens. (Presley, 1958) [40].

Dehydrogenase enzyme activity (OD Value)

Twenty five seeds in four replicates from each treatment were taken and preconditioned by soaking in water for 6 h at room temperature. The seeds were prepared by removing seed coat to expose the embryonic axis. These seeds were steeped in 0.25 per cent 2, 3, 5 – triphenyl tetrazolium chloride solution and kept in dark for 2 h at 40 ºC for staining. The stained seeds were thoroughly washed with water and then steeped in 25 ml of 2 methoxy ethanol (methyl cellosolve) and kept in dark for overnight to extract the red coloured formazon. The intensity of red colour was measured in spectronic 20 at 470 nm and OD value as recorded. The mean values for dehydrogenase activity was expressed as optical density value (Kittock and Law, 1968) [30].

Seed protein content (%)

The protein content of seed was estimated as per the method outlined by Ali-Khan and Youngs (1973) [3]. Two replicates of 100 mg of finely powdered seed material was taken in 50 ml polythene screw cap bottle and 25 ml of 1N NaOH was added.

The mixture was shaken for 30 minutes on a wrist action shaker to disperse the protein. Then 10 ml of suspension was transferred into graduated test tube and used as blank to compensate the differences in the amount of natural pigment extracted. To the remaining suspension in bottle, 0.25 ml of 10 per cent copper sulphate was added and the bottle was reshaken for five minutes to develop the colour complex. The sample solution was then poured into a separate test tube and along with its blank left overnight to allow the dispersed material to settle down. After centrifugation for ten minutes at 3000 rpm, the optical density (OD) of the clear supernatant solution was measured in spectronic 20 at 620 nm with suitable blank. The percentage of protein was calculated by using the following formula. Protein percentage = 3.78 + (61.6 × OD value)

Statistical Analysis

The data obtained from different experiments were analysed for 'F' test of significance following the methods described by Panse and Sukhatme, 1985 [37]. Wherever necessary and the per cent values were transformed to angular (Arc-sine) values before analysis. The critical differences (CD) were calculated at 5 per cent probability level. The data were tested for statistical significance (*). If F test is non-significant, it was indicated as NS.

Results and discussion

One of the factors that determine the viability of seeds in storage is the seed moisture content. The result revealed that, overall gain in moisture in nine months of storage was 0.9 per cent irrespective of treatments however, it was within the safe limit. Cloth bag being a pervious container, moisture exchange took place until it reached the equilibrium with the storage environment (Malarkodi, 1997) [32]. Increase in seed moisture might be due to the hygroscopic nature of seed (Harrington, 1972; Roberts, 1986) [15, 42]. In polythene bag container, the increase in moisture content was less due to their lesser amenability to variations in atmospheric moisture as reported by (Sumathi, 2010) [49] in karpokkarasi. Among the treatments, seeds coated with polymer @ 3ml kg^{-1} + imidacloprid @ 2ml kg^{-1} + carbendazim @ 2g kg^{-1} registered lower moisture content than untreated seeds. The moisture content was comparatively low compared to control due to the preventive mechanism in seed coated with fungicide and

insecticide (Manjunatha *et al.*, 2008) [34]. Duan and Burris (1997) [10] explained the possibilities of using polymers along with other chemicals to ensure the keeping quality of seeds. Dexter and Takao (1960) [8] and West *et al*. (1985) [58] opined that adsorption of moisture from the storage environment has been reduced by coating the seeds with polymer in soybean and sugarbeet, respectively.

The germination potential is the basic requirement for any seeds. The viability and vigour are the two important factors of seed quality and they go hand in hand while judging the seed quality. Seeds treated with polymer @ 3ml kg^{-1} + imidacloprid @ 2ml kg^{-1} + carbendazim @ 2g kg^{-1} able to maintain 88 and 90 per cent germination thoroughout the period both in cloth bag and polythene bag, respectively. On the other hand the loss in germination was to an extent of ten per cent in untreated seeds (Table 1). The decline in germination percentage over the storage period

was due to ageing effect leading to depletion of food reserves, seed deterioration, fluctuating temperature, relative humidity and seed moisture content as influenced by storage packaging materials. These findings were similar with Jitendra *et al.*, 2007 [19]. The beneficial effect of coating the seeds with polykote was reported by many researchers (Sherin, 2003; Giang and Rame Gowda, 2007; Manjunatha *et al*., 2008; Sureshvegulla, 2008) [44, 14, 34, 51]. According to Taylor and Harman (1990) [52], the coating process is worthy to be applied for many species of seeds with consequent improvement on the seed quality and maintenance of properties after storage.

Table 1: Effect of seed treatments on germination (%) of blackgram var. ADT 3

Treatments	Cloth bag (Storage period in months)					Polythene bag (Storage period in months)				
	Initial	3	6	9	Mean	Initial	3	6	9	Mean
T_1	91(72.54)	84(66.42)	74(64.89)	64(53.13)	**78(62.02)**	91(72.54)	88(69.73)	79(70.63)	70(57.26)	82(64.89)
T_2	95(77.08)	91(72.54)	83(65.65)	76(60.66)	86(68.02)	95(77.08)	92(73.57)	84(66.42)	79(62.72)	88(69.73)
T_3	94(75.82)	89(70.63)	80(63.43)	74(64.89)	84(66.42)	94(75.82)	91(72.54)	82(64.89)	77(61.34)	86(68.02)
T_4	92(73.57)	86(68.02)	77(61.34)	68(56.06)	81(64.55)	92(73.57)	88(69.73)	80(63.43)	72(58.05)	83(65.65)
T_5	96(78.46)	92(73.57)	85(67.21)	79(62.72)	88(69.73)	96(78.46)	93(74.66)	87(68.86)	82(64.89)	90(71.56)
T_6	94(75.82)	87(68.86)	79(62.72)	71(57.41)	83(65.65)	94(75.82)	89(70.63)	80(63.43)	75(60.00)	85(67.21)
Mean	94(75.82)	88(69.73)	80(63.43)	72(58.05)	83(65.65)	94(75.82)	90(71.56)	82(64.89)	76(60.66)	85(67.21)
	P	C	T	PxC	CxT	PxT	PxCxT			
SEd	(0.29)	(0.10)	(0.90)	(0.01)	(0.03)	(0.02)	(0.05)			
CD (P = 0.05)	(0.52)	(0.20)	(1.82)	(NS)	(NS)	(NS	(NS)			

(Figures in parentheses indicate arc sine transformed values)

T_1 - Control; T_2 - Vasambu rhizome powder @ 3g kg^{-1} of seed;T_3 - Turmeric rhizome powder @ 3g kg^{-1} of seed; T_4 - Polymer @ 3ml kg^{-1} of seed ;T_5 - Polymer @ 3ml kg^{-1} of seed + Imidacloprid @ 2ml kg^{-1} of seed + Carbendazim @ 2g kg^{-1} of seed;T_6 - Polymer @ 3ml kg^{-1} of seed + Halogen mixture @ 3g kg^{-1} of seed (chlorine based) ($CaCl_2$ + $CaCO_3$ in 1:1 ratio).

The observations on the evaluated vigour parameters *viz.*, seedling length, dry matter production and vigour index were in line with germination percentage which also decreased with increase in storage period. In the present study, seed treated with polymer @ 3ml kg^{-1} + imidacloprid @ 2ml kg^{-1} + carbendazim @ 2g kg^{-1} recorded highest root length (15.6 and 15.9 cm), shoot length (25.2 and 25.4 cm), drymatter production (232 and 254 mg / 10 seedlings) and vigour index (3588 and 3699) (Table 2) both in cloth bag and polythene bag, respectively during the storage period. Vishnurammethi (1996) [57] in cowpea, Vakeswaran (1998) [53] in peas, Nakka *et al*. (1999) [35] in soybean and Ananthi (2001) in cowpea, also reported similar results.

Table 2: Effect of seed treatments on vigour index of blackgram var. ADT 3

Treatments	Cloth bag (Storage period in months)					Polythene bag (Storage period in months)				
	Initial	3	6	9	Mean	Initial	3	6	9	Mean
T_1	3767	3293	2775	2246	2997	3767	3520	3026	2590	3212
T_2	4038	3740	3270	2896	3474	4038	3818	3368	3089	3570
T_3	3957	3613	3112	2768	3349	3957	3749	3255	2972	3474
T_4	3836	3423	2934	2448	3141	3836	3564	3112	2707	3293
T_5	4109	3818	3409	3057	3588	4109	3906	3550	3255	3699
T_6	3948	3506	3034	2599	3254	3948	3631	3136	2865	3384
Mean	3942	3563	3086	2663	3298	3942	3697	3239	2909	3437
	P	C	T	PxC	CxT	PxT	PxCxT			
SEd	11.56	7.02	53.92	16.05	74.92	124.61	132.42			
CD (P = 0.05)	23.15	13.94	107.8	NS	NS	NS	NS			

T_1 - Control; T_2 - Vasambu rhizome powder @ 3g kg^{-1} of seed;T_3 - Turmeric rhizome powder @ 3g kg^{-1} of seed; T_4 - Polymer @ 3ml kg^{-1} of seed ;T_5 - Polymer @ 3ml kg^{-1} of seed + Imidacloprid @ 2ml kg^{-1} of seed + Carbendazim @ 2g kg^{-1} of seed;T_6 - Polymer @ 3ml kg^{-1} of seed + Halogen mixture @ 3g kg^{-1} of seed (chlorine based) ($CaCl_2$ + $CaCO_3$ in 1:1 ratio).

In the present storage experiment, a progressive decline in vigour and viability of seeds was noticed with advancement in storage period. Deterioration of seedling vigour in stored seeds was associated with weakening of cell membrane (Heydecker, 1972) [17]. This reduction in physiological and biochemical quality might be due to depletion of stored food reserves (Koostra and Harrington, 1969) [31] and decline in synthetic activity of the embryo. This might be due to reduced seed deterioration (Natesan, 2006; Selvakumari, 2010) [36, 43] fungicidal property of carbendazim and insecticidal property of imidacloprid (Reddy, 1985) [41]. Seed treatment with carbendazim or like compounds, separately or in combination

with suitable insecticides to maintain better germination in storage was reported by many researchers (Paramasivam, 1990; Sujatha, 1994 and Arulprabhu, 1998) [38, 48, 7]. However, high seedling vigour index in polymer coat with combination of fungicide, is due to more germination, seedling length and dry weight. Similar findings were also reported with Sinta *et al.,* (2014) [45] and Kamara *et al.,* (2014) [25]. This was conformity with the results of Geethalakshmi and Venugopal (1998) [13] in greengram, Nakka *et al*. (1999) [35] in Soybean and Eevera (2000) [11] in blackgram.

Electrical conductivity of seed leachate is normally recorded as an important biochemical manifestation and this measurement in the present study increased gradually over periods of storage from 0.221 dSm^{-1} to 0.263 dSm^{-1} and 0.221 dSm^{-1} to 0.282 dSm^{-1}, irrespective of the treatments both in cloth bag and polythene bag. The increase in electrical conductivity of seeds could have been focused to loss of membrane integrity that multiplies with the advancement in storage period as detailed by Koostra and Harrington (1972) [31] and Dharmalingam *et al*. (1976) [9]. The electrical conductivity of coated seed (polymer at 3ml kg^{-1}, imidacloprid at 2ml kg^{-1} and carbendazim at 2g kg^{-1}) was indeed very low whereas it was high in untreated seed, indirecting the leaching of lesser quantum of protoplasmic substances into seed steep water due to maintanance of semi permeability.

Seeds treated with polymer @ 3ml kg^{-1} + imidacloprid @ 2ml kg^{-1} + carbendazim @ 2g kg^{-1} reduced the dehydrogenase activity with increase in storage period highlighting the biochemical protection given to this molecular enzymes for prolonged shelf life of resultant seed managed against seed quality character. The decrease in enzyme activity due to storage is in agreement with the findings of Kittock and Law (1968) [30], Woodstock (1973) [59] and Sung (1996) [50]. This biochemical test result corroborated very well with the physiological manifestations of the seed namely the seedling growth parameters. Similar results have been reported by Parameshwari (2002) in redgram and Malarkodi (2003) [33] in greengram.

During storage, protein becomes less soluble and degraded into free amino acids (Anderson, 1973) [1]. Hence, the denaturation of protein could also be one of the reason for loss of physiological vigour in the seeds during storage. The estimation of protein content of stored seed is an effective method for determination of its vigour. In line with these views the protein content, evaluated as a measure of vigour in the present investigation degraded with ageing. Dharmalingam *et al*. (1976) [9] in blackgram, Arulprabhu (1998) [7] in pole bean, Vakeswaran (1998) [53] in pea and Eevera (2000) [11] in blackgram, have observed similar results. The degradation of protein could be preserved to some extent by treating the seeds either with polymer / fungicide / insecticide / biopesticide their combination compared to untreated seed. However decrease in protein content was observed with increase in storage period. The reduction in the protein content may be attributed to Oxidation of the amino acids, due to increase in the respiratory Activity as a result of deterioration process of the stored seeds. These findings were similar with Veraja *et al.,* (2015) [54].

Conclusion

Overall results shows that, seeds of blackgram were treated with polymer @ 3ml kg^{-1} + imidacloprid @ 2ml kg^{-1} + carbendazim @ 2g kg^{-1} and packed in polythene bag stored for nine-months under ambient conditions was very effective for extending the seed longevity and maintaining the storability by safe guarding seed.

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