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Full length Research paper

Effect of botanicals in improving the performance of aged seeds in comparison with fresh seeds of blackgram variety

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An experiment was conducted in Department of Seed Science and Technology during 2012 to standardize suitable seed dry dressing treatments, using botanicals that can alleviate the deleterious effects of accelerated ageing and improve the physiological performance of both fresh and aged seeds in blackgram variety TNAU blackgram CO 6. Both fresh and four days accelerated aged seeds were dry dressed with fenugreek seed powder, custard apple and moringa leaf powder at 2, 3 and 4 g/kg of seeds with 1, 2 and 3 h of shaking and evaluated for its physiological performance of seedlings under lab condition. The result revealed that seeds treated with 3 g/kg of fenugreek seed powder with 1 h shaking registered an increased physiological performance in terms of germination percentage, shoot length, root length, dry matter production and vigour index for both fresh and aged seeds. This, which was on par with 4 g/kg of custard apple leaf powder treated seeds. Interestingly, the increase in physiological performance of identified seed treatments was more pronounced in aged seeds, indicating the efficacy of botanicals in alleviating the deleterious effect of accelerated ageing. All the botanicals found to contain higher antioxidant activity and rich in minerals such as titanium, molybdenum and iron, apart from other trace elements.

Key words: Blackgram, custard apple leaf powder, fenugreek seed powder, moringa leaf powder, seed ageing.

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. It is consumed in the form of split pulse as well as whole pulse, which is an essential supplement of cereal based diet. In India, blackgram occupies 12.7% of total area under pulses and contribute 8.4% of total pulses production. However, area and production of blackgram has declined from 3.01 million ha and 1.30 million tons in 2000 to 2001 to 2.97 million ha and 1.23 million tons, respectively in 2009 to 2010 (ASSOCHAM, 2012). This emphasized the need to increase the performance of pulse crops, particularly in developing countries, where most grain legume production is for human consumption and demand is increasing due to population increase (Jeuffroy and Ney, 1997). The poor performance of blackgram may be attributed to several factors, of which level of seed deterioration is of great importance. Invariably, the seeds have to be stored through the monsoon for the next sowing during which

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period the rise in the ambient relative humidity coupled with the prevailing high temperature can accelerate the ageing process of the seed, resulting in loss of vigour and viability.

Using the seed lot with reduced vigour and viability for sowing may affect the field performance and productivity of the resultant crops (Layek et al., 2007; Pati and Bhattacharjee, 2011). Therefore, there is a need to improve the performance of deteriorated seeds which is possible through seed treatments. Seed treatments with synthetic chemicals had effectively managed to alleviate the deteriorative effect of seed ageing (Sathish and Sundareswaran, 2010; Sathish et al., 2011). Though, many of the synthetic chemicals look effective but they are not readily degradable and yield more toxic residues (Ames et al., 1990; Moore and Waring, 2001). However, the use of chemical is still in vogue. Hence, 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. It was proven that the deteriorating effect of seed ageing was mainly due to the production of free radicals (Bailly, 2004; Bailly et al., 2008) and use of antioxidants can quench the free radicals and prolong the storability of seeds (Maeda et al., 2005; Sattler et al., 2006).

Among the several botanicals available, presence of antioxidant property along with high nutrient content was pharmacologically proved in fenugreek seed powder (Bukhari et al., 2008; Toppo et al., 2009), custard apple leaf powder (Baskar et al., 2007; Pandey and Brave, 2011) and moringa leaf powder (Fahey, 2005; Ferreira et al., 2008). Therefore, these botanicals could serve as a potential source of natural antioxidants which can be used for seed treatments to improve vigour and viability. 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 (Kalavathi, 1985).

Accelerated ageing has been widely used to study the pattern of seed deterioration in various crops instead of waiting to obtain naturally aged seeds (Jatoi et al., 2001; Scialabba et al., 2002). Hence, with this backdrop, the present investigation was formulated to study the effect of botanicals in improving the performance of aged seeds in comparison with fresh seeds of blackgram variety TNAU blackgram CO 6.

MATERIALS AND METHODS

Seed materials

Genetically, pure, freshly harvested seeds of blackgram variety TANU blackgram CO 6 obtained from the Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore were graded using a BSS 7 × 7 wire mesh sieve and used for the study. Standardization of seed dry dressing with botanicals was carried out in Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2012.

Preparation of two seed lots with different vigour levels

In order to obtain two seed lots with different germination potential and vigour level, half the quantity of fresh seeds having 98% germination was subjected to four days accelerated ageing. It was carried out by packing seeds in paper bag with uniform pin head size perforation all over and placed in ageing jar containing 100 ml double distilled water to maintain 98 ± 2% relative humidity and the whole unit was kept in incubator maintained at 40 ± 1°C for four days to reduce germination approximately around 80% (Delouche and Baskin, 1973).

Seed dry dressing with botanicals

Fenugreek seed obtained from local market, custard apple and moringa leaves collected from the orchards of Tamil Nadu Agricultural University were freeze dried at -80°C and grounded finely to obtain fine powder. Both fresh seeds (FS) and four days accelerated aged seeds (AS) were dry dressed with finely ground botanicals namely, fenugreek seed powder (FSP), custard apple leaf powder (CALP) and moringa leaf powder (MLP) at the rate of 2, 3 and 4 g/kg of seeds and shaken for 1, 2 and 3 h to find effective duration for uniform seed dressing. Treated seeds along with untreated, fresh and aged seeds which served as control were assessed for the following physiological seed quality parameters.

Seed quality evaluation

The laboratory germination test was carried out in quadruplicate using 100 seeds each with 4 sub replicates of 25 seeds in the paper medium (ISTA, 2009). The test conditions of 25 ± 2°C temperature and 95 ± 3% relative humidity were maintained in a germination room. Germination was counted in 24 h intervals and continued until no further germination occurred. At the end of seven days, the number of normal seedlings was counted and the mean was expressed as a percentage. Speed of germination was calculated based on the following formula of Maguire (1962):

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots\ldots\ldots + \frac{X_n - X_{n-1}}{Y_n}$$

Where X_1, X_2 and X_n are number of seeds germinated on first, second and n^{th} day, respectively and Y_1, Y_2 and Y_n are number of days from sowing to first, second and n^{th} count, respectively.

Root and shoot lengths were measured at the time of germination count from ten normal seedlings selected at random from each replication and the mean was expressed in centimetre and those seedlings used for growth measurement were placed in a paper cover and dried in shade for 24 h and then they were kept in an oven maintained at 85 ± 2°C for 48 h. The dried seedlings were weighed to estimate the dry matter production and the mean values were expressed in gram per 10 seedlings. The vigour index was computed as described by Abdul-Baki and Anderson (1973) with slight modifications as follows and expressed in whole numbers.

VI 1 = GP × (RL + SL)
VI 2 = GP × DMP

Where VI 1 is vigour index one, VI 2 is vigour index two, GP is germination percentage, RL is root length (cm), SL is shoot length (cm) and DMP is dry matter production (gram per 10 seedlings).

Properties of botanicals

The properties such as antioxidant activity and mineral content were analyzed as follows. The total potential antioxidant activity

Table 1. Effect of botanical treatment on speed of germination of fresh seeds of blackgram variety TNAU blackgram CO 6.

Botanicals	1 h				2 h				3 h				B × C interaction mean			Grand mean
	2 g	3 g	4 g	Mean	2 g	3 g	4 g	Mean	2 g	3 g	4 g	Mean	2 g	3 g	4 g	
FSP	24.0	24.5	24.5	24.3	23.9	23.9	24.1	24.0	23.8	24.1	24.3	24.1	23.9	24.2	24.3	24.1
CALP	24.0	24.5	24.0	24.2	24.1	24.1	24.0	24.1	23.6	23.4	24.2	23.7	23.9	24.0	24.1	24.0
MLP	24.3	25.0	23.8	24.4	23.6	24.1	24.1	23.9	22.9	23.8	24.1	23.6	23.6	24.3	24.0	24.0
Mean	24.1	24.7	24.1	24.3	23.9	24.0	24.1	24.0	23.4	23.8	24.2	23.8	23.8	24.2	24.1	24.0
Control	23.3															

	Control versus rest	D	B	C	DB	DC	BC	DBC
SEd	0.432	0.141	0.141	0.141	0.245	0.245	0.245	0.424
CD (P = 0.05)	NS	NS	NS	NS	NS	NS	NS	NS
CD (P = 0.01)	NS	NS	NS	NS	NS	NS	NS	NS

D – Shaking duration, C – Botanicals concentration, B – botanicals, FSP, fenugreek seed powder; CALP, custard apple leaf powder; MLP, moringa leaf powder.

of the aqueous acetone extract of powdered botanicals were assessed based on their scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, using a modified DPPH assay of Koleckar et al. (2007). The extract was prepared by dissolving 0.3 g of flour in 10 ml of 70% (v/v) acetone. After continuous shaking for 30 min at room temperature, the solution was centrifuged for 20 min at 13,000 rpm. An aliquot of 100 µl extract was mixed with the ethanol DPPH solution (0.5 mM, 0.25 ml) and the acetate buffer (100 mM, pH 5.5, 0.5 ml). After standing for 30 min in the dark, the absorbance was measured at 517 nm against a blank containing absolute ethanol instead of a sample aliquot. DPPH-radical scavenging activity is expressed as % of blank:

$$\text{Per cent inhibition activity} = \frac{\text{Blank OD - Sample OD}}{\text{OD}} \times 100 \text{ Blank}$$

About 18 elements namely, Al, B, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Sr, Te, Ti and Zn were estimated by following the method described by McQuaker et al. (1979) and the results of minerals having more than 0.1 ppm were presented here. Finely ground seed samples weighing 0.5 g was digested in 15 ml of concentrated nitric acid using kelplus infra digestion system (Model: KES 12IL) at 100°C for half an hour and later increased to 300°C till the solution turns colourless. To the digested solution, 100 ml of double distilled water was added and filtered through whatman's filter paper number 40 to obtain a clear colourless solution and mineral content were quantified using inductively coupled plasma spectrometer (ICP) and iTEVA software and expressed in ppm. The ICP multi-element standard solution VIII (24 elements in dilute nitric acid) obtained from Merck Chemicals, Germany was used as standard.

Statistical analysis

The experiment was carried out with four replications in factorial completely randomized block design. The data obtained were analysed by the 'F' test of significance following the methods described by Rangaswamy (2002). The percent values were transformed to arc-sine values and used for analysis. The critical differences (CD) were calculated at 1 and 5% probability level. The data were tested for statistical significance by three ways ANOVA. If the F test is non-significant, it was indicated by the letters NS.

RESULTS

Speed of germination and germination percentage

The results of the present investigation revealed that there was no significant difference in speed of germination between treatments of fresh seeds. Whereas, aged seeds registered significant difference in speed of germination between the control and other treatments but the difference was not significant within treatments and their interactions (Tables 1 and 2). Similarly, the difference was not significant for germination percentage among treatments and their interactions in fresh seeds. Whereas, the difference was highly pronounced and significant for germination percentage of aged seeds to all the factors and their interactions over control (81%). FSP (87%) registered maximum germination followed by CALP (86%). Among the shaking duration, 1 and 2 h of shaking are on par with each other and registered higher germination (86%) than 3 h of shaking (84%). Among the concentration, 4 g/kg of seeds (88%) registered maximum germination followed by 3 g/kg of seeds (86%). However, interaction between botanicals and concentration registered maximum germination in FSP at 3 and 4 g/kg of seeds and CALP at 4 g/kg of seeds (89%) which were on par with

Table 2. Effect of botanical treatment on speed of germination of aged seeds of blackgram variety TNAU blackgram CO_6.

Botanicals	1 h				2 h				3 h				B × C interaction mean			Grand
	2 g	3 g	4 g	Mean	2 g	3 g	4 g	Mean	2 g	3 g	4 g	Mean	2 g	3 g	4 g	mean
FSP	19.8	20.6	20.4	20.3	19.8	20.4	20.5	20.2	19.1	20.0	20.2	19.8	19.6	20.3	20.4	20.1
CALP	19.3	19.6	20.9	19.9	19.4	20.0	20.6	20.0	19.4	19.9	20.1	19.8	19.4	19.8	20.5	19.9
MLP	18.9	19.1	19.6	19.2	18.9	19.0	19.6	19.2	18.9	19.4	19.9	19.4	18.9	19.2	19.7	19.3
Mean	19.3	19.8	20.3	19.8	19.4	19.8	20.2	19.8	19.1	19.8	20.1	19.7	19.3	19.8	20.2	19.8
Control	17.8															

	Control versus rest	D	B	C	DB	DC	BC	DBC
SEd	0.948	0.310	0.310	0.310	0.537	0.537	0.537	0.931
CD (P = 0.05)	1.884	NS	NS	NS	NS	NS	NS	NS
CD (P = 0.01)	NS	NS	NS	NS	NS	NS	NS	NS

D – Shaking duration, C – Botanicals concentration, B – Botanicals, FSP, fenugreek seed powder; CALP, custard apple leaf powder; MLP, moringa leaf powder.

each other.

Interaction between duration of shaking (D) and botanicals (B); D and concentration of botanicals (C) and interaction between D, B and C were not significant (Figure 1).

Shoot and root length

In fresh seeds, difference was significant over control (21.07 cm) for shoot length in all the three factors, except their interactions. Higher shoot length was observed in seeds dry dressed with FSP (23.03 cm) and CALP (22.66 cm) which was on par with each other. Among concentration, 3 and 4 g/kg of seeds registered maximum shoot length and on par with each other; among the duration of shaking, 1 h (23.02 cm) and 2 h (23.17 cm) were on par and produced significantly higher shoot length than 3 h (21.71 cm). Similar trend was also observed in shoot length of aged seeds. In addition, significant difference in the interaction between botanicals and its concentration of aged seeds were also observed by producing significantly longer shoot length in seeds dry dressed with FSP at 3 g/kg of seeds (22.26 cm), 4 g/kg of seeds (22.29 cm) and CALP at 4 g/kg of seeds (22.26 cm) which are on par with each other (Figure 2). Root length of fresh seeds exhibited different trend from shoot length where no significant differences were observed due to shaking duration and interactions between D and C, D and B, and D, B and C. But significant difference was observed in seeds dry dressed with botanicals, its concentration and their interaction and also all the treatments as a whole registered significantly higher root length than control.

Interactional effect of botanicals and its concentration revealed that seeds dry dressed with FSP at 3 g/kg of seeds (18.03 cm), 4 g/kg of seeds (18.03 cm) and CALP at 4 g/kg of seeds (18.02 cm) registered higher root length which were on par with each other. In case of aged seeds, root length depicted similar trend as like that of its shoot length (Figure 3).

Dry matter production

Dry matter production of fresh seeds was significantly influenced by botanicals, concentration and duration of shaking and performed better than control (0.218 g / 10 seedlings). But there was no significant difference for interactional effect. Among the botanicals, FSP registered higher dry matter production (0.273 g / 10 seedlings) which was on par with CALP (0.268 g / 10 seedlings) while dry matter production was maximum in concentration of 3 and 4 g/kg of seeds (0.265 and 0.271 g / 10 seedlings, respectively) which was on par with each other. Among the duration of shaking, 1 h (0.268 g / 10 seedlings) and 2 h (0.270 g / 10 seedlings) was on par and produced significantly higher dry matter than 3 h (0.247 g / 10 seedlings). Similar trend was also observed in dry matter of aged seeds. In addition, a significant difference in the interaction between botanicals and its concentration were also observed by producing significantly higher dry matter in seeds dry

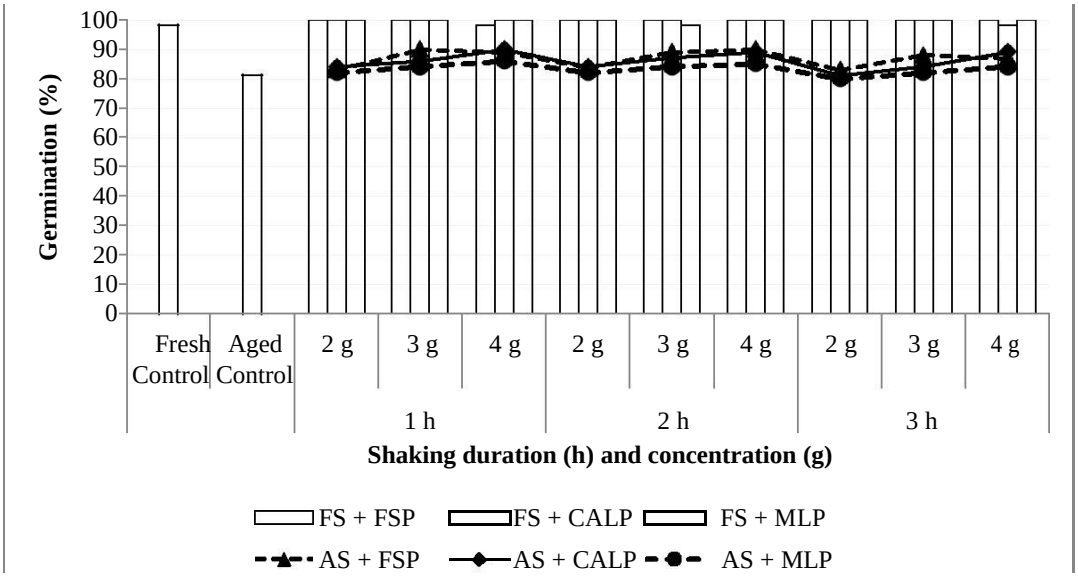

Figure 1. Effect of botanicals dry dressing on germination (%) of fresh and aged seeds of blackgram.

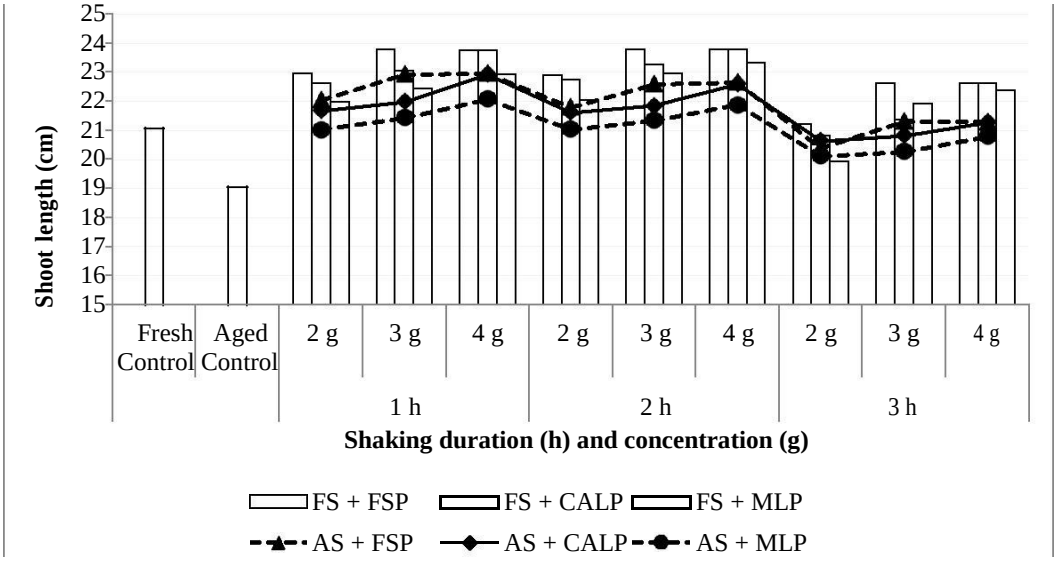

Figure 2. Effect of botanicals dry dressing on shoot length (cm) of fresh and aged seeds of blackgram.

dressed with FSP at 3 g/kg of seeds (0.262g / 10 seedlings) and 4 g / kg of seeds (0.262 g / 10 seedlings) and CALP at 4 g / kg of seeds (0.263 g / 10 seedlings) which are on par with each other (Figure 4).

Vigour index

Interestingly, vigour index 1 and 2 of both fresh and aged seeds were significantly influenced by all the three factors and their interactions. Seeds dry dressed with FSP at 3 g/kg of seeds and CALP at 4 g/kg of seeds and shaked for 1 h registered maximum vigour index 1 and 2 for both fresh and aged seeds (Figures 5 and 6).

Properties of botanicals

Antioxidant activity was higher in all the three botanicals among which FSP (95.9%) and CALP (96.0%) registered higher antioxidant activity than MLP (91.7%) (Table 3). In case of mineral content, FSP contain higher amount of Mo, Ti, Fe, Al, Zn and traces of Mn, B, Cu, Sr, Ba; while CALP was rich in Ti, Mo, Fe, Al, Sr, B and traces of Ba,

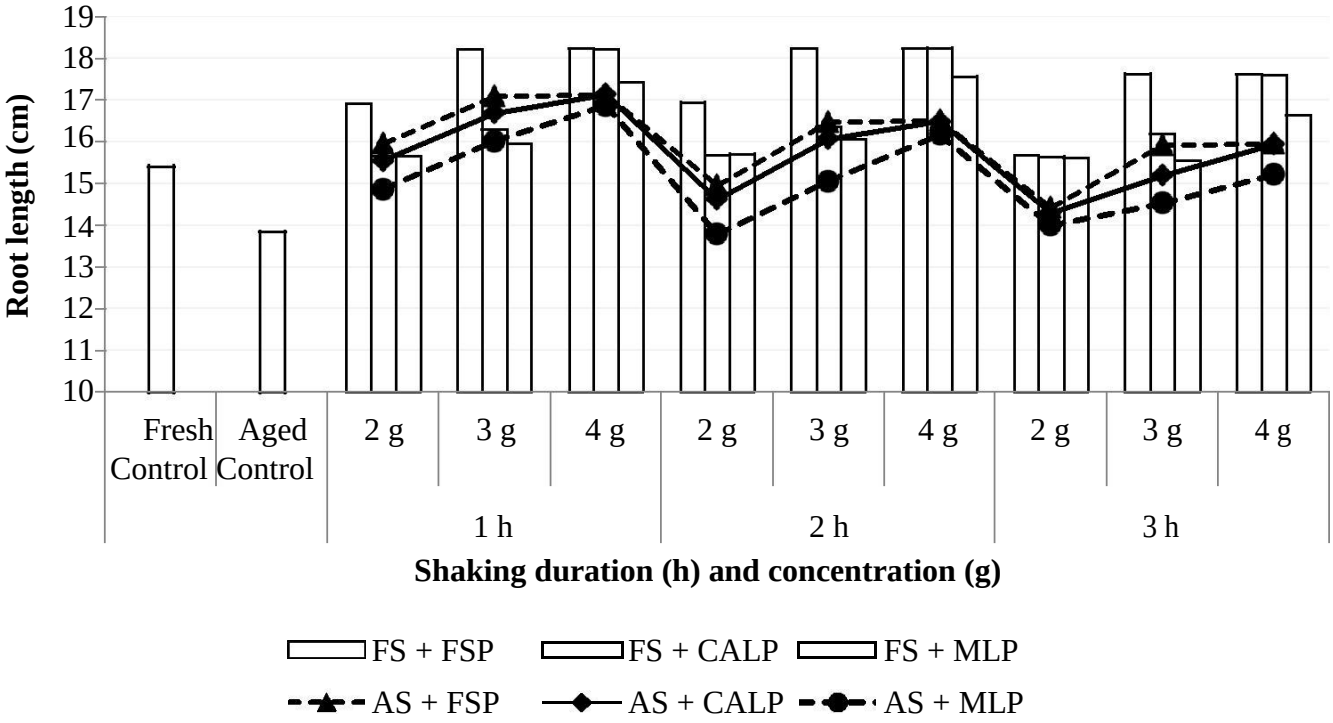

Figure 3. Effect of botanicals dry dressing on root length (cm) of fresh and aged seeds of blackgram.

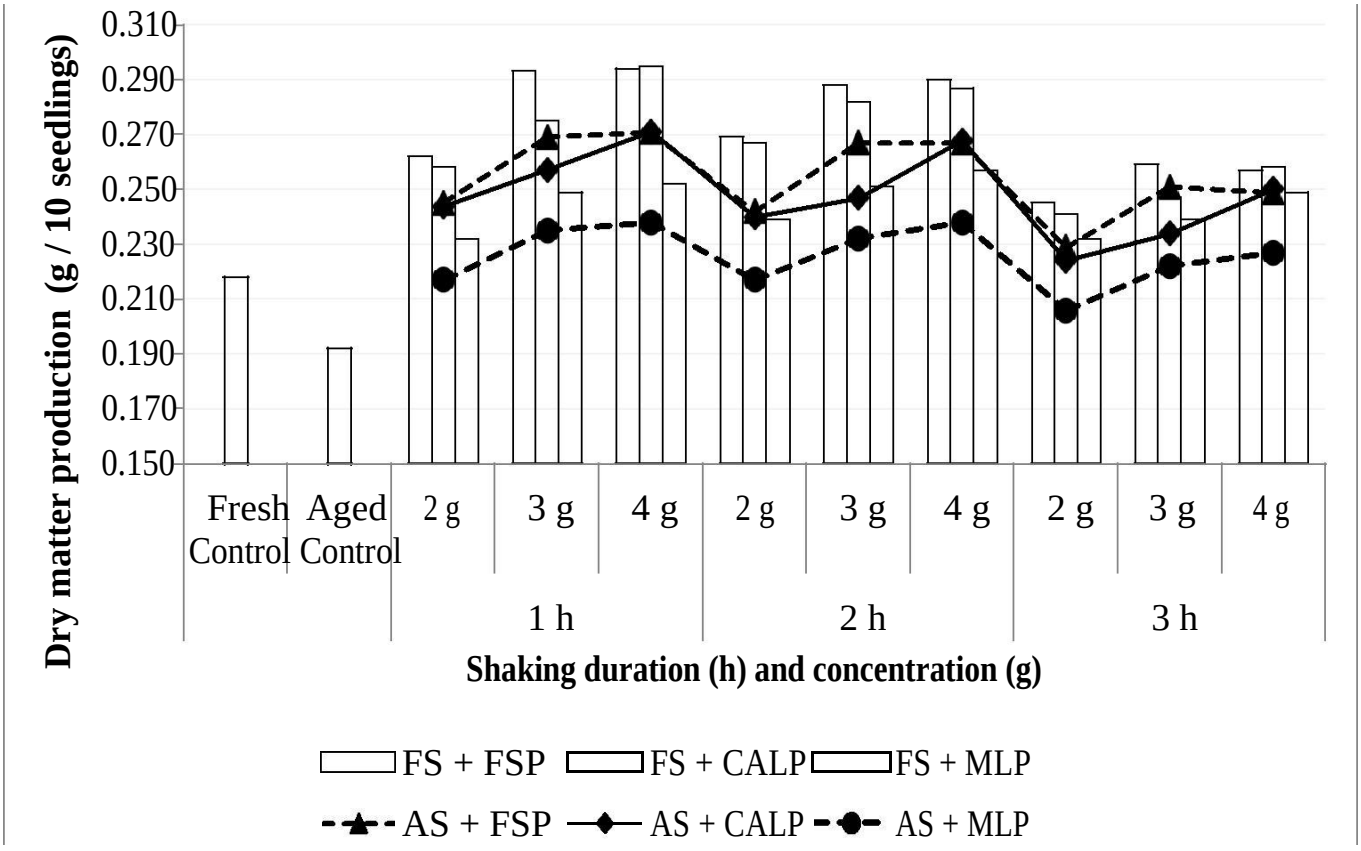

Figure 4. Effect of botanicals dry dressing on dry matter production (g / 10 seedlings) of fresh and aged seeds of blackgram.

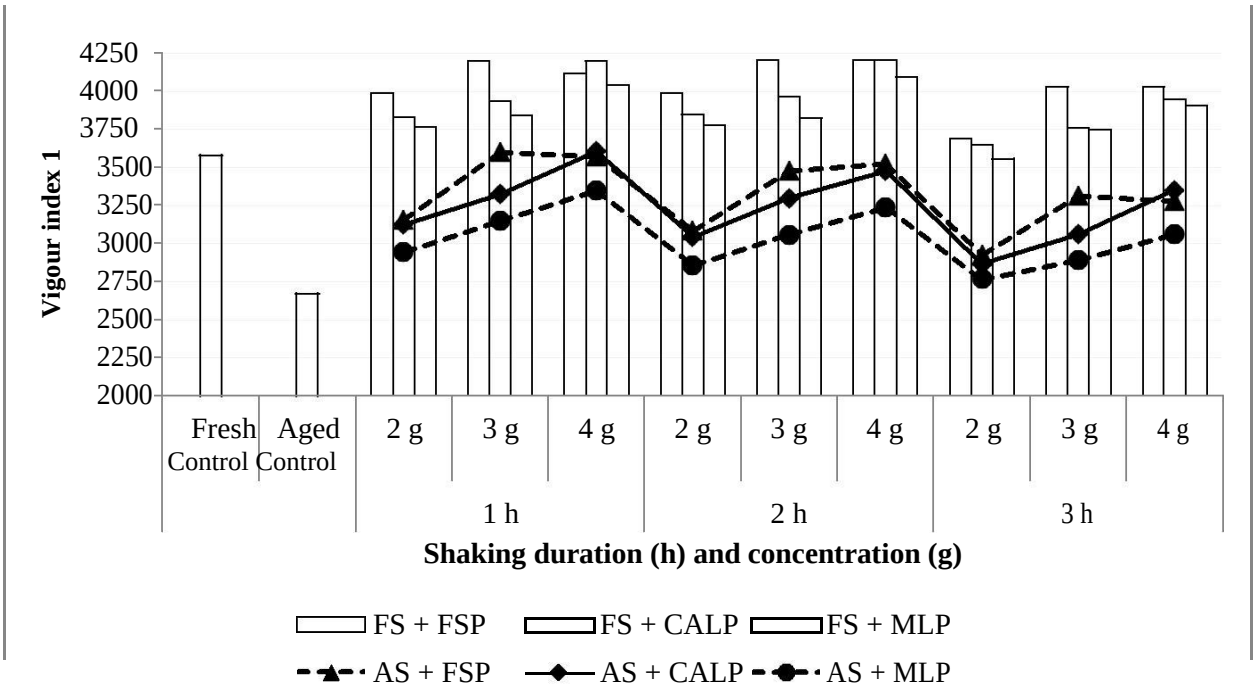

Figure 5. Effect of botanicals dry dressing on vigour index 1 of fresh and aged seeds of blackgram.

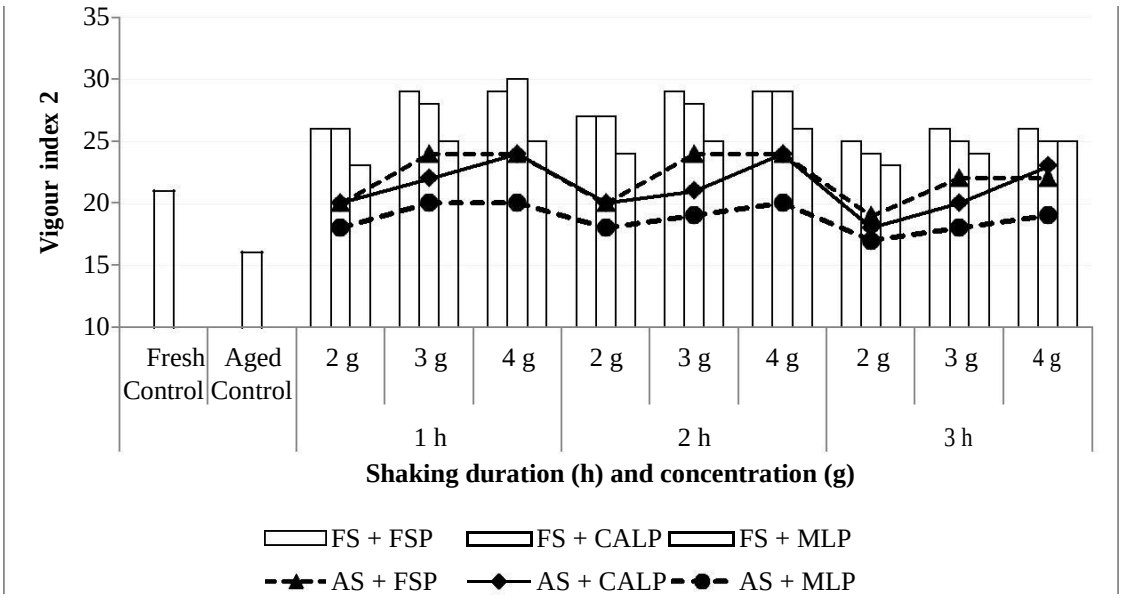

Figure 6. Effect of botanicals dry dressing on vigour index 2 of fresh and aged seeds of blackgram.

Zn, Cu, Mn. MLP was rich in Ti, Mo, Fe, Al, Sr and traces of Zn, B, Mn, Ba, Cu. However, MLP contains lesser mineral content than FLP and CALP (Table 3).

DISCUSSION

Availability of good quality seed is the key for successful agriculture and their use is an important factor for increased productivity. The seeds with good physiological potential act as catalyst for all agricultural inputs. All the botanicals, its concentration and shaking duration performed better than control of both fresh and aged seeds. In fresh seeds though, speed of germination and germination percentage was not significantly influenced by treatments by all the other parameters namely, shoot

Table 3. Antioxidant property and mineral content (ppm) of botanicals.

	FSP	CALP	MLP
Antioxidant activity (% of blank)	**95.9 (±0.36)**	**96.0 (±0.27)**	**91.7 (±0.28)**
Mineral content (ppm)			
Al	3.20 (±0.037)	6.10 (±0.512)	2.79 (±0.100)
B	0.49 (±0.006)	2.36 (±0.008)	0.55 (±0.010)
Ba	0.15 (±0.001)	0.91 (±0.003)	0.28 (±0.010)
Cu	0.40 (±0.002)	0.44 (±0.001)	0.27 (±0.004)
Fe	20.51 (±0.218)	11.68 (±0.045)	7.59 (±0.130)
Mn	0.62 (±0.004)	0.44 (±0.002)	0.49 (±0.010)
Mo	40.24 (±0.543)	24.86 (0.196)	13.73 (±0.550)
Sr	0.26 (±0.002)	3.19 (0.009)	2.78 (±0.050)
Ti	38.34 (±0.443)	107.10 (±1.852)	47.71 (±3.050)
Zn	1.35 (0.008)	0.86 (±0.004)	0.87 (±0.010)

Note: Each value in the table was the average of three replications± standard deviation.

length, root length, dry matter production, vigour index 1 and 2, were significantly influenced by the botanicals. In case of aged seeds, except speed of germination, all the other parameters were highly influenced by the seed dry dressing treatments; both in fresh and aged seeds, dry dressing with FSP at 3 g/kg of seeds and CALP at 4 g/kg of seeds with 1 h shaking performed better than other treatments; both the treatments increased germination percentage of aged seeds to 9% than control (81%) but no significant influence in germination of fresh seeds was observed.

Vigour index 1 was increased by 17 and 32%, respectively for fresh and aged seeds over their respective control. Similarly, increase in vigour index 2 was also much pronounced in aged seeds than fresh seeds. Similar results of botanicals were also reported by Vijayalakshmi (2012) in tomato. The reason for increase in germination, vigour index and other parameters can be substantiated from the result obtained by the analysis of properties of botanicals. All the botanicals have higher antioxidant property among which FSP and CALP topped (Table 3). The presence of higher antioxidant activity in FSP and CALP was also reported by several researchers (Baskar et al., 2007; Bukhari et al., 2008; Toppo et al., 2009; Pandey and Brave, 2011; Bose et al., 2011). Both these powders proposed to contain poly phenolics and flavonoids, namely vitexin, tricin, naringenin and quercetin, which act as a hydrogen donor and the OH^- scavenger (Kaviarasan et al., 2007). In addition, both FSP and CALP were rich in titanium, molybdenum and iron apart from other trace elements (Table 3). Ti plays a major role in biomass production and participate in cell metabolism as redox catalyst (Tlustos et al., 2005). Molybdenum is utilized by selected enzymes to carry out redox reactions and helps in vigorous seedling growth (Kaiser et al., 2005). Iron is also utilized by several enzymes and participates in the energy-yielding electron transfer reactions of respiration during germination (Guerinot and Yi, 1994).

The pronounced effect of FSP and CALP could be attributed to excellent proton radical scavenging property as described earlier and subsequent alleviation of deteriorative effect (Bhatia et al., 2002; Chandrashekar and Kulkarani, 2011) with substantial mineral supplement required for seed germination and vigourous seedling growth. Hence, seed dry dressing with FSP at 3 g/kg of seeds and CALP at 4 g/kg of seeds with 1 h shaking can be recommended to improve the physiological performance of blackgram seeds. Especially, it is applicable in the case of aged seeds to alleviate the deleterious effect of ageing and subsequently to improve its physiological performance during germination.

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