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Bio-molecule (Curcumin) Seed invigoration for extending the shelf-life of blackgram (*Vigna mungo*) under accelerated ageing

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Abstract

A study on curcumin seed invigoration for extending the storability of blackgram seeds under accelerated ageing was undertaken during 2021. Genetically pure seeds of blackgram (VBN 8) were treated with curcumin at different concentrations viz., 20,40,60,80,100 and 120 mg per kg. The treated seeds were subjected to accelerated ageing for 10 days. Every day of accelerated ageing, required quantity (50-100g) of seed samples were drawn and tested for physiological and biochemical seed quality attributes. The results revealed that the seeds invigorated with curcumin at 80 mg per kg of seeds significantly registered higher germination (66%), root (11.3cm) and shoot length (16.3cm) seedling vigour index I (1813) and II (12), alpha amylase (0.97 μ mol starch hydrolysed min⁻¹ mg⁻¹ protein) and dehydrogenase (1.642 OD value) enzymes activity. Overall results of this study conclude that blackgram seeds invigorated with curcumin at 80 mg per kg excelled the other treatments, irrespective of the accelerated ageing period.

Keywords: curcumin, blackgram VBN 8, seed invigoration, accelerated ageing

1. Introduction

Seeds are the primary and critical input for the profitable crop production. Quality of the seeds has to be protected for a prescribed period of storage without quality deterioration until sowing. Seed deterioration is triggered by many biotic and abiotic factors such as temperature, relative humidity, seed moisture content and storage pathogen & insects (Raja, 2003; Pallavi *et al.* 2003) [18, 1]. All these factors are directly or indirectly associated with lipid oxidation, a process happens both through enzymatic and non enzymatic pathways, that causes cell membrane disintegration and eventually causes seed death (Stewart and Bewley 1980; Oenel *et al.*, 2017) [2, 3]. Seed deterioration is an inevitable process that reduces the seed viability and vigour, which make difficulties in supplying quality seeds to the farmers at right time. Seed invigoration techniques like seed priming with, chemicals, pesticides, botanicals, halogenations, hydration and dehydration (*H-D*), dry permeation, *soaking drying (S-D)* etc., are used to reduce the seed deterioration (Jerlin *et al.*,2010; Duraimurugan *et al.*, 2011) [4, 5]. Most of the pre and mid-storage chemical seed treatments are unsafe and become ineffective moreover the excess dose damage the seed quality. By considering these limitations of existing seed treatments, an alternative seed invigoration technique has to be developed to protect seeds under ageing without any environment hazardous. Considering these established data, this study focused to develop an environmentally safe biomolecule seed invigoration technique using curcumin to extend the storability of blackgram by reducing deterioration, since blackgram is a proteinaceous crop that loss seed viability quickly as compared to cereal crops. Seeds coated with turmeric rhizome powder at rate of 10 gram per kg extended the storability of blackgram (Sakthivel, 2004) and Chilli (Mounica and Natarajan, 2015) [6]. Krishna Shyla (2016) [7] reported that the antioxidant properties of curcumin will donate free electron and pair with unpaired electron so that the free radical formation is quenched, which reduces the lipid oxidation process, protect the bilipid layer cell membrane and extend the shelf life of seeds. These published data clearly explain that curcumin biomolecules capable of extending the seed viability by means of reducing the ROS and also act as anti-fungal agent during seed ageing.

2. Materials and Methods

Genetically pure seeds of blackgram var. VBN 8 purchased from Department of pulses, TNAU, Coimbatore and high purity curcumin purchased from M/s Sigma Aldrich constituted as study materials.

2.1. Seed invigoration with curcumin

Seeds of blackgram (VBN 8) were dry dressed with curcumin at different concentrations *viz.*, 20,40,60,80,100 and 120 mg per kg. For dry dressing, seeds were taken in a screw capped bottle and added with required quantity of curcumin as detailed above. After adding curcumin, bottle along with seeds were vigorously shaken for five minutes. This was repeated five times with five minutes interval. The treated seeds were subjected to accelerated ageing for 10 days as per the procedure described by Delouche and Baskin (1973) [23], where the temperature in accelerated ageing chamber maintained at 40°C with relative humidity of 98% ± 2°C. Every day of accelerated ageing, required quantity (50-100g) of seed samples were drawn and tested for physiological and biochemical seed quality attributes.

2.2. Germination: Germination test was carried out using 4 × 100 seeds in paper medium (ISTA, 2016). The test conditions of 25 ± 2°C temperature and 95 ± 3 per cent relative humidity were maintained in the germination room. At the end of seven days, number of normal seedlings was counted and the mean was expressed as percentage.

2.3. Shoot and root length: Shoot and root length of normal seedlings from each replication of the germination test was measured and the mean was expressed in centimeter.

2.4. Dry matter production: The seedlings used for growth measurement were shade dried for 24 h (after removing the cotyledons and seed coat) and dried again in hot air oven maintained at 85 ± 2°C for 24 h and cooled in desiccators filled with silica gel for 30 min. The dry weight of seedlings was recorded using an electronic balance and expressed as g / 10 seedlings⁻¹.

2.5. Vigour index I & II: Vigour index I & II values were computed using the following formula as suggested by Abdul-Baki and Anderson (1973) [8] and the mean values were expressed in whole number.

Vigour index I = Germination (%) × Total seedling length (cm).

Vigour index II = Germination (%) × Dry matter production (g / 10 seedlings).

Biochemical seed quality parameters such as α-amylase activity (Paul *et al* 1970) [9], and dehydrogenase activity (Kittock and Law, 1968) [10] were analysed as per the established protocols.

2.6. Statistical analysis: The experiment data were analysed statistically using analysis of variance (ANOVA) as a factorial combination of treatments. The values in per cent data were arcsine converted before analysis. The critical differences (CD) were computed at 5 per cent probability level (Panse and Sukhatme, 1984) [19].

3. Results and Discussion

Seed deterioration, a natural process is expressed as the loss of quality, viability and vigour during ageing or adverse

environmental conditions. It is an irreversible degenerative process that occurs during storage. Many physiological and biochemical manifestations of seed deterioration have been extensively reported (Jatoi *et al.*, 2004). During seed ageing, physiological changes *viz.*, delayed germination, reduced seedling growth, dry weight, vigour and biochemical changes namely reduced activity of the enzymes *viz.*, catalase, peroxidase, ascorbate peroxidase, DPPH and α-amylase were notable events, which reduce the quality of seeds (Rame Gowda, 1992; Ramanadane, 2003) [15, 16]. Seed invigoration techniques like seed priming with, chemicals, pesticides, botanicals, halogenations, hydration and dehydration (*H-D*), dry permeation, *soaking drying* (*S-D*) etc., are used to reduce the seed deterioration (Jerlin *et al.*, 2010; Duraimurugan *et al.*, 2011) [4, 5]. This investigation focused to develop an environmentally safe and economically feasible seed invigoration technique using curcumin biomolecules. Seeds of blackgram (VBN 8) were dry dressed with curcumin at different concentrations *viz.*, 20,40,60,80,100 and 120 mg per kg and subjected accelerated ageing, and the accelerated aged seeds were evaluated for physiological and biochemical quality attributes under laboratory condition. The results exhibited that the invigorated with curcumin at 80 mg per kg of seeds significantly registered higher germination (66%), root (11.3cm) and shoot length (16.3cm) seedling vigour index I (1813) and II (12), alpha amylase (0.97mg maltose min⁻¹) and dehydrogenase (1.642 OD value) enzymes activity as compared to the control which recorded the minimum germination of 58 per cent, root (10.3cm) and shoot (14.9cm) length, vigor index I (1461) and II (9), alpha amylase (0.87 mg maltose min⁻¹) and dehydrogenase (1.563) OD value) enzymes activity (Table 1-4 & Figure 1-3). Overall results of this study conclude that blackgram seeds invigorated with curcumin at 80 mg per kg excelled the other treatments, irrespective of the accelerated ageing period.

The out performance of the curcumin seed invigoration in reducing the seed deterioration over a period of accelerated ageing is due to antioxidant properties of curcumin where it donate free electrons that are pair with unpaired electrons resulting in reduced free radical production. Due to this activity, the lipid bilayer membrane is protected as a result the cell membrane integrity is confined and makes the seeds to live for long time (Krishna Shyla, 2016) [7]. Botanicals act as a catalyst for production of reactive oxygen species (ROS) in a slow and sustained manner for maintenance of seed viability. The improvement in germination upon treating seeds with organic powders might also be due to activation of cells resulting in enhanced of mitochondrial activity leading to the formation of more energy compounds and vital biomolecules which are made available during the early phase of germination as reported by Renugadevi *et al.* (2001) [17] in rice. Similar results were reported by Renugadevi and Vijayageetha (2007) [12] in cluster bean. Effects of organic powder in improving the seedling vigour index are found to agree with the earlier reports of Rudrapal and Basu (2004) [13] in french bean; Mythili (2012) [20] in onion and Vijayalakshmi (2012) [21] in tomato. Malarkodi (2003) [22] reported that green gram seeds treated with vasambu rhizome powder at 100 g kg⁻¹ of seed maintained 87 per cent of germination after 21 months of storage and protected the seeds from bruchids. The reduced seed quality parameters at higher concentration of 120 mg per kg of under accelerated ageing is due to inhibitory effect on seed germination at higher concentrations the probable reason for decreased germination at higher

concentration might be the increased absorption and accumulation of these curcumin within the cells resulted in reduced cell division, cell elongation and inhibition of the hydrolytic enzymes during seed germination (Jesmin Akter 2018) ^[14].

5. Conclusion

The study infers that curcumin as an antioxidant helps in

reducing the seed deterioration in blackgram by quenching the free radicals there by lowering the oxidative damages of seed and improves the enzymatic activity eventually maintaining viability and vigour of the seeds specifically it shows significant positive result during long term storage. At the same time it is also true that usage of curcumin at higher concentration than 120 mg/kg will inhibit seed physiological and biochemical parameters.

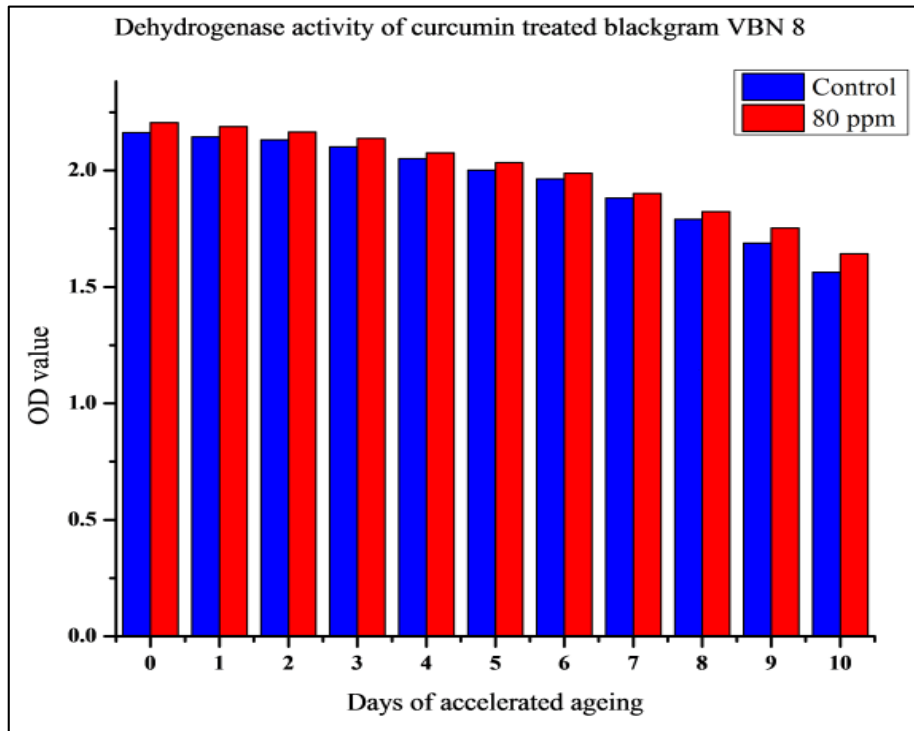


Fig 1: shows dehydrogenase activity of 80mg/kg curcumin treated and untreated blackgram

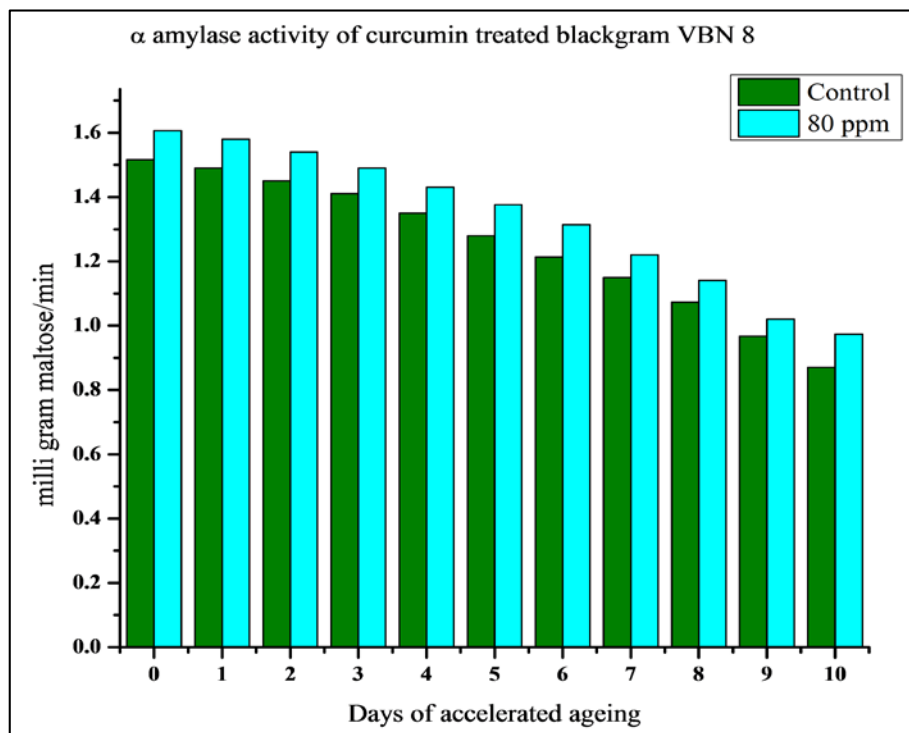


Fig 2: shows α-amylase activity of 80 mg/kg curcumin treated and untreated blackgram

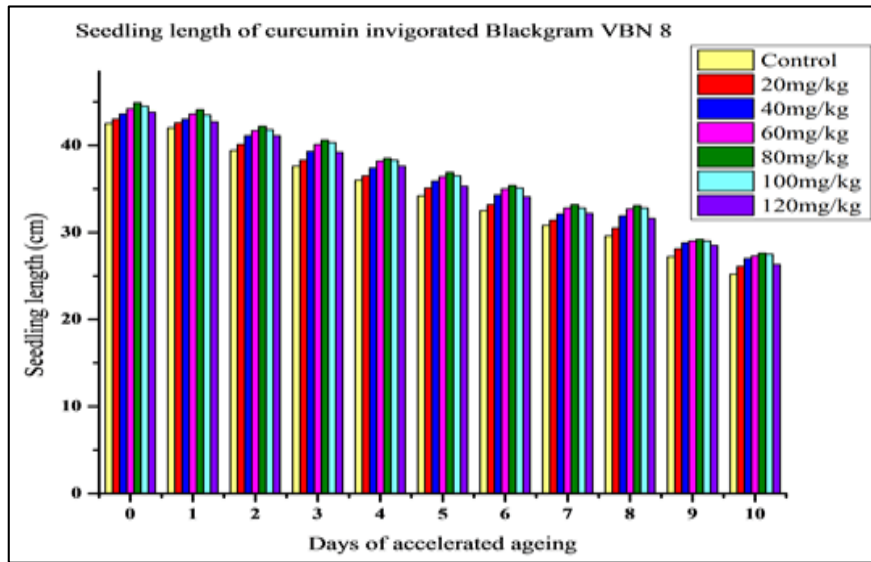


Fig 3: Shows seedling length of different concentration Curcumin treated and untreated blackgram

Table 1: Impact of Curcumin Seed invigoration on Germination (%) of Blackgram var VBN 8 under Accelerated Ageing

Treatments(T) Curcumin	Accelerated Ageing Duration In Days (D)											Mean
	Day 0	Day1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	
Control	94(75.82)	91(72.54)	87(68.86)	83(65.65)	79(62.72)	75(60.00)	72(58.05)	69(56.16)	65(53.73)	62(51.94)	58(49.60)	76(60.66)
20 mg/kg	94(75.82)	92(73.57)	87(68.86)	85(67.21)	82(64.89)	79(62.72)	75(60.00)	72(58.05)	69(56.16)	65(53.73)	63(52.53)	78(62.02)
40 mg/kg	94(75.82)	93(74.66)	88(69.73)	84(66.42)	83(65.65)	80(63.43)	76(60.66)	74(59.34)	70(56.79)	65(53.73)	65(53.73)	79(62.72)
60 mg/kg	95(77.08)	93(74.66)	90(71.56)	85(67.21)	85(67.21)	82(64.89)	78(62.02)	75(60.00)	71(57.41)	66(54.33)	65(53.73)	80(63.43)
80 mg/kg	95(77.08)	94(75.82)	91(72.54)	86(68.02)	85(67.21)	84(66.42)	79(62.72)	76(60.66)	72(58.05)	68(55.55)	66(54.33)	81(64.15)
100 mg/kg	94(75.82)	93(74.66)	89(70.63)	85(67.21)	83(65.65)	82(64.89)	76(60.66)	73(58.69)	70(56.79)	66(54.33)	62(51.94)	79(62.72)
120 mg/kg	93(74.66)	90(71.56)	86(68.02)	82(64.89)	78(62.02)	73(58.69)	70(56.79)	69(56.16)	65(53.73)	61(51.35)	57(49.02)	75(60.00)
Mean	94(75.82)	92(73.57)	88(69.73)	84(66.42)	82(64.89)	79(62.72)	75(60.00)	73(58.69)	69(56.16)	65(53.73)	62(51.94)	
	Treatment (T)			Duration(D)			TD					
SEd	0.447			0.561			1.483					
CD(0.05)	0.883			1.107			2.930					

(Figures in parenthesis indicates arc sine values)

Table 2: Impact of Curcumin Seed invigoration on Vigour Index 1 of Blackgram var VBN 8 under Accelerated ageing

Treatments (T) Curcumin	Accelerated Ageing Duration In Days (D)											Mean
	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10	
Control	3995	3822	3428	3121	2844	2564	2339	2125	1925	1685	1461	2664
20 mg/kg	4042	3919	3488	3255	2993	2772	2490	2261	2104	1826	1645	2800
40 mg/kg	4099	3999	3617	3302	3104	2871	2607	2375	2233	1872	1755	2894
60 mg/kg	4199	4054	3754	3408	3244	2985	2729	2460	2322	1914	1775	2986
80 mg/kg	4266	4146	3842	3491	3273	3099	2796	2523	2383	1986	1813	3056
100 mg/kg	4183	4045	3720	3425	3178	2992	2668	2395	2296	1914	1705	2956
120 mg/kg	4073	3842	3534	3214	2933	2575	2385	2222	2054	1739	1499	2734
Mean	4122	3975	3626	3317	3081	2837	2573	2337	2188	1848	1665	
	Treatment (T)			Duration(D)			TD					
SEd	23.176			29.053			76.866					
CD(0.05)	45.784			57.393			151.848					

Table 3: Impact of Curcumin Seed invigoration on Vigour Index 2 of Blackgram var VBN 8 under Accelerated Ageing

Treatments(T) Curcumin	Accelerated Ageing Duration In Days (D)											Mean
	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10	
Control	38	35	31	27	23	19	17	15	13	10	9	22
20 mg/kg	38	36	31	28	24	22	19	16	14	12	10	23
40 mg/kg	38	37	32	28	25	23	20	17	15	12	11	23
60 mg/kg	40	38	34	29	28	25	21	18	17	13	12	25
80 mg/kg	39	39	35	30	28	26	22	19	17	14	12	26
100 mg/kg	39	38	33	29	27	25	21	18	16	13	11	25
120 mg/kg	38	34	31	26	23	18	17	16	13	11	9	21
Mean	39	37	32	28	25	23	20	17	15	12	11	
	Treatment (T)			Duration(D)			TD					
SEd	0.231			0.290			0.767					
CD(0.05)	0.457			0.573			1.516					

Table 4: Impact of Curcumin Seed invigoration on Dry matter production of Blackgram var VBN 8 under Accelerated Ageing

Treatments(T) Curcumin	Accelerated Ageing Duration In Days (D)										Mean	
	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9		Day10
Control	0.398	0.382	0.350	0.323	0.288	0.256	0.230	0.215	0.198	0.165	0.148	0.268
20 mg/kg	0.403	0.393	0.352	0.328	0.298	0.273	0.250	0.226	0.210	0.182	0.164	0.280
40 mg/kg	0.408	0.402	0.361	0.331	0.307	0.285	0.258	0.234	0.224	0.187	0.176	0.288
60 mg/kg	0.416	0.408	0.373	0.339	0.325	0.298	0.272	0.246	0.235	0.190	0.178	0.298
80 mg/kg	0.417	0.410	0.382	0.345	0.328	0.305	0.280	0.253	0.236	0.197	0.180	0.303
100 mg/kg	0.415	0.405	0.375	0.338	0.323	0.302	0.270	0.243	0.232	0.194	0.174	0.297
120 mg/kg	0.408	0.380	0.355	0.320	0.297	0.250	0.240	0.222	0.204	0.174	0.150	0.273
Mean	0.409	0.397	0.364	0.332	0.309	0.281	0.257	0.234	0.220	0.184	0.167	
	Treatment (T)				Duration(D)				TD			
SEd	0.002				0.002				0.006			
CD(0.05)	0.004				0.005				0.012			

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