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Research Article

EFFICIENCY OF SEED TREATMENTS AND STORAGE CONTAINERS ON PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF BLACKGRAM SEEDS

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Abstract: An experiment was conducted in Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2017-18 to study the suitable seed dry dressing treatments to alleviate the deleterious effects during storage and improve the physiological and biochemical performance of seeds in blackgram CO 6. The seeds were subjected to different seed treatments with botanicals Fenugreek (*Trigonella foenum*-graecum) seed powder @ 3g/kg, Castor (*Ricinus communis*) oil @ 5ml/kg, Neem (*Azadirachata indica*) oil @ 5ml/kg; Sea weed Sargassum myricocystum @ 3g/kg, Gracilaria edulis @ 3g/kg and Nano particles of ZnO@1250mg/kg of seeds and untreated seeds served as control. Seed samples were drawn at monthly intervals and tested its physical, physiological and biochemical properties. The result revealed that blackgram CO 6 seeds treated with Nano particles of ZnO@1250mg/kg, recorded maximum seed germination (88%) than control (79%) and maintained the antioxidant enzyme activity of seeds up to 12 months of storage followed by Fenugreek (*Trigonella foenum*-graecum) seed powder @ 3 g/kg. Super grain bag performed as better storer than cloth bag and it was evident that anti oxidative enzymes and better cell membrane integrity of stored seeds increased after seed treatments. Since, these treatments contain higher antioxidant activity and rich in minerals such as titanium, molybdenum and iron, apart from other trace elements.

Keywords: Blackgram, Storage container, Botanicals, Sea weed, Nanao particles, Moisture, Germination, Electrical conductivity, Dehydrogenase activity

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Introduction

Blackgram (*Vigna mungo* L.) is a protein rich food, containing about 26% protein, which is almost three times that of cereals and ranks third among the major pulses cultivated in India. Blackgram supplies a major share of protein requirement of vegetarian population of the country. In India, blackgram occupies 12.7% of total area under pulses and contribute 8.4% of total pulses production. This emphasized the need to increase the performance of pulse crops, where most grain legume production is for human consumption and demand is increasing due to population increase [1]. The poor performance of blackgram may be attributed to several factors, of which level of seed deterioration is of great importance. It was proven that the deteriorating effect of seeds during was mainly due to the production of free radicals [2,3] and use of antioxidants can quench the free radicals and prolong the storability of seeds [4,5]. Genetically, presence of antioxidant property along with high nutrient content was pharmacologically proved in fenugreek (*Trigonella foenum*-graecum) seed powder [6,7]. However, dry dressing of seeds will be more effective rather than wet treatment; since wet treatment leads to soaking injury due to the hygroscopic nature of pulses seeds [8]. Heydecker (1972) [8] stated that deterioration of vigour in stored seeds was associated with the weakening of cell membrane. Increased leachate was related to low metabolic activity of seed [10]. Membrane integrity as a measure of vigour and viability had been reported widely. The loss of membrane integrity and a decrease in proportion of unsaturated fatty acids have been reported as causes for seed deterioration and presumably a loss in membrane permeability under unfavorable condition of storage resulting in increased leachate of seed constituents and thus loss in viability [11]. The most important factor that determines the longevity of seeds in storage is moisture content of seed, temperature and relative humidity of the storage atmosphere. As seeds being hygroscopic in nature, exhibit fluctuation in seed moisture content due to changes with atmospheric relative humidity and temperature, so it is essential to preserve them in suitable moisture proof containers which eliminates dampness, deterioration, microorganisms, and enhances the seed longevity. Storability of seed is influenced by packing materials irrespective of its storage conditions [12]. In this study, the dynamics of antioxidant enzyme bustle and germination uniqueness in storage seeds of blackgram Co-6 with different seed treatment during storage were examined to investigate the physiological and biochemical changes during 12 months of storage upon seed treatment and storage containers.

Materials and Methods

Genetically, pure, freshly harvested seeds of blackgram CO 6 obtained from the Department of Pulses, Tamil Nadu Agricultural University (TNAU) Coimbatore. The research was conducted at Department of Seed Science and Technology, TNAU Coimbatore, Tamil Nadu, India. After assessing the initial quality parameters seeds were subjected to different seed treatments viz., Fenugreek (*Trigonella foenum*-graecum) seed powder @ 3g/kg, Castor (*Ricinus communis*) oil @ 5ml/kg, Neem (*Azadirachata indica*) oil @ 5ml/kg; Sea weed Sargassum myricocystum @ 3g/kg, *Gracilaria edulis* @ 3g/kg and Nano particles of ZnO@1250mg/kg of seeds and untreated seeds served as control. After the treatment, the seeds were packed in Cloth Bag and Super Grain Bag and stored in ambient condition 25 ± 2°C temperature and 95 ± 3 % RH under room temperature. The physiological and biochemical activity of stored seeds were assessed up to 12 months of storage.

Moisture content

Five gram of ground seed material was placed in a moisture weighing bottle and kept in a hot air oven maintained at 130 ± 2°C for 1 h for drying and cooled in a desiccator containing silica gel for 30 min. The weight of seeds along with moisture bottle before and after drying was recorded in gram.

Table-1 Influence of seed treatments, storage containers and period of storage on moisture content of blackgram variety CO-6.

Seed Treatment (T)	Storage Containers (C)											
	Cloth bag						Super grain bag					
	Period of storage (p)											
	P0	P3	P6	P9	P12	MEAN	P0	P3	P6	P9	P12	MEAN
T_0	8	8.4	8.9	9.8	10.6	9.1	8	8.3	8.7	9.2	9.7	8.8
T_1	8	8.2	8.4	8.9	9.4	8.6	8	8.1	8.3	8.6	8.8	8.3
T_2	8	8.3	8.5	9.2	10.1	8.8	8	8.2	8.5	8.8	9.3	8.6
T_3	8	8.2	8.6	9.3	10.2	8.9	8	8.2	8.5	8.9	9.4	8.6
T_4	8	8.3	8.5	9.1	9.7	8.7	8	8.2	8.5	8.9	9	8.5
T_5	8	8.3	8.6	9.2	9.8	8.8	8	8.3	8.5	9	9.2	8.6
T_6	8	8.2	8.4	8.8	9.3	8.5	8	8	8.3	8.6	8.7	8.3
Mean	8	8.3	8.6	9.2	9.8		8	8.2	8.5	8.9	9.1	

Treatments (T)	TxP Interaction Mean					Treatments (Mean)
	P_0	P_3	P_6	P_9	P_{12}	
T_0	8	8.4	8.8	9.5	10.2	9
T_1	8	8.2	8.4	8.8	9.1	8.5
T_2	8	8.3	8.5	9	9.7	8.7
T_3	8	8.2	8.6	9.1	9.8	8.7
T_4	8	8.3	8.5	9	9.3	8.6
T_5	8.1	8.3	8.6	9.1	9.5	8.7
T_6	8	8.1	8.4	8.7	8.9	8.4
Mean	8	8.2	8.5	9	9.5	

	T	P	C	TxP	CxP	TxC	TxPxC
SEd	0.0548	0.0463	0.0293	0.1226	0.0655	0.0775	0.1733
CD(P=0.05)	0.1084	0.0916	0.0579	0.2424	0.1296	NS	NS

T_0 - Control seed; T1 - Fenugreek seed powder @ 3g/kg; T2 - Castor oil @ 5ml/kg; T_3 - Neem oil @ 5ml/kg; T4 - Sea weed powder *Sargassum myricocystum* @ 3g/kg; T_5 - *Gracilaria edulis* @ 3g/kg, T6 - Nano particles of ZnO @ 1250mg/kg.

Table-2 Influence of seed treatments, storage containers and period of storage on germination (%) of blackgram variety CO-6

Seed Treatment (T)	Storage Containers (C)											
	Cloth bag						Super grain bag					
	Period of storage (p)											
	P0	P3	P6	P9	P12	MEAN	P0	P3	P6	P9	P12	MEAN
T_0	99(84.26)	94(75.82)	89(70.63)	79(62.03)	70(56.79)	86(68.03)	99(84.26)	96(78.46)	92(73.57)	85(67.21)	79(62.73)	91(72.54)
T_1	100(90.00)	98(81.87)	94(75.82)	87(68.87)	80(63.43)	92(73.57)	100(90.00)	99(84.26)	98(81.87)	92(73.57)	85(67.21)	95(77.08)
T_2	99(84.26)	95(77.08)	90(71.57)	84(66.42)	78(62.03)	89(70.63)	99(84.26)	98(81.87)	97(80.03)	89(70.63)	82(64.90)	93(74.66)
T_3	99(84.26)	97(80.03)	95(77.08)	84(66.42)	77(61.34)	90(71.57)	99(84.26)	97(80.03)	96(78.46)	87(68.87)	82(64.90)	93(74.66)
T_4	99(84.26)	95(77.08)	91(72.54)	85(67.21)	78(62.03)	90(71.57)	99(84.26)	98(81.87)	97(80.03)	90(71.57)	83(65.65)	93(74.66)
T_5	99(84.26)	96(78.46)	90(71.57)	83(65.65)	75(60.00)	89(70.63)	99(84.26)	97(80.03)	96(78.46)	89(70.63)	82(64.90)	93(74.66)
T_6	100(90.00)	98(81.87)	94(75.82)	89(70.63)	80(63.43)	92(73.57)	100(90.00)	99(84.26)	98(81.87)	93(74.66)	88(69.73)	96(78.46)
Mean	99(84.26)	97(80.03)	95(77.08)	87(68.87)	81(64.16)		99(84.26)	98(81.87)	97(80.03)	90(71.57)	84(66.42)	

(*Figures in parentheses indicate arcsine transformed values*)

Treatments (T)	T x P Interaction Mean					Treatments (Mean)
	P_0	P_3	P_6	P_9	P_{12}	
T_0	99(84.26)	96(78.46)	91(75.24)	82(64.90)	75(60.00)	88(69.73)
T_1	100(90.00)	99(84.26)	96(78.46)	90(71.57)	83(65.65)	93(74.66)
T_2	99(84.26)	97(80.03)	94(75.82)	87(68.87)	80(63.43)	91(72.54)
T_3	99(84.26)	98(81.87)	96(78.46)	86(68.03)	80(63.43)	92(73.57)
T_4	99(84.26)	97(80.03)	94(75.82)	88(69.73)	81(64.16)	92(73.57)
T_5	99(84.26)	97(80.03)	94(75.82)	86(68.03)	79(62.73)	91(72.54)
T_6	100(90.00)	99(84.26)	96(78.46)	91(72.54)	84(66.42)	94(75.82)
Mean	99(84.26)	98(81.87)	96(78.46)	89(70.63)	83(65.65)	

	T	P	C	TxP	CxP	TxC	TxPxC
SEd	0.8382	0.7084	0.4480	1.8743	1.0019	1.1854	2.6507
CD(P=0.05)	1.6576	1.4009	0.8860	NS	1.9813	NS	5.2420

The moisture content was calculated using following formula and expressed as percentage [13].

$$Moisture\ content\ (\%) = \frac{M2 - M3}{M2 - M1} \times 100$$

Where,

M1 – Weight of moisture weighing bottle alone

M2 – Weight of moisture weighing bottle + seed sample before drying

M3 – Weight of moisture weighing bottle + seed sample after drying

Germination

Germination test was conducted by following the procedure outlined in ISTA Seed Testing Rules (2011) with roll towel medium using 4 x 100 seeds in a germination room maintained at 25 ± 2°C temperature and 95 ± 3 % RH. After the germination period of seven days the seedlings were evaluated. Based on normal seedlings, the germination was calculated adopting the following formula and the mean expressed as percentage.

$$Germination\ (\%) = \frac{Number\ of\ normal\ seedlings}{Total\ number\ of\ seeds\ sown} \times 100$$

Electrical conductivity of seed leachate

Four replicates of fifty seeds from each genotype and container were drawn, prewashed well with distilled water and then soaked in 75 ml of distilled water for duration 6 h at room temperature. After soaking, the seed steep water was decanted to obtain the seed leachate. Using digital conductivity meter with a cell constant of one the electrical conductivity of the seed leachate was measured and the mean expressed as µS cm^{-1} [14].

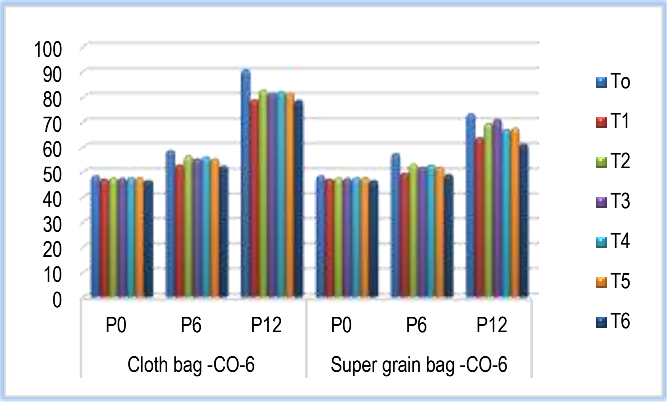

Fig-1 Influence of seed treatments, storage containers and period of storage on electrical conductivity (µS cm^{-1} g^{-1}) of blackgram variety CO-6

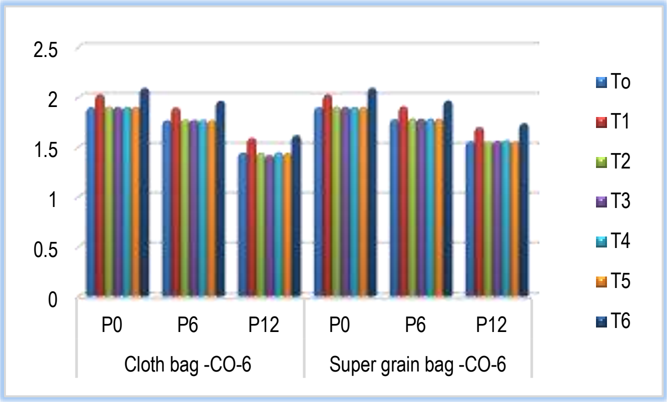

Fig-2 Influence of seed treatments, storage containers and period of storage on dehydrogenase activity (OD value) of blackgram variety CO-6

Dehydrogenase activity

Seeds were selected randomly and preconditioned by soaking in water for 6 h. Then the seeds were bisected longitudinally into two halves and were steeped in (0.1 %) 2, 3, 5 triphenyl tetrazolium chloride solution and kept in dark for 2 h at 40°C for staining. After staining, the excess solution was drained and the seeds were washed thoroughly with distilled water and transferred to a test tube containing 5 ml of 2-Methoxy Cellosolve. The test tubes were air tightly closed and kept overnight in an incubator under darkness for extracting the red coloured formazon. The coloured solution was decanted and the colour intensity was measured using Cary UV spectrophotometer at 470nm and methyl cellosolve as the blank. The OD value obtained was reported as total dehydrogenase activity [15].

Results and Discussion

Moisture content of stored seeds were significantly influenced by seed treatment, storage period, container and interaction with period of storage and container. However, interaction effect between treatment and container, treatment, period and container were non significant [Table-1]. Irrespective of period of storage and container, after 12 month of storage seed treatment with nano particles of ZnO@1250mg/kg recorded significantly minimum seed moisture content of 8.7 percent compared to untreated control seeds 9.7 percent. Between the containers, seed stored in super grain bag registered the lowest moisture of (9.1%) than cloth bag (9.8%). The interaction between treatment and period of storage revealed that the seed treatment with nano particles of ZnO@1250mg/kg registered the lower moisture of 8.9 percent at the end of the 12 months of storage while the untreated control seeds recorded the highest moisture of 10.2 percent [Table-1]. Cloth bag being a pervious container, moisture exchange took place frequently until it reaches the equilibrium status with environment where as super grain bag is impervious, the results are also accordance with Jeya, *et al.*(2014) [16]. Similar findings were reported by Lei Zhang et al. (2005) in spinach. The improvement in treated seed may be due to the quenching of free radicles by the ZnO. The interaction between treatment and period of storage revealed that the seed treatment with nano particles of ZnO@1250mg/kg registered the higher germination of 88 percent at the end of the 12 months of storage while the untreated control seeds recorded the lower germination of 79 percent [Table-2]. Between the container's seeds stored in super grain bag recorded highest germination percent (88%) compared to cloth bag (80%) at the end of the 12 months of storage [Table-2]. Antioxidant protects and prevents oxidative deterioration of lipids and maintains structural and functional integrity of cells. Free radicals are atoms, molecules or ions with unpaired electrons, which are highly reactive to chemical reactions with other molecules, in the biology system. The significant lower values of antioxidant enzyme activity indicated better membrane integrity in treated seeds as compared to untreated control during storage period.

The electrical conductivity was lowest in seeds treated with nano particles of ZnO seeds (60.7µS cm^{-1} g-1) in super grain bag at the end of 12 months of storage compared to cloth bag (78.2 cm^{-1} g^{-1}) [Fig-1]. The variation in electrical conductivity of seed leachate indicates increased membrane permeability and decreased compactness of seed coat and cellular membrane deterioration. Similar, findings were reported by Vasundhara and Bommegouda (1999) in groundnut. Stable cell membrane also rendered resistance to peroxidase and free radical reactions. The dehydrogenase activity was highest in seeds treated with nano particles of ZnO (1.731) in super grain bag at the end of 12 months of storage compared to cloth bag (1.611) [Fig-2]. The marked decrease in the seed quality parameters under advancing storage period may be attributed to seed coat characters [17], age induced physicochemical seed deterioration, lipid peroxidation leading to production of toxic metabolites that act upon cell and cell organelles denaturation of proteins and enzymes [18]. Similar decline in seed quality parameters with advancing storage period was also reported by Pramila (2003) [19] in black gram.

Conclusion

It is evident from the present investigated that the seed stored in cloth bag showed poor quality parameter i.e. decreased germination, dehydrogenase activity and higher EC value due to increase seed quantitative losses and greater fluctuations in moisture content due to permeable nature of cloth bag to moisture vapours. The seed treatments with Nano particles of ZnO@1250mg/kg and Fenugreek (*Trigonella foenum*-graecum) seed powder@3g/kg and stored in super grain bag were considered as effective seed storage management approach in blackgram. Among the seed treatments, seeds treated with Nano particles of ZnO@1250mg/kg recorded higher seed quality parameters throughout the storage period. The safest and feasible approach is the treatment of seeds with botanicals which are safe, economical, ecofriendly and non-harmful to seed, animal and human beings.

Application of research: The findings of the research will be useful for the prolonged storage, traders and farmers.

Research Category: Seed Science and Technology

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Author statement: All authors read, reviewed, agreed and approved the final manuscript

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Sample Collection: Genetically, pure, freshly harvested seeds of blackgram CO 6 obtained from the Department of Pulses, Tamil Nadu Agricultural University, Coimbatore, 641003.

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