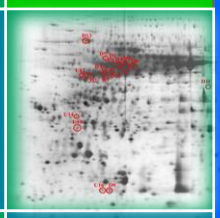
SEED VIGOUR AND VIABILITY STUDIES IN BLACKGRAM (*Vigna mungo* L.) cv. TNAU Blackgram CO 6



By

S. SATHISH, M.Sc., (Agri.)

I.D.No. 10-614-003

DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

AGRICULTURAL COLLEGE AND RESEARCH INSTITUTE

TAMIL NADU AGRICULTURAL UNIVERSITY

COIMBATORE – 641 003

2013

SEED VIGOUR AND VIABILITY STUDIES IN BLACKGRAM (*Vigna mungo* L.) cv. TNAU BLACKGRAM CO 6

Thesis submitted in part fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY (AGRICULTURE) IN SEED SCIENCE AND TECHNOLOGY

to the Tamil Nadu Agricultural University, Coimbatore – 641 003

By

S. SATHISH, M. Sc., (Ag.)

I.D.No. 10-614-003

DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

TAMIL NADU AGRICULTURAL UNIVERSITY

COIMBATORE – 641 003

2013

CERTIFICATE

This is to certify that the thesis entitled "**SEED VIGOUR AND VIABILITY STUDIES IN BLACKGRAM (*Vigna mungo* L.) cv. TNAU Blackgram CO 6"** submitted in part fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE) IN SEED SCIENCE AND TECHNOLOGY** to the Tamil Nadu Agricultural University, Coimbatore, is a record of *bonafide* research work carried out by **Mr. S. SATHISH** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles. However, part of the thesis work has been published in peer reviewed scientific journal of national/international repute (copy enclosed).

Place : Coimbatore
Date :

(Dr. M. BHASKARAN)
Chairperson

Approved by

Chairman : **(Dr. M. BHASKARAN)**

Members : **(Dr. R. UMARANI)**

(Dr. A.R. MUTHIAH)

(Dr. N. SENTHIL)

External Examiner

Date:

ACKNOWLEDGEMENT

First and foremost, I praise and thank the ***almighty*** *for giving me the strength and courage to complete this endeavour successfully in time.*

Words could not suffice to tell of my deep sense of gratitude and respect to my chairman ***Dr.M.Bhaskaran****, Special Officer (Seeds), Seed Centre, Tamil Nadu Agricultural University, Coimbatore for his adroit and peerless guidance, kind treatment, sustained inspiration, constructive criticisms and manifold help bestowed upon me throughout the progress of my work without which this thesis would not have attained its shape.*

With deep respect and esteem regards, I owe my indebtedness to the members of Advisory Committee ***Dr.R.Umarani****, Professor (SST), FC & RI, Mettupalayam,* ***Dr.N.Senthil,*** *Professor (PBG), CPBG, TNAU, Coimbatore and* ***Dr.A.R.Muthiah****, Director (CPBG) (Retd.), TNAU, Coimbatore for their valuable guidance, advice and whole hearted help, keen interest and critical perusal of the manuscript.*

I express my deep sense of gratitude and grateful regards to ***Dr.A.Vijayakumar****, Professor and Head, Department of Seed Science and Technology for his encouragement in various stages of the study.*

I express my sincere thanks to ***Dr.K.Vanangamudi, Dr.P.Balamurugan*** *and* ***Dr.P.Natesan*** *Professor (SST) (Rtd.),* ***Dr.K.Ramamoorthy****, Special Officer (Seeds) (Retd.).* ***Dr.P.Srimathi,*** *and* ***Dr.P.Selvaraju****, Professors (SST),* ***Dr.K.Prabakar****, Professor of Plant Pathology Tamil Nadu Agricultural University for their timely help during the study period.*

My sincere thanks are also due to, ***Dr. K. Sundaralingam, Dr.R.Jerlin, Dr.J.Renugadevi, Dr.V.Manonmani****,* ***Dr.P.R.Renganayagi,*** *Professors and* ***Dr.S.Lakshmi, Dr.K.Malarkodi, Dr.R.Vijayan, Dr.Nelson navamaniraj, and Dr.C.Menaka*** *Assistant Professors (SST),*

Department of Seed Science and Technology for their timely help during the study period.

I express my special thanks to ***Dr.N.Natarajan,*** *Professor (SST), Dept. of Nano Sci. & Tech. for their timely help in my biochemical analysis. My thanks are due to* ***Dr.S.Sundareswaran,*** *Professor (SST) and* ***Dr.Vakeswaran,*** *Assistant Professor (SST) ARS, Bhavanisagar, for their timely help and suggestions during the field experiments.*

I express my sincere thanks to my dear ***Cokes (M.Kokila), Scientist. Somasundaram, Scientist.H.P.Vijayakumar, M.Govindaraj, Anbarasu (SST), V.Vijayalakshmi, B.Venudevan, M. Ameer junaithal begum*** *and* ***Nithya*** *for encouragement and painful help in preparing and shaping this manuscript.*

I express my heartful gratitude to my classmates ***Karthika, Mrs.Revathi****,* ***Mrs.Vigneshwari (Vicky) and Mrs.Thirusendura Selvi*** *for their valid suggestions and untired help rendered throughout my study period.*

I also extend my heartfelt thanks to My M.Sc. classmates ***Dr.Tamilkumar, Dr.Poonguzhali*** *and* ***Gomathi****, super senior* ***Dr.Sumathi****, Junior friends* ***Anandraj Kuppiah, Ananthi, Shyla, Laxmi, Shankar****,* ***Kirubha****,* ***Anandh, Ramki****,* ***Marthandan****,* ***Mohan, Anbu****,* ***Vadivel, Nagu*** *(PAT), and my other senior and junior friends for their co-operation and help rendered time to time.*

I express my heartfelt thanks to ***Nivetha*** *and* ***Poorani,*** *Junior Research Fellows and also the non teaching staffs especially* ***Mrs. S. Selvi****, Typist****, Nagaraj, Selvaraj, Hema, Bhuvana, Logammal, Saraswathi, Karbagam Nithya and field helpers*** *at ARS, Bhavanisagar for their help during my study period.*

Thanks are due to my friends ***A.P.Ramaraj, Arun venkatesh, Anbarasu, Shoban chakravarthy,*** *and other friends for their help during my research work.*

On a personal note I felicitate my gorgeous love with adoration and wish to place my glowing gratitude to my affectionate parents ***Mr.S.Sekar****,* ***Mrs.Kousalya Sekar,*** *and brother* ***Santhosh*** *and my family members for their constant encouragement, prayers, love, care and affection showered upon me in the pursuit of my educational ambition for which I am eternally grateful.*

I express my gratitude to ***Tamil Nadu Agricultural University, Coimbatore*** *for permitting an opportunity to carry over this study.*

Thanks are due to many who might be missed by chance in this acknowledgement but rendered a permanent place in my heart. I express my indebtedness to one and all for giving their helping hand during this endeavour.

Finally, I dedicate this thesis to the almighty, my parents and relatives whose selfless love and blessings are the only reason for my success.

(S.SATHISH)

ABSTRACT

SEED VIGOUR AND VIABILITY STUDIES IN BLACKGRAM (*Vigna mungo* L.) cv. TNAU BLACKGRAM CO 6

By

S. SATHISH

Degree : **Doctor of Philosophy (Agriculture) in Seed Science and Technology**

Chairman : **Dr. M. Bhaskaran, Ph.D.**
Special Officer (Seeds)
Seed Centre
Tamil Nadu Agricultural University
Coimbatore – 641 003

2013

A study was conducted to standardize a suitable vigour test to delineate field emergence potential of seed lots, proteomics of seed deterioration, standardize botanical seed treatment for maintenance of seed quality during storage, unravel the mode of action of seed invigouration and its effect on crop productivity and standardize a low temperature treatment to curb secondary infestation of pulse beetle during storage using blackgram cv. TNAU Blackgram CO 6 seeds.

Seven seed lots of blackgram cv. TNAU Blackgram CO 6 were exposed to different vigour tests. Among the different vigour tests evaluated, accelerated ageing for 3 days and electrical conductivity test with incubation of 6 h in 75 ml distilled water at 20°C grouped seed lots similar to groupings based on field emergence. Correlation analysis showed a significantly highest positive association between accelerated ageing test (r = 0.993) and field emergence while electrical conductivity test (r = -0.962) showed a significantly highest negative association with field emergence. Hence, these two vigour tests could be considered as the suitable vigour tests for screening the vigour status of blackgram seed lots.

Evaluation of fresh and 2-10 days accelerated aged seeds revealed that germination and vigour declined gradually during accelerated ageing. Seed germination reached 14% after 10 days of accelerated ageing from 99% of initial germination. On 6^{th} day of ageing, germinability of the seeds reached 58% which is less than IMSCS of 75%.

Biochemical analysis of fresh and 2-10 days accelerated aged seeds revealed a decrease in DPPH radical scavenging activity with concomitant increase in solute leakages, protease activity and free amino acid content as ageing progresses. Total protein profile showed a reduction in intensity of protein bands in aged seeds and loss of a protein with molecular weight 60.21 kDa in the 6 and later days of accelerated aged seeds. The most interesting observation was both the viability reduction below germination standard and loss of protein band coincided on 6 days of accelerated ageing of seeds.

Comparative analysis of fresh and 6 days accelerated aged seeds using two dimensional electrophoresis and MALDI-TOF-MS revealed a total of 16 differentialy expressed proteins that can be classified into 8 functional groups. Out of 16, 12 proteins were down-regulated and 4 proteins were up-regulated indicating that artificial ageing affected the proteome of the dry seeds. The down-regulated proteins were functionally related to cell structure, followed by transcription, protein synthesis, storage protein, transporters and metabolism, while the up-regulated proteins were related to proteolytic protein defense related protein and elongation factor.

Standardization of dry dressing treatment with botanicals revealed that seed dry dressing with fenugreek seed powder @ 3 g kg^{-1} of seeds or custard apple leaf powder @ 4 g kg^{-1} of seeds with one hour shaking improved the germination by 9% over control of aged seeds and vigour index by 17 % and 32 % respectively for fresh and aged seeds over their respective control.

Standardization of wet seed treatment with botanicals revealed that the blackgram seeds soaked for 1 h in 1.0% fenugreek seed powder solution and 1.5% custard apple leaf powder solution increased the germination and vigour. Both the dry and wet treatment was highly effective in case of aged seeds than fresh seeds.

The seeds treated with fenugreek seed powder or custard apple leaf powder in dry or wet form possessed high free radical scavenging property and resulted in lower solute leakages. Also possessed higher α - amylase activity and lower protease activity and free amino acid content.

Analysis of DPPH free radical scavenging property of botanicals revealed high antioxidant property in fenugreek seed powder and custard apple leaf powder with 95.9 and 96.0%, respectively followed by moringa leaf powder (91.7%). ICP analysis of botanicals revealed the presence of Ti, Mo, Fe, Al, Sr, B, Ba, Zn, Cu, Mn in all the three botanicals. Among these minerals, availability of titanium, molybdenum and iron were higher.

The field study was carried out to evaluate the efficacy of botanical seed treatment in improving field performance of blackgram seeds. The seeds treated with fenugreek seed powder or custard apple leaf powder in dry or wet form shows its superiority in its growth attributes such as plant height, leaf area and leaf area index which were significantly higher over fresh and aged control at vegetative stage, while dry matter production was higher in all the stages *viz*., vegetative, flowering and harvesting stage. Yield attributes such as pod and seed yield was also higher both in fresh and aged seeds during *kharif* and *rabi* season. The increase in seed yield per hectare was to the tune of 9 and 17 per cent over control in fresh and aged seeds, respectively.

A study was carried out with exposure of seeds to low temperature of -18°C, a temperature found in freezer of commercial refrigerator, to curb the secondary infestation of pulse beetles. Among the different stages of pulse beetle, egg stage was more tolerant to low temperature of -18°C and adult was the most susceptible stage than other immature stages.

The blackgram seeds packed in 700 gauge polyethylene bag exposed to -18°C temperature for 6 h or more resulted in 0% seed damage after 10 months of storage and it reduced the rate of deterioration during storage which was evidenced from maintenance of moisture content at 8.1% without any further increase and slow reduction in germination and vigour till the end of 10 months of storage. Therefore, exposure of seeds packed in 700 gauge polythene bag to low temperature (-18°C) for 6 h could be sufficient to curb secondary infestation of pulse beetle during storage of blackgram seeds.

CONTENTS

LIST OF TABLES

10.	Differentially expressed proteins during seed ageing identified using MALDI-TOF. Arranged according to their functional classification	
11.	Effect of dry dressing with botanicals on speed of germination of fresh seeds of blackgram cv. TNAU Blackgram CO 6	
12.	Effect of dry dressing with botanicals on germination (%) of fresh seeds of blackgram cv. TNAU Blackgram CO 6	
13.	Effect of dry dressing with botanicals on shoot length (cm) of fresh seeds of blackgram cv. TNAU Blackgram CO 6	
14.	Effect of dry dressing with botanicals on root length (cm) of fresh seeds of blackgram cv. TNAU Blackgram CO 6	
15.	Effect of dry dressing with botanicals on dry matter production (g / 10 seedlings) of fresh seeds of blackgram cv. TNAU Blackgram CO 6	
16.	Effect of dry dressing with botanicals on vigour index I of fresh seeds of blackgram cv. TNAU Blackgram CO 6	
17.	Effect of dry dressing with botanicals on vigour index II of fresh seeds of blackgram cv. TNAU Blackgram CO 6	
18.	Effect of dry dressing with botanicals on speed of germination of aged seeds of blackgram cv. TNAU Blackgram CO 6	
19.	Effect of dry dressing with botanicals on germination (%) of aged seeds of blackgram cv. TNAU Blackgram CO 6	
20.	Effect of dry dressing with botanicals on shoot length (cm) of aged seeds of blackgram cv. TNAU Blackgram CO 6	
21.	Effect of dry dressing with botanicals on root length (cm) of aged seeds of blackgram cv. TNAU Blackgram CO 6	
22.	Effect of dry dressing with botanicals on dry matter production (g / 10 seedlings) of aged seeds of blackgram cv. TNAU Blackgram CO 6	
23.	Effect of dry dressing with botanicals on vigour index I of aged seeds of blackgram cv. TNAU Blackgram CO 6	
24.	Effect of dry dressing with botanicals on vigour index II of aged seeds of blackgram cv. TNAU Blackgram CO 6	
25.	Effect of wet treatment with botanicals on speed of germination of fresh seeds of blackgram cv. TNAU Blackgram CO 6	

26.	Effect of wet treatment with botanicals on germination (%) of fresh seeds of blackgram cv. TNAU Blackgram CO 6	
27.	Effect of wet treatment with botanicals on shoot length (cm) of fresh seeds of blackgram cv. TNAU Blackgram CO 6	
28.	Effect of wet treatment with botanicals on root length (cm) of fresh seeds of blackgram cv. TNAU Blackgram CO 6	
29.	Effect of wet treatment with botanicals on dry matter production (g / 10 seedlings) of fresh seeds of blackgram cv. TNAU Blackgram CO 6	
30.	Effect of wet treatment with botanicals on vigour index I of fresh seeds of blackgram cv. TNAU Blackgram CO 6	
31.	Effect of wet treatment with botanicals on vigour index II of fresh seeds of blackgram cv. TNAU Blackgram CO 6	
32.	Effect of wet treatment with botanicals on speed of germination of aged seeds of blackgram cv. TNAU Blackgram CO 6	
33.	Effect of wet treatment with botanicals on germination (%) of aged seeds of blackgram cv. TNAU Blackgram CO 6	
34.	Effect of wet treatment with botanicals on shoot length (cm) of aged seeds of blackgram cv. TNAU Blackgram CO 6	
35.	Effect of wet treatment with botanicals on root length (cm) of aged seeds of blackgram cv. TNAU Blackgram CO 6	
36.	Effect of wet treatment with botanicals on dry matter production (g / 10 seedlings) of aged seeds of blackgram cv. TNAU Blackgram CO 6	
37.	Effect of wet treatment with botanicals on vigour index I of aged seeds of blackgram cv. TNAU Blackgram CO 6	
38.	Effect of wet treatment with botanicals on vigour index II of aged seeds of blackgram cv. TNAU Blackgram CO 6	
39.	Evaluation of the effect of dry dressing with botanicals on speed of germination of blackgram seeds through accelerated ageing test	
40.	Evaluation of the effect of dry dressing with botanicals on seed germination (%) of blackgram seeds through accelerated ageing test	
41.	Evaluation of the effect of dry dressing with botanicals on shoot length (cm) of blackgram seeds through accelerated ageing test	

42.	Evaluation of the effect of dry dressing with botanicals on root length (cm) of blackgram seeds through accelerated ageing test	
43.	Evaluation of the effect of dry dressing with botanicals on dry matter production (g / 10 seedlings) of blackgram seeds through accelerated ageing test	
44.	Evaluation of the effect of dry dressing with botanicals on vigour index I of blackgram seeds through accelerated ageing test	
45.	Evaluation of the effect of dry dressing with botanicals on vigour index II of blackgram seeds through accelerated ageing test	
46.	Evaluation of the effect of wet treatment with botanicals on speed of germination of blackgram seeds through accelerated ageing test	
47.	Evaluation of the effect of wet treatment with botanicals on germination (%) of blackgram seeds through accelerated ageing test	
48.	Evaluation of the effect of wet treatment with botanicals on shoot length (cm) of blackgram seeds through accelerated ageing test	
49.	Evaluation of the effect of wet treatment with botanicals on root length (cm) of blackgram seeds through accelerated ageing test	
50.	Evaluation of the effect of wet treatment with botanicals on dry matter production (g / 10 seedlings) of blackgram seeds through accelerated ageing test	
51.	Evaluation of the effect of wet treatment with botanicals on vigour index I of blackgram seeds through accelerated ageing test	
52.	Evaluation of the effect of wet treatment with botanicals on vigour index II of blackgram seeds through accelerated ageing test	
53.	Effect of botanical treatments on biochemical characters of fresh seeds of blackgram cv. TNAU Blackgram CO 6.	
54.	Effect of botanical treatments on biochemical characters of aged seeds of blackgram cv. TNAU Blackgram CO 6.	
55.	Antioxidant property and mineral content (ppm) of botanicals	
56.	Effect of botanicals seed treatments on plant height (cm) in *kharif* 2012	
57.	Effect of botanicals seed treatments on leaf area (cm^2) in *kharif* 2012	

78.	Effect of low temperature (-18°C) exposure duration on pulse beetle infestation (%) in stored seeds of blackgram cv. TNAU Blackgram CO 6.	
79.	Effect of low temperature (-18°C) exposure duration on moisture content (%) in stored seeds of blackgram cv. TNAU Blackgram CO 6.	
80.	Effect of low temperature (-18°C) exposure duration on seed germination (%) in stored seeds of blackgram cv. TNAU Blackgram CO 6.	
81.	Effect of low temperature (-18°C) exposure duration on shoot length (cm) in stored seeds of blackgram cv. TNAU Blackgram CO 6.	
82.	Effect of low temperature (-18°C) exposure duration on root length (cm) in stored seeds of blackgram cv. TNAU Blackgram CO 6.	
83.	Effect of low temperature (-18°C) exposure duration on dry matter production (g / 10 seedlings) in stored seeds of blackgram cv. TNAU Blackgram CO 6.	
84.	Effect of low temperature (-18°C) exposure duration on vigour index I in stored seeds of blackgram cv. TNAU Blackgram CO 6.	
85.	Effect of low temperature (-18°C) exposure duration on vigour index II in stored seeds of blackgram cv. TNAU Blackgram CO 6.	

LIST OF FIGURES

14.	Effect of wet treatment with botanicals on vigour index I of blackgram seeds through accelerated ageing test	
15.	Effect of botanical seed treatment on electrical conductivity and antioxidant activity of fresh and aged seeds of blackgram	
16.	Mineral content in botanicals as analyzed using ICP	
17.	Effect of botanical seed treatments on dry matter production (g plant^{-1}) of blackgram in *kharif* 2012	
18.	Effect of botanical seed treatments on dry matter production (g plant^{-1}) of blackgram in *rabi* 2012	
19.	Effect of botanical seed treatments on seed yield (kg ha^{-1}) of blackgram in *kharif* and *rabi* 2012	
20.	Effect of low temperature (-18°C) exposure on mortality of pulse beetle	
21.	Effect of low temperature (-18°C) exposure on pulse beetle infestation in stored seeds of blackgram	
22.	Effect of low temperature (-18°C) exposure on seed germination in stored seeds of blackgram	

LIST OF PLATES

LIST OF PUBLICATIONS

S.No	Title of the paper	Name of the journal
1	Seed dry dressing with botanicals to improve physiological performance of fresh and aged seeds of blackgram (*Vigna mungo* L.)	African Journal of Agricultural Research. 8(29): 4049 - 4057
2	Effect of seed invigouration with botanicals on physiological performance of aged seeds of blackgram (*Vigna mungo* L.)	Madras Agricultural Journal, 100(10-12).

CHAPTER I

INTRODUCTION

Pulses are the second most important group of crops, worldwide. India ranks first in terms of pulses production, consumption and acreage. Nevertheless, the country continues to face endemic shortage in pulses. Though pulses account for 25 to 30 per cent protein of its dry weight as against 8 to 12 per cent in cereals, larger proportion of dietary protein is contributed only from cereals, due to shortage in pulses production. World Health Organization actually recommends 80 grams of pulses per person per day. Based on this recommendation, India needs to produce 35 million tonnes of pulses annually to meet the national requirement. But, the estimated pulses production during 2011-12 was only 17.02 million tonnes. Even more disturbing factor is the alarmingly poor per capita availability of pulses in our country during the last two decades. As per the latest economic survey, per capita net availability of pulses has come down from 41.6 grams per day in 1991 to 31.6 grams per day in 2010, which highlights the consequences of sluggish production in pulses in the country on the face of growing population (ASSOCHAM, 2012).

Blackgram (*Vigna mungo* L) is a protein rich seed, containing about 26 per cent protein, which is almost three times that of cereals and ranks fourth 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 per cent of total area under pulses and contribute 8.4 per cent of total pulses production. However, area and production of blackgram has declined from 3.01 million hectares and 1.30 million tonnes in 2000-01 to 2.97 million hectare and 1.23 million tonnes, respectively in 2009-10. In Tamil Nadu, it is cultivated in an area of 0.26 million hectares with a production of 0.09 million tonnes and ranks only 5^{th} in terms of area and production preceded by Maharashtra, Andhra Pradesh, Madhya Pradesh and Uttar Pradesh (ASSOCHAM, 2012).

The gradual reduction in area of cultivation of blackgram in India in the previous decades is attributed to the poor seed storability, low seed quality and low productivity.

In any crop, seeds have to be invariably stored as carryover seed for sowing in next season. During the storage period, seed deterioration is inevitable, resulting in loss of seed vigour and viability (McDonald, 1999). Reduced vigour and viability of seeds may affect field performance and productivity of the subsequent crops (TeKrony and Egli, 1991). Therefore, use of high quality seed is mandatory, which necessitates the evaluation of its quality before introducing into market. The evaluation of physiological potential is officially based on the germination test, which is done under optimum environmental conditions as described by ISTA (2007) and aims to determine the actual germination potential of the seed lot. However, studies on germination and vigour of seeds shows that seed lots which have similar laboratory germination often differ in field emergence (Marshall and Naylor, 1985), as field conditions are rarely ideal (Hampton, 1991). Therefore, more efficient vigour tests should be employed to detect differences in potential performance among the seed lots with a similar germination.

Seed vigour is defined as an index of the extent of the physiological deterioration and/or mechanical integrity of a high germinating seed lot which governs its ability to perform in a wide range of environments (Hampton, 1998). In order to obtain a more realistic picture, it is recommended that seed vigour must be evaluated with two or more tests so as to evaluate different aspects of seed behavior (Marcos Filho, 2005). The vigour tests, including accelerated ageing, controlled deterioration test, complex stress vigour test, seedling growth rate and electrical conductivity have the capacity to classify seed lots and safely evaluate potential performance in the field (Hampton and TeKrony, 1995).

Development of technologies to improve the quality of seeds will go a long way in improving crop productivity. In order to develop such a technology, obtaining critical knowledge about the seed deterioration is extremely important. Although there is no concrete evidence to suggest definite molecular mechanism of seed deterioration yet, mounting research data pinpoints the production of free radicals during storage as a major cause for deterioration. Free radical damage of cell organelles leads to disruption of cellular membranes and damage to protein and nucleic acid, ultimately resulting in seed ageing (Bailly, 2004; Kibinza *et al.*, 2006). It is well proven that proteins are the major targets for free radicals due to their abundance in biological systems and their high rate constants for reaction (Davies, 2005). Recently, information is available on the possible

changes of proteome in dry seeds during storage. In *Arabidopsis thaliana*, Rajjou *et al*. (2008) identified 18 spots differentially expressed in dry mature seeds after controlled deterioration treatment (85% relative humidity, 20°C). While Xin *et al*. (2011) identified 40 spots differentially expressed in dry mature seed after accelerated ageing treatment in maize seeds and demonstrated that the proteome change could take place at the dry state of aged seeds.

With an insight on the mechanism of seed ageing, it is possible to understand the vigour potential of a seed lot. If a seed lot has been identified to have poor quality, to prevent further quantitative and qualitative losses due to biotic and abiotic factors, different seed management practices are being adopted. One such safe and feasible seed treatment is using suitable botanicals for maintaining vigour and viability during storage. The development of eco-friendly, easy to-do technique of dry treatment with botanicals would be of great advantage to reduce the problems in maintenance of seed quality during storage. Layek *et al.* (2006) found that dry treatment with red chilli powder improved storability and field performance of high vigour seeds of gram. To improve the performance of seed, dry seed treatment purely with crude plant materials in powder form have been showed to significantly slow down the deterioration of seeds under various ageing conditions (De *et al.,* 2004 in wheat; Rudrapal and Basu, 2004 in french bean; Sengupta *et al.,* 2005 in onion; Kundagrami *et al.,* 2008 in rice).

Wet seed invigouration treatments are physiological treatments that imply an improvement in physiological status of the seed, thereby resulting in improved germinability, greater storability and better performance. The yield potential of the seeds can be improved by various invigouration treatments. Lowell (2005) stated that seed treatment with extracts from fresh moringa leaves increased yield by 25-30 per cent in onion, bell pepper, soyabean, maize, sorghum, coffee, tea, chilli and melon. Seed fortification with prosopis (*Prosopis juliflora*), pungam (*Pongamia pinnata*) and arappu *(Albizia amara)* leaf extract improved germination, vigour and field performance in cluster bean (Renugadevi *et al.,* 2008). The yield increase by wet seed invigouration in cowpea, groundnut and beans was reported by Phiri and Mbewe (2010).

It has been proven that the deteriorative effect of seed ageing is mainly due to production of free radicals (Bailly, 2004; Bailly *et al.*, 2008) and use of antioxidants can quench the free radicals and retain seed vigour during germination (Maeda *et al.*, 2005; Sattler *et al.*, 2006). Among the several botanicals available, it has been pharmacologically proved that 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) possess appreciable antioxidant property along with high nutrient content. Therefore, these botanicals can be used as a potential source of natural antioxidants which can be attempted for seed treatments for vigour and viability maintenance in their commercial form.

Apart from rapid deterioration of vigour due to ageing, quality of seed is also greatly affected by pest during storage. Pulse beetle (*Callosobruchus maculatus*) is the major storage pest of blackgram. In general, the damage starts in the field, where adult female lays eggs on the green pods. The grubs feed through the pod cover and remain concealed within the developing seed (Southgate, 1979). When such seeds are harvested and stored, the insect continues to feed as hidden infestation and emerges as an adult, cause secondary infestation and may result in total destruction within a period of 3-4 months making the seed unfit for sowing purposes (Singh and Jackai, 1985). Gujar and Yadav (1978) recorded 55 to 60 per cent loss in seed weight and 45 to 66 per cent loss in protein content due to the damage by the pulse weevil. Pulse beetle infestation also leads to increase in moisture content and micro-organisms infections that adds to further deterioration (Patro *et al.*, 2007). Use of pulse beetle damaged seeds will reduce germination, plant development and negatively impact yield components with increased fungal disease severity (Chipungahelo *et al.*, 2001).

Conventional chemicals *viz*. grain protectants or fumigants have been extensively used all over the world to check infestation. However, presently the use of chemicals are being restricted globally because of the problems related to persistence of toxic residues in food grains, the development of insect resistance and adverse environmental impacts. Therefore, a need was felt to investigate the ecologically safe physical methods to control insect pests of pulses. Temperature is the most important environmental factor, determining the rate of metabolism, growth, development, reproduction, general

behaviour and distribution of insect pests. Each insect pest has a particular requirement of temperature to carry out its activities. Any temperature lower/ higher will affect insect's activities. Therefore, low temperature have been exploited for pulse beetle control without affecting the quality of seeds (Johnson and Valero, 2003; Bhalla *et al.*, 2008).

With these backdrops, the present investigation was taken up with the following objectives in blackgram cv. TNAU Blackgram CO 6.

1. To evaluate various seed vigour tests and ascertain the most suitable vigour tests for blackgram.
2. To delineate the pattern of seed deterioration in blackgram by employing the proteomic approach.
3. To optimize appropriate botanical seed treatments to improve the vigour of fresh and aged seeds of blackgram.
4. To elucidate the mode of action of botanical seed treatment and evaluate their effect on crop productivity in fresh and aged seeds of blackgram.
5. To assess the influence of low temperature treatment on control of pulse beetle infestation during storage

CHAPTER II

REVIEW OF LITERATURE

Maintenance of seed vigour and viability from harvest until planting is important in any crop production programme. Ageing is an inevitable physiological phenomenon occurring in all living organisms including seeds (Crocker and Barton, 1953; Barton, 1961 and Strehler, 1962). Seed deterioration leads to reduction in vigour and ultimately become less viable leading to poor stand establishment (Shelar, 2007) and crop performance (Nutile, 1964; Egli and TeKrony, 1979; Pallavi *et al.,* 2003). The mechanism of ageing that differ with cultivars is still an enigma. Understanding the seed senescence phenomenon is required to develop suitable technologies to slow down the processes of ageing. Apart from seed ageing, loss of quality of seeds is also caused by pest infestation during seed storage. Hence, the literature pertaining to longevity and storability of seed, the physiological and biochemical aspects of seed deterioration, tests which are used to detect the vigour status of seed lots, enhancement of vigour and viability of aged seeds using different botanicals treatments to improve the seed quality and finally physical method of pulse beetle control with reference to pulses and other crops have been reviewed hereunder.

2.1. Seed vigour and its importance

Testing for vigour becomes more important for carryover seeds, especially if seeds are stored under unknown conditions or unfavorable storage conditions. Seed vigour is used as indicator of storage potential of a seed lot and seed vigour testing helps in ranking various seed lots with different qualities.

Seed vigour is qualitative in concept than a quantifiable seed quality character but it is often evaluated through various vigour tests where, the vigour status is often indicated through comparative values. Vanderlip *et al.* (1973) observed that seed lots differed widely between each variable measured on standard germination and other vigour tests. A practical seed vigour test should give a good indication of field performance potential of the seed lot and the test results should be reproducible (Hampton and TeKrony, 1995).

2.2. Determination of seed vigour

Evaluation of seed vigour is important to predict the planting value of seed lot. A large number of researchers use developmental stage of seedling for seed vigour determination like length of whole seedling, individual either stem or root dry matter production (Abdul-Baki and Anderson, 1973) and rate of germination (Maguire, 1962). Since individual method does not satisfy all requirements for seed vigour determination, a combination of several tests should be used.

2.2.1. Standard germination test

The object of the germination test is to determine the maximum germination potential of a seed lot, which can in turn be used to compare the quality of different lots and also estimate the field planting value (ISTA, 1993). The major limitation of the germination test as an assessment of seed lot potential performance is its inability to detect differences among high germinating seed lots. Because of the nature of the normal distribution on which the seed survival curve is based, a small difference in percentage germination represents a large variation in the progress of deterioration (Ellis and Roberts, 1980).

Norton (1986) pointed out that standard seed germination test is not good indicator of field emergence and show poor correlation with field emergence, mainly due to varied environmental factors. In these circumstances, a more sensitive differentiation of potential seed performance (*i.e.* seed vigour testing) is necessary (Hampton and Coolbear, 1990). McDonald (1993) observed that vigour tests are designed to reveal the field performance of the seeds and also suggested that in seed testing laboratories, routinely one or more number of vigour tests ought to be conducted.

Ferguson (1993) emphasized that vigour tests are not designed to predict the exact number of seedlings that will emerge and survive in the field, although many of the vigour tests do correlate well with field emergence. Several vigour tests have been developed successfully and used to evaluate the seed quality of different seed lots with similar germination percentage in major agricultural crops (Hampton and TeKrony, 1995).

2.2.2. Accelerated ageing test

Accelerated ageing (AA) was developed as a test to estimate the longevity of seed in commercial storage (Delouche and baskin, 1973). The test is recommended for soybean seeds by the Association of Official Seed Analysts (AOSA) in the seed vigour testing handbook (AOSA, 1983) and has been used to predict the life span of a number of different species. The test has subsequently been evaluated as an indicator of seed vigour in a wide range of crop species and has been successfully related to field emergence and stand establishment (Hampton and TeKrony, 1995). The accelerated aged seeds generally show a marked reduction in their ability to germinate (McDonald, 1999), which is also associated to increased leaching of organic and inorganic constituents.

Cookson *et al.* (2001) reported that seed lots of perennial ryegrass harvested from soils with different N concentration differed significantly in their laboratory emergence at autumn and winter temperatures and concluded that these differences could be effectively determined by vigour test such as accelerated ageing and seedling dry weight.

Rodo and Filho (2003a) suggested that saturated salt accelerated ageing at 41°C for 48 and 72 h and controlled deterioration at moisture content adjusted to 24% at 45ºC for 24 h were the best procedures to assess the physiological potential of onion seeds and are indicated for use in quality control programs.

Mavi and Demir (2007) found that the optimum accelerated ageing conditions of 120 h at 45 to 47°C and controlled deterioration conditions of 48 h and 20% moisture content at 45°C could predict the field emergence of 22 melon seed lots under different stress conditions and suggested as suitable vigour test to estimate relative seedling emergence of melon seeds.

Demir and Mavi (2008) reported that the most consistent regimens for accelerated ageing tests was 96 h at 45°C and for controlled deterioration tests was 72 h with 20% moisture content at 45°C to predict the storage life in cucurbit seed lots.

Havstad *et al.* (2011) revealed that difference in the storage potential of six different timothy seed lots can be identified by accelerated ageing for 56 h at 45°C while

in red clover seed lots, accelerated ageing for 24 h at 43 - 45°C was appropriate to assess the seed vigour.

Lopes *et al.* (2012) concluded that the physiological potential of scarlet eggplant seeds can be evaluated using accelerated ageing at 41°C for 48 h and controlled deterioration at 24 % moisture content for 24 h at 45°C and seed lots can be classified into different vigour levels.

2.2.3. Controlled deterioration test

Controlled deterioration was initially developed as a test for detecting storage potential of seed lots of small seeded vegetable species (carrot, onion, lettuce, brassicas) with poor field emergence potential (Matthews, 1980; Powell and Matthews, 1981, 1984) and later used to rank the seed lots of large number of species for potential performance and well correlated with field emergence than standard germination test for a wide range of species including sugar beet, carrot, lettuce and onion (Matthews and Powell, 1987), pea (Bustamante *et al.*, 1984) broccoli (Mendonca *et al.*, 2000) and *Brassica* species (Powell, 2009).

Rodo and Filho (2003b) recommended 24% moisture content, 45°C temperature and 24 h incubation period for controlled deterioration of onion.

Mavi and Demir (2005) revealed that germination of three seed lots of winter squash after subjecting to controlled deterioration for 96 h at 45°C and 24 % moisture content were positively and significantly related to mean seedling emergence time, seedling fresh and dry weight, hypocotyl length and cotyledon width and concluded that controlled deterioration test can be used to predict seedling performance of winter squash seed lots under salt stress.

Basak *et al.* (2006) concluded that controlled deterioration at 45°C and 22% moisture content for 24 h of 13 pepper seed lots were strongly correlated with high and low temperature emergence while initial laboratory germination test was not significantly related to either of the temperature emergence. They also concluded that laboratory germination after 4 and 8 month storage at 5°C and 8% moisture content were closely correlated with initial controlled deterioration germination.

Kavak *et al.* (2008) reported that germination after controlled deterioration at 24 % seed moisture content for 24 h at 45°C highly correlated with field emergence and clearly revealed the differences in seed vigour of pepper seed lots.

Eksi and Demir (2011) revealed that controlled deterioration test has potent use to predict field emergence and longevity of onion seed lots and also concluded that the duration of test can be reduced to 5 days from 14 days by increasing the initial moisture content of seeds to 24 % and ageing temperature to 47°C instead of 20% and 45°C, respectively in validated controlled deterioration test and counting the radicle at 74 or 89 h instead of final count at 14 days.

2.2.4. Complex stressing vigour test

The complex stressing vigour test was first developed for wheat and later modified for maize, to provide an indication of the minimum expected range of emergence under the stress condition imposed by Hungarian soils (Szirtes and Barla-Szabo, 1981). Later Barla-Szabo and Dolinka (1984) modified the method to simulate several different stress conditions in which the seed lot may be subjected to the field, as opposed to other vigour tests which concentrate on a single form of stress. The test has been widely used in Hungary for over 10 years and has consistently identified low and high vigour seed lots, with the result that desired populations of maize can be more frequently achieved (Barla-Szabo and Dolinka, 1988; Barla-Szabo *et al.*, 1990).

The test imposes temperature and oxygen deficiency stress to certain duration during which seeds with weaker cell membrane progressively lose their biochemical functions and leaching of cell contents occurs and the results are well correlated with field emergence (Lovato and Balboni, 1997; Maree *et al.*, 2007 in maize; Van de Venter *et al.*, 1993 in wheat).

2.2.5. Seedling growth test

While all the seedlings judged to be morphologically normal are included in the germination test result (ISTA, 1993), no account is taken on the rate of germination or growth, nor of the strength or sturdiness of the seedling in making this decision (Perry, 1987). Differences in these characteristics are frequently observed and there is abundant

literature available on various aspects of seed deterioration and their relationship to seedling development (Burris *et al.*, 1969; Edje and Burris, 1970; Perry and Harrison, 1977). Linear growth and seedling dry weight have been used as growth tests. The linear measurement of plumule growth was first suggested as a vigour test by Germ (1949) and has been successfully used on maize (Woodstock, 1969), barley and wheat (Perry and Harrison, 1977). Edje and Burris (1970) found that soybean seedling dry weight (excluding the cotyledons) was closely related to seed vigour.

Demir *et al.* (2008b) revealed that mean germination time of 11 pepper seed lots at 18 and 25°C predicted the final emergence, mean size and variability of seedlings in transplant production.

Mavi *et al.* (2010) proposed that mean germination time can be used to estimate the field emergence cucurbit seed lots and confirmed that the test result of mean germination time correlated well with results of accelerated ageing and controlled deterioration test.

Matthews *et al*. (2010) suggested that measurements of rate of germination such as mean germination time was effective in discriminating vigour status of 6 lots in maize.

Matthews *et al*. (2011) reported that single count of radicle emergence after 6 days at 13°C and 66 h at 20°C consistently ranked the vigour status of 9 lots of maize and were significantly related to field emergence. He also suggested that it could be used as an alternative to cold test.

2.2.6. Electrical conductivity test

The conductivity test provides a measurement of electrolyte leakage from plant tissues and was first recognized for seeds of several crop species by Hibbard and Miller (1928). It was later developed into a routine vigour test to predict field emergence of garden pea (Matthews and Bradnock, 1968). The basis of the conductivity test is the solute leakage from seeds into water. The extent of solute leakage can be attributed to impaired membrane integrity and development of dead tissue on the living cotyledons as a result of seed ageing or imbibition damage (Matthews and Powell, 2006).

Tajbakhsh (2000) revealed that electrical conductivity of seed soak water can be used for measuring viability and seedling vigour of differently aged wheat seed lots.

Demir *et al.* (2008a) reported that electrical conductivity of seed soak water has the potential to predict the germination of cabbage seed lots developed by different duration of controlled deterioration and concluded that electrical conductivity of seed soak water could be used to predict normal laboratory germination and germination after the controlled deterioration test.

Matthews *et al.* (2009) proposed that electrical conductivity of 24 or 17 h seed soak water of fresh seeds and electrical conductivity of 2 to 3 days controlled deteriorated seeds for 17 h could be used as relatively quick vigour tests for the cabbage cultivar Yalova.

Powell (2010) suggested that electrical conductivity test is effective in determining the vigour status of soyabean seed lots with high reproducibility and it could be used as vigour test to assess the vigour status of soyabean seed lots.

Demir *et al.* (2012) revealed that electrical conductivity test for 8 h of soaking has potential to detect radish seed lots with unacceptably low germination levels and concluded that this method might help the seed industry to decide the renewal time of seed lots during storage in the retailer points and can be used as a routine test in checking germination quality of unsold seeds produced in previous years before marketing.

2.3. Physiological and biochemical changes associated with seed deterioration

The exact mechanisms that lead to the loss of seed viability are by no means completely elucidated and the susceptibility of seeds to ageing varies among families and species (Walters *et al.,* 2005; Niedzielski *et al.,* 2009; Nagel and Borner, 2010).

Kharab and Dahiya (2000) reported that changes in colour, delayed germination, reduced tolerance to adverse storage and germination conditions and reduction in seedling growth during ageing in pigeon pea.

Murali *et al.* (2002) stated that germination and field emergence of the pulses seed decreased while the electrical conductivity of seed leachate increased with increase in

storage period. The loss of vigour might be the outcome of reduction in the synthesis of enzymes, nucleic acid and amino acid in blackgram (Kavitha, 2002).

Changes in the levels of dehydrogenase, catalase, peroxidase, amylase, phosphatase and glutamic acid decarboxylase have been found to be associated with seed viability during storage in soybean was noticed by Anuja and Aneja (2004).

Bailly (2004) stated that reactive oxygen species (ROS) accumulation and lipid peroxidation are generally considered as the major contributors to seed deterioration.

Sujatha and Srimathi (2006) described that seed deterioration alters the differential permeability properties of the membranes. Increase in conductivity might be due to loss of membrane permeability and leaching of the electrolytes such as sugars, amino acids and organic acids in blackgram. When poor seeds are planted in soil, electrolytes probably provide food material for soil fungi causing seed decay and poor stand establishment in bitter gourd (Bellard *et al.*, 2006).

Paramasivam and Balamurugan (2007) reported that in groundnut seeds, oil and protein content decreased and free fatty acid content was increased with progress in storage period.

Freitas *et al.* (2009) reported that the cotton seeds stored under ambient laboratory conditions and in a cold room (10 ± 2°C) led to a decline in vigour, viability, lipoxygenase and phosphatase activities. Higher reduction on viability was observed in the seeds stored under ambient conditions.

Kaewnaree *et al.* (2011) opined that seed deterioration during storage is a complex physiological and biochemical process leading to loss of germination ability.

2.4. Accelerated ageing

Many factors like genetics, mechanical damage, relative humidity and temperature of the storage environment, seed water content, presence of micro flora, seed maturity, etc. contributes to seed ageing. The rate of loss of seed viability is mainly a function of temperature and seed moisture content (McDonald, 1999). During ageing, seed viability and vigour decreases. Furthermore, the losses of viability and vigour in seeds differ with species and cultivars.

Al-Maskri *et al.* (2003) found that accelerated ageing not only affects germination percentage but also decreased the seed vigour in carrot.

Khan *et al.* (2003) recorded reduced radicle length and germination speed of the onion seeds with increase in period of ageing and indicated that when ageing is progressed beyond a critical period, there was a carryover effect resulting in much reduced germination and radicle length. Increased conductance of the leachate of aged seeds has been documented by Goel and Sheoran (2003).

Vanitha *et al.* (2005) reported that artificial ageing reduced the rate of radicle extension and shoot growth in maize, blackgram and sunflower due to non-availability of food reserves. The activity of enzymes like acid phosphatase, phosphomonoesterase, dehydrogenase, amylase, catalase, and peroxidase were also decreased during accelerated ageing.

Dutra and Vieira (2006) confirmed that the accelerated ageing test was widely used to evaluate seed vigour in various species including vegetable crops.

2.4.1. Physiological changes during accelerated ageing

Basra *et al.* (2000) reported decrease in germination percentage and increase in root and shoot length up to three days of ageing in cotton.

Al-Maskri *et al.* (2002) studied that the effect of accelerated ageing in cucumber seeds and found that accelerated ageing not only affected germination percentage but also decreased the seed vigour.

Krishnaveni (2003) stated that eight and twelve days of artificial ageing of paddy seeds might be similar to six and ten months of storage under ambient conditions as observed through progressive decrease in germination and other related parameters. Al-Maskri *et al.* (2003**)** concluded that carrot seeds aged rapidly with significant reduction in the seed viability and seedling growth.

Khan *et al.* (2004) found loss of viability and vigour in onion seeds after ageing and related it to loss of membrane integrity.

Atici *et al.* (2007) and Cakmak *et al.* (2009) confirmed that aged seeds showed decreased vigour and produced weak seedlings that were unable to survive once reintroduced into a habitat.

Sathish *et al*. (2011) indicated that germination potential, root and shoot length, seedling dry matter and vigour index declined due to accelerated ageing of maize seeds. However, the poor performance can be alleviated by seed priming treatments.

2.4.2. Biochemical changes during accelerated ageing

Ageing coincides with protein denaturation and degradation, inactivation of enzymes, breakdown of phospholipids and depository lipids, lipid peroxidation and alteration of membrane permeability. Super oxide dismutase (SOD) and catalase (CAT) activities in seeds were observed to decrease with ageing.

Membrane permeability was strongly related to loss of seed viability as reported in cultivated Brassicaceae species (Verma *et al.*, 2001; Bedi *et al.*, 2006). They also found significant increase in electrical conductivity after ageing at 45°C and 90% RH.

Goel *et al.* (2003) studied the mechanism of seed deterioration in cotton and reported that membrane deterioration, electrical conductivity of seed leachates increased with ageing and it was closely related to decrease in activities of various peroxide scavenging enzymes such as peroxidase, catalase, ascorbate peroxidase, glutathione reductase and superoxide dismutase.

Hsu *et al.* (2003) studied the accelerated ageing test in bitter gourd seeds and observed that accelerated ageing resulted in increased lipid peroxidation with concomitant decrease in activities of several free radical and peroxide scavenging enzymes both in the embryonic axes and cotyledons and also reported reduced amount of soluble protein due to induced ageing.

Khan *et al.* (2003) stated that the degree of cell membrane damage in response to ageing could be measured in terms of rate of seed electrolyte leakage.

Murthy *et al.* (2003) confirmed the causes of seed ageing as lipid peroxidation mediated by free radicals, inactivation of enzymes or decrease in proteins, disintegration of cell membranes and genetic damage.

McDonough *et al.* (2004) reported that physical, structural and chemical changes occur during accelerated ageing. Physically, hardness and density of maize and sorghum decreased due to voids and cracks developed during the ageing process. Interactions within and between starch, protein and cell walls increased within the endosperm. Protein solubility patterns changed. Amounts of soluble proteins decreased and insoluble proteins increased because of increased protein interactions, possibly disulfide bonds.

Bailly (2004) indicated that the loss of viability during seed ageing was mainly related to the loss of plasma membrane integrity, due to the production of free radicals and reactive oxygen species (ROS) during storage. ROS was the major molecules responsible for reducing seed longevity after prolonged storage and could also be a factor inhibiting germination during stressful conditions.

Krishnan *et al.* (2004) found that loss of viability and increase in soybean seed leachate conductivity indicates the changes in thermodynamic properties of seed water which reflects the seed deterioration during storage under accelerated ageing.

Pukacka and Ratajczak (2005) concluded that the loss of germinability of peach seeds during storage at temperatures above 0°C was due to excessive production of ROS and an inefficient system to scavenge free radicals that appears after 6 weeks of incubation. Kibinza *et al.* (2006) demonstrated that the H_2O_2 induced ATP depletion could trigger cytochrome release, which in turn might lead to loss of viability and germinability in sunflower. Increased ROS production was due to mitochondrial alteration during ageing (Cash *et al.*, 2007).

Tommasi *et al.* (2006) concluded that *Ginkgo biloba* stored at 4°C preserves tissue viability, but only part of seeds had germinability. The embryo seems more equipped with antioxidant systems than endosperm. However, the antioxidant enzymes were scarcely regulated and unable to counteract oxidative stress occur during the long-term storage.

Rao *et al.* (2006) reported that absence of active enzymes to scavenge free radicals leads to degradation products of thermo-labile lipid peroxidation accumulate in the aged seeds, finally resulting in complete loss of onion seed viability.

Bailly *et al.* (2008) reported that to control free radical-induced cellular damage, seeds have developed a detoxification mechanism. This detoxification system includes a number of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydro ascorbate reductase (DHAR), glutathione peroxidase (GSHPx) and glutathione reductase (GSSGR).

Sital *et al.* (2008) stated that biochemical changes like contents of lipids, sugars and activities of certain enzymes in relation to seed viability were determined under artificial ageing and storage conditions in sunflower (*Helianthus annuus* L.) seeds. They found that catalase and peroxidase activities decreased whereas invertase and sucrose synthase activities increased with ageing.

Rutzke *et al.* (2008) reported that in aged cabbage seeds, degradation of respiratory pathway (at cytochrome C) leading to fermentation and high ethanol production, resulting in reduced dehydrogenase activity.

Rajjou and Debeaujon (2008) suggested on the contribution of testa to seed longevity for maintaining the weakest metabolic activity and protection against various environmental stresses. Free radical-counteracting processes and detoxification mechanisms are closely related to the control of the pro-oxidant/antioxidant balance both during seed storage and germination. When the pro-oxidant scavenging systems are saturated, detoxification mechanisms might be affected that irreparably will lead to seed death.

Niakan and Sabari (2009) reviewed that reactive oxygen species (ROS) is a major cause of lipid peroxidation on unsaturated fatty acid of cell membrane of Eucalyptus seed.

Cakmak *et al.* (2010) reported that long term storage (42 years) reduced the germination capability and caused delay in the germination of alfalfa seeds. In addition, antioxidant enzymes activities of catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) were also low and total phenolic matter content and lipid peroxidation were high in the aged dry seeds.

Demirkaya *et al.* (2010) reported that inactivation of free radical scavenging enzymes (*i.e.*, SOD and CAT) during ageing and showed a direct relationship with the germination efficiency of ageing onion seeds. Moreover, a high level of correlation was found between the loss of seed viability and the decreases in CAT and SOD activities during seed ageing.

Khanahmadi *et al.* (2010) suggested that almost all organisms are well protected against free radical damage by antioxidant. When the mechanism of antioxidant protection becomes unbalanced by the deterioration of cell, oxidation can occur which result in accumulation of free radical. The antioxidants are important compounds that prevent oxidation.

Sathish and Sundareswaran (2010) reported that accelerated ageing of maize seeds resulted in decrease in protein content and hydrolyzing enzymes activity such as α-amylase and dehydrogenase with increase in electrical conductivity of seed leachates.

Kaewnaree *et al.* (2011), in sweet pepper (*Capsicum annuum* L.) seeds, observed that a decrease in germination ability during ageing was well correlated with increase in membrane deterioration, as assayed by electrical conductivity and electrolyte leakage in soaked seeds. Malondialdehyde (MDA), the major product of lipid peroxidation, has rapidly increased from 0 to 75 mg g^{-1} within 10 days of accelerated ageing time.

2.4.3. Proteomics of seed ageing

Proteins are the functional components of a biological system. Earlier researches in seed ageing clearly indicated quantitative reduction in protein content (Anderson, 1970 in barley; Trawatha *et al.,* 1995 in soybean) and loss of hydrolyzing (Thangaraj *et al.,* l973, in cotton, Thornberry and Smith, 1995 in corn; Kavitha *et al.,* 2003, in blackgram) and free radical scavenging enzyme activities (Chhabra, 1984 in wheat and mungbean; Dal, 1984 in maize and pigeon pea; Ramamoorthy *et al.,* 1992 in rice; Das and Sen-Mandi, 1992 in wheat) during the process of seed deterioration and correlated the increased deteriorative process during ageing to elevated free radical activity due to oxidative stress (Wilson and McDonald 1986; Hendry, 1993; McDonald, 1999; Corbineau *et al.,* 2000; Bailly *et al.,* 2002; Bailly, 2004).

Recently, due to availability of genomic sequence information and based on the progress achieved in sensitive and rapid separation of proteins and in their high-throughput identification by electrophoresis and mass spectrometry, proteomic approaches have opened up new perspectives to analyze the complex functions of model plants and crop species (Canovas *et al.,* 2004; Park, 2004; Agrawal *et al.,* 2005a; 2005b; 2005c; Rossignol *et al.,* 2006; Jorrin *et al.,* 2007).

The extent of oxidative damage to nucleic acids, lipids and proteins has been found to increase with age (Levine and Stadtman, 2001). Indeed, a progressive accumulation of oxidative damage of these macromolecules in aged tissues is thought to contribute to the decline in biological functions characteristic of the aged phenotype (Stadtman, 2001; 2004). There is strong evidence that proteins are the most important targets for oxidants (Davies, 2005). Protein carbonylation has been widely used as an indicator of oxidative damage in several organisms and has been shown to increase in aged tissues (Nystrom, 2005; Moller *et al.,* 2007). It results from oxidative attack on Arg, Lys, Pro, or Thr residues of proteins (Levine *et al.,* 1990), which can affect enzyme activities or alter susceptibility of the modified proteins to proteolysis (Berlett and Stadtman, 1997; Davies, 2005).

Rajjou *et al*. (2008) studied the molecular basis of seed longevity through proteome approach in fresh, naturally aged and controlled deteriorated seeds of *Arabidopsis* and revealed that loss in seed vigour can be accounted for protein changes in the dry seeds and by an inability of the low-vigour seeds to display a normal proteome during germination. Furthermore, concluded that controlled deterioration strongly increased the extent of protein oxidation / carbonylation of α- and β-subunits of the 12S cruciferins (legumin-type seed storage proteins), chaperone proteins and isoforms of late embryogenesis abundant (LEA) proteins which induced loss of functional properties of seed proteins and enzymes and enhanced their susceptibility toward proteolysis.

Wu *et al.* (2011) identified specific proteins related to maize seed viability from highly viable seeds showing deep red embryo staining (R type) and dead seeds with white embryo (W type) after tetrazolium test. Comparative proteomic analysis revealed that 28 protein spots identified were differently expressed between R and W embryos, of which

20 were up-regulated and 8 down-regulated in R embryos. It includes proteins involved in stress response, protein folding and stabilization, as wells as proteins related to nutrient reservoir and metabolism. Prominently, small heat shock proteins, LEA proteins and antioxidant enzymes were highly up-regulated, while two proteases were highly down-regulated in R embryos compared to W embryos. They concluded that one of LEA proteins, EMB564, which declined in abundance during artificial ageing, was highly associated with maize seed viability and could be used to develop quick tests for seed quality.

Xin *et al*. (2011) investigated the effect of seed ageing on maize dry seeds through proteome analysis. Fresh, 5 and 13 days accelerated aged seeds were used for proteome analysis and identified 40 differentially expressed proteins, in which 16 proteins were up-regulated, indicating that artificial ageing affected the proteome of the dry seeds. They also revealed that the signal transduction and transcription were disturbed by artificial ageing and led to reduced protection against ageing. Proteins involved in metabolism and energy were the largest down regulated protein group.

2.5. Seed enhancement treatment with botanicals

In recent years, attempts have been made to replace synthetic chemicals with natural products of plant origin which are cheaper, safer and eco-friendly, less persistent and more specific. Among the various methods followed, use of botanicals has been a traditional method and is being received much attention, to prevent the loss of seed during storage. Earlier reports suggested that seeds treated with botanicals, both in dry and wet form were protected from faster deterioration, which has resulted in better maintenance of seed germinability and seedling vigour (Vadivelu *et al.,* 1985; Ravichandran, 1991; Umarani, 1999).

2.5.1. Seed invigouration with dry treatment

Arati (2000) reported that bengal gram seeds treated with neem leaf powder recorded higher germination and vigour index compared to control at the end of 10 months of storage.

Mandal *et al.* (2000) confirmed that freshly harvested soybean seeds dry dressed with finely powdered dry red chilli fruit at one g kg^{-1} and catharanthus leaf powder at 2 g $kg^{-1}$of seeds also improved germinability over control.

Vyakaranahal *et al.* (2000) inferred that pungam leaf powder 4g kg^{-1} seed treatment maintained significantly higher seed germination, root length, shoot length, vigour index compared to control after accelerated ageing at 45± 1°C temperature and 95 ± 1 per cent RH for 4 days in sunflower.

Rudrapal and Basu (2002) described that dry dressing of high vigour mustard seed with finely powdered tablets of aspirin at 100 mg kg^{-1}, vitamin C at 0.5 g kg^{-1} of seed could retain better vigour and viability after natural ageing under ambient relatively hot and humid conditions.

Maraddi (2002) observed that cowpea seeds treated with neem leaf powder 5g kg-^{1} recorded higher germination and vigour index compared to control at the end of 10 months of storage period.

De *et al.* (2003) reported that aspro and fenugreek seed powder treated wheat seeds showed better results in improving storability, yield and other yield attributes and also noticed that the treatments were equally effective in all seed sizes (large, medium and small) of the same seed lot.

Kapri *et al.* (2003) found that dry treatments showed better germinability than the wet treatments when both were given as a pre-storage treatment. The invigouration effect of the dry treatments was particularly noticeable with ibucon, celin, fenugreek seed powder and periwinkle leaf powder in okra seeds.

Malarkodi (2003) reported that greengram 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.

De *et al.* (2004) observed that finely powdered dry red chilli fruit at 1 g kg^{-1} and *Trigonella* seed powder at 1 g kg^{-1} of seed improved storability over control in wheat seeds. The final emergence of seedlings in the field was significantly improved by aspirin at 100 mg kg^{-1} of seed.

High vigour seeds were more responsive to the dry treatments than the medium vigour seeds in french bean. Rudrapal and Basu (2004) reported that dry treatments given to high vigour seeds resulted in better post-ageing performance which would indicate that the beneficial treatments reduced degradative reactions responsible for the loss of seed vigour and viability.

Sengupta *et al.* (2005) demonstrated that pre-storage dry seed invigouration treatments of high vigour onion with red chilli powder and aspro improved the storability and field performance.

Layek *et al.* (2006) observed that dry treatments in high vigour gram seeds with aspirin at 50 mg kg^{-1} of seed and red chilli powder at 1 g kg^{-1} of seed improved storability and field performance over control.

Kundagrami *et al.* (2008) suggested that dry dressing with aspirin and fenugreek seed powder were very effective for the improvement of storability and field performance of rice seeds.

Channabasanagowda *et al.* (2008) reported that seed treatment with sweet flag rhizome powder at 10 g kg^{-1} of seed improved storability of wheat seeds by recording significantly higher germination percentage and vigour index with lower electrical conductivity than control at the end of 10 months of storage.

Recent studies on seed treatment with botanicals revealed that, dry dressing of seeds with nano size leaf powder of custard apple at 2 g kg^{-1} of seed and shaken for 1h enhanced germination, vigour and field emergence in onion (Mythili, 2012). Seeds dry dressed with near nano size fenugreek seed powder at 2g kg^{-1} of seed for 2 h shaking found to be effective in tomato seeds (Vijayalakshmi, 2012).

2.5.2. Seed invigouration with wet treatment

Vadivelu *et al.* (2001) reported that seed fortification was a process of soaking the seeds in bioactive chemicals especially growth regulators, organic acids and nutrients to invigourate the seeds which were expressed through increase in germinability and vigour and ultimate establishment and yield. The effectiveness of treatment can further be enhanced by the use of botanicals *viz*. leaf extracts of *Albizia amara, Prosopis juliflora,*

Pongamia pinnata, Cynadon dactylon, Calotropis gigantia and *Tamarindus indica* in different crops.

Hydration-dehydration treatment in the maintenance of vigour and viability of seeds has been demonstrated by several workers (Mandal *et al.,* 2000 in soybean; Kapri *et al.,* 2003 in okra).

Menaka *et al.* (2003) reported that sorghum seeds soaked in 10 per cent prosopis leaf extract for 6 h excelled others in producing vigourous seedlings, which recorded maximum vigour index, plant height, panicle length and yield.

Suma (2005) revealed that sesamum seeds fortified with tamarind leaf extract at one and two per cent maximized the seed germination by 88 and 85 per cent, respectively.

Sundaralingam (2005) revealed that rice seeds soaked in 4 per cent panchakavya for 4 h recorded maximum germination, speed of germination, root and shoot length of seedling, dry matter production and vigour index.

Vijayan (2005) stated that in rice, organic seed treatment with coconut water 75 per cent to be the best in enhancing germination and vigour.

Manimekalai (2006) revealed that soaking black gram seeds in 4 per cent panchakavya or 2 per cent moringa leaf extract for 4 h improved germination and seedling quality characters and also confirmed that seed germination percentage and seedling vigour of one year old seed could be improved by soaking the seeds in 2 per cent prosopis leaf extract for 4 h.

Renugadevi *et al.* (2008) concluded that seed fortification with prosopis (*Prosopis juliflora*), pungam (*Pongamia pinnata*) and arappu (*Albizia amara*) leaf extract at one and two per cent concentrations for 3 h were superior to control in terms of germination, vigour and field emergence in cluster bean.

Jayanthi (2008) and Vijayalakshmi (2009) confirmed that seed fortification with 3 per cent of sprouted horse gram and cowpea extract improved seed performance in terms of germination and vigour than control and water soaked seeds.

Phiri and Mbewe (2010) stated that leaf extracts of moringa had induced beans to germinate early and increased germination percentage of cowpea by 4% and increased hypocotyl length by 16.6% in groundnut.

Dawale *et al.* (2011) reported that in mango, the stones pre-soaked with 5% of custard apple leaf extract performed better in terms of all seed quality characters.

Vijayalakshmi (2012) revealed that seeds wet invigourated either with 1 % commercial size custard apple leaf powder solution for 6 h or 0.25 % near nano size fenugreek seed powder solution for 12 h improved germination and vigour both in fresh and aged seeds of tomato.

2.5.3. Antioxidant and nutritional properties of botanicals

2.5.3.1. Fenugreek

The seeds of fenugreek contain lysine and L-tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline (Sauvaire *et al.*, 1991; Billaud, 2001)

Kaviarasan *et al.* (2007) reported that the extract of fenugreek seeds exhibit scavenging of hydroxyl radicals (OH) and inhibition of hydrogen peroxide-induced lipid peroxidation, these antioxidants properties protects the cellular structures from oxidative damage. Bukhari *et al.* (2008) reported that fenugreek seed extract exhibit antioxidant activity that could act as potent source of antioxidant. Subhashini *et al*. (2011) found that ethanol extract of fenugreek seed offered strong antioxidant activity in a concentration dependent manner.

2.5.3.2. Custard apple leaf

Stem and leaf constituents include anonaine, roemerine, corydine, isocorydine, apoprine alkaloids (Brever, 1986).

Baskar *et al.* (2007) revealed that leaf extract of anona possessed potent *in vitro* free radical scavenging of hydroxyl ions with moderate lipid peroxidation inhibition activity.

Chandrashekar and Kulkarni (2011) concluded that custard apple leaf powder has antioxidant properties which are comparable to that of synthetic antioxidant butylated hydroxyl anisole. Bose *et al.* (2011) observed that methanol extracts of custard apple leaf extract is a good free radical scavenger and also showed presence of terpenoids, glycosides and carbohydrates in the extract.

2.5.3.3. Moringa leaf

Moringa leaves are rich source of α and γ-tocopherol, vitamin C, phenolic compounds and total proteins including the essential sulfur amino acids, methionine and cysteine. Among these, α-tocopherol and vitamin C are potential source of antioxidants. It also consists of appreciable amount of vitamins A and B, potassium, calcium, iron, ascorbate and traces of carotenoids, mainly β-carotene and xanthins. Apart from these, moringa leaf extract is also rich in growth regulating hormones like zeatin and act as an ideal plant growth enhancer (Makkar and Becker, 1997; Ferreira *et al.,* 2008).

Tocopherol, an antioxidant substance, helps to halt lipid peroxidation chain reactions generated by free radicals from cellular and subcellular membranes, which are rich in polyunsaturated lipids. Vitamin C can also act as a scavenger of free radicals and indirectly regenerate vitamin E (Kummar *et al.,* 2004). Zeatin is the most naturally occurring cytokinin that not only promotes the growth of plants but also has anti-ageing potential and protective effects in plants (Marcu, 2005).

2.6. Effect of seed ageing on crop performance

In rapid ageing treatments, Harrison (1966) observed a significant decrease in growth, when the viability dropped to about 50 per cent. Ageing of seeds during storage is considered to be a major cause for loss of vigour and can result in reductions in both the rate and final emergence of field plantings (Smith *et al.,* 1973 in lettuce; Ellis, 1989 in onion).

The reduction in yield potential due to ageing could be attributed to the extent of deterioration of seeds, loss in vigour and decline in initial growth and yield contributing characters (Roberts, 1983).

According to Hussaini *et al.* (1988) increase in the period of accelerated ageing in maize significantly decreased the vigour, 1000 seed weight and seed yield. Manjunathaswamy and Narayanaswamy (1996) reported that the decrease in yield attributes were positively associated with ageing of the seeds in maize. The reduction in filled seeds, 1000 seed weight and seed yield was mainly due to seed ageing in sunflower (Kharab and Dahiya, 2000 in Pigeon pea; Pallavi *et al.*, 2003 in sunflower; Rajkumar *et al.*, 2004 in field pea; Poonguzhali, 2012 in black gram).

2.7. Effect of botanical seed treatment on crop performance

Lowell (2005) opined that seed treatment with juice from fresh moringa leaves increases yield by 25-30 per cent in onion, bell pepper, soya, maize, sorghum, coffee, tea, chili, melon and reported that moringa leaf juice contains cytokinin group hormone namely zeatin, which favors increased seed yield.

Manimekalai (2006) indicated that seed invigouration with 2 per cent neem leaf extract or 2 per cent moringa leaf extract for 4 h resulted in better morphological characters, yield components, shelling percentage and seed yield in black gram.

Vijayalakshmi (2009) convincingly proved that seed fortification with 3% sprouted cowpea extract and subsequent foliar spray with 2 % cowpea extract resulted in 31 per cent yield increase in paddy varieties and hybrid.

Nouman *et al.* (2012) reported that seed priming with moringa leaf extract (1:30) produced vigourous root in *Cenchrus ciliaris* and *Panicum antidotale* while it improved the number of leaves, number of tillers and shoot vigour in *Echinochloa crusgalli*.

Vijayalakshmi (2012) concluded that the plant and yield attributing factors of tomato were higher in seeds soaked for 6 h in 1% custard apple leaf powder solutions or in 0.25% near nano size fenugreek seed powder solution for 12 h.

2.8. Effect of low temperature treatment on pulse beetle control

Cowpea weevil larvae feed hidden within the seeds of many different legumes and populations may grow unnoticed to severely damaging levels. Cosmopolitan in distribution, the cowpea weevil and related pulse beetle cause serious product loss throughout the world, particularly in developing countries where legumes may serve as a

significant source of nutrition. Rising popularity of organic product lines have created interest in nonchemical disinfestation treatments. One alternative is the use of cold storage.

Product storage at temperatures of -7 to 1°C was recommended for control of *Callosobruchus* species early in the previous century (Duvel, 1905; Larson and Simmons, 1924). Using very rapid cooling rates, Mullen and Arbogast (1979) determined the LD_{95} of *C. maculatus* eggs after exposure to -15°C to be approximately 5 h, noting that this species was among the more cold tolerant stored product insects.

Sorensen (1994) and Lyon (1997) recommended cold storage of cowpea seeds for 4 days at -18°C for control of cowpea weevil in home pantries.

Johnson and Valero (2003) determined the use of commercial freezers to control cowpea weevil, *Callosobruchus maculatus* in organic garbanzo beans. Laboratory studies showed that, among different stages, egg stage was most tolerant to -18°C and that adults were most susceptible. Finally concluded that 14 days of cold storage at -18°C is required for uniform cooling of the bin and complete mortality of eggs.

Kumar and Bhalla (2007) and Bhalla *et al.* (2008) exposed green gram seeds infested with different stages of *C. maculatus viz*. egg, early larva, late larva, pupa to low temperatures *viz*. 20 ± 1°C, 9 ± 1°C and –14 ± 1°C for 24h and revealed that all the stages were highly sensitive to a temperature of –14 ± 1°C. Adult mortality at this temperature occurred within 12 min.

Upadhyay and Ahmad (2011) reviewed that low temperature treatment of stored grains is a best physical method which successfully kills several life stages of insects at a time and provides long term effect on stored seed and keeps them free of insect infestation. The insects become inactive and eventually die at a temperature below 12°C while super cooling point at low temperature causes very high mortality in stored grain and also maintains seed viability.

Loganathan *et al.* (2011) studied the effect of low temperature for the control of cowpea beetle in chickpeas and found that pupa was the most cold tolerant stage. The lethal time to reduce survival by 50% (LT_{50}) at 0°C for eggs, larvae, pupae and adults were 3, 8, 10 and 4 days, respectively. While the LT_{50} of pupae at 0, -5, -10 and -15°C were 274, 122, 7 and 2 h, respectively.

CHAPTER III

MATERIALS AND METHODS

3.1. MATERIALS

Seeds from seven different lots of blackgram cv. TNAU Blackgram CO 6 obtained from the Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore were used for standardization of vigour tests. Freshly harvested seeds of blackgram cv. TNAU Blackgram CO 6 collected from the Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore were graded using a BSS 7 x 7 wire mesh sieve and used for the study of seed deterioration process in blackgram through proteomic approach, standardization of botanical seed treatment study and standardization of low temperature treatments to curb secondary infestation in pulse beetles. The laboratory experiments were conducted at Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2011 - 2013. Field experiment was carried out in the Agricultural Research Station, Bhavanisagar, Erode district. Two dimensional electrophoresis and MALDI-TOF experiments were conducted at Laboratory of Biochemistry, College of Veterinary Medicine, Gyeongsang National University, Jinju, Gyeongsang, South Korea.

3.2. METHODS

3.2.1. Evaluation of initial quality of seed lots

Seeds from seven different lots of blackgram cv. TNAU Blackgram CO 6 obtained from the Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore were subjected to germination test and evaluated for seed quality parameters as follows.

3.2.1.1. Germination

The laboratory germination test was carried out in using 4 × 100 seeds in paper medium (ISTA, 2007). 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.

3.2.1.2. Shoot length

Shoot length of normal seedlings from each replication of the germination test was measured from collar region to the shoot apex and the mean was expressed in centimeter.

3.2.1.3. Root length

Root length of normal seedlings from each replication of the germination test was measured from collar region to the root tip and the mean was expressed in centimeter.

3.2.1.4. 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 desiccator 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}.

3.2.1.5. Vigour index I

Vigour index I values were computed using the following formula as suggested by Abdul-Baki and Anderson (1973) and the mean values were expressed in whole number.

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

3.2.1.6. Vigour index II

Vigour index II values were computed using the following formula as suggested by Reddy and Khan (2001) and the mean values were expressed in whole number.

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

3.2.1.7. Field emergence

Hundred seeds replicated four times were sown in flat beds in field condition. The number of seedlings emerged were counted on 7th day and the mean was expressed in percentage.

3.2.2. Standardization of vigour test for delineation of field emergence potential of different seed lots

Seven different lots of blackgram cv. TNAU Blackgram CO 6 seeds having germination between 95 and 100 per cent were imposed with various vigour tests *viz*., accelerated ageing test (AA), controlled deterioration test (CDT), complex stressing vigour test (CSVT), electrical conductivity test (EC) and mean germination time (MGT) test and evaluated for seed quality characters *viz*., germination percentage and the results were correlated with field emergence.

3.2.2.1. Standardization of duration for accelerated ageing test (Delouche and Baskin, 1973)

Fifteen gram of seeds from seven lots having moisture content between 8 and 9% were subjected to accelerated ageing for 2, 3 and 4 days. Seeds were packed in paper bag with uniform pin head size perforation all over and placed in an ageing jar containing 100 ml of distilled water to maintain 98 ± 2% relative humidity and incubated at a temperature of 40 ± 1° C. At the end of fourth day, seeds aged from two to four days were drawn and tested for the following seed quality characters.

3.2.2.1.1. Germination as detailed in 3.2.1.1.

3.2.2.2. Standardization of controlled deterioration test (Matthews and Powell, 1987)

The seed moisture content of seven lots was increased to 25 and 30 per cent using petriplate method and exposed to 40°C for durations of 12, 24, 36, 48 h and evaluated for seed germination. The increase in moisture content was determined as quoted by ISTA (2007).

3.2.2.2.1. Moisture content

Increase in moisture content was determined as described by ISTA (2007). About five gram of samples from each lot were ground and transferred to a weighing bottle and placed in a hot air oven maintained at 130 ± 2°C temperature for one hour. Then, it was cooled in a desiccator for 30 min and weighed. The estimations were done in duplicate. The moisture content was calculated and expressed as percentage using the following formula.

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

Where,

M_1 = Weight of the bottle (g)

M_2 = Weight of the bottle with sample before drying (g)

M_3 = Weight of the bottle with sample after drying (g)

3.2.2.2.2. Germination as detailed in 3.2.1.1.

3.2.2.3. Standardization of Complex Stressing Vigour Test (CSVT) (Szirtes and Barla - Szabo, 1981)

Hundred seeds were soaked in 100 ml of distilled water (oxygen deficiency stress) and kept initially at 40°C for 1, 2 and 3 h and then at 5°C for 1 and 2 h to create temperature stress. The seeds were then dried in a blotter paper to remove the excess moisture. Germination test was conducted by employing roll towel method in a dark germinator maintained at 25°C. After 96 h, normal seedlings germinated were recorded and the seedlings were evaluated for seedling length (cm) as follows.

3.2.2.3.1. Seedling length (Hampton and TeKrony, 1995)

After 96 h, normal seedlings from the germination test were measured from shoot apex to the root tip and average length of the five longest seedlings were determined and multiplied with 0.25. The normal seedlings measuring longer than the product of 0.25 cm and average length of the five longest seedlings expressed in centimeter were classified as high vigour seeds and those with lesser seedling length were classified as medium vigour and the results were reported as percentage of high and medium vigour seedlings. The vigour status of the seed lots was assessed based on the percentage of high vigour seedlings as follows.

80 – 100% normal seedlings are high vigour = high vigour seed lot

48 – 79% normal seedlings are high vigour = medium vigour seed lot

<48% normal seedlings are high vigour = low vigour seed lot

3.2.2.4. Standardization of volume of distilled water and soaking duration for electrical conductivity test (Hampton and TeKrony, 1995)

To identify appropriate volume of distilled water and soaking duration required to obtain peak electrical conductivity of seed leachates without splitting of seeds, four replications of fifty seeds was drawn, prewashed well with distilled water to remove the adhering particles and the surface water was removed by blotting with tissue paper. Cleaned seeds were weighed and then soaked in pre-cooled 50, 75 and 100 ml of distilled water for 2, 4, 6, 8, 10 and 12 h at 20°C temperature. After completion of soaking duration, the beaker was swirled for 10 – 15 seconds and electrical conductivity of seed leachates was measured using Conductivity Bench Meter by immersing dip-type cell of a digital conductivity meter with a cell constant of one. Care was taken to avoid direct placement of the cell on the seeds or touching the walls of the beaker. The electrical conductivity of the seed leachate was expressed as $\mu S\ cm^{-1}\ g^{-1}$. The split seeds in each duration of soaking were counted and expressed in percentage.

3.2.2.4.1. Evaluation of seed lots by electrical conductivity test

Four replications of fifty seeds were drawn, prewashed well with distilled water to remove the adhering particles and the surface water was removed by blotting with tissue paper. Cleaned seeds were weighed and then soaked in standardized soaking volume (75 ml) and duration (6 h) and electrical conductivity of seed leachates was measured as detailed in 3.2.2.4. The lots were grouped based on the electrical conductivity of seed leachates. Higher the electrical conductivity of seed leachates, lower will be the vigour of the lot and vice versa.

3.2.2.5. Mean germination time (Mavi *et al.*, 2010)

The germination of four replicates of 50 seeds from each seed lot was assessed using the between paper method (ISTA, 2007) at 25 ± 2°C. Between the first and third day of the germination period frequent counts of germination was taken. The germination was defined as the appearance of a 2 mm radicle. Each day counts were made on five occasions, at 07.00 h, 11.00 h, 15.00 h, 19.00 h and 22.00 h and the mean germination time (MGT) was calculated for each lot using the formula as given below.

$$MGT = \frac{\sum (nT)}{\sum n}$$

Where,

n = number of seeds newly germinated (2mm, radicle emergence) at time T

T = hours from the beginning of the germination test

Σn = final germination

3.2.3. Study of seed deterioration process in blackgram through proteomic approach

3.2.3.1. Accelerated ageing

Twenty gram of fresh seeds having 98 per cent germination was subjected to accelerated ageing every day for up to 10 days as detailed in 3.2.2.1. During the period of accelerated ageing, seed packets were shuffled daily to ensure uniform exposure and at the end of tenth day seeds aged from two to ten days were drawn and subjected to the following physiological and biochemical analysis along with fresh seeds to delineate the process of seed deterioration and to find the days required to obtain germination significantly lesser than Indian minimum seed certification standard (IMSCS) of 75%.

3.2.3.1.1. Physiological analysis

3.2.3.1.1.1. Moisture content (ISTA, 2007)

About five gram of samples from each fresh and two to ten days accelerated aged seeds were ground and moisture content was estimated as detailed in 3.2.2.2.1.

3.2.3.1.1.2. Germination as detailed in 3.2.1.1.

3.2.3.1.1.3. Shoot length as detailed in 3.2.1.2.

3.2.3.1.1.4. Root length as detailed in 3.2.1.3.

3.2.3.1.1.5. Dry matter production as detailed in 3.2.1.4.

3.2.3.1.1.6. Vigour index I as detailed in 3.2.1.5.

3.2.3.1.1.7. Vigour index II as detailed in 3.2.1.6.

3.2.3.1.2. Biochemical analysis

3.2.3.1.2.1. Electrical conductivity of seed leachate

The membrane integrity of fresh and two to ten days accelerated aged seeds were analyzed by assessing the electrical conductivity of seed leachates as detailed in 3.2.2.4.1. using 75 ml of distilled water and soaked for 6 h at 20°C temperature.

3.2.3.1.2.2. DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging assay

The total potential antioxidant activity of the aqueous acetone seed extracts of fresh and two to ten days accelerated aged blackgram seeds were assessed based on their ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, using a modified DPPH assay of Koleckar *et al*. (2007). Seed 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 keeping it 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.

$$\text{The free radical scavenging activity} = \frac{\text{Blank OD - Sample OD}}{\text{Blank OD}} \times 100$$

3.2.3.1.2.3. Protease activity

Endopeptidase activity was measured by using chromogenic substrate, azocasein, following the method described by Ramakrishna and Rao (2005) with slight modifications. 250 µl of 1% azocasein (prepared in 0.02 M sodium acetate buffer pH 5.5 containing 2 mM mercaptoethanol) was mixed with 150 µl of enzyme extract. The reaction mixture was incubated at 40° C for 1 h. The reaction was arrested by adding 1.2 ml of 10 % trichloroacetic acid and mixed thoroughly. The contents were centrifuged, 1.2 ml of supernatant was transferred to a tube containing 1.4 ml of 1 M NaOH, mixed and the absorbance was read at 440 nm against the reagent blank using UV / Vis Spectrophotometer model OPtizen 2120 UV plus and expressed in OD value.

3.2.3.1.2.4. Free amino acid content

Four replications of fifty seeds from each treatment was drawn, prewashed well with distilled water to remove the adhering particles and soaked in 100 ml distilled water for 6 h to obtain the seed leachates. One ml of 0.2 per cent ninhydrin was added to one ml of seed leachate and boiled for 15 min. in a water bath. Then, it was cooled in running water and diluted to 10 ml and intensity of colour developed was measured in an Optima UV-VIS Spectrophotometer (Model SP-3000) at 620 nm against a leucine standard curve and expressed in µg 50 seeds^{-1}100 ml^{-1} (Ching and Ching, 1964).

3.2.3.1.2.5. Analysis of total seed proteins through Sodium Dodecyl Sulphate - Polyacrylamide Gel Electrophoresis (SDS - PAGE)

Fresh and two to ten days accelerated aged seeds were taken in required quantities and used for protein profile analysis through SDS - PAGE.

Preparation of total protein extracts for SDS - PAGE

One gram of seeds from fresh and 2 to 10 days accelerated aged seeds were ground separately with pestle and mortar. One ml of extraction buffer consisting of 50 mM HEPES pH 8.0, 1 mM EDTA and SDS 2% (v/v) was added to the seed powder. This suspension was stirred at 5°C for 15 min and was then centrifuged at 13000 rpm for 15 min at 4°C. Supernatant was withdrawn and subjected to a second, clarifying centrifugation at 13000 rpm for 10 min at 4°C. Aliquots were stored at - 20°C till further use.

Casting of gel

The gel plates were cleaned with distilled water and a wash was given with ethyl alcohol and dried. The plates with spacers were assembled using gel casting stand. The spacers used between the glass plates were of one mm thickness. The plates were made liquid proof by vacuum pressure. The 12.5% separating gel mixture consisting of 12.5 ml stock acrylamide solution (30% acrylamide + 0.8% bis-acrylamide), 6 ml Tris-HCl buffer (1.875 M, pH 8.8), 11.1 ml distilled water, 300 µl of 10% SDS, 160 µl of 10% ammonium persulphate (APS) and 20 µl N,N,N',N'-Tetramethylethylenediamine (TEMED) was poured to the glass sandwich assembly to a level of 11.5 cm from the

notch. One ml of isoamyl alcohol was gently applied over the separating gel and allowed to polymerize for 45 min. After polymerization the outer layer of isoamyl alcohol was washed off by inverting the casting gel and washed 3 to 4 times with distilled water to remove traces of isoamyl alcohol. The water droplets were removed using a filter paper without touching the separating gel.

The 4% stacking gel mixture consisting of 1.35 ml stock acrylamide solution (30% acrylamide + 0.8% bis-acrylamide), 1 ml Tris-HCl buffer (0.6 M, pH 6.8), 7.5 ml distilled water, 100 µl of 10% SDS, 50 µl of 10% APS, 10 µl TEMED was poured on the top of separating gel. An acrylic well forming comb was inserted, ensuring that no air bubble was trapped beneath. The gel was allowed to polymerize for 30 min. Then, the acrylic comb was removed carefully not to distort the wells. The gel was then installed by removing the gasket in the electrophoresis apparatus to which electrode buffer (pH 8.2 – 8.4) consisting of 0.05 M Tris base, 0.192 M glycine, 0.1% SDS was poured and pre run for 10 min.

Electrophoresis

SDS-PAGE of the protein extracts was carried out according to Laemmli (1970), using ATTO AE-8450 vertical electrophoresis unit. Samples were mixed with the 1X of 5X sample buffer containing 5 ml Tris-HCl (0.6 M, pH 6.8), 0.5 g SDS, 5 g sucrose, 0.25 ml mercaptoethanol and 0.01 % (w/v) bromophenol blue made to 10 ml with distilled water and then loaded on to gels. Electrophoresis was conducted at a constant voltage intensity of 100 V until the bromophenol blue dye reaches the bottom. Proteins were revealed by staining with Coomassie brilliant blue R 250.

Staining

The gel removed after run was rinsed with distilled water and immersed in the staining solution containing 0.1% Coomassie brilliant blue R 250 dissolved in methanol, glacial acetic acid and distilled water mixture taken in the ratio of 4:1:5. Staining was done till the bands appeared. Then the stain was tripped off and the gel was destained keeping in destaining solution (staining solution without Coomassie brilliant blue R 250) till the background become clear.

3.2.3.1.2.6. Analysis of total seed proteins through two dimensional electrophoresis (2-DE)

Fresh seeds and six days (day on which germination per cent reached below the IMSCS) accelerated aged seeds from which significant changes were observed in SDS-PAGE were taken in required quantities and used for the protein profile analysis on 2-DE.

Preparation of total protein extracts for 2-DE

The fresh and 6 days accelerated aged seeds were homogenized separately in a pestle and mortar using liquid nitrogen. The ground sample was further extracted using 500µl of cell lysis buffer (7 M urea, 2 M thiourea, and 4% (w/v) CHAPS, 0.5% (v/v) IPG buffer, and 1% dithiothreitol (DTT)). The extract was centrifuged at 14,000 rpm for 15 min at 4°C and supernatant was collected. To precipitate the proteins in the supernatant, 20% TCA was added to the supernatant (1:1 ratio) and samples were incubated at 4^{o}C for 30 minutes. Protein concentrations in various extracts were quantified by the Non-Interfering™ protein assay kit (G-Biosciences, St. Louis, MO, USA), in accordance to the manufacturers protocol.

Two-dimensional electrophoresis (2-DE)

For the first dimension, 100µg of proteins were rehydrated using 18 cm immobilized linear pH gradient (IPG) strips, pH 4–7, in a rehydration buffer (7 M urea, 2 M thiourea, 4% (w/v) CHAPS and 0.002% Bromophenol blue). Isoelectric focusing was performed in the Ettan IPGphor 3 system (GE Healthcare) with following subsequent steps: 50 V for 1 h, 200 V for 1h, 500 V for 30 min, 4000 V for 30 min, 4000 V for 1 h 10,000 V for 1 h, 10,000 V for 13 h and 50 V for 3 h. Prior to the second dimension, the IPG strips were equilibrated twice for 30min each in 5 ml/strip of equilibration solution containing 6 M urea, 30% (v/v) glycerol, 2.5% (w/v) SDS, 0.15 M BisTris, and 0.1 M HCl, DTT (50 mM) was added to the first equilibration solution, and iodoacetamide (4% [w/v]) was added to the second. Equilibrated gel strips were placed on top of vertical sodium dodecyl sulphate-polyacrylamide gels (12%) (10% acrylamide, 0.33% bisacrylamide, 15 ml of 4x resolving buffer, 10% Sodium dodecyl sulphate, 10% APS and 60 µl TEMED). A denaturing solution (agarose sealing solution (0.075 g of low-melting agarose [Gibco BRL], 15 ml of SDS) was loaded onto gel strips. The

electrophoresis was performed at 20°C in a 1x electrophoresis SDS buffer at 30 mA/gel constant current. For each condition analyzed, 2D gels were made at least in duplicate and from two independent protein extractions. 2D gels were stained with silver nitrate according to Blum *et al.* (1987) for densitometric analyses. Image analysis was carried out with Progenesis Samespots software (Nonlinear Dynamics, Newcastle, UK) & Image Master 2D Elite version 4.01 software (Amerschem Biosciences).

Sample preparation for Matrix Assisted Laser Desorption Ionization – Time Of Flight Mass Spectrometry (MALDI-TOF- MS)

Protein spots were picked up manually from the gel and protein digestion was done as described by Shevchenko *et al*. (1996) with slight modifications. The excised gel pieces were washed using 100 µl of destaining solution (30 mM potassium ferricyanide 0.00987 g/ml, 100 mM sodium thiosulfate 0.02482 g/ml 1:1 mix) for 5 minutes. Gel pieces were vortexed with 500 µl of pure water and the water was removed by speed vacuum. Gel pieces were incubated with NH_4HCO_3 for 10 minutes. Incubated gel pieces were first vortexed with 30 µl of reduction solution for 45 minutes in 56°C and then vortexed with 100 µl of acetonitrile (ACN) and again vortexed with 100 µl of 100 mM NH_4HCO_3 solution followed by 100 µl of ACN each for 10 minutes and the gel pieces were subjected to speed vacuum for ten minutes. The gel plug was digested with 10 µl of trypsin solution (20 µg trypsin/ 1ml 50 mM NH_4HCO_3) for 14 h at 37°C. After digestion the protein peptides were extracted from the gel plugs using 10 µl of extraction buffer. The tryptic peptides were collected into siliconized tubes. The extraction procedure was repeated with the same amount of extraction solution. Extracted tryptic peptides were dried by vacuum evaporation and stored at -80°C until analysis or used immediately.

MALDI-TOF- MS targeting and protein identification by database searching

Tryptic peptides of the extracts were dried and re-dissolved in 1 µl of extraction buffer and 1 µl of matrix solution (α-acyano- 4-hydroxycinnamic acid) and targeted onto a matrix assisted laser desorption ionization time of flight (MALDI-TOF) plate. MALDI-TOF experiments were performed on a Voyager- DE STR mass spectrometer (Applied Biosystems, Franklin Lakes, NJ, USA). For identification of proteins, the peptide mass fingerprinting data were used to search against the database using the

Mascot program (http://www.matrixscience.com). The following parameters were used for database searches: taxonomy, viridiplantae (green plants); cleavage specificity, trypsin with one missed cleavage allowed; peptide tolerance of 100ppm for the fragment ions; and allowed modifications, Cys Carbamidomethyl (fixed), and oxidation of Met (variable). The protein score was −10*log (P), where P is the probability that the observed match is a random event.

3.2.4. Effect of botanical seed treatment on seed quality of blackgram cv. TNAU Blackgram CO 6

Two lots having 98 per cent and 80 per cent which is near IMSCS for germination of 75% were obtained. For this half of the fresh seeds of blackgram cv. TNAU Blackgram CO 6 having 98 per cent germination were subjected to accelerated ageing to bring down the germination to 80 per cent and both fresh and aged seeds were used for standardization studies.

3.2.4.1. Accelerated ageing: Accelerated ageing was done for four days as detailed in 3.2.3.1.

3.2.4.2. Seed enhancement treatment

Fenugreek seed obtained from local market, custard apple and moringa leaves collected from orchards of Tamil Nadu Agricultural University were freeze dried at -80°C and grounded to obtain fine powder (Plate 1). Fresh and four days accelerated aged seeds were treated in both dry and wet conditions as follows.

Seed lot	Botanicals	Dry treatment		Wet treatment	
		Dosage	Shaking duration	Concentration	Soaking duration
i. Fresh seeds ii. Aged seeds (four days accelerated ageing)	Fenugreek seed powder	2, 3 & 4 g kg^{-1} of seeds	1, 2 & 3 h	0.5, 1.0, 1.5 & 2.0 %	1, 2 & 3 h
	Custard apple leaf powder				
	Moringa leaf powder				

Plate 1. Different botanicals used for seed enhancement treatement

For wet treatment seeds were soaked in equal volume solutions and at the end of treatment duration, seeds were removed from the solutions, shade dried at room temperature and both dry as well as wet treated seeds were assessed for the following seed quality parameters. The untreated seed was used as control for fresh seeds and untreated, four days accelerated aged seed was used as control for aged seeds.

The experiment was carried out with four replications in factorial completely randomised block design. Dry and wet treatments of both fresh and aged seeds were analyzed separately.

3.2.4.2.1. Speed of germination (Maguire, 1962)

Four replicates of twenty five seeds each were used to test the speed of germination of seeds from different treatments. The seeds showing radicle protrusion were counted daily from third day after sowing until seventh day. From the number of seeds germinated on each day, the speed of germination was calculated using the following formula and the result was expressed in number.

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

Where,

X_1 - number of seeds germinated at first count

X_2 - number of seeds germinated at second count

X_n - number of seeds germinated on n^{th} day

Y_1 - number of days from sowing to first count

Y_2 - number of days from sowing to second count

Y_n - number of days from sowing to n^{th} count

3.2.4.2.2. Germination as detailed in 3.2.1.1.

3.2.4.2.3. Shoot length as detailed in 3.2.1.2.

3.2.4.2.4. Root length as detailed in 3.2.1.3.

3.2.4.2.5. Dry matter production as detailed in 3.2.1.4.

3.2.4.2.6. Vigour index I as detailed in 3.2.1.5.

3.2.4.2.7. Vigour index II as detailed in 3.2.1.6.

3.2.4.3. Assessment of seed vigour status in seeds subjected to botanical seed treatment in blackgram cv. TNAU Blackgram CO 6

3.2.4.3.1. Accelerated ageing test

Fresh seeds treated with wet and dry treatments as detailed in 3.2.4.2. were subjected to accelerated ageing test for three days as detailed in 3.2.3.1. and assessed for following seed quality parameters.

3.2.4.3.1.1. Speed of germination as detailed in 3.2.4.2.1.

3.2.4.3.1.2. Germination as detailed in 3.2.1.1.

3.2.4.3.1.3. Shoot length as detailed in 3.2.1.2.

3.2.4.3.1.4. Root length as detailed in 3.2.1.3.

3.2.4.3.1.5. Dry matter production as detailed in 3.2.1.4.

3.2.4.3.1.6. Vigour index I as detailed in 3.2.1.5.

3.2.4.3.1.7. Vigour index II as detailed in 3.2.1.6.

3.2.4.4. Biochemical analysis

3.2.4.4.1. Biochemical analysis of treated seeds

The fresh and accelerated aged seeds (for four days) treated with the best invigouration treatments were subjected to the following biochemical analysis to delineate the mode of action of the treatments.

3.2.4.4.1.1. Electrical conductivity of seed leachate as detailed in 3.2.2.4.1.

3.2.4.4.1.2. α-amylase activity (Simpson and Naylor, 1962)

Two grams of agar shred and one gram of potato starch was mixed together in water to form a paste and the volume was made up to 100 ml. The homogenous solution of agar and starch mixture after boiling was poured into sterilized petridishes and allowed to settle in the form of gel after cooling. The presoaked (8 h) blackgram seeds were

placed in the petridishes in such a way that the endospermic part remained in contact with agar starch gel and was incubated in dark for 24 h. Then the dishes were uniformly poured with potassium iodide solution (0.44 g iodine crystal + 20.008 g potassium iodide in 500 ml of distilled water) and excess solution was drained off after few minutes. The diameter of halo (clear zone) formed around the seed was measured in mm.

3.2.4.4.1.3. DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging assay as detailed in 3.2.3.1.2.2.

3.2.4.4.1.4. Protease activity as detailed in 3.2.3.1.2.3.

3.2.4.4.1.5. Free amino acid content as detailed in 3.2.3.1.2.4.

3.2.4.4.1.6. Total protein profile as detailed in 3.2.3.1.2.5.

3.2.4.4.2. Biochemical analysis of botanicals

3.2.4.4.2.1. DPPH (1, 1-diphenyl -2-picryl hydrazyl) free radical scavenging assay as detailed in 3.2.3.1.2.2.

3.2.4.4.2.2. Minerals content (McQuaker *et al.,* 1979)

Random samples of germinating seeds were drawn from each treatment, finely ground using bio-homogenizer and the minerals content *viz*., boron, copper, magnesium, manganese, iron, zinc and potassium content were estimated.

Fifteen ml of concentrated nitric acid, sulphuric acid and perchloric acid in the ratio of 9:2:1 was added to 0.5 g of finely ground seed sample taken in digestion tubes. The digestion tubes were then placed in kelplus infra digestion system (Model: KES 12IL) at low temperature initially and then increased to the maximum of 300°C when frothing subsides and the digestion was continued till the solution turns colourless.

Initially during digestion, the solution was turned from clear to yellow colour and after digestion for nearly 3 h, it turned to transparent clear solution. To the digested solution, 100 ml of double distilled water was added and filtered through whatman's filter paper number 40 into a 250 ml volumetric flask to obtain a clear colourless solution and used for minerals content estimation.

Minerals were quantified by using inductively coupled plasma spectrometer (ICP) and iTEVA software and the minerals content were expressed in mg per 100 gram of seed sample. The ICP multi-element standard solution VIII (24 elements in dilute nitric acid) obtained from Merck chemicals, Germany was used as standard.

3.2.4.5. Effect of botanical seed treatment on crop yield and productivity in fresh and aged seeds of blackgram cv. TNAU Blackgram CO 6

The best performed botanical seed treatments standardized in experiment 3.2.4.2. and 3.2.4.3. as detailed below were forwarded to assess the crop growth and productivity.

Treatment details: 14 treatments

Factor 1 (Age of seeds)	A_0	Non aged seeds (Fresh seeds)
	A_1	Accelerated aged for 4 days
Factor 2 (Botanical seed treatment)	T_0	Control
	T_1	Fenugreek seed powder @ 3 g kg^{-1} of seeds shaken for 1h
	T_2	Custard apple leaf powder @ 4 g kg^{-1} of seeds shaken for 1h
	T_3	Moringa leaf powder @ 4 g kg^{-1} of seeds shaken for 1h
	T_4	1% Fenugreek seed powder solution soaked for 1h
	T_5	1.5% Custard apple leaf powder solution soaked for 1h
	T_6	1.5% Moringa leaf powder solution soaked for 1h

The field experiment was carried out in field number 2 at Pungar block, Agricultural Research Station, Tamil Nadu Agricultrual University, Bhavanisagar (Fig. 1 and Plate 2).

Season : *Kharif* and *Rabi*, 2012

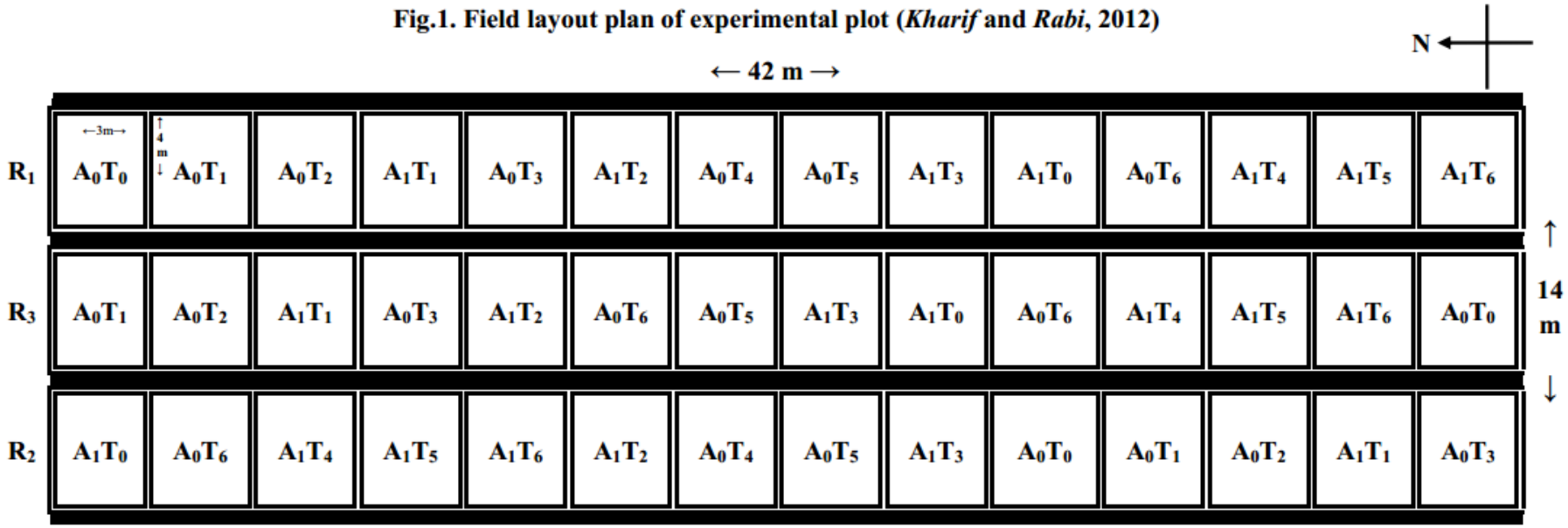
Plot size : 4 m x 3 m

Spacing : 30 cm x 10 cm

Replication : Three

Design : FRBD

Fig.1. Field layout plan of experimental plot (*Kharif* and *Rabi*, 2012)



Factor 1 (Age of seeds)	A_0 : Non aged seeds (Fresh seeds) A_1 : Accelerated aged for 4 days	
Factor 2 (Botanical seed treatment)	T_0 : Control T_1 : Fenugreek seed powder @ 3 g kg^{-1} of seeds shaken for 1h T_2 : Custard apple leaf powder @ 4 g kg^{-1} of seeds shaken for 1h T_3 : Moringa leaf powder @ 4 g kg^{-1} of seeds shaken for 1h	T_4 : 1% Fenugreek seed powder solution soaked for 1h T_5 : 1.5% Custard apple leaf powder solution soaked for 1h T_6 : 1.5% Moringa leaf powder solution soaked for 1h

***Kharif* season**

***Rabi* season**

Plate 2. General view of experimental fields

The crop was raised with recommended package of practices and observations on the following characters were recorded replication wise on five random plants.

3.2.4.5.1. Plant height

The height of five randomly selected plants from each plot was measured from the ground level to the tip of the main stem at vegetative stage (20 DAS), flowering stage (40 DAS) and harvesting stage (60 DAS) and the mean was expressed in centimeter.

3.2.4.5.2. Dry weight

After recording the observation at each stage, the shoots were cut into pieces and shade dried for two days followed by drying in oven at 80°C for 24 h and mean weight expressed as g plant$^{1.}$

3.2.4.5.3. Days to 50 % flowering

The number of days required for 50 per cent flowering of plants in five rows from the date of sowing was recorded.

3.2.4.5.4. Leaf area and leaf area index

Leaf area per plant was worked out by the length and breadth measurement method. The observation was recorded at vegetative stage (20 DAS), flowering stage (40 DAS) and harvesting stage (60 DAS) and expressed in cm^2.

$$\text{Leaf area} = L \times B \times K \times n$$

Where,

L = Length of the leaf (cm)

B = Maximum breadth of the leaf (cm)

K (Constant value) = 0.631 (Montgomery, 1911)

n = Number of leaves

Based on the leaf area plant^{-1}, the LAI was calculated by using the following formula.

$$\text{LAI} = \frac{\text{Leaf area per plant } (cm^2)}{\text{Ground area occupied } (cm^2)}$$

3.2.4.5.5. Chlorophyll content

Chlorophyll content was observed at vegetative stage (20 DAS), flowering stage (40 DAS) and harvesting stage (60 DAS) using chlorophyll meter (Model CCM 200 plus).

3.2.4.5.6. Crop growth rate (CGR)

Crop growth rate was estimated using the following formula suggested by Watson (1958) and expressed in g $m^{-2}d^{-1}$.

$$CGR = \frac{W_2 - W_1}{P\,(t_2 - t_1)}$$

Where,

W_1 : Whole plant dry weight at t_1 stage (g)

W_2 : Whole plant dry weight at t_2 stage (g)

$(t_2 – t_1)$: Time interval in days between stages (g)

P : Area occupied by the plant (m^2)

3.2.4.5.7. Seed yield attributes

At harvest, the pods were collected and the following observations were recorded:

3.2.4.5.7.1. Number of pods $plant^{-1}$

Total number of pods in ten randomly selected plants was counted at maturity and their mean was expressed in whole number.

3.2.4.5.7.2. Pod yield $plant^{-1}$

Pods from ten randomly selected plants were harvested separately and weighed and their mean was expressed in gram.

3.2.4.5.7.3. Pod yield $plot^{-1}$

Pods from the plants in the plot area were harvested and weighed and expressed in gram.

3.2.4.5.7.4. Pod yield ha^{-1}

Pod yield per hectare was computed based on pod yield per plot and mean was expressed in kg ha^{-1}.

3.2.4.5.7.5. Seed yield plant^{-1}

Weight of seeds extracted from the pods of ten randomly selected plants were recorded after drying to 8 ± 0.5 per cent moisture content and their mean was expressed in gram.

3.2.4.5.7.6. Seed yield plot^{-1}

The seeds obtained from the plants in the plot area were weighed after drying to 8 ± 0.5% moisture content on wet basis and expressed in gram.

3.2.4.5.7.7. Seed yield ha^{-1}

Seed yield per hectare was computed based on seed yield per plot and their mean was expressed in kg ha^{-1}.

3.2.4.5.7.8. Seed recovery percentage

Seed recovery percentage was computed from the seeds using a sample of 100 g pod obtained from individual treatments.

$$\text{Seed recovery percentage} = \frac{\text{Weight of seed (g)}}{\text{Weight of pod (g)}} \times 100$$

3.2.4.5.8. Quality of the resultant seeds

To find out the quality of the resultant seed, the cleaned and graded seeds were evaluated for the following seed and seedling characters,

3.2.4.5.8.1. Germination as detailed in 3.2.1.1.

3.2.4.5.8.2. Shoot length as detailed in 3.2.1.2.

3.2.4.5.8.3. Root length as detailed in 3.2.1.3.

3.2.4.5.8.4. Dry matter production as detailed in 3.2.1.4.

3.2.4.5.8.5. Vigour index I as detailed in 3.2.1.5.

3.2.4.5.8.6. Vigour index II as detailed in 3.2.1.6.

3.2.5. Standardization of low temperature treatments to curb secondary infestation of pulse beetles (*Callosobruchus maculatus*) in blackgram

3.2.5.1. Pulse beetle culture development

250 g of freshly harvested seeds of blackgram cv. TNAU Blackgram CO 6 were packed in plastic container and incubated under ambient condition to develop secondary infestation. After 3 to 4 weeks, secondary infested seeds were transferred to another 250 g of fresh seeds to allow multiplication of pulse beetles.

3.2.5.2. Effect of low temperature treatment on different life stages of pulse beetle

3.2.5.2.1. Tolerance of adult weevils to freezing

Fifteen recently emerged adults from the culture developed as detailed in 3.2.4.1. were exposed to freezer of commercial refrigerator (-18°C) for 0, 10, 20, 30, 40, 50, 60 and 90 minutes in three replicates. At the end of each treatment duration, adults were withdrawn and held at ambient condition. Adults were evaluated 2 h after treatment and again 24 h later. Adults were counted as normal if they were actively moving, moribund if they were moving but unable to walk and dead if no movement was seen.

3.2.5.2.2. Tolerance of immature stages to freezing

Fifteen numbers of different stages of life cycle namely egg (1-3 days old), first - second instar larvae (4-6 days old), third instar larvae (8-9 days old), fourth instar larvae (13-14 days old), and pupae (19-20 days old) in three replicates were exposed to freezer of commercial refrigerator (-18°C) for 0, 30, 60, 90, 120, 150, 180, 210 and 240 minutes along with the infested seeds. At the end of each treatment duration, insects at each immature stage were withdrawn and held at ambient condition separately for adult emergence. Number of adult emerged from each stages and dead were calculated and expressed as % mortality.

3.2.5.3. Effect of low temperature treatment on control of secondary infestation of pulse beetle and seed quality of blackgram cv. TNAU Blackgram CO 6

Five hundred gram of fresh untreated seeds of blackgram cv. TNAU Blackgram CO 6 (moisture content of 8 %) containing 10 seeds infested with pulse beetle eggs were packed air tight in 700 gauge polyethylene bag and subjected to low temperature treatments in the freezer of commercial refrigerator (-18°C) for 0, 2, 4, 6, 8 and 10 hours. At the end of treatment duration seed packets were withdrawn and stored under ambient condition along with insecticide (chlorpyriphos 2 ml kg^{-1} of seeds) treated seed packets and evaluated for following quality parameters. 0 h treated seeds served as absolute control.

3.2.5.3.1. Level of seed damage due to pulse beetle infestation

At monthly interval packets of different treatment were cut opened and numbers of seeds damaged were counted and expressed in percentage.

3.2.5.3.2. Seed quality parameters

Following seed quality parameters were evaluated at bimonthly interval.

3.2.5.3.2.1. Moisture content as detailed in 3.2.2.2.1.

3.2.5.3.2.2. Germination as detailed in 3.2.1.1.

3.2.5.3.2.3. Shoot length as detailed in 3.2.1.2.

3.2.5.3.2.4. Root length as detailed in 3.2.1.3.

3.2.5.3.2.5. Dry matter production as detailed in 3.2.1.4.

3.2.5.3.2.6. Vigour index I as detailed in 3.2.1.5.

3.2.5.3.2.7. Vigour index II as detailed in 3.2.1.6.

Statistical analysis

The data obtained from different experiments were analysed by the ‘F’ test of significance following the methods described by Rangaswamy (2002). Wherever necessary, 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 the F test is non-significant it was indicated by the letters NS.

CHAPTER IV

RESULTS

4.1. Standardization of vigour test for delineation of field emergence potential of different seed lots

4.1.1. Initial quality of seed lots

4.1.1.1. Seed germination (%)

Significant difference was observed among the seven seed lots for seed germination. Among the seven seed lots L1, L2, L5, L6 and L7 registered significantly higher germination than L3 and L4 and were on par with each other (Table 1).

4.1.1.2. Shoot length (cm)

There was no significant difference was observed among the shoot length of seven lots (Table 1).

4.1.1.3. Root length (cm)

Root length did not differ significantly among the seven lots (Table 1).

4.1.1.4. Dry matter production (g / 10 seedlings)

There was no significant difference among the seed lots with respect to dry matter production of seedlings (Table 1).

4.1.1.5. Vigour index I

Significant difference was observed among seven seed lots of blackgram with respect to vigour index I. Among the seven seed lots L1, L2, L5, L6 and L7 registered significantly higher vigour index I than L3 and L4 and were on par with each other (Table 1).

4.1.1.6. Vigour index II

Significant difference was observed with respect to vigour index II of seven lots. Among the seven seed lots L1, L2, L5, L6 and L7 registered significantly higher vigour index II than L3 and L4 and were on par with each other (Table 1).

Table 1. Initial evaluation of quality parameters of seven seed lots of blackgram cv. TNAU Blackgram CO 6

Lot no.	Germination (%)	Shoot length (cm)	Root length (cm)	Dry matter production (mg / 10 seedlings)	Vigour index I	Vigour index II	Field emergence (%)
L1	98 (81.87)	21.07	15.41	0.218	3575	21	92 (73.57)
L2	98 (81.87)	21.16	15.63	0.228	3605	22	92 (73.57)
L3	95 (77.08)	20.54	15.59	0.196	3432	19	86 (68.03)
L4	96 (78.47)	20.82	15.32	0.205	3469	20	87 (68.87)
L5	97 (80.03)	20.90	15.38	0.212	3519	21	90 (71.57)
L6	98 (81.87)	20.96	15.42	0.216	3565	21	91 (72.54)
L7	99 (84.26)	21.15	15.65	0.225	3643	22	95 (77.08)
Mean	**97 (80.03)**	**20.94**	**15.49**	**0.214**	**3544**	**21**	**90 (71.57)**
SEd	1.98	0.346	0.171	0.0069	40.41	0.68	1.10
CD (P = 0.05)	4.25	NS	NS	NS	86.69	1.46	2.39
CD (P = 0.01)	NS	NS	NS	NS	NS	NS	3.36

(Figures in parenthesis indicate arcsine values)

4.1.1.7. Field emergence (%)

Significant difference among the field emergence of seven lots was observed. The seven seed lots can be grouped into three groups based on the significantly higher field emergence. Among the lots L7 (95%) recorded significantly higher field emergence followed by L1, L2 and L5, L6 which were on par with each other. Two lots, L3 (86%) and L4 (87%) recorded the lowest field emergence than other lots and were on par with each other (Table 1).

4.1.2. Accelerated ageing test

Accelerated ageing test established significant difference among the seed lots. Among the duration of accelerated ageing, the results obtained after 3 days of ageing was more similar to the grouping established through the field emergence. The results revealed that lot L7 (95%) recorded significantly higher seed germination than other lots followed by L1, L2 and L5, L6 which were on par with each other. Two lots, L3 (86%) and L4 (87%) recorded the lowest germination and were on par with each other (Table 2a).

The correlation coefficient between different duration of ageing and field emergence reveals significant positive association between different accelerated ageing duration and field emergence. However, 3 days of accelerated aged seeds showed higher correlation of r = 0.993 with field emergence (Table 2b).

4.1.3. Controlled deterioration test

Significant difference among the seed lots was revealed through controlled deterioration test. The interaction effect of seed moisture content and duration of test revealed all the treatments grouped the seed lots in to two groups in which lots L1, L2, L5, L6 and L7 recorded significantly higher seed germination and on par with each other followed by L3 and L4 which recorded the lowest germination. The grouping had no similarity to field emergence (Table 3a).

The correlation coefficient between different controlled deterioration test and field emergence revealed significant positive association between different controlled deterioration test and field emergence. However, test condition with 25 and 30% seed

Table 2a. Standardization of accelerated ageing test conditions for blackgram cv. TNAU Blackgram CO 6 seeds

Lot number (L)	Duration of accelerated ageing test in days (A)			Mean
	2 days	3 days	4 days	
L1	97 (80.03)	92 (73.57)	86 (68.03)	**92 (73.57)**
L2	98 (81.87)	92 (73.57)	86 (68.03)	**92 (73.57)**
L3	91 (72.54)	86 (68.03)	80 (63.44)	**86 (68.03)**
L4	93 74.66)	87 (68.87)	82 (64.90)	**87 (68.87)**
L5	94 (75.82)	90 (71.57)	85 (67.22)	**89 (70.63)**
L6	96 (78.47)	90 (71.57)	85 (67.22)	**90 (71.57)**
L7	99 (84.26)	95 (77.08)	89 (70.63)	**94 (75.82)**
Mean	**95 (77.08)**	**90 (71.57)**	**85 (67.22)**	**90 (71.57)**
	A	L	A × L	
SEd	0.306	0.467	0.810	
CD (P = 0.05)	0.618	0.943	1.634	
CD (P = 0.01)	0.826	1.261	NS	

(Figures in parenthesis indicate arcsine values)

Table 2b. Correlation coefficients among accelerated ageing durations and field emergence in seven lots of blackgram cv. TNAU Blackgram CO 6 seeds.

	Ageing for 2 days	Ageing for 3 days	Ageing for 4 days	Field emergence
Ageing for 2 days	1	0.957**	0.946**	0.965*
Ageing for 3 days		1	0.986**	0.993**
Ageing for 4 days			1	0.989**
Field emergence				1

** Correlation is significant at 1 % level

* Correlation is significant at 5 % level

Table 3a. Standardization of controlled deterioration test conditions for blackgram cv. TNAU Blackgram CO 6 seeds

Lot no. (L)	Seed moisture (%) (M)									
	25%					30%				
	Duration of incubation (h) (D)									
	12 h	**24 h**	**36 h**	**48 h**	**Mean**	**12 h**	**24 h**	**36 h**	**48 h**	**Mean**
L1	94 (75.82)	88 (69.73)	80 (63.44)	71 (57.42)	**83 (65.65)**	93 (74.66)	85 (67.22)	70 (56.17)	39 (38.65)	**72 (58.05)**
L2	94 (75.82)	88 (69.73)	81 (64.16)	72 (58.05)	**84 (66.42)**	93 (74.66)	85 (67.22)	70 (56.17)	39 (38.65)	**72 (58.05)**
L3	91 (72.54)	83 (65.65)	77 (61.34)	68 (55.55)	**80 (63.44)**	90 (71.57)	82 (64.90)	65 (53.73)	30 (33.21)	**67 (54.94)**
L4	92 (73.57)	86 (68.03)	79 (62.73)	68 (55.55)	**81 (64.16)**	91 (72.54)	83 (65.65)	68 (55.55)	36 (36.87)	**70 (56.17)**
L5	93 (74.66)	87 (68.87)	80 (63.44)	71 (57.42)	**83 (65.65)**	92 (73.57)	84 (66.42)	69 (56.17)	38 (38.06)	**71 (57.42)**
L6	94 (75.82)	88 (69.73)	81 (64.16)	72 (58.05)	**84 (66.42)**	93 (74.66)	85 (67.22)	70 (56.17)	39 (38.65)	**72 (58.05)**
L7	95 (77.08)	89 (70.63)	82 (64.90)	73 (58.70)	**85 (67.22)**	94 (75.82)	86 (68.03)	71 (57.42)	40 (39.23)	**73 (58.70)**
Mean	**93 (74.66)**	**87 (68.87)**	**80 (63.44)**	**71 (57.42)**	**83 (65.65)**	**92 (73.57)**	**84 (66.42)**	**69 (56.17)**	**37 (37.47)**	**71 (57.42)**
	M	D	L	M × D	M × L	D × L	M × D × L			
SEd	0.26	0.37	0.48	0.52	0.68	0.97	1.37			
CD (P = 0.05)	0.51	0.72	0.96	1.03	1.36	1.92	2.71			
CD (P = 0.01)	0.68	0.96	1.27	1.36	NS	NS	NS			

(Figures in parenthesis indicate arcsine values)

Table 3b. Correlation coefficients among different controlled deterioration test conditions and field emergence in seven seed lots of blackgram cv. TNAU Blackgram CO 6.

Seed moisture content	Incubation duration	25 % moisture content				30 % moisture content				Field emergence
		12 h	24 h	36 h	48 h	12 h	24 h	36 h	48 h	
25 %	12 h	1	0.966**	0.961**	0.952**	1.000**	1.000**	0.946**	0.925**	0.979**
	24 h		1	0.970**	0.886**	0.966**	0.966**	0.982**	0.990**	0.914**
	36 h			1	0.930**	0.961**	0.961**	0.994**	0.947**	0.921**
	48 h				1	0.952**	0.952**	0.895**	0.845**	0.948**
30 %	12 h					1	1.000**	0.946**	0.925**	0.979**
	24 h						1	0.946**	0.925**	0.969**
	36 h							1	0.972**	0.893**
	48 h								1	0.859**
Field emergence										1

** Correlation is significant at 1 % level

moisture content incubated for 12 h showed higher correlation of r = 0.979 with field emergence (Table 3b).

4.1.4. Complex stressing vigour test

Significant difference among the seed lots was established by complex stressing vigour test. However, the grouping of seed lots was not similar to the field emergence (Table 4a). Grouping of seed lots based on the seedling length shows that all the seed lots belongs to the high vigour status (Table 4c).

The correlation coefficient between different complex stressing vigour test and field emergence revealed significant positive association between different test condition and field emergence. However, test condition of 2 h soaking at 40°C followed by 2 h soaking at 5°C showed higher correlation of r = 0.877 with field emergence (Table 4b).

4.1.5. Standardization of electrical conductivity test

Electrical conductivity of seed leachates was significantly influenced by different soaking durations, volume of distilled water and their interaction effect. Electrical conductivity of seed leachates increased gradually with increase in duration of soaking. However, steep increase was observed from 8 h of soaking which coincided with the appearance of split seeds (Table 5). Therefore 6 h soaking duration could be optimum. Among the volume of distilled water used, electrical conductivity of seed leachates was decreased with increase in volume of distilled water. Therefore, 75 ml of distilled water taken in 100 ml beaker which can accommodate the electrode cell was optimum for soaking seeds to estimate electrical conductivity.

4.1.5.1. Electrical conductivity test for seven lots

Significant difference among the seed lots was established by electrical conductivity test. The seed lots were grouped into three groups similar to the results obtained through field emergence. Seed lot L7 (51.0 µS cm^{-1} g^{-1}) recorded significantly lower electrical conductivity followed by L1, L2 and L5, L6 which were on par with each other. Two lots, L3 (93.2 µS cm^{-1} g^{-1}) and L4 (89.8 µS cm^{-1} g^{-1}) recorded the highest electrical conductivity and were on par with each other (Table 6).

Table 4a. Standardization of complex stressing vigour test conditions for blackgram cv. TNAU Blackgram CO 6 seeds

Lot no. (L)	1 h in 40°C followed by 1 h in 5° C	1 h in 40°C followed by 2 h in 5° C	2 h in 40°C followed by 1 h in 5° C	2 h in 40°C followed by 2 h in 5° C	3 h in 40°C followed by 1 h in 5° C	3 h in 40°C followed by 2 h in 5° C	Mean
L1	96 (78.47)	93 (74.66)	92 (73.57)	84 (66.42)	64 (53.13)	58 (49.60)	**81 (64.16)**
L2	97 (80.03)	94 (75.82)	95 (77.08)	85 (67.22)	67 (54.94)	58 (49.60)	**83 (65.65)**
L3	72 (58.05)	62 (51.94)	68 (55.55)	56 (48.45)	48 (43.86)	39 (38.65)	**58 (49.60)**
L4	86 (68.03)	80 (63.44)	84 (66.42)	72 (58.05)	59 (50.19)	50 (45.00)	**72 (58.05)**
L5	96 (78.47)	88 (69.73)	94 (75.82)	79 (62.73)	66 (54.33)	55 (47.87)	**80 (63.44)**
L6	96 (78.47)	86 (68.03)	94 (75.82)	77 (61.34)	66 (54.33)	53 (46.72)	**79 (62.73)**
L7	98 (81.87)	94 (75.82)	97 (80.03)	85 (67.22)	68 (55.55)	58 (49.60)	**83 (65.65)**
Mean	**92 (73.57)**	**85 (67.22)**	**89 (70.63)**	**77 (61.34)**	**63 (52.54)**	**53 (46.72)**	**76 (60.67)**

	D	L	D × L
SEd	0.52	0.56	1.37
CD (P = 0.05)	1.03	1.11	2.73
CD (P = 0.01)	1.37	1.48	3.62

(Figures in parenthesis indicate arcsine values)

Table 4b. Correlation coefficients among different complex stressing vigour test conditions and field emergence in seven seed lots of blackgram cv. TNAU Blackgram CO 6.

	1 h in 40°C followed by 1 h in 5° C	1 h in 40°C followed by 2 h in 5° C	2 h in 40°C followed by 1 h in 5° C	2 h in 40°C followed by 2 h in 5° C	3 h in 40°C followed by 1 h in 5° C	3 h in 40°C followed by 2 h in 5° C	Field emergence
1 h in 40°C followed by 1 h in 5° C	1	0.976**	0.995**	0.970**	0.993**	0.972**	0.854*
1 h in 40°C followed by 2 h in 5° C		1	0.965**	1.000**	0.960**	0.999**	0.872*
2 h in 40°C followed by 1 h in 5° C			1	0.958**	0.999**	0.959**	0.851*
2 h in 40°C followed by 2 h in 5° C				1	0.953**	0.999**	0.877**
2 h in 40°C followed by 1 h in 5° C					1	0.953**	0.846*
2 h in 40°C followed by 2 h in 5° C						1	0.859*
Field emergence							1

** Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

Table 4c. Vigour status of seed lots based on seedling length obtained in complex stressing vigour test in blackgram cv. TNAU Blackgram CO 6 seeds

C1	C2	C3	C4	C5	C6
CSVT	**Lot no.**	**Mean of 5 longest seedlings**	**C3 × 0.25**	**% of normal seedlings > C4**	**Vigour status**
1 h @ 40°C + 1 h @ 5° C	L1	29.24	7.31	100	High vigour
	L2	29.45	7.36	100	High vigour
	L3	26.82	6.70	100	High vigour
	L4	26.02	6.50	100	High vigour
	L5	29.71	7.43	100	High vigour
	L6	28.22	7.05	100	High vigour
	L7	27.77	6.94	100	High vigour
1 h @ 40°C + 2 h @ 5°C	L1	30.14	7.54	100	High vigour
	L2	29.33	7.33	100	High vigour
	L3	26.68	6.67	95	High vigour
	L4	26.70	6.67	97	High vigour
	L5	29.30	7.33	100	High vigour
	L6	27.01	6.75	100	High vigour
	L7	28.13	7.03	100	High vigour
2 h @ 40°C + 1 h @ 5°C	L1	29.91	7.48	100	High vigour
	L2	29.75	7.44	100	High vigour
	L3	25.90	6.48	100	High vigour
	L4	27.41	6.85	100	High vigour
	L5	28.06	7.02	100	High vigour
	L6	26.64	6.66	100	High vigour
	L7	28.88	7.22	100	High vigour

Table 4c continued

C1	C2	C3	C4	C5	C6
CSVT	**Lot no.**	**Mean of 5 longest seedlings**	**C3 × 0.25**	**% of normal seedlings > C4**	**Vigour status**
2 h @ 40°C + 2 h @ 5°C	L1	28.82	7.21	100	High vigour
	L2	29.03	7.26	100	High vigour
	L3	26.43	6.61	90	High vigour
	L4	25.64	6.41	95	High vigour
	L5	29.28	7.32	100	High vigour
	L6	27.81	6.95	100	High vigour
	L7	27.37	6.84	100	High vigour
3 h @ 40°C + 1 h @ 5°C	L1	29.71	7.43	100	High vigour
	L2	28.91	7.23	100	High vigour
	L3	26.30	6.57	100	High vigour
	L4	26.32	6.58	100	High vigour
	L5	28.88	7.22	100	High vigour
	L6	26.63	6.66	100	High vigour
	L7	27.73	6.93	100	High vigour
3 h @ 40°C + 2 h @ 5°C	L1	29.48	7.37	100	High vigour
	L2	29.33	7.33	100	High vigour
	L3	25.53	6.38	84	High vigour
	L4	27.01	6.75	87	High vigour
	L5	27.66	6.92	96	High vigour
	L6	26.25	6.56	100	High vigour
	L7	28.47	7.12	100	High vigour

Criteria for allotting vigour status to seed lots

Seedlings longer than column 4 are high vigour and less than that are medium vigour

80 – 100 % normal seedlings are high vigour = high vigour seed lot
48 – 79 % normal seedlings are high vigour = medium vigour seed lot
<48 % normal seedlings are high vigour = low vigour seed lot

Table 5. Standardization of electrical conductivity test conditions for blackgram cv. TNAU Blackgram CO 6 seeds

Volume of dH_2O (V) / Soaking duration (D)	Electrical conductivity of seed leachates ($\mu S\ cm^{-1}\ g^{-1}$)				Split / sprouted seeds (%)			
	50 ml	**75 ml**	**100 ml**	**Mean**	**50 ml**	**75 ml**	**100 ml**	**Mean**
2 h	28.5	25.8	21.7	**25.3**	0	0	0	**0.0**
4 h	54.1	50.4	42.3	**48.9**	0	0	0	**0.0**
6 h	63.1	57.2	48.1	**56.1**	0	0	0	**0.0**
8 h	78.4	70.6	61.4	**70.1**	8	10	12	**10.0**
10 h	148.0	133.4	116.1	**132.5**	60	60	64	**61.3**
12 h	156.0	145.5	126.6	**142.7**	100	100	100	**100.0**
Mean	**88.0**	**80.5**	**69.4**	**79.3**	**28.0**	**28.3**	**29.3**	**28.6**
	V	D	V × D		V	D	V × D	
SEd	1.51	2.14	3.71		0.58	0.82	1.43	
CD (P = 0.05)	3.07	4.34	7.52		1.18	1.67	2.89	
CD (P = 0.01)	4.12	5.82	10.09		1.58	2.24	3.88	

Table 6. Electrical conductivity test and mean germination time for blackgram cv. TNAU Blackgram CO 6 seed lots

Lot No.	Electrical conductivity test ($\mu S\ cm^{-1}\ g^{-1}$)	Mean germination time
L1	56.2	21.5
L2	55.3	21.4
L3	93.2	24.5
L4	89.8	23.9
L5	58.2	22.2
L6	58.7	22.0
L7	51.0	21.1
Mean	**66.1**	**22.4**
SEd	1.63	0.65
CD (P = 0.05)	3.50	1.40
CD (P = 0.01)	4.86	1.94
r value	-0.962**	-0.928**

The correlation coefficient between electrical conductivity test and field emergence revealed a significant negative association with correlation of r = -0.962 which indicates electrical conductivity and field emergence was inversely related (Table 6).

4.1.6. Mean germination time

Significant difference among the seed lots was established by mean germination time. The seed lots were grouped in to two groups. The results revealed that lots L1, L2, L5, L6 and L7 recorded significantly lower mean germination time while lots, L3 and L4 recorded the highest mean germination time and were on par with each other (Table 6).

The correlation coefficient between mean germination time and field emergence revealed a significant negative association with correlation of r = -0.928 which indicated that mean germination time and field emergence was inversely related (Table 6).

4.1.7. Correlation of vigour tests with field emergence

Among the vigour tests, accelerated ageing test showed the maximum positive association with field emergence (r = 0.993) while the electrical conductivity test showed consistent maximum negative association with field emergence (r = -0.962) (Table 7). Therefore, these two vigour tests can be used to predict the field emergence of blackgram seed lots.

4.2. Study on pattern of seed deterioration in black gram through proteomic approach

Physiological and biochemical changes that take place during accelerated ageing were studied to delineate the pattern of seed deterioration. Based on these changes, duration of ageing required to obtain seeds with germination below Indian minimum seed certification standard (IMSCS) of 75% was identified and used for comparative proteomic analysis of fresh and aged seeds.

4.2.1. Physiological changes due to accelerated ageing of blackgram seeds.

4.2.1.1. Moisture content (%)

Increase in moisture content was observed with advancement of ageing duration. Moisture content of fresh seeds was 8.1%. Significant increase was observed from 3rd day

Table 7. Correlation coefficients among vigour tests and field emergence of blackgram cv. TNAU Blackgram CO 6 seeds.

	Accelerated ageing test	Control deterioration test	Complex stressing vigour test	Electrical conductivity test	Mean germination time	Field emergence
Accelerated ageing test	1	0.954**	0.878**	-0.908**	-0.949**	0.993**
Control deterioration test		1	0.911**	-0.935**	-0.969**	0.979**
Complex stressing vigour test			1	-0.897**	-0.938**	0.877**
Electrical conductivity test				1	0.984**	-0.962**
Mean germination time					1	-0.928**
Field emergence						1

** Correlation is significant at the 0.01 level.

of ageing (8.7%). Steep increase in moisture content was observed from 6th day (10.8%) onwards and reached the maximum of 17.2% in ten days aged seeds (Table 8).

4.2.1.2. Seed germination (%)

Germinability declined gradually due to accelerated ageing. Fresh seeds showed higher germination (99%), and during ageing, germination declined and reached 14% at the 10th day. The decline was significant from 3rd day of accelerated ageing and steep reduction was observed from 5th day onwards. At the 6th day, the germinability of the seeds decreased to 58%, which is significantly lesser than the IMSCS for germination of blackgram *viz*., 75% (Table 8).

4.2.1.3. Shoot length (cm)

The control seeds recorded the highest shoot length of 25.29 cm. It declined from 2 days accelerated aged seeds (25.25). However, significant reduction was recorded only after 10 days of accelerated ageing (Table 8).

4.2.1.4. Root length (cm)

Root length started to decline from 2nd day of accelerated ageing (15.25). However, the reduction in root length was significant only from 10 days accelerated aged seeds. The initial root length recorded was 15.74 cm (Table 8).

4.2.1.5. Dry matter production (g / 10 seedlings)

Dry matter of seedlings decreased with increase in duration of accelerated ageing. However, even after 9 days of accelerated ageing, the dry matter production recorded was not statistically significant when compared to control (0.242 g / 10 seedlings) (Table 8).

4.2.1.6. Vigour index I

The control seeds recorded the highest vigour index I (4085). Vigour index I was declined from 2 days of accelerated ageing (4030). But significant reduction was observed from 3 days accelerated aged seeds (3690) and the minimum of 495 was recorded in 10 days accelerated aged seeds (Table 8).

Table 8. Physiological changes due to accelerated ageing of blackgram cv. TNAU Blackgram CO 6 seeds.

Duration of accelerated ageing	Moisture content (%)	Germination (%)	Shoot length (cm)	Root length (cm)	Dry matter production (g / 10 seedlings)	Vigour index I	Vigour index II
Control - Fresh	8.1	99 (84.26)	25.29	15.74	0.242	4085	24
2 Days	8.3	99 (84.26)	25.25	15.25	0.243	4030	24
3 Days	8.7	92 (73.57)	25.15	14.95	0.242	3690	22
4 Days	9.1	86 (68.03)	25.00	14.30	0.242	3380	21
5 Days	9.6	74 (59.34)	24.75	14.20	0.240	2884	18
6 Days	10.8	58 (49.60)	24.15	14.10	0.240	2230	14
7 Days	11.9	42 (40.40)	24.25	13.95	0.238	1592	10
8 Days	13.4	40 (39.23)	23.84	13.40	0.238	1491	9
9 Days	15.0	30 (33.21)	23.02	12.35	0.220	1090	7
10 Days	17.2	14 (21.97)	16.80	9.05	0.201	495	3
Mean	**11.2**	**63 (53.13)**	**23.75**	**13.73**	**0.235**	**2497**	**15**
SEd	0.177	2.83	1.40	1.22	0.011	176	1.06
CD (P=0.05)	0.370	5.91	2.92	2.54	NS	367	2.22
CD (P=0.01)	0.504	8.05	3.99	NS	NS	501	3.03

(Figures in parenthesis indicate arcsine values)

4.2.1.7. Vigour index II

Vigour index II of control and 2 days aged seeds were on par with each other (24). However, significant reduction in vigour index II than control was observed only on the 4th day (21) and reached the minimum of 3 in 10th day (Table 8).

4.2.2. Biochemical changes in blackgram seeds subjected to accelerated ageing.

4.2.2.1. Electrical conductivity (µS cm^{-1} g^{-1})

Electrical conductivity of seed leachates in control was 14.1 µS cm^{-1} g^{-1}. It recorded an increase in seeds aged for 2 days. But significant increase than control was observed only in 4 days aged seeds. The maximum value of 71.6 µS cm^{-1} g^{-1} was recorded in 10 days aged seeds (Table 9).

4.2.2.2. Antioxidant activity (% of blank)

Results of DPPH test showed a gradual reduction in antioxidant activity with increase in accelerated ageing duration. Antioxidant activity of control was 87.5% and it was on par with 2 days accelerated aged seeds (85.7%). Significant reduction was observed on 3rd day of accelerated ageing (79.9%) and the minimum of 47.5% was recorded in 10 days aged seeds (Table 9).

4.2.2.3. Protease activity (OD value)

Protease activity significantly increased with increase in accelerated ageing duration. Protease activity of control was 0.152. It increased significantly from second day onwards and reaches the maximum on 10th day of accelerated ageing (0.338) (Table 9).

4.2.2.4. Free amino acid content (µg 50 seeds^{-1} 100 ml^{-1})

Free amino acid content showed a concomitant increase with increase in ageing duration. Free amino acid content of control seeds was 0.121 µg 50 seeds^{-1} 100 ml^{-1}. It increased significantly from second day of accelerated ageing and reached the maximum on 10th day of ageing (0.256) (Table 9).

4.2.2.5. Changes in protein pattern during accelerated ageing

There was no major difference in protein pattern of seeds up to 5 days of accelerated ageing. However, significant differences in protein pattern were observed

Table 9. Biochemical changes due to accelerated ageing of blackgram cv. TNAU Blackgram CO 6 seeds.

Duration of accelerated ageing	Electrical conductivity ($\mu S\ cm^{-1}\ g^{-1}$)	Antioxidant activity (% of blank)	Protease activity (OD value)	Free amino acid ($\mu g\ 50seeds^{-1}\ 100\ ml^{-1}$)
Control - Fresh	14.1	87.5	0.152	0.121
2 days	17.7	85.7	0.215	0.156
3 days	18.3	79.9	0.222	0.162
4 days	20.9	74.1	0.230	0.171
5 days	21.2	73.6	0.252	0.184
6 days	21.3	71.6	0.265	0.191
7 days	22.1	69.4	0.285	0.210
8 days	30.9	62.6	0.310	0.226
9 days	59.8	56.1	0.315	0.234
10 days	71.6	47.5	0.338	0.256
Mean	**29.8**	**70.8**	**0.258**	**0.191**
SEd	2.30	2.12	0.015	0.009
CD (P=0.05)	4.80	4.43	0.032	0.019
CD (P=0.01)	6.55	6.04	0.044	0.026

from sixth days onwards. Most of the changes were observed in proteins of molecular weight ranging between 31.00 and 66.20 kDa. The changes were mostly reduction in band intensity. There was a disappearance of protein band at 60.21 kDa in 6 to 10 days of accelerated ageing (Plate 3). This disappearance of protein band and significant reduction in germination below Indian minimum seed certification standards was recorded in 6 days aged seeds.

4.2.3. Proteome changes due to accelerated ageing of blackgram cv. TNAU Blackgram CO 6 seeds.

Totally 782 spots were detected both in fresh and 6-days accelerated aged seeds. Among these, 16 spots were differentially expressed due to accelerated ageing. Out of 16, four were up- regulated spots and 12 were down-regulated spots (Table 10).

4.2.3.1. MALDI-TOF/MS identification of proteins affected by ageing

MALDI-TOF/MS analysis of the 16 differentially expressed spots revealed the identity of the proteins (Table 10). On the basis of putative protein functions, these proteins were grouped into eight categories as described by Bevan *et al*. (1998). Three spots identified as actin (D03), actin-101-like protein (D11) and predicted protein containing LIM domain (D06) were related to cell structure contributing major portion of the total identified protein with known functions (18.75%). Two spots identifed as 8S globulin alpha' isoform precursor (D10) and putative 26S proteasome regulatory subunit (U16) comes under protein destination and storage (12.50% of total identified protein). Two spots which was identifed as Valyl-tRNA synthetase (D05) and Elongation factor Tu (U15) comes under protein synthesis (12.50%). Another two spots such as, hypothetical protein containing Sec14p-like lipid-binding domain (D08) and SNARE domain containing protein (D09) were related to transporters (12.50%). One spot identifed as largest subunit of RNA polymerase II (D01) was related to transcription (16.25%). Another one spot identified as, 5' nucleotides, deoxy (Pyrimidine), cytosolic type C protein (D02) comes under metabolism (6.25%). One spot namely pheophorbide a oxygenase (U12) related to disease/defence (6.25%), and finally two spots which were identifed as Retrotransposon protein, putative (D04) and Retrotransposon protein, putative, Ty3-gypsy subclass (D13) has unclear functions while another two spots such as

Plate 3. Changes in protein profile during accelerated ageing of blackgram cv. TNAU Blackgram CO 6 seeds

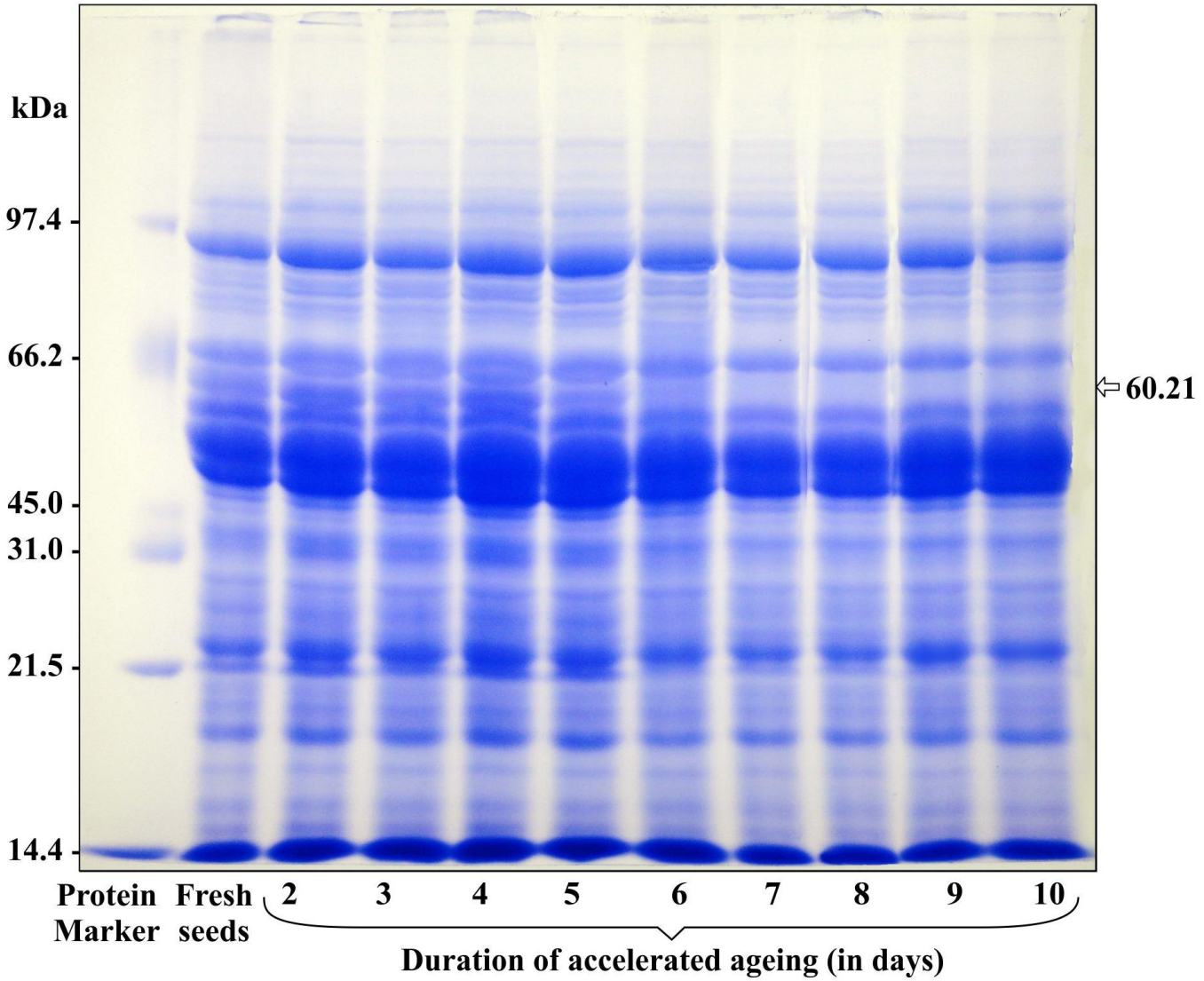


Table 10. Differentially expressed proteins during seed ageing identified using MALDI-TOF. Arranged according to their functional classification

Spot No.	NCBI accession no.	Protein name	Theoretical MW (kDa) / pI	Experimental MW (kDa) / pI	MASCOT score	Matched peptides	Sequence coverage (%)	Relative abundance*
Metabolism (6.25%)								
D02	297802676	5' nucleotidase, deoxy (Pyrimidine), cytosolic type C protein	40.4/9.05	40/5.2	81	9	30	0.78
Transcription (6.25 %)								
D01	168010051	Largest subunit of RNA polymerase II	99.1/9.34	47/5.5	80	24	29	0.95
Protein synthesis (12.50 %)								
D05	255077874	Valyl-tRNA synthetase	16.5/5.23	47/5.4	82	32	24	0.47
U15	336285879	Elongation factor Tu	25.4/4.74	25/5.1	74	6	36	1.03
Protein destination and storage (12.50 %)								
D10	108743974	8S globulin alpha' isoform precursor	51.6/5.58	43/5.5	77	7	20	0.44
U16	308800992	putative 26S proteasome regulatory subunit	41.6/8.51	14/5.4	71	7	20	2.85
Transporters (12.50 %)								
D08	242062694	hypothetical protein (contains Sec14p-like lipid-binding domain)	70.8/7.31	44/5.7	51	5	10	0.52
D09	219362943	SNARE domain containing protein	26.1/8.73	14/5.5	40	4	27	0.92
Cell structure (18.75 %)								
D03	357503463	Actin	40.2/5.56	43/5.4	69	8	36	0.38
D11	356558578	Actin-101-like protein	41.8/5.31	36/6.8	98	12	47	0.59
D06	167997611	Predicted protein (Contains LIM domain)	20.0/6.09	44/5.8	40	3	29	0.93
Disease / defense (6.25 %)								
U12	1935914	Pheophorbide a oxygenase	62.0/8.81	41/5.1	78	7	15	1.09
Unclear / uncharacterized (25.00 %)								
D04	77554262	Retrotransposon protein, putative	12.8/8.87	44/5.6	40	5	5	0.67
D13	108710077	Retrotransposon protein, putative, Ty3-gypsy sub class	15.0/8.87	57/5.2	82	29	28	0.62
D07	303279362	predicted protein	89.2/8.15	42/5.4	75	12	19	0.51
U14	168058176	Predicted protein	46.2/9.18	28/5.1	77	15	42	1.47

* Normalized spot volume in the deteriorated seeds (6 days accelerated aged) divided by the normalized spot volume in the nondeteriorated control seeds (Fresh seeds)

D07 and U14 whose protein function was uncharacterized. These four spots contributes 25.00% of the 16 differentially expressed protein spots. One spots each from protein destination and storage (spot U16), protein synthesis (U15), disease/defence (U12) related proteins and one unclassified proteins were up-regulated, while the others were down-regulated (Table 10).

4.3. Standardization of seed enhancement treatment for extending vigour and viability of blackgram seeds

4.3.1. Effect of seed dry dressing with botanicals on fresh seeds of blackgram

4.3.1.1. Speed of germination

The speed of germination of fresh seeds was not significantly influenced by botanical treatments (Table 11).

4.3.1.2. Seed germination (%)

Germination of fresh seeds was also not significantly influenced by botanical seed treatments (Table 12).

4.3.1.3. Shoot length (cm)

The fresh seeds treated with botanicals registered significantly higher shoot length compared to control (21.07 cm). Among the factors, shoot length was significantly influenced by botanicals, dosage and shaking duration but not by their interactions. Higher shoot length was observed in seeds dry dressed with fenugreek seed powder (23.03 cm) and custard apple leaf powder (22.66 cm) which were on par with each other. Among the dosages, 3 and 4 g kg^{-1} of seeds registered maximum shoot length and were on par with each other. Among the durations of shaking, 1 h (23.02 cm) and 2 h (23.17 cm) were on par and significantly higher than 3 h (21.71 cm) (Table 13).

4.3.1.4. Root length (cm)

Significant difference in root length was observed in seeds dry dressed with botanicals, its dosage and their interaction. Irrespective of the treatments, treated seeds registered significantly higher root length than control (15.41 cm). Among the botanicals, fenugreek seed powder was superior (17.52 cm) and among the dosage 4 g kg^{-1} of seeds

Table 11. Effect of dry dressing with botanicals on speed of germination of fresh seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (Dry dressing)	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 Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
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 (h) B - Botanicals C – Botanical dosage (g)

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 12. Effect of dry dressing with botanicals on germination (%) of fresh seeds of blackgram cv. TNAU Blackgram CO 6

B	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	100 (89.72)	100 (89.72)	98 (81.87)	**99 (84.26)**	100 (89.72)	100 (89.72)	100 (89.72)	**100 (89.72)**	100 (89.72)	100 (89.72)	100 (89.72)	**100 (89.72)**	100 (89.72)	100 (89.72)	99 (84.26)	**100 (89.72)**
CALP	100 (89.72)	100 (89.72)	100 (89.72)	**100 (89.72)**	100 (89.72)	100 (89.72)	100 (89.72)	**100 (89.72)**	100 (89.72)	100 (89.72)	98 (81.87)	**99 (84.26)**	100 (89.72)	100 (89.72)	99 (84.26)	**100 (89.72)**
MLP	100 (89.72)	100 (89.72)	100 (89.72)	**100 (89.72)**	100 (89.72)	98 (81.87)	100 (89.72)	**99 (84.26)**	100 (89.72)	100 (89.72)	100 (89.72)	**100 (89.72)**	100 (89.72)	99 (84.26)	100 (89.72)	**100 (89.72)**
Mean	**100 (89.72)**	**100 (89.72)**	**99 (84.26)**	**100 (89.72)**	**100 (89.72)**	**99 (84.26)**	**100 (89.72)**	**100 (89.72)**	**100 (89.72)**	**100 (89.72)**	**99 (84.26)**	**100 (89.72)**	**100 (89.72)**	**100 (89.72)**	**100 (89.72)**	**100 (89.72)**
Control	98 (81.87)															

(Figures in parenthesis indicate arcsine values)

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	2.33	0.76	0.76	0.76	1.32	1.32	1.32	2.29
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 (h) B - Botanicals C – Botanical dosage (g)

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 13. Effect of dry dressing with botanicals on shoot length (cm) of fresh seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (Dry dressing)	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	22.95	23.76	23.75	**23.49**	22.90	23.76	23.78	**23.48**	21.19	22.60	22.62	**22.14**	22.35	23.37	23.38	**23.03**
CALP	22.61	23.04	23.75	**23.13**	22.74	23.27	23.78	**23.26**	20.81	21.37	22.61	**21.60**	22.05	22.56	23.38	**22.66**
MLP	21.97	22.42	22.92	**22.44**	22.03	22.95	23.32	**22.77**	19.92	21.90	22.36	**21.39**	21.31	22.42	22.87	**22.20**
Mean	**22.51**	**23.07**	**23.47**	**23.02**	**22.56**	**23.33**	**23.63**	**23.17**	**20.64**	**21.96**	**22.53**	**21.71**	**21.90**	**22.79**	**23.21**	**22.63**
Control	21.07															

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	0.644	0.211	0.211	0.211	0.365	0.365	0.365	0.633
CD (P = 0.05)	1.281	0.419	0.419	0.419	NS	NS	NS	NS
CD (P = 0.01)	1.698	0.556	NS	0.556	NS	NS	NS	NS

D – Shaking duration (h) B - Botanicals C – Botanical dosage (g)

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

(17.76 cm) registered significantly higher root length. The interaction effect revealed that seeds dry dressed with fenugreek seed powder @ 3 g kg^{-1} of seeds (18.03 cm), 4 g kg^{-1} of seeds (18.03 cm) and custard apple leaf powder @ 4 g kg^{-1} of seeds (18.02 cm) registered higher root length which were on par with each other (Table 14).

4.3.1.5. Dry matter production (g / 10 seedlings)

Dry matter production of fresh seeds was significantly influenced by botanicals seed treatment, dosage and duration of shaking. All the treated seeds performed better than control (0.218 g / 10 seedlings). But there was no significant difference due to their interaction effect. Among the botanicals, fenugreek seed powder registered higher dry matter production (0.273 g / 10 seedlings) which was on par with custard apple leaf powder (0.268 g / 10 seedlings). Among the dosages, dry matter production was the maximum in 3 and 4 g kg^{-1} of seeds (0.265 and 0.271g / 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) were on par and produced significantly higher dry matter than 3 h (0.247 g / 10 seedlings) (Table 15).

4.3.1.6. Vigour index I

Vigour index I of fresh seed was significantly influenced by all the three factors and their interactions and were significantly higher than control (3575). Among the botanicals, fenugreek seed powder registered the maximum vigour (4047). Among the dosages, 4 g kg^{-1} of seeds recorded the maximum vigour index (4078). With respect to shaking durations, vigour index I was maximum due to 2 h shaking followed by 1 h shaken seeds. In case of their interactional effects, seeds dry dressed with fenugreek seed powder @ 3 g kg^{-1} and custard apple leaf powder @ 4 g kg^{-1} and shaken for 1 h registered maximum vigour index I of 4197 and was on par with each other (Table 16).

4.3.1.7. Vigour index II

All the treatments recorded significantly higher vigour index II than control (21). Among the botanicals, fenugreek seed powder and custard apple leaf powder was on par and registered the maximum vigour index II of 27. Among the dosages, 3 and 4 g kg^{-1} of seeds were on par and recorded the maximum vigour index II (27). In case of shaking

Table 14. Effect of dry dressing with botanicals on root length (cm) of fresh seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (Dry dressing)	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	16.91	18.21	18.24	**17.79**	16.95	18.24	18.24	**17.81**	15.68	17.64	17.61	**16.98**	16.51	18.03	18.03	**17.52**
CALP	15.67	16.29	18.22	**16.73**	15.68	16.36	18.25	**16.76**	15.64	16.19	17.60	**16.48**	15.66	16.28	18.02	**16.66**
MLP	15.66	15.96	17.43	**16.35**	15.71	16.06	17.57	**16.45**	15.61	15.55	16.64	**15.93**	15.66	15.86	17.21	**16.24**
Mean	**16.08**	**16.82**	**17.96**	**16.95**	**16.11**	**16.89**	**18.02**	**17.01**	**15.64**	**16.46**	**17.28**	**16.46**	**15.95**	**16.72**	**17.76**	**16.81**
Control	15.41															

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	0.686	0.225	0.225	0.225	0.389	0.389	0.389	0.674
CD (P = 0.05)	1.364	NS	0.446	0.446	NS	NS	0.773	NS
CD (P = 0.01)	NS	NS	0.592	0.592	NS	NS	NS	NS

D – Shaking duration (h) B - Botanicals C – Botanical dosage (g)

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 15. Effect of dry dressing with botanicals on dry matter production (g / 10 seedlings) of fresh seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (Dry dressing)	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	0.262	0.293	0.294	**0.283**	0.269	0.288	0.290	**0.282**	0.245	0.259	0.257	**0.254**	0.259	0.280	0.280	**0.273**
CALP	0.258	0.275	0.295	**0.276**	0.267	0.282	0.287	**0.279**	0.241	0.247	0.258	**0.249**	0.255	0.268	0.280	**0.268**
MLP	0.232	0.249	0.252	**0.244**	0.239	0.251	0.257	**0.249**	0.232	0.239	0.249	**0.240**	0.234	0.246	0.253	**0.244**
Mean	**0.251**	**0.272**	**0.280**	**0.268**	**0.258**	**0.274**	**0.278**	**0.270**	**0.239**	**0.248**	**0.255**	**0.247**	**0.249**	**0.265**	**0.271**	**0.262**
Control	0.218															

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	0.0142	0.0047	0.0047	0.0047	0.0081	0.0081	0.0081	0.0140
CD (P = 0.05)	0.0283	0.0093	0.0093	0.0093	NS	NS	NS	NS
CD (P = 0.01)	0.0375	0.0123	0.0123	0.0123	NS	NS	NS	NS

D – Shaking duration (h) B - Botanicals C – Botanical dosage (g)

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 16. Effect of dry dressing with botanicals on vigour index I of fresh seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (Dry dressing)	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	3986	4197	4115	**4099**	3985	4200	4202	**4129**	3687	4024	4023	**3911**	3886	4140	4113	**4047**
CALP	3828	3933	4197	**3986**	3842	3963	4203	**4003**	3645	3756	3941	**3781**	3772	3884	4114	**3923**
MLP	3763	3838	4035	**3879**	3774	3823	4089	**3895**	3553	3745	3900	**3733**	3697	3802	4008	**3836**
Mean	**3859**	**3989**	**4116**	**3988**	**3867**	**3995**	**4165**	**4009**	**3628**	**3842**	**3955**	**3808**	**3785**	**3942**	**4078**	**3935**
Control	3575															

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	11.22	3.67	3.67	3.67	6.36	6.36	6.36	11.02
CD (P = 0.05)	22.32	7.30	7.30	7.30	12.65	12.65	12.65	21.91
CD (P = 0.01)	29.58	9.68	9.68	9.68	16.77	16.77	16.77	29.04

D – Shaking duration (h) B - Botanicals C – Botanical dosage (g)

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

durations, 1 and 2 h of shaking was on par and recorded the maximum vigour index II. Among the interactional effects, vigour index II of fresh seeds treated with fenugreek seed powder (29) and custard apple leaf powder shaken for 1 (30) and 2 h (29) were significantly higher and on par with each other while the other interaction effects were not significant (Table 17).

4.3.2. Effect of seed dry dressing with botanicals on aged seeds of blackgram

4.3.2.1. Speed of germination

In aged seeds, though there was no significant difference in speed of germination among the treatments and their interactions, treated seeds as a whole registered significantly higher speed of germination than control, indicating their overall positive influence in aged seeds. (Table 18).

4.3.2.2. Seed germination (%)

Germination was significant in all the factors and their interactions over control (81%). Among the treatments, fenugreek seed powder (87%) registered maximum germination followed by custard apple leaf powder (86%). Among the shaking durations, 1 and 2 hours of shaking were on par with each other and registered higher germination (86%) than 3 h of shaking (84%). Among the dosages, 4 g kg^{-1} of seeds (88%) registered maximum germination followed by 3 g kg^{-1} of seeds (86%). However, interaction effect between botanicals and dosage revealed the maximum germination in fenugreek seed powder @ 3 and 4 g kg^{-1} of seeds and custard apple leaf powder @ 4 g kg^{-1} of seeds (89%) which were on par with each other. Other interaction effects were not significant (Table 19).

4.3.2.3. Shoot length (cm)

In aged seeds, shoot length of all the treated seeds were significantly higher than control (19.05). Among the botanicals, higher shoot length was observed in seeds dry dressed with fenugreek seed powder (21.98 cm) and custard apple leaf powder (21.70 cm) which were on par with each other. Among the dosages, 4 g kg^{-1} of seeds (22.04 cm) registered maximum shoot length followed by 3 and 4 g kg^{-1} of seeds (21.61 cm). Among the durations of shaking, 1 h (22.11 cm) recorded significantly higher

Table 17. Effect of dry dressing with botanicals on vigour index II of fresh seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (Dry dressing)	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	26	29	29	**28**	27	29	29	**28**	25	26	26	**26**	26	28	28	**27**
CALP	26	28	30	**28**	27	28	29	**28**	24	25	25	**25**	26	27	28	**27**
MLP	23	25	25	**24**	24	25	26	**25**	23	24	25	**24**	23	25	25	**24**
Mean	**25**	**27**	**28**	**27**	**26**	**27**	**28**	**27**	**24**	**25**	**25**	**25**	**25**	**27**	**27**	**26**
Control	21															

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	0.831	0.272	0.272	0.272	0.471	0.471	0.471	0.816
CD (P = 0.05)	1.653	0.541	0.541	0.541	0.937	NS	NS	NS
CD (P = 0.01)	2.191	0.717	0.717	0.717	NS	NS	NS	NS

D – Shaking duration (h) B - Botanicals C – Botanical dosage (g)

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 18. Effect of dry dressing with botanicals on speed of germination of aged seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (Dry dressing)	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	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 Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
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 (h) B - Botanicals C – Botanical dosage (g)

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 19. Effect of dry dressing with botanicals on germination (%) of aged seeds of blackgram cv. TNAU Blackgram CO 6

B	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	83 (65.65)	90 (71.57)	89 (70.63)	**87 (68.87)**	84 (66.42)	89 (70.63)	90 (71.57)	**88 (69.73)**	83 (65.65)	88 (69.73)	87 (68.87)	**86 (68.03)**	83 (65.65)	89 (70.63)	89 (70.63)	**87 (68.87)**
CALP	84 (66.42)	86 (68.03)	90 (71.57)	**87 (68.87)**	84 (66.42)	87 (68.87)	89 (70.63)	**87 (68.87)**	81 (64.16)	84 (66.42)	89 (70.63)	**85 (67.22)**	83 (65.65)	86 (68.03)	89 (70.63)	**86 (68.03)**
MLP	82 (64.90)	84 (66.42)	86 (68.03)	**84 (66.42)**	82 (64.90)	84 (66.42)	85 (67.22)	**84 (66.42)**	81 (64.16)	82 (64.90)	84 (66.42)	**82 (64.90)**	81 (64.16)	83 (65.65)	85 (67.22)	**83 (65.65)**
Mean	**83 (65.65)**	**87 (68.87)**	**88 (69.73)**	**86 (68.03)**	**83 (65.65)**	**87 (68.87)**	**88 (69.73)**	**86 (68.03)**	**81 (64.16)**	**85 (67.22)**	**87 (68.87)**	**84 (66.42)**	**83 (65.65)**	**86 (68.03)**	**88 (69.73)**	**85 (67.22)**
Control	81 (64.16)															

(Figures in parenthesis indicate arcsine values)

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	0.69	0.22	0.22	0.22	0.39	0.39	0.39	0.67
CD (P = 0.05)	1.37	0.45	0.45	0.45	NS	NS	0.77	NS
CD (P = 0.01)	1.81	0.59	0.59	0.59	NS	NS	1.03	NS

D – Shaking duration (h) B - Botanicals C – Botanical dosage (g)

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

shoot length than 2 and 3 h. In addition, significant difference in the interaction between botanicals and its dosage of aged seeds were also observed in accelerated aged seeds. Significantly longer shoot were recorded in seeds dry dressed with fenugreek seed powder @ 3 g kg^{-1} of seeds (22.26 cm), 4 g kg^{-1} of seeds (22.29 cm) and custard apple leaf powder @ 4 g kg^{-1} of seeds (22.26 cm) which were on par with each other (Table 20).

4.3.2.4. Root length (cm)

All the treated seeds recorded significantly longer root than control (13.85 cm) and also it was significantly influenced by botanicals, its dosage, shaking duration and interaction between botanicals and its dosage while other interactions were not significant. Among the botanicals, seeds dry dressed with fenugreek seed powder and custard apple leaf powder was on par with each other and registered longer root than moringa leaf powder. Among dosages, 4 g kg^{-1} of seeds registered significantly higher root length (16.38 cm). Among the durations of shaking, higher root length was registered by 1 h shaken seeds. Interactional effect between botanicals and its dosage revealed that fenugreek seed powder @ 3 g kg^{-1} of seeds (16.50 cm), 4 g kg^{-1} of seeds (16.54 cm) and custard apple leaf powder @ 4 g kg^{-1} of seeds (16.52 cm) were superior (Table 21).

4.3.2.5. Dry matter production (g / 10 seedlings)

Dry matter production of aged seeds was significantly influenced by botanicals, dosage, duration of shaking and interaction between botanicals and its dosage. Irrespective of the treatments, treated seeds performed better than control (0.192 g / 10 seedlings). However, other interaction effects were not significant. Among the botanicals, fenugreek seed powder registered higher dry matter production (0.254 g / 10 seedlings) which was on par with custard apple leaf powder (0.248 g / 10 seedlings). Among the dosages, dry matter production was the maximum in seed dry dressing @ 3 and 4 g kg^{-1} of seeds (0.246 and 0.253 g / 10 seedlings, respectively) which was on par with each other. Among the duration of shaking, 1 h (0.250 g / 10 seedlings) and 2 h (0.246 g / 10 seedlings) was on par and produced significantly higher dry matter than 3 h (0.232 g / 10 seedlings). The interaction effect of botanicals and its dosage shows higher dry matter production in fenugreek seed powder @ 3 g kg^{-1} of seeds (0.262 g / 10 seedlings) and

Table 20. Effect of dry dressing with botanicals on shoot length (cm) of aged seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (Dry dressing)	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	22.03	22.91	22.95	**22.63**	21.78	22.58	22.63	**22.33**	20.39	21.30	21.29	**20.99**	21.40	22.26	22.29	**21.98**
CALP	21.66	21.99	22.92	**22.19**	21.61	21.83	22.59	**22.01**	20.62	20.81	21.28	**20.90**	21.30	21.54	22.26	**21.70**
MLP	21.02	21.42	22.06	**21.50**	21.03	21.34	21.87	**21.41**	20.12	20.27	20.79	**20.39**	20.72	21.01	21.57	**21.10**
Mean	**21.57**	**22.11**	**22.64**	**22.11**	**21.47**	**21.92**	**22.36**	**21.92**	**20.38**	**20.79**	**21.12**	**20.76**	**21.14**	**21.61**	**22.04**	**21.60**
Control	19.05															

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	0.437	0.143	0.143	0.143	0.247	0.247	0.247	0.429
CD (P = 0.05)	0.868	0.284	0.284	0.284	NS	NS	0.492	NS
CD (P = 0.01)	1.150	0.377	0.377	0.377	NS	NS	NS	NS

D – Shaking duration (h) B - Botanicals C – Botanical dosage (g)

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 21. Effect of dry dressing with botanicals on root length (cm) of aged seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (Dry dressing)	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	15.97	17.10	17.14	**16.74**	14.97	16.49	16.51	**15.99**	14.42	15.92	15.96	**15.43**	15.12	16.50	16.54	**16.05**
CALP	15.55	16.69	17.13	**16.46**	14.62	16.06	16.49	**15.72**	14.31	15.19	15.94	**15.15**	14.83	15.98	16.52	**15.78**
MLP	14.87	16.02	16.87	**15.92**	13.79	15.05	16.18	**15.01**	13.99	14.53	15.22	**14.58**	14.22	15.20	16.09	**15.17**
Mean	**15.46**	**16.60**	**17.05**	**16.37**	**14.46**	**15.87**	**16.39**	**15.57**	**14.24**	**15.21**	**15.71**	**15.05**	**14.72**	**15.89**	**16.38**	**15.67**
Control	13.85															

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	0.486	0.159	0.159	0.159	0.276	0.276	0.276	0.478
CD (P = 0.05)	0.967	0.317	0.317	0.317	NS	NS	0.548	NS
CD (P = 0.01)	1.282	0.420	0.420	0.420	NS	NS	NS	NS

D – Shaking duration (h) B - Botanicals C – Botanical dosage (g)

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 22. Effect of dry dressing with botanicals on dry matter production (g / 10 seedlings) of aged seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (Dry dressing)	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	0.245	0.269	0.271	**0.262**	0.242	0.267	0.267	**0.259**	0.229	0.251	0.249	**0.243**	0.239	0.262	0.262	**0.254**
CALP	0.244	0.257	0.271	**0.257**	0.240	0.247	0.268	**0.252**	0.224	0.234	0.250	**0.236**	0.236	0.246	0.263	**0.248**
MLP	0.217	0.235	0.238	**0.230**	0.217	0.232	0.238	**0.229**	0.206	0.222	0.227	**0.218**	0.213	0.230	0.234	**0.226**
Mean	**0.235**	**0.254**	**0.260**	**0.250**	**0.233**	**0.249**	**0.258**	**0.246**	**0.220**	**0.236**	**0.242**	**0.232**	**0.229**	**0.246**	**0.253**	**0.243**
Control	0.192															

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	0.0141	0.0046	0.0046	0.0046	0.0080	0.0080	0.0080	0.0139
CD (P = 0.05)	0.0281	0.0092	0.0092	0.0092	NS	NS	0.0159	NS
CD (P = 0.01)	0.0372	0.0122	0.0122	0.0122	NS	NS	NS	NS

D – Shaking duration (h) B - Botanicals C – Botanical dosage (g)

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

4 g kg^{-1} of seeds (0.262 g / 10 seedlings) and custard apple leaf powder @ 4 g kg^{-1} of seeds (0.263 g / 10 seedlings) which are on par with each other (Table 22).

4.3.2.6. Vigour index I

Vigour index I of aged seeds was significantly influenced by all the three factors and their interactions. Among the botanicals, fenugreek seed powder registered the maximum vigour index I (3325). Among the dosages, 4 g kg^{-1} of seeds recorded the maximum vigour index I (3383). While the vigour index I was maximum in shaking duration of 1 h (3313). In case of their interaction effects, seeds dry dressed with fenugreek seed powder @ 3 g kg^{-1} of seeds and custard apple leaf powder @ 4 g kg^{-1} of seeds shaken for 1 h registered the maximum vigour index I (Table 23).

4.3.2.7. Vigour index II

Vigour index II was significantly influenced only by botanicals, its dosage and duration of shaking while the interaction effect was significant only for botanicals and its dosage in aged seeds. However, all the treatments recorded significantly higher vigour index II than control (16).

Among the botanicals, seeds fenugreek seed powder was superior. Among the dosages, 4 g kg^{-1} of seeds topped. In case of shaking durations 1 and 2 h of shaking was on par and recorded the maximum vigour index II. Among the interactional effects, custard apple leaf powder @ 4 g kg^{-1} of seeds followed by fenugreek seed powder @ 3 and 4 g kg^{-1} of seeds recorded the maximum vigour index II. (Table 24).

4.3.3. Effect of wet treatment with botanical solutions on fresh seeds of blackgram

4.3.3.1. Speed of germination

The speed of germination was not significantly influenced by the treatments or their interactions (Table 25).

4.3.3.2. Seed germination (%)

Seed germination of fresh seeds was also not significantly influenced by the treatments or their interactions (Table 26).

Table 23. Effect of dry dressing with botanicals on vigour index I of aged seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (Dry dressing)	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	3154	3601	3568	**3441**	3087	3477	3523	**3362**	2924	3313	3278	**3172**	3055	3464	3456	**3325**
CALP	3126	3326	3605	**3352**	3043	3296	3478	**3272**	2864	3060	3350	**3091**	3011	3227	3478	**3239**
MLP	2943	3145	3348	**3145**	2855	3057	3234	**3049**	2763	2888	3061	**2904**	2854	3030	3214	**3033**
Mean	**3074**	**3357**	**3507**	**3313**	**2995**	**3277**	**3412**	**3228**	**2850**	**3087**	**3230**	**3056**	**2973**	**3240**	**3383**	**3199**
Control	2665															

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	10.81	3.54	3.54	3.54	6.13	6.13	6.13	10.61
CD (P = 0.05)	21.49	7.03	7.03	7.03	12.18	12.18	12.18	21.10
CD (P = 0.01)	28.48	9.32	9.32	9.32	16.15	16.15	16.15	27.97

D – Shaking duration (h) B - Botanicals C – Botanical dosage (g)

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 24. Effect of dry dressing with botanicals on vigour index II of aged seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (Dry dressing)	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	20	24	24	**23**	20	24	24	**23**	19	22	22	**21**	20	23	23	**22**
CALP	20	22	24	**22**	20	21	24	**22**	18	20	23	**20**	19	21	24	**21**
MLP	18	20	20	**19**	18	19	20	**19**	17	18	19	**18**	18	19	20	**19**
Mean	**19**	**22**	**23**	**21**	**19**	**21**	**23**	**21**	**18**	**20**	**21**	**20**	**19**	**21**	**22**	**21**
Control	16															

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	0.416	0.136	0.136	0.136	0.236	0.236	0.236	0.408
CD (P = 0.05)	0.826	0.271	0.271	0.271	NS	NS	0.469	NS
CD (P = 0.01)	1.095	0.359	0.359	0.359	NS	NS	0.621	NS

D – Shaking duration (h) B - Botanicals C – Botanical dosage (g)

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 25. Effect of wet treatment with botanicals on speed of germination of fresh seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	24.4	24.1	24.0	**24.2**
FSP 0.5%	24.6	24.4	24.4	**24.5**
FSP 1.0%	25.0	24.5	24.5	**24.7**
FSP 1.5%	24.6	24.6	24.4	**24.5**
FSP 2.0%	24.6	24.6	24.5	**24.6**
CALP 0.5%	24.6	24.6	24.4	**24.5**
CALP 1.0%	24.6	24.5	24.4	**24.5**
CALP 1.5%	25.0	24.8	24.5	**24.8**
CALP 2.0%	24.6	24.6	24.2	**24.5**
MLP 0.5%	24.6	24.4	24.2	**24.4**
MLP 1.0%	24.6	24.5	24.5	**24.5**
MLP 1.5%	24.7	24.5	24.4	**24.5**
MLP 2.0%	24.4	24.2	24.2	**24.3**
Mean	**24.6**	**24.5**	**24.4**	**24.5**
Control	23.3			
	Control Vs. Rest	D	B	D × B
SEd	0.550	0.151	0.313	0.543
CD (P = 0.05)	NS	NS	NS	NS
CD (P = 0.01)	NS	NS	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 26. Effect of wet treatment with botanicals on germination (%) of fresh seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	100 (89.72)	98 (81.87)	98 (81.87)	**99 (84.26)**
FSP 0.5%	99 (84.26)	98 (81.87)	98 (81.87)	**98 (81.87)**
FSP 1.0%	100 (89.72)	99 (84.26)	98 (81.87)	**99 (84.26)**
FSP 1.5%	99 (84.26)	99 (84.26)	98 (81.87)	**99 (84.26)**
FSP 2.0%	99 (84.26)	98 (81.87)	98 (81.87)	**98 (81.87)**
CALP 0.5%	98 (81.87)	98 (81.87)	98 (81.87)	**98 (81.87)**
CALP 1.0%	100 (89.72)	98 (81.87)	97 (80.03)	**98 (81.87)**
CALP 1.5%	100 (89.72)	99 (84.26)	99 (84.26)	**99 (84.26)**
CALP 2.0%	100 (89.72)	98 (81.87)	98 (81.87)	**99 (84.26)**
MLP 0.5%	98 (81.87)	99 (84.26)	98 (81.87)	**98 (81.87)**
MLP 1.0%	98 (81.87)	98 (81.87)	98 (81.87)	**98 (81.87)**
MLP 1.5%	100 (89.72)	98 (81.87)	98 (81.87)	**99 (84.26)**
MLP 2.0%	99 (84.26)	98 (81.87)	98 (81.87)	**98 (81.87)**
Mean	**98 (81.87)**	**98 (81.87)**	**98 (81.87)**	**98 (81.87)**
Control	98 (81.87)			
	Control Vs. Rest	D	B	D × B
SEd	1.703	0.566	0.971	1.682
CD (P = 0.05)	NS	NS	NS	NS
CD (P = 0.01)	NS	NS	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

(Figures in parenthesis indicate arcsine values)

4.3.3.3. Shoot length (cm)

Shoot length of fresh seeds was significantly influenced by wet treatment with botanicals solution and their soaking duration and registered significantly longer shoot than control (21.07 cm). But the interactions were not significant. Among the botanical solutions, seeds treated with 1% fenugreek seed powder (23.80 cm), 1.5% custard apple leaf powder (23.79 cm) and 1.5% moringa leaf powder solution (23.68 cm) recorded the maximum shoot length and was on par with each other. Among the soaking durations, seeds soaked for 1 and 2 h recorded significantly longer shoot than 3 h soaking and were on par with each other (Table 27).

4.3.3.4. Root length (cm)

Root length of fresh seeds was significantly influenced by wet treatments with botanicals solution and their soaking durations. Over all treatment mean for root length was greater than control (15.41 cm). Among the botanical solutions, seeds treated with 1% fenugreek seed powder (17.75 cm), 1.5% custard apple leaf powder (17.73 cm) and 1.5% moringa leaf powder solution (17.67 cm) recorded the maximum root length and was on par with each other. Among the soaking durations, seeds soaked for 1 h (18.28 cm) recorded significantly longer root than 2 and 3 h soaking. The interaction effects were not significant (Table 28).

4.3.3.5. Dry matter production (g / 10 seedlings)

Significant difference in dry matter production of fresh seeds was observed for botanicals and soaking durations but their interaction was not significant. However, the overall treatment mean was significantly higher than control (0.218). Among the botanical solutions, 1% fenugreek seed powder (0.287), 1.5% custard apple leaf powder (0.286) and 1.5% moringa leaf powder solution (0.281) treated seeds recorded higher dry matter and was on par with each other. Significantly higher dry matter production due to soaking duration was observed in 1 h soaked seeds (0.283) (Table 29).

4.3.3.6. Vigour index I

Significant difference in vigour index I was observed due to botanicals, soaking duration and their interaction. Also overall treatment mean recorded was significantly

Table 27. Effect of wet treatment with botanicals on shoot length (cm) of fresh seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	22.81	22.78	20.98	**22.19**
FSP 0.5%	23.11	22.99	22.03	**22.71**
FSP 1.0%	24.28	24.01	23.11	**23.80**
FSP 1.5%	23.76	23.59	22.89	**23.41**
FSP 2.0%	23.14	22.99	22.16	**22.76**
CALP 0.5%	22.90	22.83	21.39	**22.37**
CALP 1.0%	23.15	23.03	22.59	**22.92**
CALP 1.5%	24.26	23.96	23.15	**23.79**
CALP 2.0%	23.96	23.26	22.94	**23.39**
MLP 0.5%	22.59	22.38	21.67	**22.21**
MLP 1.0%	23.07	23.00	22.21	**22.76**
MLP 1.5%	24.20	23.60	23.24	**23.68**
MLP 2.0%	23.25	22.95	22.88	**23.03**
Mean	**23.42**	**23.18**	**22.40**	**23.00**
Control		**21.07**		
	Control Vs. Rest	D	B	D × B
SEd	0.598	0.164	0.341	0.590
CD (P = 0.05)	1.183	0.324	0.674	NS
CD (P = 0.01)	1.564	0.428	0.891	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

Table 28. Effect of wet treatment with botanicals on root length (cm) of fresh seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	17.97	16.76	16.45	**17.06**
FSP 0.5%	18.22	16.91	16.68	**17.27**
FSP 1.0%	18.52	17.86	16.86	**17.75**
FSP 1.5%	18.45	17.55	16.53	**17.51**
FSP 2.0%	17.98	17.02	16.58	**17.19**
CALP 0.5%	18.02	16.86	16.52	**17.13**
CALP 1.0%	18.45	17.55	16.56	**17.52**
CALP 1.5%	18.56	17.88	16.76	**17.73**
CALP 2.0%	18.28	17.22	16.58	**17.36**
MLP 0.5%	17.98	16.84	16.44	**17.09**
MLP 1.0%	18.42	17.45	16.44	**17.44**
MLP 1.5%	18.52	17.77	16.72	**17.67**
MLP 2.0%	18.22	17.11	16.50	**17.28**
Mean	**18.28**	**17.29**	**16.59**	**17.38**
Control		15.41		
	Control Vs. Rest	D	B	D × B
SEd	0.285	0.078	0.163	0.282
CD (P = 0.05)	0.565	0.155	0.322	NS
CD (P = 0.01)	0.747	0.204	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

Table 29. Effect of wet treatment with botanicals on dry matter production (g / 10 seedlings) of fresh seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	0.269	0.265	0.261	**0.265**
FSP 0.5%	0.280	0.273	0.268	**0.274**
FSP 1.0%	0.299	0.284	0.277	**0.287**
FSP 1.5%	0.290	0.279	0.272	**0.280**
FSP 2.0%	0.281	0.273	0.269	**0.274**
CALP 0.5%	0.271	0.267	0.262	**0.267**
CALP 1.0%	0.288	0.277	0.272	**0.279**
CALP 1.5%	0.300	0.282	0.276	**0.286**
CALP 2.0%	0.285	0.277	0.270	**0.277**
MLP 0.5%	0.270	0.264	0.261	**0.265**
MLP 1.0%	0.275	0.266	0.265	**0.269**
MLP 1.5%	0.286	0.283	0.274	**0.281**
MLP 2.0%	0.281	0.277	0.266	**0.275**
Mean	**0.283**	**0.274**	**0.269**	**0.275**
Control		0.218		
	Control Vs. Rest	D	B	D × B
SEd	0.0095	0.0026	0.0054	0.0094
CD (P = 0.05)	0.0188	0.0052	0.0107	NS
CD (P = 0.01)	0.0249	0.0068	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

higher than control (3575). Among the botanical solutions, 1% fenugreek seed powder and 1.5% custard apple leaf powder solution treated seeds recorded the maximum vigour index I and was on par with each other. Among the soaking durations, significantly higher vigour index I was recorded in 1 h soaked seeds (4122). Among the interactional effect, higher vigour index I was observed in seeds treated with 1% fenugreek seed powder, 1.5% custard apple leaf powder and 1.5% moringa leaf powder solution for 1 h soaking (Table 30).

4.3.3.7. Vigour index II

Vigour index II was significantly influenced only by botanicals and soaking duration but not their interaction. However, all the treatments in fresh seeds recorded significantly higher vigour index II than control. Among the botanicals, seeds treated with 1% and 1.5% fenugreek seed powder, 1.0 and 1.5% custard apple leaf powder and 1.5% moringa leaf powder solution recorded the maximum vigour index II of 28 which was on par with each other. While the higher vigour index II due to soaking duration was observed in 1 h soaked seeds (Table 31).

4.3.4. Effect of wet treatment with botanical solutions on aged seeds of blackgram

4.3.4.1. Speed of germination

In aged seeds, speed of germination was not significantly influenced among the treatments and their interactions. But the treated seeds on the whole registered significantly higher speed of germination than control (17.8), indicating their overall positive influence in aged seeds similar to dry dressing (Table 32).

4.3.4.2. Seed germination (%)

Seed germination of aged seeds were highly influenced by botanicals, duration of soaking and their interactions and also registered highly significant germination percentage over control (81%). Among the botanicals, seeds treated with 1% fenugreek seed powder (89%), 1.5% custard apple leaf powder (89%) and 1.5% moringa leaf powder solution (88%) recorded the maximum germination and was on par with each other. Among the duration of soaking, seeds soaked for 1 h recorded the maximum germination of 88%. The interactional effects shows the higher germination for 1.0 and

Table 30. Effect of wet treatment with botanicals on vigour index I of fresh seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	3996	3875	3743	**3871**
FSP 0.5%	4050	3950	3794	**3931**
FSP 1.0%	4280	4145	3917	**4114**
FSP 1.5%	4179	4073	3863	**4038**
FSP 2.0%	4071	3921	3797	**3930**
CALP 0.5%	4010	3890	3715	**3872**
CALP 1.0%	4160	3977	3837	**3991**
CALP 1.5%	4282	4142	3951	**4125**
CALP 2.0%	4140	3967	3952	**4020**
MLP 0.5%	3976	3883	3735	**3865**
MLP 1.0%	4066	3964	3788	**3939**
MLP 1.5%	4272	4054	3916	**4081**
MLP 2.0%	4106	3926	3859	**3964**
Mean	**4122**	**3982**	**3836**	**3980**
Control		3575		
	Control Vs. Rest	D	B	D × B
SEd	16.12	4.42	9.19	15.92
CD (P = 0.05)	31.93	8.74	18.20	31.52
CD (P = 0.01)	42.20	11.56	24.06	41.67

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

Table 31. Effect of wet treatment with botanicals on vigour index II of fresh seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	26	26	26	**26**
FSP 0.5%	27	27	26	**27**
FSP 1.0%	30	28	27	**28**
FSP 1.5%	29	28	27	**28**
FSP 2.0%	28	27	26	**27**
CALP 0.5%	27	26	26	**26**
CALP 1.0%	29	27	27	**28**
CALP 1.5%	30	28	27	**28**
CALP 2.0%	28	27	27	**27**
MLP 0.5%	26	26	26	**26**
MLP 1.0%	27	26	26	**26**
MLP 1.5%	29	28	27	**28**
MLP 2.0%	28	27	26	**27**
Mean	**28**	**27**	**26**	**27**
Control		21		
	Control Vs. Rest	D	B	D × B
SEd	0.827	0.226	0.471	0.816
CD (P = 0.05)	1.637	0.448	0.933	NS
CD (P = 0.01)	2.164	0.593	1.234	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

Table 32. Effect of wet treatment with botanicals on speed of germination of aged seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	19.9	19.5	19.7	**19.7**
FSP 0.5%	20.5	20.5	20.4	**20.5**
FSP 1.0%	21.0	20.6	20.7	**20.8**
FSP 1.5%	20.8	20.8	20.6	**20.7**
FSP 2.0%	20.5	20.4	19.9	**20.3**
CALP 0.5%	20.1	20.0	19.8	**20.0**
CALP 1.0%	20.9	20.4	20.2	**20.5**
CALP 1.5%	21.0	20.7	20.6	**20.8**
CALP 2.0%	20.8	20.8	20.6	**20.7**
MLP 0.5%	20.5	20.3	20.1	**20.3**
MLP 1.0%	20.5	20.3	20.0	**20.3**
MLP 1.5%	21.0	20.5	20.5	**20.7**
MLP 2.0%	20.8	20.4	20.3	**20.5**
Mean	**20.6**	**20.4**	**20.3**	**20.4**
Control		17.8		
	Control Vs. Rest	D	B	D × B
SEd	0.413	0.113	0.236	0.408
CD (P = 0.05)	0.819	NS	NS	NS
CD (P = 0.01)	1.082	NS	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

Table 33. Effect of wet treatment with botanicals on germination (%) of aged seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	86 (68.03)	84 (66.42)	83 (65.65)	**84 (66.42)**
FSP 0.5%	86 (68.03)	85 (67.22)	84 (66.42)	**85 (67.22)**
FSP 1.0%	90 (71.57)	88 (69.73)	88 (69.73)	**89 (70.63)**
FSP 1.5%	90 (71.57)	87 (68.87)	85 (67.22)	**87 (68.87)**
FSP 2.0%	88 (69.73)	86 (68.03)	84 (66.42)	**86 (68.03)**
CALP 0.5%	85 (67.22)	84 (66.42)	84 (66.42)	**84 (66.42)**
CALP 1.0%	88 (69.73)	86 (68.03)	85 (67.22)	**86 (68.03)**
CALP 1.5%	90 (71.57)	88 (69.73)	88 (69.73)	**89 (70.63)**
CALP 2.0%	89 (70.63)	87 (68.87)	85 (67.22)	**87 (68.87)**
MLP 0.5%	86 (68.03)	84 (66.42)	83 (65.65)	**84 (66.42)**
MLP 1.0%	88 (69.73)	85 (67.22)	83 (65.65)	**85 (67.22)**
MLP 1.5%	89 (70.63)	88 (69.73)	88 (69.73)	**88 (69.73)**
MLP 2.0%	86 (68.03)	86 (68.03)	85 (67.22)	**86 (68.03)**
Mean	**88 (69.73)**	**86 (68.03)**	**85 (67.22)**	**86 (68.03)**
Control	81 (64.16)			
	Control Vs. Rest	D	B	D × B
SEd	1.045	0.286	0.596	1.032
CD (P = 0.05)	2.070	0.567	1.180	2.044
CD (P = 0.01)	2.736	0.749	1.560	2.701

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

(Figures in parenthesis indicate arcsine values)

1.5% fenugreek seed powder, 1.5 and 2.0% custard apple leaf powder and 1.5% moringa leaf powder solution soaked for 1 h and were on par with each other (Table 33).

4.3.4.3. Shoot length (cm)

Shoot length was significantly influenced by botanicals solution and their soaking duration and registered significantly longer shoot than control (19.05 cm). But it was not significant for their interactions. Higher shoot length in seeds treated with botanicals were recorded for 1% fenugreek seed powder (22.98 cm), 1.5% custard apple leaf powder (22.97 cm) and 1.5% moringa leaf powder solution (22.70 cm) which was on par with each other. Among the soaking duration, seeds soaked for 1 and 2 h recorded significantly longer shoot than 3 h soaking and on par with each other (Table 34).

4.3.4.4. Root length (cm)

Root length was also significantly influenced by botanicals solution and their soaking duration and registered significantly longer root than control (13.85 cm). But it was not significant for their interactions. Higher root length in seeds treated with botanicals were recorded for 1% fenugreek seed powder (16.56 cm), 1.5% custard apple leaf powder (16.56 cm) and 1.5% moringa leaf powder solution (16.47 cm) which was on par with each other. Among the soaking durations, seeds soaked for 1 h (17.04 cm) recorded significantly longer root than 2 and 3 h soaking (Table 35).

4.3.4.5. Dry matter production (g / 10 seedlings)

Significant influence in dry matter production was observed due to botanicals and soaking duration and performed better than control (0.192 g). But the interaction effect was not significant. Among the botanical solutions, 1% fenugreek seed powder (0.267 g), 1.5% fenugreek seed powder (0.260 g), 1.5% custard apple leaf powder (0.265 g) and 1.5% moringa leaf powder solution (0.259 g) treated seeds recorded the maximum dry matter and was on par with each other. Significantly higher dry matter production due to soaking duration was observed in 1 h soaked seeds (0.261 g) (Table 36).

4.3.4.6. Vigour index I

Significant difference in vigour index I was observed due to botanicals, soaking duration and their interaction. Also all the treatments recorded significantly higher vigour

Table 34. Effect of wet treatment with botanicals on shoot length (cm) of aged seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	21.73	21.70	19.90	**21.11**
FSP 0.5%	22.03	21.91	20.95	**21.63**
FSP 1.0%	23.53	23.16	22.26	**22.98**
FSP 1.5%	22.91	22.74	22.04	**22.56**
FSP 2.0%	22.19	22.04	21.21	**21.81**
CALP 0.5%	21.73	21.66	20.22	**21.20**
CALP 1.0%	22.20	22.08	21.64	**21.97**
CALP 1.5%	23.51	23.13	22.26	**22.97**
CALP 2.0%	23.03	22.53	22.01	**22.52**
MLP 0.5%	21.66	21.45	20.74	**21.28**
MLP 1.0%	21.99	21.92	21.13	**21.68**
MLP 1.5%	23.15	22.65	22.29	**22.70**
MLP 2.0%	22.40	22.10	22.03	**22.18**
Mean	**22.50**	**22.27**	**21.47**	**22.08**
Control		19.05		
	Control Vs. Rest	D	B	D × B
SEd	0.454	0.124	0.259	0.448
CD (P = 0.05)	0.898	0.246	0.512	NS
CD (P = 0.01)	1.187	0.325	0.677	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

Table 35. Effect of wet treatment with botanicals on root length (cm) of aged seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	16.49	15.28	14.93	**15.57**
FSP 0.5%	17.03	15.72	15.19	**15.98**
FSP 1.0%	17.33	16.67	15.67	**16.56**
FSP 1.5%	17.26	16.36	15.34	**16.32**
FSP 2.0%	16.79	15.83	15.39	**16.00**
CALP 0.5%	16.83	15.67	15.21	**15.90**
CALP 1.0%	17.26	16.36	15.34	**16.32**
CALP 1.5%	17.37	16.67	15.63	**16.56**
CALP 2.0%	17.09	16.03	15.39	**16.17**
MLP 0.5%	16.69	15.55	15.11	**15.78**
MLP 1.0%	17.13	16.16	15.15	**16.15**
MLP 1.5%	17.32	16.57	15.52	**16.47**
MLP 2.0%	16.93	15.82	15.21	**15.99**
Mean	**17.04**	**16.05**	**15.31**	**16.14**
Control		13.85		
	Control Vs. Rest	D	B	D × B
SEd	0.294	0.080	0.167	0.290
CD (P = 0.05)	0.581	0.159	0.331	NS
CD (P = 0.01)	0.768	0.210	0.438	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

Table 36. Effect of wet treatment with botanicals on dry matter production (g / 10 seedlings) of aged seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	0.242	0.238	0.234	**0.238**
FSP 0.5%	0.259	0.252	0.247	**0.253**
FSP 1.0%	0.279	0.264	0.257	**0.267**
FSP 1.5%	0.270	0.259	0.252	**0.260**
FSP 2.0%	0.261	0.253	0.249	**0.254**
CALP 0.5%	0.249	0.245	0.240	**0.245**
CALP 1.0%	0.267	0.256	0.251	**0.258**
CALP 1.5%	0.279	0.261	0.255	**0.265**
CALP 2.0%	0.264	0.256	0.249	**0.256**
MLP 0.5%	0.247	0.242	0.239	**0.243**
MLP 1.0%	0.253	0.244	0.243	**0.247**
MLP 1.5%	0.264	0.261	0.252	**0.259**
MLP 2.0%	0.259	0.255	0.244	**0.253**
Mean	**0.261**	**0.253**	**0.247**	**0.254**
Control		0.192		
	Control Vs. Rest	D	B	D × B
SEd	0.007	0.002	0.004	0.007
CD (P = 0.05)	0.015	0.004	0.008	NS
CD (P = 0.01)	0.019	0.005	0.011	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

index I than control (2665). Among the botanical solutions, 1% fenugreek seed powder (3507) and 1.5% custard apple leaf powder solution (3505) treated seeds recorded the maximum vigour index I and was on par with each other. Significantly higher vigour index I due to soaking duration was recorded in 1 h soaked seeds (3469). Interactional effect shows a higher vigour index I in seeds treated with 1% fenugreek seed powder and 1.5% custard apple leaf powder solution for 1 h soaking (Table 37).

4.3.4.7. Vigour index II

In aged seeds, vigour index II was significantly influenced by botanicals, soaking duration and by their interactions and also performed better than control (16). Among the botanicals, seeds treated with 1% fenugreek seed powder (24) followed by 1.5% custard apple leaf powder (23) and 1.5% moringa leaf powder solution (23) recorded the maximum vigour index II. While the higher vigour index II due to soaking duration was observed in 1 h soaked seeds. The interactional effect on vigour index II was the maximum in 1% fenugreek seed powder and 1.5% custard apple leaf powder solution soaked for 1 h (25) (Table 38).

4.3.5. Assessment of seed vigour status in blackgram seeds subjected to botanical seed treatment

4.3.5.1. Accelerated ageing test for dry dressed seeds

4.3.5.1.1. Speed of germination

The results of the present investigation revealed that the speed of germination did not differ significantly among the treatments and their interactions. But treated seeds as a whole registered significantly higher speed of germination than control (20.6) (Table 39).

4.3.5.1.2. Seed germination (%)

Seed germination was highly superior and significant due to all the factors and interaction between botanicals and its dosage over control (89%). However, other interaction effects were not significant. Among the treatments, fenugreek seed powder (94%) registered maximum germination followed by custard apple leaf powder (93%). Among the shaking duration 1 and 2 h of shaking were on par with each other and registered the highest germination (93%) than 3 h of shaking (92%). Among the dosages,

Table 37. Effect of wet treatment with botanicals on vigour index I of aged seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	3287	3106	2891	**3095**
FSP 0.5%	3359	3199	3036	**3198**
FSP 1.0%	3677	3505	3338	**3507**
FSP 1.5%	3615	3402	3177	**3398**
FSP 2.0%	3430	3257	3074	**3254**
CALP 0.5%	3278	3136	2976	**3130**
CALP 1.0%	3472	3306	3143	**3307**
CALP 1.5%	3679	3502	3334	**3505**
CALP 2.0%	3571	3355	3179	**3368**
MLP 0.5%	3298	3108	2976	**3127**
MLP 1.0%	3443	3237	3011	**3230**
MLP 1.5%	3602	3451	3327	**3460**
MLP 2.0%	3382	3261	3165	**3269**
Mean	**3469**	**3294**	**3125**	**3296**
Control		2665		
	Control Vs. Rest	D	B	D × B
SEd	14.88	4.08	8.49	14.70
CD (P = 0.05)	29.47	8.07	16.80	29.10
CD (P = 0.01)	38.95	10.67	22.21	38.46

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

Table 38. Effect of wet treatment with botanicals on vigour index II of aged seeds of blackgram cv. TNAU Blackgram CO 6

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	21	20	19	**20**
FSP 0.5%	22	21	21	**21**
FSP 1.0%	25	23	23	**24**
FSP 1.5%	24	23	21	**23**
FSP 2.0%	23	22	21	**22**
CALP 0.5%	21	21	20	**21**
CALP 1.0%	23	22	21	**22**
CALP 1.5%	25	23	22	**23**
CALP 2.0%	23	22	21	**22**
MLP 0.5%	21	20	20	**20**
MLP 1.0%	22	21	20	**21**
MLP 1.5%	23	23	22	**23**
MLP 2.0%	22	22	21	**22**
Mean	**23**	**22**	**21**	**22**
Control		16		
	Control Vs. Rest	D	B	D × B
SEd	0.413	0.113	0.236	0.408
CD (P = 0.05)	0.819	0.224	0.467	NS
CD (P = 0.01)	1.082	0.296	0.617	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

Table 39. Evaluation of the effect of dry dressing with botanicals on speed of germination of blackgram seeds through accelerated ageing test

Botanicals (B) (Dry dressing)	Shaking duration (D)												B × C interaction mean			Grand mean
	1 h				2 h				3 h							
	Concentration (C)															
	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	22.2	22.9	22.8	**22.6**	22.2	22.5	22.6	**22.4**	21.8	22.4	22.6	**22.3**	22.1	22.6	22.7	**22.4**
CALP	22.0	22.4	22.8	**22.4**	22.1	22.4	22.6	**22.4**	21.8	22.0	22.5	**22.1**	22.0	22.3	22.6	**22.3**
MLP	21.9	22.4	22.0	**22.1**	21.6	21.9	22.2	**21.9**	21.2	21.9	22.3	**21.8**	21.6	22.1	22.2	**21.9**
Mean	**22.0**	**22.6**	**22.5**	**22.4**	**22.0**	**22.3**	**22.5**	**22.2**	**21.6**	**22.1**	**22.5**	**22.1**	**21.9**	**22.3**	**22.5**	**22.2**
Control	20.6															

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	0.780	0.255	0.255	0.255	0.442	0.442	0.442	0.766
CD (P = 0.05)	1.550	NS	NS	NS	NS	NS	NS	NS
CD (P = 0.01)	NS	NS	NS	NS	NS	NS	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

4 g kg^{-1} of seeds (94%) registered maximum germination followed by 3 g kg^{-1} of seeds (93%). However, interaction between botanicals and dosages showed that the maximum germination was recorded by fenugreek seed powder @ 3 and 4 g kg^{-1} of seeds and custard apple leaf powder @ 4 g kg^{-1} of seeds (95%) which were on par with each other (Table 40).

4.3.5.1.3. Shoot length (cm)

All the treated seeds registered significantly higher shoot length over control (20.06 cm). Among the botanicals, higher shoot length was observed in seeds dry dressed with fenugreek seed powder (22.52 cm) followed by custard apple leaf powder (22.20 cm). Among the dosages, 4 g kg^{-1} of seeds registered maximum shoot length (22.64 cm) followed by 3 g kg^{-1} of seeds (22.21 cm). Among the durations of shaking, 1 and 2 h produced significantly higher shoot length which was on par with each other. However, interactions between the factors were not significant (Table 41).

4.3.5.1.4. Root length (cm)

Root length of all the treatments registered significantly longer root than control (14.63 cm) and also root length was significantly influenced by botanicals, its dosage, shaking duration and interaction between botanicals and its dosage while other interactions were not significant. Among the botanicals, seeds dry dressed with fenugreek seed powder registered longer root (16.81 cm) followed by custard apple leaf powder (16.24 cm). While among dosages, 4 g kg^{-1} of seeds registered significantly higher root length (17.09 cm) followed by 3 g kg^{-1} of seeds (16.33 cm). Among the duration of shaking higher root length was observed in 1 h shaken seeds. Interaction effect between botanicals and its dosage registered higher root length for seeds treated with fenugreek seed powder @ 3 g kg^{-1} of seeds (17.29 cm), 4 g kg^{-1} of seeds (17.31 cm) and custard apple leaf powder @ 4 g kg^{-1} of seeds (17.29 cm) (Table 42).

4.3.5.1.5. Dry matter production (g / 10 seedlings)

Dry matter production was significantly influenced by botanicals, dosages and durations of shaking and performed better than control (0.205 g / 10 seedlings). But there was no significant difference due to their interactional effect. Among the botanicals,

Table 40. Evaluation of the effect of dry dressing with botanicals on seed germination (%) of blackgram seeds through accelerated ageing test

B	Shaking duration (D)												B × C interaction mean			Grand mean
	1 h				2 h				3 h							
	Concentration (C)															
	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	92 (73.57)	95 (77.08)	95 (77.08)	**94 (75.82)**	92 (73.57)	95 (77.08)	95 (77.08)	**94 (75.82)**	92 (73.57)	94 (75.82)	94 (75.82)	**93 (74.66)**	92 (73.57)	95 (77.08)	95 (77.08)	**94 (75.82)**
CALP	92 (73.57)	93 (74.66)	95 (77.08)	**93 (74.66)**	92 (73.57)	94 (75.82)	95 (77.08)	**94 (75.82)**	91 (72.54)	92 (73.57)	94 (75.82)	**92 (73.57)**	92 (73.57)	93 (74.66)	95 (77.08)	**93 (74.66)**
MLP	91 (72.54)	92 (73.57)	93 (74.66)	**92 (73.57)**	91 (72.54)	91 (72.54)	93 (74.66)	**92 (73.57)**	90 (71.57)	91 (72.54)	92 (73.57)	**91 (72.54)**	91 (72.54)	91 (72.54)	93 (74.66)	**92 (73.57)**
Mean	**92 (73.57)**	**93 (74.66)**	**94 (75.82)**	**93 (74.66)**	**92 (73.57)**	**93 (74.66)**	**94 (75.82)**	**93 (74.66)**	**91 (72.54)**	**92 (73.57)**	**93 (74.66)**	**92 (73.57)**	**91 (72.54)**	**93 (74.66)**	**94 (75.82)**	**93 (74.66)**
Control	89 (70.63)															

(Figures in parenthesis indicate arcsine values)

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	0.78	0.25	0.25	0.25	0.44	0.44	0.44	0.76
CD (P = 0.05)	1.54	0.50	0.50	0.50	NS	NS	0.87	NS
CD (P = 0.01)	2.04	0.67	0.67	0.67	NS	NS	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 41. Evaluation of the effect of dry dressing with botanicals on shoot length (cm) of blackgram seeds through accelerated ageing test

Botanicals (B) (Dry dressing)	Shaking duration (D)												B × C interaction mean			Grand mean
	1 h				2 h				3 h							
	Concentration (C)															
	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	22.50	23.35	23.36	**23.07**	22.35	23.18	23.22	**22.92**	20.80	21.96	21.97	**21.58**	21.88	22.83	22.85	**22.52**
CALP	22.15	22.53	23.35	**22.68**	22.19	22.56	23.20	**22.65**	20.73	21.10	21.96	**21.26**	21.69	22.06	22.84	**22.20**
MLP	21.51	21.93	22.50	**21.98**	21.54	22.16	22.61	**22.10**	20.03	21.10	21.59	**20.91**	21.03	21.73	22.23	**21.66**
Mean	**22.05**	**22.60**	**23.07**	**22.58**	**22.03**	**22.63**	**23.01**	**22.56**	**20.52**	**21.39**	**21.84**	**21.25**	**21.53**	**22.21**	**22.64**	**22.13**
Control	20.06															

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	0.437	0.143	0.143	0.143	0.247	0.247	0.247	0.429
CD (P = 0.05)	0.868	0.284	0.284	0.284	NS	NS	NS	NS
CD (P = 0.01)	1.150	0.377	0.377	0.377	NS	NS	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 42. Evaluation of the effect of dry dressing with botanicals on root length (cm) of blackgram seeds through accelerated ageing test

Botanicals (B) (Dry dressing)	Shaking duration (D)												B × C interaction mean			Grand mean
	1 h				2 h				3 h							
	Concentration (C)															
	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	16.46	17.68	17.71	**17.28**	15.98	17.39	17.40	**16.92**	15.07	16.80	16.81	**16.23**	15.84	17.29	17.31	**16.81**
CALP	15.63	16.51	17.70	**16.61**	15.17	16.23	17.39	**16.26**	15.00	15.71	16.79	**15.83**	15.27	16.15	17.29	**16.24**
MLP	15.29	16.01	17.17	**16.16**	14.77	15.58	16.90	**15.75**	14.82	15.06	15.95	**15.28**	14.96	15.55	16.67	**15.73**
Mean	**15.79**	**16.73**	**17.53**	**16.68**	**15.31**	**16.40**	**17.23**	**16.31**	**14.96**	**15.86**	**16.52**	**15.78**	**15.35**	**16.33**	**17.09**	**16.26**
Control	14.63															

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	0.420	0.137	0.137	0.137	0.238	0.238	0.238	0.412
CD (P = 0.05)	0.835	0.273	0.273	0.273	NS	NS	0.473	NS
CD (P = 0.01)	1.106	0.362	0.362	0.362	NS	NS	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

fenugreek seed powder registered higher dry matter production (0.264 g / 10 seedlings) which was on par with custard apple leaf powder (0.258 g / 10 seedlings). Among the dosages, dry matter production was the maximum in 3 and 4 g kg^{-1} of seeds (0.256 and 0.262 g / 10 seedlings, respectively) which was on par with each other. Among the durations, of shaking 1 and 2 h was on par (0.259 g / 10 seedlings) and produced significantly higher dry matter than 3 h shaking (0.240 g / 10 seedlings) (Table 43).

4.3.5.1.6. Vigour index I

Vigour index I was significantly influenced by all the three factors and their interactions and recorded significantly higher than control (3087). Among the botanicals, fenugreek seed powder registered the maximum vigour index (3690) followed by custard apple leaf powder (3581). Among the dosages, 4 g kg^{-1} of seeds recorded the maximum vigour index of 3736 followed by 3 g kg^{-1} of seeds (3586). Among the shaking durations, vigour index I was maximum for 1 h shaken seeds (3658). In case of their interaction effects, seeds dry dressed with fenugreek seed powder @ 3 and 4 g kg^{-1} of seeds and custard apple leaf powder @ 4 g kg^{-1} of seeds shaken for one hour registered maximum vigour index I (Table 44).

4.3.5.1.7. Vigour index II

Vigour index II was significantly influenced only by botanicals, its dosage and duration of shaking while their interactions were not significant. However, all the treatments recorded significantly higher vigour index II than control (18). Among the botanicals, fenugreek seed powder registered the maximum vigour index II followed by custard apple leaf powder. Among the dosages, 4 g kg^{-1} of seeds recorded the maximum vigour index II followed by 3 g kg^{-1} of seeds. In case of shaking durations, 1 and 2 h shaking was on par and recorded the maximum vigour index II (Table 45).

4.3.5.2. Accelerated ageing test on seeds subjected to wet treatment with botanicals

4.3.5.2.1. Speed of germination

The speed of germination was not significantly influenced among different wet treatments and their interactions. However, irrespective of the treatments, treated seeds registered significantly higher speed of germination than control (20.6) (Table 46).

Table 43. Evaluation of the effect of dry dressing with botanicals on dry matter production (g / 10 seedlings) of blackgram seeds through accelerated ageing test

Botanicals (B) (Dry dressing)	Shaking duration (D)												B × C interaction mean			Grand mean
	1 h				2 h				3 h							
	Concentration (C)															
	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	0.254	0.281	0.283	**0.273**	0.256	0.278	0.279	**0.271**	0.237	0.255	0.253	**0.248**	0.249	0.271	0.272	**0.264**
CALP	0.251	0.266	0.283	**0.267**	0.254	0.265	0.278	**0.266**	0.233	0.241	0.254	**0.243**	0.246	0.257	0.272	**0.258**
MLP	0.225	0.242	0.245	**0.237**	0.228	0.242	0.248	**0.239**	0.219	0.231	0.238	**0.229**	0.224	0.238	0.244	**0.235**
Mean	**0.243**	**0.263**	**0.270**	**0.259**	**0.246**	**0.262**	**0.268**	**0.259**	**0.230**	**0.242**	**0.248**	**0.240**	**0.240**	**0.256**	**0.262**	**0.253**
Control	0.205															

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	0.0141	0.0046	0.0046	0.0046	0.0080	0.0080	0.0080	0.0139
CD (P = 0.05)	0.0281	0.0092	0.0092	0.0092	NS	NS	NS	NS
CD (P = 0.01)	0.0372	0.0122	0.0122	0.0122	NS	NS	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 44. Evaluation of the effect of dry dressing with botanicals on vigour index I of blackgram seeds through accelerated ageing test

Botanicals (B) (Dry dressing)	Shaking duration (D)												B × C interaction mean			Grand mean
	1 h				2 h				3 h							
	Concentration (C)															
	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	3584	3898	3902	**3795**	3526	3854	3859	**3746**	3300	3643	3645	**3529**	3470	3798	3802	**3690**
CALP	3476	3631	3900	**3669**	3437	3646	3856	**3646**	3251	3387	3643	**3427**	3388	3555	3800	**3581**
MLP	3349	3490	3689	**3509**	3304	3434	3674	**3471**	3137	3291	3454	**3294**	3263	3405	3606	**3425**
Mean	**3470**	**3673**	**3830**	**3658**	**3422**	**3645**	**3796**	**3621**	**3229**	**3440**	**3581**	**3417**	**3374**	**3586**	**3736**	**3565**
Control	3087															

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	11.64	3.81	3.81	3.81	6.60	6.60	6.60	11.43
CD (P = 0.05)	23.14	7.57	7.57	7.57	13.12	NS	13.12	22.72
CD (P = 0.01)	30.67	10.04	10.04	10.04	17.39	NS	17.39	30.12

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 45. Evaluation of the effect of dry dressing with botanicals on vigour index II of blackgram seeds through accelerated ageing test

Botanicals (B) (Dry dressing)	Shaking duration (D)												B × C interaction mean			Grand mean
	1 h				2 h				3 h							
	Concentration (C)															
	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	23	27	27	**26**	24	26	27	**26**	22	24	24	**23**	23	26	26	**25**
CALP	23	25	27	**25**	23	25	26	**25**	21	22	24	**22**	22	24	26	**24**
MLP	20	22	23	**22**	21	22	23	**22**	20	21	22	**21**	20	22	23	**22**
Mean	**22**	**25**	**26**	**24**	**23**	**24**	**25**	**24**	**21**	**22**	**23**	**22**	**22**	**24**	**25**	**23**
Control	18															

	Control Vs. Rest	D	B	C	D × B	D × C	B × C	D × B × C
SEd	0.641	0.210	0.210	0.210	0.363	0.363	0.363	0.629
CD (P = 0.05)	1.274	0.417	0.417	0.417	NS	NS	NS	NS
CD (P = 0.01)	1.688	0.553	0.553	0.553	NS	NS	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 46. Evaluation of the effect of wet treatment with botanicals on speed of germination of blackgram seeds through accelerated ageing test

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	22.6	22.3	22.3	**22.4**
FSP 0.5%	23.0	22.9	22.9	**22.9**
FSP 1.0%	23.5	23.0	23.1	**23.2**
FSP 1.5%	23.2	23.2	23.0	**23.1**
FSP 2.0%	23.0	23.0	22.7	**22.9**
CALP 0.5%	22.8	22.8	22.6	**22.7**
CALP 1.0%	23.2	22.9	22.8	**23.0**
CALP 1.5%	23.5	23.2	23.0	**23.2**
CALP 2.0%	23.2	23.2	22.9	**23.1**
MLP 0.5%	23.0	22.8	22.6	**22.8**
MLP 1.0%	23.0	22.9	22.7	**22.9**
MLP 1.5%	23.3	23.0	22.9	**23.1**
MLP 2.0%	23.1	22.8	22.7	**22.9**
Mean	**23.1**	**22.9**	**22.8**	**22.9**
Control		20.6		
	Control Vs. Rest	D	B	D × B
SEd	0.372	0.102	0.212	0.367
CD (P = 0.05)	0.737	NS	NS	NS
CD (P = 0.01)	0.974	NS	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

4.3.5.2.2. Seed germination (%)

Seed germination was significantly influenced by botanicals and durations of soaking however, their interaction was not significant. Irrespective of the treatments, treated seeds registered significantly higher germination percentage over control (89%). Among the botanicals, seeds treated with 1 and 1.5% fenugreek seed powder, 1.5 and 2.0% custard apple leaf powder and 1.5% moringa leaf powder solution recorded the maximum germination which was on par with each other. Among the durations of soaking, seeds soaked for 1 h recorded the maximum germination of 95% (Table 47).

4.3.5.2.3. Shoot length (cm)

Shoot length of seedlings was also significantly influenced by botanicals and soaking durations and registered significantly longer shoot than control (20.06 cm). Higher shoot length was recorded in seeds treated with 1% fenugreek seed powder (24.05 cm), 1.5% custard apple leaf powder (24.03 cm) and 1.5% moringa leaf powder solution (23.84 cm) which were on par with each other. Among the soaking durations, seeds soaked for 1 h recorded significantly longer shoots (23.60 cm). However, its interaction effect was not significant (Table 48).

4.3.5.2.4. Root length (cm)

Root length was also significantly influenced by wet seed treatment with botanical solutions and their soaking duration and registered significantly longer root than control (14.63 cm). However, its interaction effect was not significant. Higher root length in seeds treated with botanicals was observed in 1% fenugreek seed powder (17.16 cm), 1.5% custard apple leaf powder (17.15 cm) and 1.5% moringa leaf powder solution (17.08 cm) which was on par with each other. Among the soaking durations, seeds soaked for 1 h (17.66 cm) recorded significantly longer root than 2 and 3 h soaking. (Table 49).

4.3.5.2.5. Dry matter production (g / 10 seedlings)

Dry matter production was also found to be significantly influenced due to botanicals and soaking duration and performed better than control (0.205 g). But the interactional effect was not significant. Among the botanical solutions, 1 and 1.5%

Table 47. Evaluation of the effect of wet treatment with botanicals on germination (%) of blackgram seeds through accelerated ageing test

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	93 (74.66)	93 (74.66)	92 (73.57)	**93 (74.66)**
FSP 0.5%	93 (74.66)	93 (74.66)	92 (73.57)	**93 (74.66)**
FSP 1.0%	96 (78.47)	95 (77.08)	94 (75.82)	**95 (77.08)**
FSP 1.5%	96 (78.47)	94 (75.82)	93 (74.66)	**94 (75.82)**
FSP 2.0%	95 (77.08)	93 (74.66)	92 (73.57)	**93 (74.66)**
CALP 0.5%	93 (74.66)	92 (73.57)	92 (73.57)	**92 (73.57)**
CALP 1.0%	95 (77.08)	93 (74.66)	92 (73.57)	**93 (74.66)**
CALP 1.5%	96 (78.47)	95 (77.08)	95 (77.08)	**95 (77.08)**
CALP 2.0%	95 (77.08)	94 (75.82)	93 (74.66)	**94 (75.82)**
MLP 0.5%	93 (74.66)	93 (74.66)	92 (73.57)	**93 (74.66)**
MLP 1.0%	94 (75.82)	93 (74.66)	92 (73.57)	**93 (74.66)**
MLP 1.5%	96 (78.47)	94 (75.82)	94 (75.82)	**95 (77.08)**
MLP 2.0%	94 (75.82)	93 (74.66)	93 (74.66)	**93 (74.66)**
Mean	**95 (77.08)**	**93 (74.66)**	**93 (74.66)**	**94 (75.82)**
Control	89 (70.63)			
	Control Vs. Rest	D	B	D × B
SEd	0.93	0.25	0.53	0.91
CD (P = 0.05)	1.83	0.50	1.04	NS
CD (P = 0.01)	2.42	0.66	1.38	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

Table 48. Evaluation of the effect of wet treatment with botanicals on shoot length (cm) of blackgram seeds through accelerated ageing test

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	22.92	22.89	21.09	**22.30**
FSP 0.5%	23.22	23.10	22.14	**22.82**
FSP 1.0%	24.56	24.24	23.34	**24.05**
FSP 1.5%	23.99	23.82	23.12	**23.64**
FSP 2.0%	23.32	23.17	22.34	**22.94**
CALP 0.5%	22.97	22.90	21.46	**22.44**
CALP 1.0%	23.33	23.21	22.77	**23.10**
CALP 1.5%	24.54	24.20	23.36	**24.03**
CALP 2.0%	24.15	23.55	23.13	**23.61**
MLP 0.5%	22.78	22.57	21.86	**22.40**
MLP 1.0%	23.18	23.11	22.32	**22.87**
MLP 1.5%	24.33	23.78	23.42	**23.84**
MLP 2.0%	23.48	23.18	23.11	**23.26**
Mean	**23.60**	**23.36**	**22.57**	**23.18**
Control		20.06		
	Control Vs. Rest	D	B	D × B
SEd	0.447	0.122	0.255	0.441
CD (P = 0.05)	0.884	0.242	0.504	NS
CD (P = 0.01)	1.169	0.320	0.666	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

Table 49. Evaluation of the effect of wet treatment with botanicals on root length (cm) of blackgram seeds through accelerated ageing test

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	17.24	16.03	15.70	**16.32**
FSP 0.5%	17.63	16.32	15.94	**16.63**
FSP 1.0%	17.93	17.27	16.27	**17.16**
FSP 1.5%	17.86	16.96	15.94	**16.92**
FSP 2.0%	17.39	16.43	15.99	**16.60**
CALP 0.5%	17.43	16.27	15.87	**16.52**
CALP 1.0%	17.86	16.96	15.96	**16.93**
CALP 1.5%	17.97	17.28	16.20	**17.15**
CALP 2.0%	17.69	16.63	15.99	**16.77**
MLP 0.5%	17.34	16.20	15.78	**16.44**
MLP 1.0%	17.78	16.81	15.80	**16.80**
MLP 1.5%	17.93	17.18	16.13	**17.08**
MLP 2.0%	17.58	16.47	15.86	**16.64**
Mean	**17.66**	**16.68**	**15.96**	**16.77**
Control		14.63		
	Control Vs. Rest	D	B	D × B
SEd	0.302	0.083	0.172	0.298
CD (P = 0.05)	0.598	0.164	0.341	NS
CD (P = 0.01)	0.790	0.216	0.450	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

fenugreek seed powder, 1.5 and 2.0% custard apple leaf powder and 1.5% moringa leaf powder solution treated seeds recorded the maximum dry matter and were on par with each other. Significantly higher dry matter production (0.278 g) due to soaking duration was observed in 1 h soaked seeds (Table 50).

4.3.5.2.6. Vigour index I

Significant difference in vigour index I was observed due to botanicals, soaking duration and their interaction. Also all the treatments recorded significantly higher vigour index I than control (3087). Among the botanical solutions, 1% fenugreek seed powder (3915) and 1.5% custard apple leaf powder solution (3927) treated seeds recorded the maximum vigour index I and was on par with each other. Among the soaking durations higher vigour index I was recorded in 1 h soaked seeds (3902). Significantly higher vigour index I with respect to interaction effect was observed in seeds treated with 1% fenugreek seed powder (4079), 1.5% custard apple leaf powder (4081) and 1.5% moringa leaf powder solution (4057) for 1 h soaking which was on par with each other (Table 51).

4.3.5.2.7. Vigour index II

Vigour index II was significantly influenced by botanicals, soaking duration and performed better than control (18). But their interaction was not significant. Among the botanicals, seeds treated with 1% fenugreek seed powder and 1.5% custard apple leaf powder solution recorded higher value of vigour index II and were on par with each other. While among soaking durations, higher vigour index II was observed in 1 h soaked seeds (Table 52).

4.3.6. Effect of botanical seed treatment on biochemical characters of fresh seeds of blackgram.

4.3.6.1. Electrical conductivity (µS cm^{-1} g^{-1})

The electrical conductivity of seed leachates was significantly influenced by seed treatment with botanicals. Electrical conductivity was significantly reduced in treated seeds than control (15.2 µS cm^{-1} g^{-1}). The lowest electrical conductivity was observed in seeds treated with 1.0% fenugreek seed powder solution (12.8 µS cm^{-1} g^{-1}), fenugreek seed powder @ 3 g kg^{-1} of seeds (13.0 µS cm^{-1} g^{-1}), 1.5% custard apple leaf powder

Table 50. Evaluation of the effect of wet treatment with botanicals on dry matter production (g / 10 seedlings) of blackgram seeds through accelerated ageing test

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	0.261	0.257	0.253	**0.257**
FSP 0.5%	0.275	0.268	0.263	**0.269**
FSP 1.0%	0.295	0.280	0.273	**0.283**
FSP 1.5%	0.286	0.275	0.268	**0.276**
FSP 2.0%	0.277	0.269	0.265	**0.270**
CALP 0.5%	0.266	0.262	0.257	**0.262**
CALP 1.0%	0.283	0.272	0.267	**0.274**
CALP 1.5%	0.295	0.277	0.271	**0.281**
CALP 2.0%	0.280	0.272	0.265	**0.272**
MLP 0.5%	0.264	0.259	0.256	**0.260**
MLP 1.0%	0.270	0.261	0.260	**0.264**
MLP 1.5%	0.281	0.278	0.269	**0.276**
MLP 2.0%	0.276	0.272	0.261	**0.270**
Mean	**0.278**	**0.269**	**0.264**	**0.270**
Control		0.205		
	Control Vs. Rest	D	B	D × B
SEd	0.0103	0.0028	0.0059	0.0102
CD (P = 0.05)	0.0205	0.0056	0.0117	NS
CD (P = 0.01)	0.0270	0.0074	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

Table 51. Evaluation of the effect of wet treatment with botanicals on vigour index I of blackgram seeds through accelerated ageing test

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	3735	3581	3421	**3579**
FSP 0.5%	3799	3666	3503	**3656**
FSP 1.0%	4079	3943	3723	**3915**
FSP 1.5%	4018	3833	3633	**3828**
FSP 2.0%	3867	3683	3526	**3692**
CALP 0.5%	3757	3604	3434	**3598**
CALP 1.0%	3913	3736	3563	**3737**
CALP 1.5%	4081	3941	3758	**3927**
CALP 2.0%	3975	3737	3677	**3796**
MLP 0.5%	3731	3606	3463	**3600**
MLP 1.0%	3850	3713	3507	**3690**
MLP 1.5%	4057	3850	3718	**3875**
MLP 2.0%	3860	3687	3624	**3724**
Mean	**3902**	**3737**	**3581**	**3740**
Control		3087		
	Control Vs. Rest	D	B	D × B
SEd	15.30	4.19	8.72	15.11
CD (P = 0.05)	30.29	8.30	17.27	29.91
CD (P = 0.01)	40.03	10.96	22.82	39.53

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

Table 52. Evaluation of the effect of wet treatment with botanicals on vigour index II of blackgram seeds through accelerated ageing test

Botanicals (B) (Wet treatment)	Soaking duration (D)			Mean
	1h	2h	3h	
Water	24	24	24	**24**
FSP 0.5%	26	25	24	**25**
FSP 1.0%	28	27	26	**27**
FSP 1.5%	27	26	25	**26**
FSP 2.0%	26	25	24	**25**
CALP 0.5%	25	24	24	**24**
CALP 1.0%	27	25	25	**26**
CALP 1.5%	28	26	26	**27**
CALP 2.0%	27	25	25	**26**
MLP 0.5%	25	24	24	**24**
MLP 1.0%	25	24	24	**24**
MLP 1.5%	27	26	25	**26**
MLP 2.0%	26	25	24	**25**
Mean	**26**	**25**	**25**	**25**
Control		18		
	Control Vs. Rest	D	B	D × B
SEd	0.620	0.170	0.354	0.612
CD (P = 0.05)	1.228	0.336	0.700	NS
CD (P = 0.01)	1.623	0.444	0.925	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder

MLP: Moringa Leaf Powder

solution (13.2 µS cm^{-1} g^{-1}) and custard apple leaf powder @ 4 g kg^{-1} of seeds (13.4 µS cm^{-1} g^{-1}) which were on par with each other (Table 53).

4.3.6.2. Antioxidant activity (% of blank)

Antioxidant activity was significantly increased due to botanical seed treatments. The highest antioxidant activity was observed in 1.0% fenugreek seed powder solution (94.5%), fenugreek seed powder @ 3 g kg^{-1} of seeds (94.2%), 1.5% custard apple leaf powder solution (93.9%) and custard apple leaf powder @ 4 g kg^{-1} of seeds (93.8%) which were on par with each other (Table 53).

4.3.6.3. α-Amylase activity (mm)

Significant increase in α- amylase activity than control was observed due to botanical seed treatments. In fresh seeds, the highest α-amylase activity was observed in 1.0% fenugreek seed powder solution (22.0 mm), 1.5% custard apple leaf powder solution (21.2 mm) and fenugreek seed powder @ 3 g kg^{-1} of seeds (19.5 mm) which was on par with each other followed by custard apple leaf powder @ 4 g kg^{-1} of seeds (18.8 mm) and 1.5% moringa leaf powder solution (18.1 mm) (Table 53).

4.3.6.4. Protease activity (OD value)

Protease activity was significantly lower in seeds treated with botanicals compared to untreated control seeds (0.318). In fresh seeds the lowest protease activity was observed in 1.0% fenugreek seed powder solution (0.228) and fenugreek seed powder @ 3 g kg^{-1} of seeds (0.231) which was on par with each other (Table 53).

4.3.6.5. Free amino acid content (µg 50 $seeds^{-1}$ 100 ml^{-1})

Free amino acid content was significantly lower than control (0.132) due to botanical seed treatments. In fresh seeds the minimum free amino acid content was observed in 1.0% fenugreek seed powder solution (0.110 µg 50 $seeds^{-1}$ 100 ml^{-1}) and 1.5% custard apple leaf powder solution (0.111 µg 50 $seeds^{-1}$ 100 ml^{-1}) which was on par with each other (Table 53).

Table 53. Effect of botanical treatments on biochemical characters of fresh seeds of blackgram cv. TNAU Blackgram CO 6.

Treatments	Electrical conductivity ($\mu S\ cm^{-1}\ g^{-1}$)	Antioxidant activity (% of blank)	α-amylase activity (mm)	Protease activity (OD value)	Free amino acid ($\mu g\ 50seeds^{-1}\ 100ml^{-1}$)
Control	15.2	84.7	13.4	0.318	0.132
FSP @ 3g kg^{-1}	13.0	94.2	19.5	0.231	0.118
CALP @ 4g kg^{-1}	13.4	93.8	18.8	0.268	0.121
MLP @ 4g kg^{-1}	14.3	89.2	15.3	0.293	0.126
1% FSP	12.8	94.5	22.0	0.228	0.110
1.5% CALP	13.2	93.9	21.2	0.256	0.111
1.5% MLP	13.5	90.9	18.1	0.269	0.119
Mean	**13.6**	**91.6**	**18.3**	**0.266**	**0.120**
SEd	0.29	0.35	1.56	0.012	0.0046
CD (P = 0.05)	0.62	0.74	3.19	0.025	0.0099
CD (P = 0.01)	0.86	1.03	4.30	0.034	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

4.3.6.6. Total protein profile

Both dry dressing and wet treatment influenced the total protein profile of fresh seeds of blackgram. The intensity of protein bands of seeds dry dressed with fenugreek seed powder @ 3 g kg^{-1} of seeds, custard apple leaf powder @ 4 g kg^{-1} of seeds and moringa leaf powder @ 4 g kg^{-1} of seeds were slightly higher than untreated control. However, the intensity of protein bands of seeds wet treated with 1.0% fenugreek seed powder, 1.5% custard apple and moringa leaf powder solution were predominantly higher than dry dressed seeds and untreated control (Plate 4), indicating synthesis of new proteins during wet treatment.

4.3.7. Effect of botanical seed treatment on biochemical characters of aged seeds of blackgram cv. TNAU Blackgram CO 6.

4.3.7.1. Electrical conductivity (µS cm^{-1} g^{-1})

Electrical conductivity was significantly lower in treated seeds than control (21.2 µS cm^{-1} g^{-1}). Except moringa leaf powder @ 4 g kg^{-1} of seeds all the other treatments were on par with each other and recorded lower electrical conductivity (Table 54).

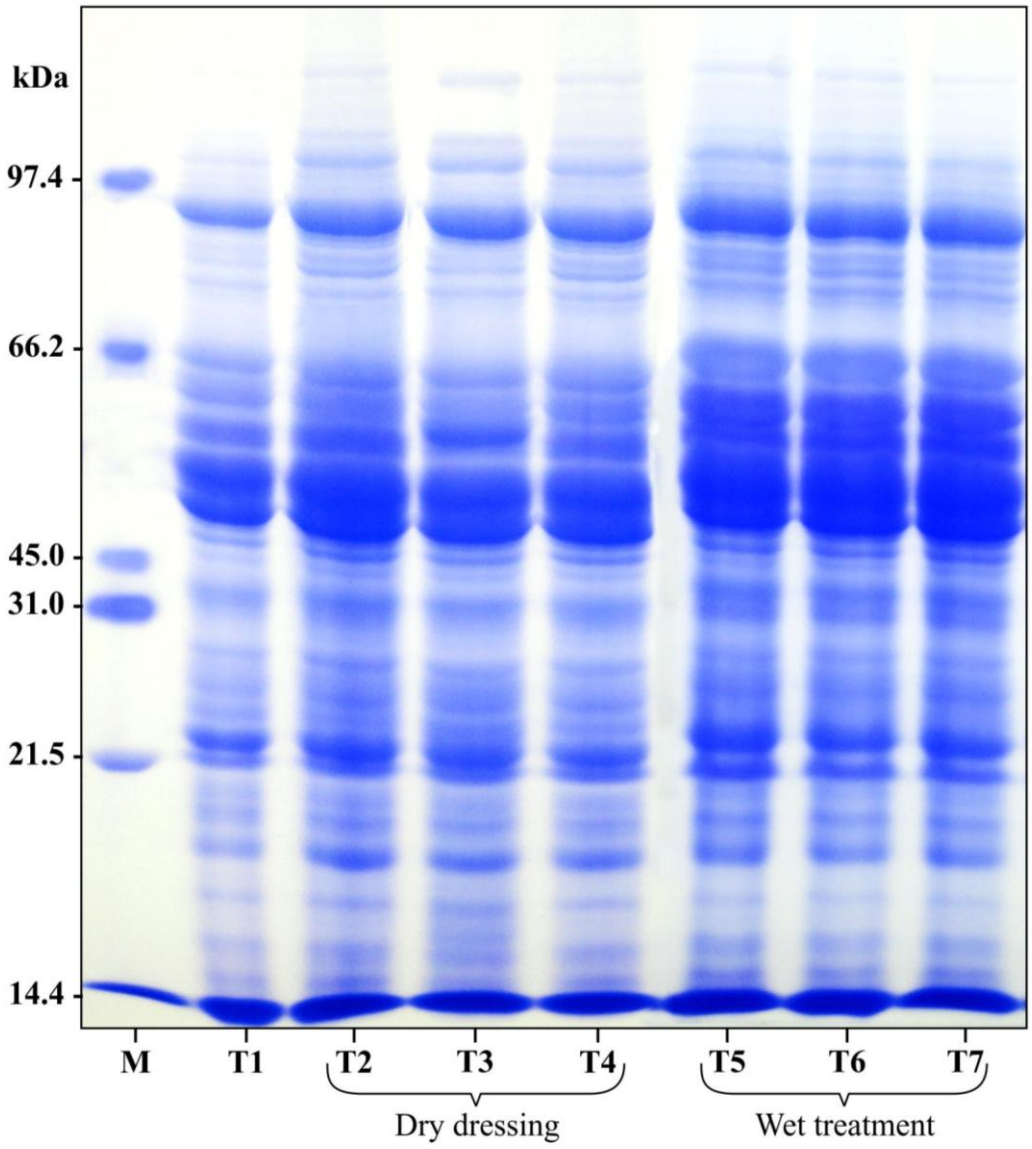
4.3.7.2. Antioxidant activity (% of blank)

In contrast to electrical conductivity, antioxidant activity was significantly higher than control (61.4%) due to botanical seed treatments. The highest antioxidant activity was observed in 1.0% fenugreek seed powder solution (90.2%) and fenugreek seed powder @ 3 g kg^{-1} of seeds (89.8%) which were on par with each other. They were followed by 1.5% custard apple leaf powder solution (88.3%) and custard apple leaf powder @ 4 g kg^{-1} of seeds (88.4%) which was on par with each other (Table 54).

4.3.7.3. α-amylase activity (mm)

Alpha amylase activity was significantly higher than control (8.2 mm) due to botanical seed treatments. In aged seeds 1.0% fenugreek seed powder solution (15.8 mm) significantly superior to other treatments. It was followed by fenugreek seed powder @ 3 g kg^{-1} of seeds (14.5 mm), 1.5% custard apple leaf powder solution (14.7 mm) and custard apple leaf powder @ 4 g kg^{-1} of seeds (13.9 mm) which were on par with each other (Table 54).

Plate 4. Effect of botanical seed treatment on protein profile of fresh seeds of blackgram cv. TNAU Blackgram CO 6



M : Protein marker

T1 : Untreated control (fresh seeds)

T2 : Seeds dry dressed with fenugreek seed powder @ 3 g kg^{-1} of seeds

T3 : Seeds dry dressed with custard apple leaf powder @ 4 g kg^{-1} of seeds

T4 : Seeds dry dressed with moringa leaf powder @ 4 g kg^{-1} of seeds

T5 : Seeds wet treated with 1.0% fenugreek seed powder solution for 1h

T6 : Seeds wet treated with 1.5% custard apple leaf powder solution for 1h

T7 : Seeds wet treated with 1.5% moringa leaf powder solution for 1h

Table 54. Effect of botanical treatments on biochemical characters of aged seeds of blackgram cv. TNAU Blackgram CO 6.

Treatments	Electrical conductivity (µS cm^{-1} g^{-1})	Antioxidant activity (% of blank)	α-amylase activity (mm)	Protease activity (OD value)	Free amino acid (µg $50seeds^{-1}$ $100ml^{-1}$)
Control	21.2	61.4	8.2	0.695	0.176
FSP @ 3g kg^{-1}	16.6	89.8	14.5	0.434	0.152
CALP @ 4g kg^{-1}	16.6	88.4	13.9	0.448	0.158
MLP @ 4g kg^{-1}	19.2	77.2	10.2	0.557	0.167
1% FSP	16.4	90.2	15.8	0.412	0.149
1.5% CALP	16.5	88.3	14.7	0.433	0.151
1.5% MLP	17.3	84.6	13.1	0.464	0.159
Mean	**17.7**	**82.8**	**12.9**	**0.492**	**0.159**
SEd	0.58	0.46	0.76	0.017	0.0040
CD (P = 0.05)	1.24	0.99	1.56	0.037	0.0087
CD (P = 0.01)	1.72	1.38	2.11	0.052	0.0120

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

4.3.7.4. Protease activity (OD value)

Protease activity was significantly reduced by botanical seed treatments compared to control (0.695). In aged seeds 1.0% fenugreek seed powder solution (0.412), 1.5% custard apple leaf powder solution (0.433), fenugreek seed powder @ 3 g kg^{-1} of seeds (0.434) and custard apple leaf powder @ 4 g kg^{-1} of seeds (0.448) registered the lower protease activity and were on par with each other (Table 54).

4.3.7.5. Free amino acid content (µg 50 $seeds^{-1}$ 100 ml^{-1})

Free amino acid content was also significantly lower due to botanical seed treatments than control (0.176 µg 50 $seeds^{-1}$ 100 ml^{-1}). In aged seeds 1.0% fenugreek seed powder solution (0.149 µg 50 $seeds^{-1}$ 100 ml^{-1}), 1.5% custard apple leaf powder solution (0.151 µg 50 $seeds^{-1}$ 100 ml^{-1}), fenugreek seed powder @ 3 g kg^{-1} of seeds (0.152 µg 50 $seeds^{-1}$ 100 ml^{-1}) and custard apple leaf powder @ 4 g kg^{-1} of seeds (0.158 µg 50 $seeds^{-1}$ 100 ml^{-1}) registered the lower free amino acid content and were on par with each other (Table 54).

4.3.7.6. Total protein profile

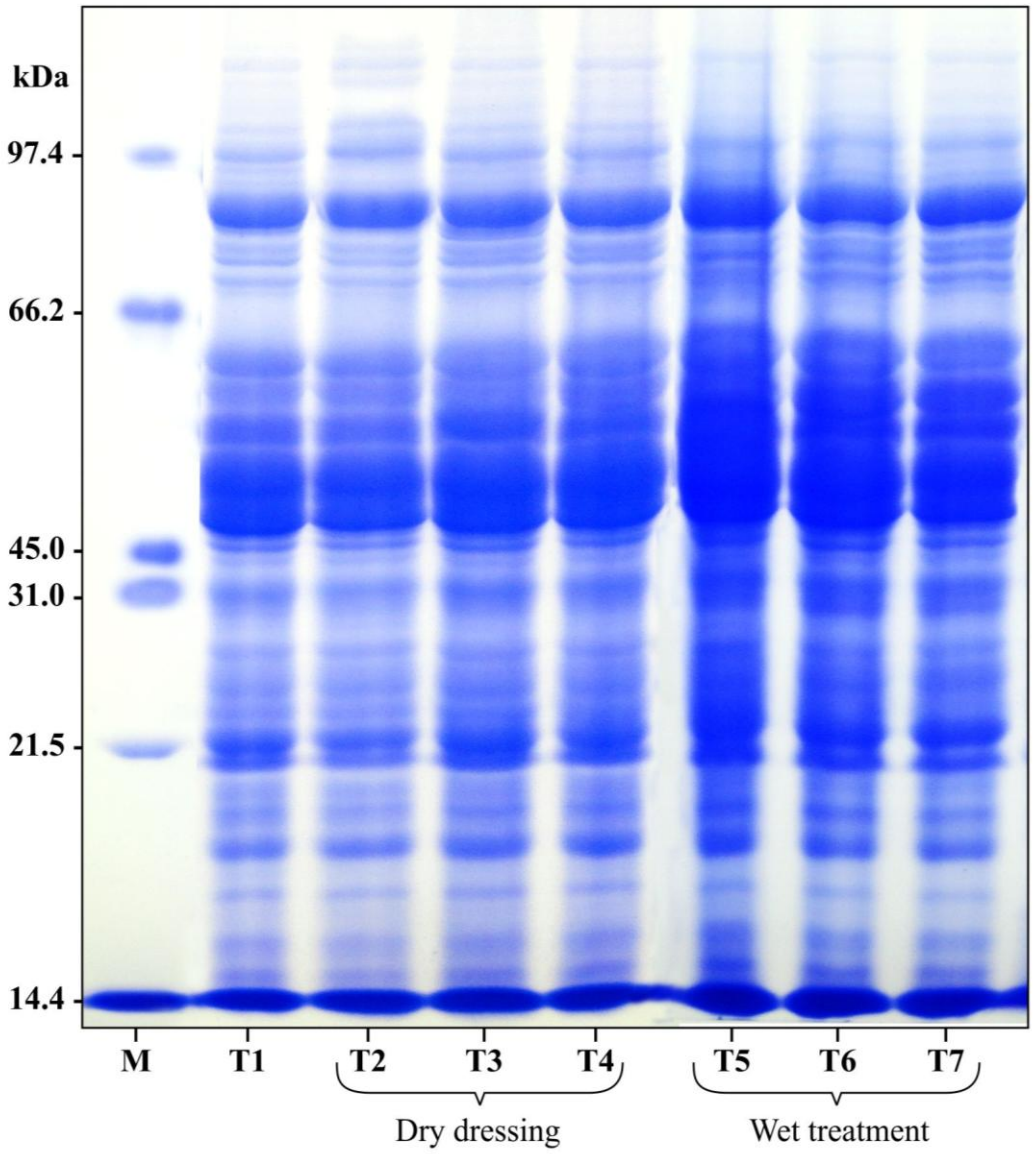
Both dry dressing and wet treatment influenced the total protein profile of aged seeds of blackgram. The intensity of protein bands of seeds dry dressed with fenugreek seed powder @ 3 g kg^{-1} of seeds, custard apple leaf powder @ 4 g kg^{-1} of seeds and moringa leaf powder @ 4 g kg^{-1} of seeds were slightly higher than untreated control. However, the intensity of protein bands of seeds wet treated with 1.0% fenugreek seed powder, 1.5% custard apple and moringa leaf powder solution were predominantly higher than dry dressed seeds and untreated control, indicating synthesis of new proteins during wet treatment (Plate 5).

4.3.8. Biochemical properties of botanicals

4.3.8.1. Antioxidant property of botanicals

All the three botanicals showed higher antioxidant activity. The highest antioxidant activity was observed in custard apple leaf powder (96.0%) and fenugreek seed powder (95.9%) which was followed by moringa leaf powder (91.7%) (Table 55).

Plate 5. Effect of botanical seed treatment on protein profile of aged seeds of blackgram cv. TNAU Blackgram CO 6



M : Protein marker

T1 : Untreated control (4 days accelerated aged seeds)

T2 : Seeds dry dressed with fenugreek seed powder @ 3 g kg^{-1} of seeds

T3 : Seeds dry dressed with custard apple leaf powder @ 4 g kg^{-1} of seeds

T4 : Seeds dry dressed with moringa leaf powder @ 4 g kg^{-1} of seeds

T5 : Seeds wet treated with 1.0% fenugreek seed powder solution for 1h

T6 : Seeds wet treated with 1.5% custard apple leaf powder solution for 1h

T7 : Seeds wet treated with 1.5% moringa leaf powder solution for 1h

Table 55. Antioxidant property and mineral content (ppm) of botanicals

Parameters	Fenugreek seed powder	Custard apple leaf powder	Moringa leaf powder
Antioxidant activity (% of blank)	95.9 (±0.36)	96.0 (±0.27)	91.7 (±0.28)
Mineral content (ppm)			
Aluminium (Al)	3.20 (±0.037)	6.10 (±0.512)	2.79 (±0.100)
Boron (B)	0.49 (±0.006)	2.36 (±0.008)	0.55 (±0.010)
Barium (Ba)	0.15 (±0.001)	0.91 (±0.003)	0.28 (±0.010)
Copper (Cu)	0.40 (±0.002)	0.44 (±0.001)	0.27 (±0.004)
Iron (Fe)	20.51 (±0.218)	11.68 (±0.045)	7.59 (±0.130)
Manganese (Mn)	0.62 (±0.004)	0.44 (±0.002)	0.49 (±0.010)
Molybdenum (Mo)	40.24 (±0.543)	24.86 (0.196)	13.73 (±0.550)
Strontium (Sr)	0.26 (±0.002)	3.19 (0.009)	2.78 (±0.050)
Titanium (Ti)	38.34 (±0.443)	107.10 (±1.852)	47.71 (±3.050)
Zinc (Zn)	1.35 (0.008)	0.86 (±0.004)	0.87 (±0.010)

± Values indicates the standard deviation

4.3.8.2. Mineral content of botanicals

All the botanicals also acted as a good source for minerals as revealed by the ICP analysis. Fenugreek seed powder recorded higher amount of Mo (40.24 ppm), Ti (38.34 ppm), Fe (20.51 ppm), Al (3.20 ppm), Zn (1.35 ppm) and traces of Mn, B, Cu, Sr, Ba. While custard apple leaf powder was rich in Ti (107.1 ppm), Mo (24.86 ppm), Fe (11.68 ppm), Al (6.10 ppm), Sr (3.19 ppm), B (2.36 ppm) and traces of Ba, Zn, Cu, Mn. moringa leaf powder was rich in Ti (47.71 ppm), Mo (13.73 ppm), Fe (7.59 ppm), Al (2.79 ppm), Sr (2.78 ppm) and traces of Zn, B, Mn, Ba, Cu. However, Moringa leaf powder contains lesser total mineral content than fenugreek seed powder and custard apple leaf powder. Among all these minerals the availability of titanium, molybdenum and iron were higher in all the three botanicals (Table 55).

4.4. Effect of botanical seed treatment of fresh and aged seeds on crop productivity in black gram

4.4.1. Effect of botanical seed treatment of fresh and aged seeds on crop productivity in blackgram cv. TNAU Blackgram CO 6 during *kharif* 2012

4.4.1.1. Physiological attributes

4.4.1.1.1. Plant height (cm)

Plants raised from treated seeds were taller in all the three stages of growth *viz*., 20, 40 and 60 days after sowing compared to control. However, significant differences were observed for treatments, only up to 20 days after sowing. Thereafter, the difference was not significant. At 20 days after sowing, seeds treated with 1% fenugreek seed powder solution exhibited maximum height of 11.7 cm followed by fenugreek seed powder @ 3 g kg^{-1} of seeds (11.5 cm) and 1.5% custard apple leaf powder solution (11.4 cm). Interaction effect revealed that 1% fenugreek seed powder solution exhibited maximum height for both fresh (13.1 cm) and aged seeds (10.2 cm) followed by fenugreek seed powder @ 3 g kg^{-1} of seeds (13.0 and 9.9 cm, respectively) and 1.5% custard apple leaf powder solution (12.9 and 9.9 cm, respectively). The same trend with minor variations was evident after 40 and 60 days after sowing, however, the values were

not significant except in the case of plant height (cm) between fresh and aged seeds (Table 56).

4.4.1.1.2. Leaf area (cm^2)

Results showed significant differences for treatments and their interactions up to 20 days after sowing. No significant differences were evident after 40 and 60 days of sowing except between fresh and aged seeds. After 20 days of sowing, 1% fenugreek seed powder solution treated seeds produced broader leaves with leaf area of 42.0 cm^2 followed by 1.5% custard apple leaf powder solution (40.6 cm^2). Among the interaction effect, the maximum leaf area was produced by 1% fenugreek seed powder solution in fresh seeds (47.3 cm^2). While in aged seeds both 1% fenugreek seed powder solution and 1.5% custard apple leaf powder solution produced the maximum leaf area of 36.6 cm^2 (Table 57).

4.4.1.1.3. Leaf area index

Results showed significant differences for treatments and their interactions up to 20 days after sowing. No significant differences were evident after 40 and 60 days of sowing except between fresh and aged seeds. After 20 days of sowing, 1% fenugreek seed powder solution treated seeds registered the maximum leaf area index of 0.140 followed by 1.5% custard apple leaf powder solution (0.135). The interaction effect reveals the maximum leaf area index by 1% fenugreek seed powder solution in fresh seeds (0.158). While in aged seeds both 1% fenugreek seed powder solution and 1.5% custard apple leaf powder solution produced the maximum leaf area index of 0.122 (Table 58).

4.4.1.1.4. Dry matter production (g $plant^{-1}$)

Significant differences were observed due to treatments and ageing of seeds, at all the stages (20, 40 and 60 days after sowing). At vegetative and flowering stage, seeds soaked with 1% fenugreek seed powder solution for 1h recorded higher dry weight (1.99 g at 20 days after sowing and 7.09 g at 40 days after sowing). Same trend was observed at 60 days after sowing also. The interaction effect was significant only at 20 days after sowing and not in later stages. At 20 days after sowing, 1% fenugreek seed

Table 56. Effect of botanicals seed treatments on plant height (cm) in *kharif* 2012

Age of seeds (A) / Botanicals (B)	Vegetative stage (20 DAS)			Flowering stage (40 DAS)			Harvesting stage (60 DAS)		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	10.7	8.2	**9.5**	27.3	24.9	**26.1**	36.9	34.6	**35.8**
FSP @ 3g kg^{-1}	13.0	9.9	**11.5**	27.9	25.8	**26.9**	37.5	35.4	**36.5**
CALP @ 4g kg^{-1}	12.7	9.6	**11.2**	27.6	25.2	**26.4**	37.2	34.8	**36.0**
MLP @ 4g kg^{-1}	12.1	8.7	**10.4**	27.5	25.4	**26.5**	37.1	35.0	**36.1**
1% FSP	13.1	10.2	**11.7**	28.1	25.9	**27.0**	37.7	35.5	**36.6**
1.5% CALP	12.9	9.9	**11.4**	28.0	25.4	**26.7**	37.6	35.0	**36.3**
1.5% MLP	12.3	9.4	**10.9**	27.7	25.0	**26.4**	37.3	34.6	**36.0**
Mean	**12.4**	**9.4**	**10.9**	**27.7**	**25.4**	**26.6**	**37.3**	**35.0**	**36.2**
	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.009	0.016	0.023	0.126	0.235	0.333	0.124	0.232	0.328
CD (P = 0.05)	0.018	0.033	0.047	0.258	NS	NS	0.255	NS	NS
CD (P = 0.01)	0.024	0.045	0.063	0.349	NS	NS	0.344	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 57. Effect of botanicals seed treatments on leaf area (cm^2) in *kharif* 2012

Age of seeds (A) / Botanicals (B)	Vegetative stage (20 DAS)			Flowering stage (40 DAS)			Harvesting stage (60 DAS)		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	18.7	16.5	**17.6**	437.0	360.3	**398.7**	1938.8	1572.8	**1755.8**
FSP @ 3g kg^{-1}	43.4	35.7	**39.6**	453.3	375.6	**414.5**	1940.3	1681.8	**1811.1**
CALP @ 4g kg^{-1}	40.5	34.2	**37.4**	444.6	370.6	**407.6**	1940.3	1668.4	**1804.4**
MLP @ 4g kg^{-1}	37.1	26.4	**31.8**	434.8	365.4	**400.1**	1903.3	1639.8	**1771.6**
1% FSP	47.3	36.6	**42.0**	456.7	377.0	**416.9**	1966.3	1682.9	**1824.6**
1.5% CALP	44.5	36.6	**40.6**	456.7	368.9	**412.8**	1959.5	1665.0	**1812.3**
1.5% MLP	34.6	30.0	**32.3**	442.1	365.5	**403.8**	1928.9	1658.2	**1793.6**
Mean	**38.0**	**30.9**	**34.4**	**446.5**	**369.0**	**407.8**	**1939.6**	**1652.7**	**1796.2**
	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.321	0.601	0.850	5.67	10.61	15.01	15.10	28.24	39.94
CD (P = 0.05)	0.661	1.236	1.748	11.66	NS	NS	31.04	NS	NS
CD (P = 0.01)	0.893	1.671	2.363	15.76	NS	NS	41.95	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 58. Effect of botanicals seed treatments on leaf area index (LAI) in *kharif* 2012

Age of seeds (A) / Botanicals (B)	Vegetative stage (20 DAS)			Flowering stage (40 DAS)			Harvesting stage (60 DAS)		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	0.062	0.055	**0.059**	1.457	1.201	**1.329**	6.463	5.243	**5.853**
FSP @ 3g kg^{-1}	0.145	0.119	**0.132**	1.511	1.252	**1.382**	6.468	5.606	**6.037**
CALP @ 4g kg^{-1}	0.135	0.114	**0.125**	1.482	1.235	**1.359**	6.468	5.561	**6.015**
MLP @ 4g kg^{-1}	0.124	0.088	**0.106**	1.449	1.218	**1.334**	6.344	5.466	**5.905**
1% FSP	0.158	0.122	**0.140**	1.522	1.257	**1.390**	6.554	5.610	**6.082**
1.5% CALP	0.148	0.122	**0.135**	1.522	1.230	**1.376**	6.532	5.550	**6.041**
1.5% MLP	0.115	0.100	**0.108**	1.474	1.218	**1.346**	6.430	5.527	**5.979**
Mean	**0.127**	**0.103**	**0.115**	**1.488**	**1.230**	**1.359**	**6.466**	**5.509**	**5.987**
	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.0010	0.0020	0.0028	0.019	0.035	0.050	0.050	0.094	0.133
CD (P = 0.05)	0.0021	0.0040	0.0057	0.039	NS	NS	0.103	NS	NS
CD (P = 0.01)	0.0029	0.0054	0.0077	0.052	NS	NS	0.140	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 59. Effect of botanicals seed treatments on dry matter production (g plant^{-1}) in *kharif* 2012

Age of seeds (A) / Botanicals (B)	Vegetative stage (20 DAS)			Flowering stage (40 DAS)			Harvesting stage (60 DAS)		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	1.87	1.57	**1.72**	6.53	5.47	**6.00**	11.43	9.57	**10.50**
FSP @ 3g kg^{-1}	2.09	1.80	**1.95**	7.17	6.43	**6.80**	11.75	9.95	**10.85**
CALP @ 4g kg^{-1}	2.03	1.74	**1.89**	7.03	6.28	**6.66**	11.57	9.89	**10.73**
MLP @ 4g kg^{-1}	1.90	1.65	**1.78**	6.69	5.71	**6.20**	11.49	9.61	**10.55**
1% FSP	2.11	1.86	**1.99**	7.49	6.68	**7.09**	11.84	9.98	**10.91**
1.5% CALP	2.06	1.81	**1.94**	7.31	6.49	**6.90**	11.63	9.88	**10.76**
1.5% MLP	1.98	1.78	**1.88**	6.94	6.28	**6.61**	11.52	9.68	**10.60**
Mean	**2.01**	**1.74**	**1.88**	**7.02**	**6.19**	**6.61**	**11.60**	**9.79**	**10.70**
	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.0046	0.0087	0.0123	0.026	0.049	0.069	0.052	0.098	0.139
CD (P = 0.05)	0.0095	0.0178	0.0252	0.054	0.101	NS	0.108	0.202	NS
CD (P = 0.01)	0.0129	0.0241	0.0341	0.073	0.136	NS	0.146	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

powder solution for 1h recorded the maximum dry weight of 2.11 g in fresh seeds and 1.86 g in aged seeds. The improvement was 13 and 18 per cent at 20 days after sowing over fresh and aged control, respectively (Table 59).

4.4.1.1.5. Chlorophyll content

Ageing of seeds, botanical treatments and their interaction effects did not show significant differences for chlorophyll content at flowering (40 days after sowing) and harvesting stage (60 days after sowing). However, at vegetative stage (20 days after sowing) significant difference in chlorophyll content between fresh and aged seeds was observed, while there was no significant difference among the treatments and its interactions (Table 60).

4.4.1.1.6. Crop Growth Rate (CGR) (g $m^{-2}d^{-1}$)

Significant differences were observed due to treatments and ageing of seeds. Among the treatments, maximum crop growth rate was recorded for seeds soaked in 1% fenugreek seed powder solution (8.51 g m^{-2} d^{-1}), 1.5% custard apple leaf powder solution for 1h (8.28 g $m^{-2}d^{-1}$) and dry dressed with fenugreek seed powder @ 3 g kg^{-1} of seeds (8.10 g $m^{-2}d^{-1}$) which were on par with each other. The interaction effect was not significant (Table 61).

4.4.1.1.7. Days to 50% flowering

Treatments have not shown any significant effect on days to 50 per cent flowering. But, crop raised from fresh seeds flowered earlier than crop raised from aged seeds. Crop raised from aged seeds, flowering was delayed one day. The interaction effect was also not significant for days to 50 per cent flowering (Table 61).

4.4.1.2. Effect of botanical seed treatments on yield attributes

4.4.1.2.1. Number of pods per plant

Significant differences in number of pods per plant could be observed in botanicals treatment and ageing of seeds. However, interaction effect was not significant. Among the botanicals seed treatment, all the treatments registered significantly higher number of pods per plant than control (28) except seeds dry dressed with moringa leaf powder @ 4 g kg^{-1} of seeds which was on par with control. Between fresh and aged

Table 60. Effect of botanicals seed treatments on chlorophyll content (SPAD value) in *kharif* 2012

Age of seeds (A) / Botanicals (B)	Vegetative stage (20 DAS)			Flowering stage (40 DAS)			Harvesting stage (60 DAS)		
	Fresh seeds	Aged seeds	Mean	Fresh seeds	Aged seeds	Mean	Fresh seeds	Aged seeds	Mean
Control	31.6	28.4	**30.0**	42.9	42.5	**42.7**	30.0	29.8	**29.9**
FSP @ 3g kg^{-1}	32.0	29.0	**30.5**	43.5	43.2	**43.4**	30.5	30.2	**30.4**
CALP @ 4g kg^{-1}	31.8	28.9	**30.4**	43.2	43.0	**43.1**	30.3	30.1	**30.2**
MLP @ 4g kg^{-1}	31.7	28.4	**30.1**	43.0	42.3	**42.7**	30.1	29.6	**29.9**
1% FSP	32.2	29.2	**30.7**	43.7	43.4	**43.6**	30.6	30.4	**30.5**
1.5% CALP	31.9	29.0	**30.5**	43.4	43.1	**43.3**	30.4	30.2	**30.3**
1.5% MLP	31.8	28.9	**30.4**	43.2	43.0	**43.1**	30.3	30.1	**30.2**
Mean	**31.9**	**28.8**	**30.3**	**43.3**	**42.9**	**43.1**	**30.3**	**30.1**	**30.2**
	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.130	0.242	0.343	0.173	0.324	0.459	0.119	0.223	0.316
CD (P = 0.05)	0.266	NS	NS	NS	NS	NS	NS	NS	NS
CD (P = 0.01)	0.360	NS	NS	NS	NS	NS	NS	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 61. Effect of botanicals seed treatments on crop growth rate (CGR) and days to 50% flowering in *kharif* 2012

Age of seeds (A) / Botanicals (B)	CGR (g m^{-2} d^{-1})			Days to 50% flowering		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	7.78	6.51	**7.15**	39	40	**40**
FSP @ 3g kg^{-1}	8.46	7.73	**8.10**	38	39	**39**
CALP @ 4g kg^{-1}	8.33	7.57	**7.95**	39	40	**40**
MLP @ 4g kg^{-1}	7.98	6.77	**7.38**	39	41	**40**
1% FSP	8.97	8.04	**8.51**	38	39	**39**
1.5% CALP	8.76	7.80	**8.28**	38	40	**39**
1.5% MLP	8.27	7.50	**7.89**	39	40	**40**
Mean	**8.36**	**7.42**	**7.89**	**39**	**40**	**39**
	A	B	A × B	A	B	A × B
SEd	0.077	0.144	0.204	0.180	0.337	0.477
CD (P = 0.05)	0.159	0.297	NS	0.371	NS	NS
CD (P = 0.01)	0.214	0.401	NS	0.501	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 62. Effect of botanicals seed treatments on pod yield attributes in *kharif* 2012

Age of seeds (A) / Botanicals (B)	No. of pods per plant			Pod yield per plant (g)			Pod yield per plot (kg)			Pod yield per ha (kg)		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	31	26	**28**	15.39	12.72	**14.06**	1.31	1.08	**1.20**	1089.7	900.8	**995.3**
FSP @ 3g kg^{-1}	34	30	**32**	16.73	14.78	**15.76**	1.43	1.26	**1.35**	1184.1	1046.1	**1115.1**
CALP @ 4g kg^{-1}	33	30	**32**	16.31	14.67	**15.49**	1.39	1.25	**1.32**	1155.1	1038.9	**1097.0**
MLP @ 4g kg^{-1}	31	27	**29**	15.49	12.83	**14.16**	1.32	1.09	**1.21**	1097.0	908.1	**1002.6**
1% FSP	34	30	**32**	16.83	14.98	**15.91**	1.43	1.28	**1.36**	1191.4	1060.7	**1126.1**
1.5% CALP	33	30	**32**	16.52	14.57	**15.55**	1.41	1.24	**1.33**	1169.6	1031.6	**1100.6**
1.5% MLP	33	29	**31**	16.01	14.78	**15.40**	1.36	1.26	**1.31**	1133.3	1046.1	**1089.7**
Mean	**33**	**29**	**31**	**16.18**	**14.19**	**15.19**	**1.38**	**1.21**	**1.29**	**1145.7**	**1004.6**	**1075.2**
	A	B	A × B	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.482	0.902	1.276	0.252	0.472	0.667	0.022	0.040	0.057	17.85	33.40	47.23
CD (P = 0.05)	0.991	1.854	NS	0.518	0.970	NS	0.045	0.083	NS	36.70	68.67	NS
CD (P = 0.01)	1.340	NS	NS	0.701	NS	NS	0.060	NS	NS	49.61	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

seeds, fresh seeds (33) registered higher number of pods per plant than aged seeds (29) (Table 62)

4.4.1.2.2. Pod yield per plant (g)

Pod yield per plant was significantly differed in botanicals treatment and ageing of seeds. However, interaction effect was not significant. Among the botanicals seed treatment, all the treatments registered significantly higher pod yield per plant than control except seeds dry dressed with moringa leaf powder @ 4 g kg^{-1} of seeds which was on par with control. Between the fresh and aged seeds, fresh seeds (16.18 g) registered higher pod yield per plant than aged seeds (14.19 g) (Table 62).

4.4.1.2.3. Pod yield per plot (kg)

Pod yield per plot was also significantly influenced by botanicals treatment and ageing of seeds. However, interaction effect was not significant. Among the botanical seed treatments, all the treatments registered significantly maximum pod yield per plot than control except seeds dry dressed with moringa leaf powder @ 4 g kg^{-1} of seeds which was on par with control. Between fresh and aged seeds, fresh seeds (1.38 kg) registered higher pod yield per plot than aged seeds (1.21 kg) (Table 62).

4.4.1.2.4. Pod yield per hectare (kg)

Pod yield per hectare was also significantly influenced by botanicals treatment and ageing of seeds. However, interaction effect was not significant. Among the botanical seed treatments, all the treatments registered significantly maximum pod yield per hectare than control except seeds dry dressed with moringa leaf powder @ 4 g kg^{-1} of seeds which was on par with control. Between fresh and aged seeds, fresh seeds (1145.7 kg) registered higher pod yield hectare than aged seeds (1004.6 kg). The increase in pod yield per hectare was 9 and 17 per cent over control of fresh and aged seeds respectively (Table 62).

4.4.1.2.5. Seed yield per plant (g)

Seed yield per plant was significantly differed due to botanicals treatment and ageing of seeds. However, interaction effect was not significant. Among the botanicals seed treatment, seeds treated with 1.0% fenugreek seed powder solution (8.92 g) registered significantly higher seed yield per plant which was on par with seeds dry

dressed with fenugreek seed powder @ 3 g kg^{-1} of seeds, 1.5% custard apple and moringa leaf powder solution and custard apple leaf powder @ 4 g kg^{-1} of seeds. The lowest seed yield per plant was observed in control (7.89 g) which was on par with seeds dry dressed with moringa leaf powder @ 4 g kg^{-1} of seeds. Between fresh and aged seeds, fresh seeds (9.12 g) registered higher seed yield per plant than aged seeds (7.96 g) (Table 63).

4.4.1.2.6. Seed yield per plot (kg)

Seed yield per plot was also significantly influenced by botanicals treatment and ageing of seeds. However, interaction effect was not significant. Among the botanicals seed treatment, all the treatments registered significantly maximum seed yield per plot than control except seeds dry dressed with moringa leaf powder @ 4 g kg^{-1} of seeds which was on par with control. Between fresh and aged seeds, fresh seeds (0.775 kg) registered higher seed yield per plot than aged seeds (0.677 kg) (Table 63).

4.4.1.2.7. Seed yield per hectare (kg)

Seed yield per hectare was also significantly influenced by botanicals treatment and ageing of seeds. However, interaction effect was not significant. Among the botanicals seed treatment, seeds treated with 1.0% fenugreek seed powder solution (631.6 kg) registered significantly higher seed yield per hectare which was on par with seeds dry dressed with fenugreek seed powder @ 3 g kg^{-1} of seeds, 1.5% custard apple and moringa leaf powder solution and custard apple leaf powder @ 4 g kg^{-1} of seeds. The lowest seed yield per hectare was observed in control (558.3 kg) which was on par with seeds dry dressed with moringa leaf powder @ 4 g kg^{-1} of seeds. Between fresh and aged seeds, fresh seeds (645.8 kg) registered higher seed yield per hectare than aged seeds (563.7 kg). The increase in seed yield per hectare was 9 and 17 per cent over control of fresh and aged seeds, respectively (Table 63).

4.4.1.2.8. Seed recovery (%)

Seed recovery was not significantly influenced by the botanicals seed treatment. However, significant differences was observed between fresh and aged seeds. In which, fresh seeds registered higher seed recovery of 56.38% than aged seeds (56.11%) (Table 63).

Table 63. Effect of botanicals seed treatments on seed yield attributes in *kharif* 2012

Age of seeds (A) / Botanicals (B)	Seed yield per plant (g)			Seed yield per plot (kg)			Seed yield per ha (kg)			Seed recovery (%)		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	8.64	7.14	**7.89**	0.734	0.607	**0.671**	611.4	505.2	**558.3**	56.11	56.09	**56.10**
FSP @ 3g kg^{-1}	9.50	8.29	**8.90**	0.807	0.704	**0.756**	672.4	586.8	**629.6**	56.46	56.09	**56.28**
CALP @ 4g kg^{-1}	9.20	8.24	**8.72**	0.782	0.700	**0.741**	651.5	583.2	**617.4**	56.43	56.14	**56.29**
MLP @ 4g kg^{-1}	8.74	7.20	**7.97**	0.743	0.612	**0.678**	618.5	509.5	**564.0**	56.40	56.11	**56.26**
1% FSP	9.44	8.40	**8.92**	0.802	0.714	**0.758**	668.1	595.0	**631.6**	56.44	56.10	**56.27**
1.5% CALP	9.32	8.18	**8.75**	0.792	0.695	**0.744**	659.8	578.9	**619.4**	56.44	56.12	**56.28**
1.5% MLP	9.03	8.29	**8.66**	0.767	0.705	**0.736**	639.2	587.1	**613.2**	56.40	56.13	**56.27**
Mean	**9.12**	**7.96**	**8.54**	**0.775**	**0.677**	**0.726**	**645.8**	**563.7**	**604.8**	**56.38**	**56.11**	**56.25**
	A	B	A × B	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.141	0.264	0.373	0.012	0.022	0.032	9.99	18.69	26.44	0.028	0.052	0.074
CD (P = 0.05)	0.290	0.542	NS	0.025	0.046	NS	20.54	38.43	NS	0.057	NS	NS
CD (P = 0.01)	0.392	NS	NS	0.033	NS	NS	27.77	NS	NS	0.077	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

4.4.1.3. Effect of botanical seed treatments on quality of resultant seeds

The resultant seeds of different treatments recorded non-significant values for seed germination, shoot length, root length, dry matter production, vigour index I and vigour index II (Table 64 and 65).

4.4.2. Effect of botanical seed treatment of fresh and aged seeds on crop productivity in black gram cv. TNAU Blackgram CO 6 during *rabi* 2012

4.4.2.1. Physiological attributes

4.4.2.1.1. Plant height (cm)

Plants raised from treated seeds were taller in all the three stages of growth *viz*., 20, 40 and 60 days after sowing compared to control. However, significant differences were observed for treatments, only up to 20 days after sowing. Thereafter, the difference was not significant. At 20 days after sowing, seeds treated with 1% fenugreek seed powder solution exhibited maximum height of 12.1 cm followed by 1.5% custard apple leaf powder solution (11.8 cm) and fenugreek seed powder @ 3 g kg^{-1} of seeds (11.8 cm). Interaction effect revealed that 1% fenugreek seed powder solution exhibited maximum height for both fresh (13.6 cm) and aged seeds (10.5 cm) followed by 1.5% custard apple leaf powder solution (13.4 and 10.2 cm, respectively) and fenugreek seed powder @ 3 g kg^{-1} of seeds (13.4 and 10.2 cm, respectively). The same trend with minor variations was evident after 40 and 60 days after sowing, where the values were not significantly different except for the difference in the plant height between fresh and aged seeds (Table 66).

4.4.2.1.2. Leaf area (cm^2)

Results showed significant differences for treatments and their interactions up to 20 days after sowing. No significant differences were evident after 40 and 60 days of sowing except between fresh and aged seeds. After 20 days of sowing, 1% fenugreek seed powder solution treated seeds produced broader leaves with leaf area of 42.5 cm^2 followed by 1.5% custard apple leaf powder solution (41.1 cm^2) and fenugreek seed powder @ 3 g kg^{-1} of seeds (40.1 cm^2). Among the interaction effect, the maximum leaf area was produced by 1% fenugreek seed powder solution in fresh seeds (47.9 cm^2).

Table 64. Effect of botanicals seed treatments on resultant seed quality in *kharif* 2012

Age of seeds (A) / Botanicals (B)	Germination (%)			Shoot length (cm)			Root length (cm)		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	98 (81.87)	98 (81.87)	**98 (81.87)**	21.30	21.30	**21.30**	15.87	15.87	**15.87**
FSP @ 3g kg^{-1}	99 (84.26)	98 (81.87)	**99 (84.26)**	21.36	21.34	**21.35**	15.93	15.91	**15.92**
CALP @ 4g kg^{-1}	98 (81.87)	99 (84.26)	**99 (84.26)**	21.36	21.33	**21.35**	15.90	15.90	**15.90**
MLP @ 4g kg^{-1}	99 (84.26)	98 (81.87)	**99 (84.26)**	21.31	21.30	**21.31**	15.88	15.87	**15.88**
1% FSP	98 (81.87)	99 (84.26)	**99 (84.26)**	21.37	21.36	**21.37**	15.94	15.93	**15.94**
1.5% CALP	99 (84.26)	98 (81.87)	**99 (84.26)**	21.35	21.33	**21.34**	15.92	15.90	**15.91**
1.5% MLP	98 (81.87)	99 (84.26)	**99 (84.26)**	21.33	21.30	**21.32**	15.90	15.87	**15.89**
Mean	**98 (81.87)**	**98 (81.87)**	**98 (81.87)**	**21.34**	**21.32**	**21.33**	**15.91**	**15.89**	**15.90**
	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.233	0.435	0.716	0.039	0.074	0.104	0.039	0.073	0.103
CD (P = 0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD (P = 0.01)	NS	NS	NS	NS	NS	NS	NS	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 65. Effect of botanicals seed treatments on resultant seed quality in *kharif* 2012

Age of seeds (A) / Botanicals (B)	Dry matter production (g / 10 seedlings)			Vigour index I			Vigour index II		
	Fresh seeds	Aged seeds	Mean	Fresh seeds	Aged seeds	Mean	Fresh seeds	Aged seeds	Mean
Control	0.221	0.220	**0.221**	3643	3643	**3643**	21.7	21.5	**21.6**
FSP @ 3g kg^{-1}	0.225	0.224	**0.225**	3673	3651	**3662**	22.1	21.9	**22.0**
CALP @ 4g kg^{-1}	0.223	0.222	**0.223**	3651	3667	**3659**	21.8	21.8	**21.8**
MLP @ 4g kg^{-1}	0.222	0.221	**0.222**	3663	3643	**3653**	21.8	21.6	**21.7**
1% FSP	0.226	0.225	**0.226**	3656	3672	**3664**	22.1	22.1	**22.1**
1.5% CALP	0.224	0.223	**0.224**	3670	3648	**3659**	22.0	21.8	**21.9**
1.5% MLP	0.223	0.222	**0.223**	3649	3661	**3655**	21.8	21.8	**21.8**
Mean	**0.223**	**0.222**	**0.223**	**3658**	**3655**	**3656**	**21.9**	**21.8**	**21.8**
	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.0008	0.0015	0.0021	8.58	16.04	22.69	0.093	0.173	0.245
CD (P = 0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD (P = 0.01)	NS	NS	NS	NS	NS	NS	NS	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 66. Effect of botanicals seed treatments on plant height (cm) in *rabi* 2012

Age of seeds (A) / Botanicals (B)	Vegetative stage (20 DAS)			Flowering stage (40 DAS)			Harvesting stage (60 DAS)		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	11.1	8.4	**9.8**	28.1	25.7	**26.9**	38.0	35.7	**36.9**
FSP @ 3g kg^{-1}	13.4	10.2	**11.8**	28.7	26.6	**27.7**	38.6	36.5	**37.6**
CALP @ 4g kg^{-1}	13.2	9.9	**11.6**	28.6	26.1	**27.4**	38.5	36.0	**37.3**
MLP @ 4g kg^{-1}	12.5	9.0	**10.8**	28.3	26.2	**27.3**	38.2	36.0	**37.1**
1% FSP	13.6	10.5	**12.1**	29.0	26.7	**27.9**	38.8	36.6	**37.7**
1.5% CALP	13.4	10.2	**11.8**	29.0	26.2	**27.6**	39.0	36.0	**37.5**
1.5% MLP	12.8	9.9	**11.4**	28.8	26.3	**27.6**	38.8	36.3	**37.6**
Mean	**12.9**	**9.7**	**11.3**	**28.6**	**26.3**	**27.5**	**38.6**	**36.2**	**37.4**
	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.007	0.014	0.020	0.129	0.242	0.342	0.127	0.238	0.336
CD (P = 0.05)	0.015	0.029	0.040	0.265	NS	NS	0.261	NS	NS
CD (P = 0.01)	0.021	0.039	0.055	0.359	NS	NS	0.353	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 67. Effect of botanicals seed treatments on leaf area (cm^2) in *rabi* 2012

Age of seeds (A) / Botanicals (B)	Vegetative stage (20 DAS)			Flowering stage (40 DAS)			Harvesting stage (60 DAS)		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	18.9	16.7	**17.8**	442.4	364.8	**403.6**	1962.6	1592.2	**1777.4**
FSP @ 3g kg^{-1}	43.9	36.2	**40.1**	458.9	380.3	**419.6**	1964.0	1702.8	**1833.4**
CALP @ 4g kg^{-1}	41.0	34.6	**37.8**	450.1	375.1	**412.6**	1964.0	1688.7	**1826.4**
MLP @ 4g kg^{-1}	37.5	26.8	**32.2**	440.1	370.0	**405.1**	1926.6	1660.0	**1793.3**
1% FSP	47.9	37.1	**42.5**	462.2	381.7	**422.0**	1990.2	1703.6	**1846.9**
1.5% CALP	45.1	37.1	**41.1**	462.3	373.4	**417.9**	1983.5	1685.4	**1834.5**
1.5% MLP	35.0	30.4	**32.7**	447.5	370.1	**408.8**	1952.5	1678.8	**1815.7**
Mean	**38.5**	**31.3**	**34.9**	**451.9**	**373.6**	**412.8**	**1963.3**	**1673.1**	**1818.2**
	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.322	0.602	0.851	5.73	10.71	15.15	15.22	28.47	40.27
CD (P = 0.05)	0.661	1.237	1.750	11.77	NS	NS	31.29	NS	NS
CD (P = 0.01)	0.894	1.672	2.365	15.91	NS	NS	42.30	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

While in aged seeds both 1% fenugreek seed powder solution and 1.5% custard apple leaf powder solution produced the maximum leaf area of 37.1 cm^2 (Table 67).

4.4.2.1.3. Leaf area index

Results showed significant differences for treatments and their interactions up to 20 days after sowing. No significant differences were evident after 40 and 60 days of sowing except between fresh and aged seeds. After 20 days of sowing, 1% fenugreek seed powder solution treated seeds registered the maximum leaf area index of 0.142 followed by 1.5% custard apple leaf powder solution (0.137). The interaction effect reveals the maximum leaf area index by 1% fenugreek seed powder solution in fresh seeds (0.160). While in aged seeds both 1% fenugreek seed powder solution and 1.5% custard apple leaf powder solution produced the maximum leaf area index of 0.124 (Table 68).

4.4.2.1.4. Dry matter production (g $plant^{-1}$)

Significant differences were exhibited due to treatments and ageing of seeds at all the stages (20, 40 and 60 days after sowing). At vegetative and flowering stages, seeds soaked with 1% fenugreek seed powder solution for 1h recorded higher dry weight (2.01, 7.19 and 11.06 g at 20, 40 and 60 days after sowing, respectively). The interaction effect was significant only at 20 days after sowing and not in later stages. At 20 days after sowing, soaking in 1% fenugreek seed powder solution for 1h recorded the maximum dry weight of 2.14 g in fresh seeds and 1.88 g in aged seeds. The improvement was 13 and 18 per cent at 20 days after sowing over fresh and aged control, respectively (Table 69).

4.4.2.1.5. Chlorophyll content

Ageing of seeds, botanical treatments and their interaction effects did not show significant differences for chlorophyll content at flowering (40 days after sowing) and harvesting stage (60 days after sowing). However, at vegetative stage (20 days after sowing) significant difference in chlorophyll content between fresh and aged seeds was observed, while there was no significant difference among the treatments and its interactions (Table 70).

Table 68. Effect of botanicals seed treatments on leaf area index (LAI) in *rabi* 2012

Age of seeds (A) / Botanicals (B)	Vegetative stage (20 DAS)			Flowering stage (40 DAS)			Harvesting stage (60 DAS)		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	0.063	0.056	**0.060**	1.475	1.216	**1.346**	6.542	5.307	**5.925**
FSP @ 3g kg^{-1}	0.146	0.121	**0.134**	1.530	1.268	**1.399**	6.547	5.676	**6.112**
CALP @ 4g kg^{-1}	0.137	0.115	**0.126**	1.500	1.250	**1.375**	6.547	5.629	**6.088**
MLP @ 4g kg^{-1}	0.125	0.089	**0.107**	1.467	1.233	**1.350**	6.422	5.533	**5.978**
1% FSP	0.160	0.124	**0.142**	1.541	1.272	**1.407**	6.634	5.679	**6.157**
1.5% CALP	0.150	0.124	**0.137**	1.541	1.245	**1.393**	6.612	5.618	**6.115**
1.5% MLP	0.117	0.101	**0.109**	1.492	1.234	**1.363**	6.508	5.596	**6.052**
Mean	**0.128**	**0.104**	**0.116**	**1.507**	**1.245**	**1.376**	**6.545**	**5.577**	**6.061**
	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.0011	0.0020	0.0029	0.019	0.036	0.051	0.051	0.095	0.134
CD (P = 0.05)	0.0022	0.0042	0.0059	0.039	NS	NS	0.104	NS	NS
CD (P = 0.01)	0.0030	0.0057	0.0080	0.053	NS	NS	0.141	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 69. Effect of botanicals seed treatments on dry matter production (g plant^{-1}) in *rabi* 2012

Age of seeds (A) / Botanicals (B)	Vegetative stage (20 DAS)			Flowering stage (40 DAS)			Harvesting stage (60 DAS)		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	1.90	1.59	**1.75**	6.62	5.55	**6.09**	11.59	9.70	**10.65**
FSP @ 3g kg^{-1}	2.12	1.82	**1.97**	7.27	6.52	**6.90**	11.91	10.09	**11.00**
CALP @ 4g kg^{-1}	2.06	1.76	**1.91**	7.12	6.37	**6.75**	11.73	10.02	**10.88**
MLP @ 4g kg^{-1}	1.93	1.67	**1.80**	6.78	5.79	**6.29**	11.64	9.74	**10.69**
1% FSP	2.14	1.88	**2.01**	7.59	6.78	**7.19**	12.00	10.12	**11.06**
1.5% CALP	2.09	1.83	**1.96**	7.42	6.58	**7.00**	11.79	10.01	**10.90**
1.5% MLP	2.01	1.80	**1.91**	7.03	6.37	**6.70**	11.67	9.81	**10.74**
Mean	**2.04**	**1.76**	**1.90**	**7.12**	**6.28**	**6.70**	**11.76**	**9.93**	**10.84**
	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.005	0.009	0.012	0.027	0.050	0.070	0.053	0.100	0.141
CD (P = 0.05)	0.010	0.018	0.025	0.054	0.102	NS	0.110	0.205	NS
CD (P = 0.01)	0.013	0.024	0.034	0.074	0.138	NS	0.148	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 70. Effect of botanicals seed treatments on chlorophyll content (SPAD value) in *rabi* 2012

Age of seeds (A) / Botanicals (B)	Vegetative stage (20 DAS)			Flowering stage (40 DAS)			Harvesting stage (60 DAS)		
	Fresh seeds	Aged seeds	Mean	Fresh seeds	Aged seeds	Mean	Fresh seeds	Aged seeds	Mean
Control	32.2	29.0	**30.6**	44.7	44.4	**44.6**	30.3	30.1	**30.2**
FSP @ 3g kg^{-1}	32.7	29.6	**31.2**	45.3	45.0	**45.2**	30.8	30.6	**30.7**
CALP @ 4g kg^{-1}	32.5	29.5	**31.0**	45.1	44.8	**45.0**	30.6	30.4	**30.5**
MLP @ 4g kg^{-1}	32.3	29.0	**30.7**	44.9	44.1	**44.5**	30.4	29.9	**30.2**
1% FSP	32.8	29.8	**31.3**	45.5	45.3	**45.4**	30.9	30.7	**30.8**
1.5% CALP	32.6	29.6	**31.1**	45.2	45.0	**45.1**	30.7	30.5	**30.6**
1.5% MLP	32.5	29.5	**31.0**	45.1	44.8	**45.0**	30.6	30.4	**30.5**
Mean	**32.5**	**29.4**	**31.0**	**45.1**	**44.8**	**44.9**	**30.6**	**30.4**	**30.5**
	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.134	0.252	0.356	0.183	0.343	0.484	0.119	0.223	0.316
CD (P = 0.05)	0.276	NS	NS	NS	NS	NS	NS	NS	NS
CD (P = 0.01)	0.374	NS	NS	NS	NS	NS	NS	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

4.4.2.1.6. Crop Growth Rate (CGR) (g $m^{-2}d^{-1}$)

Significant differences were observed due to treatments and ageing of seeds. Among the treatments, maximum crop growth rate was recorded for seeds soaked in 1% fenugreek seed powder solution (8.63 g m^{-2} d^{-1}) and 1.5% custard apple leaf powder solution for 1h (8.40 g $m^{-2}d^{-1}$) which were on par with each other. The interaction effect was not significant (Table 71).

4.4.2.1.7. Days to 50% flowering

Treatments have not shown any significant effect on days to 50 per cent flowering. But, crop raised from fresh seeds flowered earlier than crop raised from aged seeds. Crop raised from aged seeds, flowering was delayed one day. The interaction effect was also not significant for days to 50% flowering (Table 71).

4.4.2.2. Effect of botanical seed treatments on yield attributes

4.4.2.2.1. Number of pods per plant

Significant difference in number of pods per plant could be observed in botanicals treatment and ageing of seeds. However, interaction effect was not significant. Among the botanicals seed treatment, all the treatment registered significantly higher number of pods per plant than control except seeds dry dressed with moringa leaf powder @ 4 g kg^{-1} of seeds which was on par with control. Between fresh and aged seeds, fresh seeds (34) registered higher number of pods per plant than aged seeds (30) (Table 72)

4.4.2.2.2. Pod yield per plant (g)

Pod yield per plant was significantly differed in botanicals treatment and ageing of seeds. However, interaction effect was not significant. Among the botanicals seed treatment, all the treatment registered significantly higher pod yield per plant than control except seeds dry dressed with moringa leaf powder @ 4 g kg^{-1} of seeds which was on par with control. Between fresh and aged seeds, fresh seeds (16.96 g) registered higher pod yield per plant than aged seeds (14.90 g) (Table 72).

Table 71. Effect of botanicals seed treatments on crop growth rate (CGR), and days to 50% flowering in *rabi* 2012

Age of seeds (A) / Botanicals (B)	CGR (g m^{-2} d^{-1})			Days to 50% flowering		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	7.88	6.61	**7.25**	39	40	**40**
FSP @ 3g kg^{-1}	8.58	7.83	**8.21**	38	39	**39**
CALP @ 4g kg^{-1}	8.44	7.68	**8.06**	39	40	**40**
MLP @ 4g kg^{-1}	8.08	6.87	**7.48**	39	41	**40**
1% FSP	9.09	8.16	**8.63**	38	39	**39**
1.5% CALP	8.88	7.91	**8.40**	38	40	**39**
1.5% MLP	8.38	7.62	**8.00**	39	40	**40**
Mean	**8.48**	**7.53**	**8.00**	**39**	**40**	**39**
	A	B	A × B	A	B	A × B
SEd	0.078	0.146	0.206	0.180	0.337	0.477
CD (P = 0.05)	0.160	0.300	NS	0.371	NS	NS
CD (P = 0.01)	0.217	0.406	NS	0.501	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 72. Effect of botanicals seed treatments on pod yield attributes in *rabi* 2012

Age of seeds (A) / Botanicals (B)	No. of pods per plant			Pod yield per plant (g)			Pod yield per plot (kg)			Pod yield per ha (kg)		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	32	27	**30**	16.11	13.24	**14.68**	1.37	1.13	**1.25**	1140.5	937.2	**1038.9**
FSP @ 3g kg^{-1}	35	31	**33**	17.49	15.53	**16.51**	1.49	1.32	**1.41**	1238.2	1099.8	**1169.0**
CALP @ 4g kg^{-1}	34	32	**33**	17.08	15.39	**16.24**	1.45	1.31	**1.38**	1209.0	1090.0	**1149.5**
MLP @ 4g kg^{-1}	33	28	**31**	16.21	13.54	**14.88**	1.38	1.16	**1.27**	1147.9	959.0	**1053.5**
1% FSP	36	32	**34**	17.86	15.70	**16.78**	1.52	1.34	**1.43**	1264.1	1111.5	**1187.8**
1.5% CALP	35	31	**33**	17.24	15.60	**16.42**	1.47	1.33	**1.40**	1220.5	1104.2	**1162.4**
1.5% MLP	34	30	**32**	16.73	15.29	**16.01**	1.42	1.30	**1.36**	1184.2	1082.5	**1133.4**
Mean	**34**	**30**	**32**	**16.96**	**14.90**	**15.93**	**1.44**	**1.27**	**1.36**	**1200.6**	**1054.9**	**1127.8**
	A	B	A × B	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.53	1.00	1.41	0.276	0.516	0.729	0.023	0.043	0.061	19.5	36.5	51.6
CD (P = 0.05)	1.09	2.05	NS	0.567	1.060	NS	0.048	0.089	NS	40.1	75.1	NS
CD (P = 0.01)	1.48	NS	NS	0.766	NS	NS	0.065	NS	NS	54.2	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

4.4.2.2.3. Pod yield per plot (kg)

Pod yield per plot was also significantly influenced due to botanical treatments and ageing of seeds. However, interaction effect was not significant. Among the botanical seed treatments, all the treatments registered significantly maximum pod yield per plot than control except seeds dry dressed with moringa leaf powder @ 4 g kg^{-1} of seeds which was on par with control. Between fresh and aged seeds, fresh seeds (1.44 kg) registered higher pod yield per plot than aged seeds (1.27 kg) (Table 72).

4.4.2.2.4. Pod yield per hectare (kg)

Pod yield per hectare was also significantly influenced by botanicals treatment and ageing of seeds. However, interaction effect was not significant. Among the botanicals seed treatment, all the treatments registered significantly maximum pod yield per hectare than control except seeds dry dressed with moringa leaf powder @ 4 g kg^{-1} of seeds which was on par with control. Between fresh and aged seeds, fresh seeds (1200.6 kg) registered higher pod yield per hectare than aged seeds (1054.9 kg). The increase in pod yield per hectare was 11 and 19 per cent over control of fresh and aged seeds respectively (Table 72).

4.4.2.2.5. Seed yield per plant (g)

Seed yield per plant was significantly differed due to botanicals treatment and ageing of seeds. However, interaction effect was not significant. Among the botanicals seed treatment, seeds treated with 1.0% fenugreek seed powder solution (9.41 g) registered significantly higher seed yield per plant which was on par with seeds dry dressed with fenugreek seed powder @ 3 g kg^{-1} of seeds, 1.5% custard apple and moringa leaf powder solution and custard apple leaf powder @ 4 g kg^{-1} of seeds. The lowest seed yield per plant was observed in control (8.24 g) which was on par with seeds dry dressed with moringa leaf powder @ 4 g kg^{-1} of seeds. Between fresh and aged seeds, fresh seeds (9.54 g) registered higher seed yield per plant than aged seeds (8.36 g) (Table 73).

Table 73. Effect of botanicals seed treatments on seed yield attributes in *rabi* 2012

Age of seeds (A) / Botanicals (B)	Seed yield per plant (g)			Seed yield per plot (kg)			Seed yield per ha (kg)			Seed recovery (%)		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	9.04	7.43	**8.24**	0.768	0.631	**0.700**	640.0	526.0	**583.0**	58.82	58.37	**58.60**
FSP @ 3g kg^{-1}	9.84	8.69	**9.27**	0.837	0.739	**0.788**	696.9	615.4	**656.2**	58.74	58.88	**58.81**
CALP @ 4g kg^{-1}	9.60	8.64	**9.12**	0.816	0.734	**0.775**	679.9	611.4	**645.7**	58.95	58.85	**58.90**
MLP @ 4g kg^{-1}	9.15	7.60	**8.38**	0.778	0.646	**0.712**	647.7	538.1	**592.9**	59.10	59.24	**59.17**
1% FSP	10.00	8.81	**9.41**	0.850	0.749	**0.800**	708.3	623.6	**666.0**	59.71	58.83	**59.27**
1.5% CALP	9.72	8.75	**9.24**	0.826	0.743	**0.785**	688.2	619.3	**653.8**	58.94	58.99	**58.97**
1.5% MLP	9.43	8.58	**9.01**	0.802	0.729	**0.766**	668.0	607.5	**637.8**	58.91	58.90	**58.91**
Mean	**9.54**	**8.36**	**8.95**	**0.811**	**0.710**	**0.761**	**675.6**	**591.6**	**633.6**	**59.02**	**58.87**	**58.95**
	A	B	A × B	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.154	0.288	0.408	0.013	0.024	0.035	10.91	20.40	28.85	0.028	0.052	0.074
CD (P = 0.05)	0.317	0.592	0.838	0.027	0.050	NS	22.42	41.95	NS	0.057	NS	NS
CD (P = 0.01)	0.428	NS	NS	0.036	NS	NS	30.31	NS	NS	0.077	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

4.4.2.2.6. Seed yield per plot (kg)

Seed yield per plot was also significantly influenced by botanicals treatment and ageing of seeds. However, interaction effect was not significant. Among the botanicals seed treatment, all the treatments registered significantly maximum seed yield per plot than control except seeds dry dressed with moringa leaf powder @ 4 g kg^{-1} of seeds which was on par with control. Between fresh and aged seeds, fresh seeds (0.811 kg) registered higher seed yield per plot than aged seeds (0.710 kg) (Table 73).

4.4.2.2.7. Seed yield per hectare (kg)

Seed yield per hectare was also significantly influenced by botanicals treatment and ageing of seeds. However, interaction effect was not significant. Among the botanicals seed treatment, seeds treated with 1.0% fenugreek seed powder solution (666.0 kg) registered significantly higher seed yield per hectare which was on par with seeds dry dressed with fenugreek seed powder @ 3 g kg^{-1} of seeds, 1.5% custard apple leaf powder solution and custard apple leaf powder @ 4 g kg^{-1} of seeds. The lowest seed yield per hectare was observed in control (583.0 kg) which was on par with seeds dry dressed with moringa leaf powder @ 4 g kg^{-1} of seeds. Between fresh and aged seeds, fresh seeds (675.6 kg) registered higher seed yield per hectare than aged seeds (591.6 kg). The increase in seed yield per hectare was 9 and 17 per cent over control of fresh and aged seeds, respectively (Table 73).

4.4.2.2.8. Seed recovery (%)

Seed recovery was not significantly influenced by the botanicals seed treatment. However, significant difference was observed between fresh and aged seeds. In which, fresh seeds registered higher seed recovery of 59.02% than aged seeds (58.87%) (Table 73).

4.4.2.3. Effect of botanical seed treatments on quality of resultant seeds

The resultant seeds of different treatments recorded non-significant values for seed germination, shoot length, root length, dry matter production, vigour index I and vigour index II (Table 74 and 75).

Table 74. Effect of botanicals seed treatments on resultant seed quality in *rabi* 2012

Age of seeds (A) / Botanicals (B)	Germination (%)			Shoot length (cm)			Root length (cm)		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	98 (81.87)	98 (81.87)	**98 (81.87)**	21.32	21.31	**21.32**	15.89	15.88	**15.89**
FSP @ 3g kg^{-1}	99 (84.26)	98 (81.87)	**99 (84.26)**	21.37	21.35	**21.36**	15.94	15.92	**15.93**
CALP @ 4g kg^{-1}	98 (81.87)	99 (84.26)	**99 (84.26)**	21.37	21.34	**21.36**	15.91	15.91	**15.91**
MLP @ 4g kg^{-1}	99 (84.26)	98 (81.87)	**99 (84.26)**	21.33	21.31	**21.32**	15.90	15.88	**15.89**
1% FSP	98 (81.87)	99 (84.26)	**99 (84.26)**	21.38	21.37	**21.38**	15.95	15.94	**15.95**
1.5% CALP	99 (84.26)	98 (81.87)	**99 (84.26)**	21.36	21.34	**21.35**	15.93	15.91	**15.92**
1.5% MLP	98 (81.87)	99 (84.26)	**99 (84.26)**	21.35	21.32	**21.34**	15.92	15.89	**15.91**
Mean	**98 (81.87)**	**98 (81.87)**	**98 (81.87)**	**21.35**	**21.33**	**21.34**	**15.92**	**15.90**	**15.91**
	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.233	0.435	0.716	0.037	0.069	0.098	0.036	0.068	0.096
CD (P = 0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD (P = 0.01)	NS	NS	NS	NS	NS	NS	NS	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

Table 75. Effect of botanicals seed treatments on resultant seed quality in *rabi* 2012

Age of seeds (A) / Botanicals (B)	Dry matter production (g / 10 seedlings)			Vigour index I			Vigour index II		
	Fresh seeds	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**	**Fresh seeds**	**Aged seeds**	**Mean**
Control	0.225	0.223	**0.224**	3645	3645	**3645**	22.0	21.9	**22.0**
FSP @ 3g kg^{-1}	0.228	0.227	**0.228**	3675	3652	**3664**	22.5	22.2	**22.4**
CALP @ 4g kg^{-1}	0.226	0.225	**0.226**	3652	3669	**3661**	22.1	22.2	**22.2**
MLP @ 4g kg^{-1}	0.225	0.224	**0.225**	3667	3645	**3656**	22.2	22.0	**22.1**
1% FSP	0.229	0.228	**0.229**	3658	3674	**3666**	22.4	22.5	**22.5**
1.5% CALP	0.227	0.226	**0.227**	3672	3650	**3661**	22.4	22.1	**22.3**
1.5% MLP	0.226	0.225	**0.226**	3652	3665	**3659**	22.1	22.2	**22.2**
Mean	**0.227**	**0.225**	**0.226**	**3660**	**3657**	**3659**	**22.2**	**22.2**	**22.2**
	A	B	A × B	A	B	A × B	A	B	A × B
SEd	0.0007	0.0013	0.0019	8.11	15.18	21.46	0.109	0.204	0.289
CD (P = 0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD (P = 0.01)	NS	NS	NS	NS	NS	NS	NS	NS	NS

FSP: Fenugreek Seed Powder CALP: Custard Apple Leaf Powder MLP: Moringa Leaf Powder

4.5. Standardization of low temperature treatment (-18°C) to curb secondary infestation of pulse beetles (*Callosobruchus maculatus*) in blackgram seeds

4.5.1. Effect of low temperature treatment (-18°C) on different life stages of pulse beetle

4.5.1.1. Tolerance of adult weevils to low temperature

Dead adults were noticed from 10 min of exposure to -18°C amounting to 13% reduction and the number of dead adults increased with increase in exposure duration. After 30 min of exposure, no active adults were noticed, but 40% of adults were moribund and the remaining 60% were dead. After 40 min of exposure 100% of adults were dead (Table 76).

4.5.1.2. Tolerance of immature stages to low temperature

Among the immature stages, egg stage was more tolerant to low temperature than the other immature stages *viz.*, 1^{st} – 2^{nd}, 3^{rd} and 4^{th} instar larva and pupa. Mortality of eggs was not noticed up to 30 min of exposure to low temperature. However, 20% mortality was observed in eggs exposed for 60 min and the mortality rate increased gradually with increase in exposure duration and reached 100% mortality after 180 min exposure (Table 77). Mortality in all the larval stages and pupae were started after 30 min of exposure and reached 100% mortality after 120 min of exposure to low temperature (Table 77).

4.5.2. Effect of low temperature treatment (-18°C) on seed quality parameters during storage

4.5.2.1. Level of seed damage due to pulse beetle infestation during storage

Level of pulse beetle infestation was significantly influenced by the low temperature treatment, storage period and their interaction effect. Among the treatments, the maximum seed damage was observed in untreated control seeds (74.24%) followed by 2 h exposed seeds (51.91%). In 4 h exposed seeds only 0.01% seed damage was observed. After 6 h exposure there was no pulse beetle infestation which was on par with chlorpyriphos @ 2 ml kg-1 treated seeds. Among the storage periods, significant seed

Table 76. Effect of low temperature (-18°C) exposure duration on tolerance of pulse beetle adults to freezing

Exposure (min)	Active	Moribund	Dead	% Reduction
0	15	0	0	0
10	13 (±1)	0	2 (±1)	13
20	7 (±1)	5 (±1)	3 (±1)	20
30	0	6 (±1)	9 (±1)	60
40	0	0	15	100
50	0	0	15	100
60	0	0	15	100
90	0	0	15	100

(Figures in parenthesis indicate ± of standard deviation values)

Table 77. Effect of low temperature (-18°C) exposure duration on tolerance of immature stages of pulse beetle to freezing

Exposure (h)	Immature stages									
	Egg		1st - 2nd instar		3rd instar		4th instar		Pupae	
	Adult emerged	Mortality (%)	Adult emerged	Mortality (%)	Adult emerged	Mortality (%)	Adult emerged	Mortality (%)	Adult emerged	Mortality (%)
0	15	0	15	0	15	0	15	0	15	0
30	15	0	12 (±2)	20 (±10)	13 (±1)	13 (±7)	13 (±1)	13 (±7)	13 (±1)	13 (±3)
60	12 (±1)	20 (±3)	5 (±1)	67 (±7)	8 (±1)	47 (±3)	9 (±1)	40 (±7)	9 (±1)	40 (±7)
90	8 (±2)	47 (±10)	1 (±1)	93 (±3)	2 (±1)	87 (±7)	3 (±1)	80 (±3)	3 (±1)	80 (±3)
120	4 (±1)	73 (±7)	0	100	0	100	0	100	0	100
150	1 (±1)	93 (±3)	0	100	0	100	0	100	0	100
180	0	100	0	100	0	100	0	100	0	100
210	0	100	0	100	0	100	0	100	0	100
240	0	100	0	100	0	100	0	100	0	100

(Figures in parenthesis indicate ± of standard deviation values)

damage was noticed from first month (0.16%) onwards which reached the peak in sixth month (28.58%).

The interaction effect between low temperature treatment and storage period reveals that, untreated control seed was significantly damaged from first month of storage (1.12%) and reached 100% damage after four months of storage. Whereas, in 2 h exposed seeds, significant seed damage was observed after four months storage and reached 100% damage after six months storage. While slight damage was observed in 4 h exposed seeds after three month storage and no significant increase in seed damage was found thereafter. In 6 h exposed seeds and later duration there was no pulse beetle infestation and no seed damage throughout the storage period was observed and it was on par with chlorpyriphos @ 2 ml kg^{-1} treated seeds (Table 78).

4.5.2.2. Seed moisture content (%)

Seed moisture content was significantly influenced by the low temperature treatment, storage period and their interaction effect. Among the treatments, the maximum moisture content was observed in untreated control seeds (9.4%) followed by 2 h exposed seeds (9.0%). Seeds exposed for 4 to 10 h registered lowest moisture content of 8.1% which was on par with chlorpyriphos @ 2 ml kg^{-1} treated seeds. Among the storage periods significant increase in moisture content was noticed from fourth month (8.4%) onwards.

The interaction effect between low temperature treatment and storage period reveals that, in untreated control seeds significant increase in moisture content was recorded from second month of storage (8.3%). While in 2 h exposed seeds, significant increase in moisture content was observed after four months storage (8.4%). However, in 4 to 10 h exposed seeds there were no significant increase in moisture content (Table 79).

4.5.2.3. Seed germination (%)

Seed germination was significantly influenced by the low temperature treatment, storage period and their interaction effect. Among the treatments, the maximum seed germination of 95% was maintained in 4 to 10 h treated seeds and chlorpyriphos @ 2 ml kg^{-1} treated seeds which was on par with each other while the lowest was recorded

Table 78. Effect of low temperature (-18°C) exposure duration on pulse beetle infestation (%) in stored seeds of blackgram cv. TNAU Blackgram CO 6.

Low temperature exposure duration (T)	Storage period (Months) (M)											Mean
	Initial	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
0 h	0.00	1.12	24.05	91.29	100.00	100.00	100.00	100.00	100.00	100.00	100.00	**74.24**
2 h	0.00	0.00	0.02	0.14	1.33	69.42	100.00	100.00	100.00	100.00	100.00	**51.91**
4 h	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	**0.01**
6 h	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
8 h	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
10 h	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
Chlorpyriphos @ 2 ml kg^{-1}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
Mean	**0.00**	**0.16**	**3.44**	**13.06**	**14.48**	**24.21**	**28.58**	**28.58**	**28.58**	**28.58**	**28.58**	**18.02**
	M					T				M × T		
SEd	0.041					0.033				0.109		
CD (P = 0.05)	0.082					0.065				0.216		
CD (P = 0.01)	0.108					0.086				0.286		

Table 79. Effect of low temperature (-18°C) exposure duration on moisture content (%) in stored seeds of blackgram cv. TNAU Blackgram CO 6.

Low temperature treatment (T)	Storage period (Months) (M)						Mean
	Initial	2	4	6	8	10	
0 h	8.1	8.3	9.9	9.9	10.0	10.0	**9.4**
2 h	8.1	8.1	8.4	9.8	9.8	10.0	**9.0**
4 h	8.1	8.1	8.1	8.1	8.1	8.1	**8.1**
6 h	8.1	8.1	8.1	8.1	8.1	8.1	**8.1**
8 h	8.1	8.1	8.1	8.1	8.1	8.1	**8.1**
10 h	8.1	8.1	8.1	8.1	8.1	8.1	**8.1**
Chlorpyriphos @ 2 ml kg^{-1}	8.1	8.1	8.1	8.1	8.1	8.1	**8.1**
Mean	**8.1**	**8.1**	**8.4**	**8.6**	**8.6**	**8.6**	**8.4**
	M		T		M × T		
SEd	0.028		0.031		0.075		
CD (P = 0.05)	0.057		0.061		0.150		
CD (P = 0.01)	0.075		0.081		0.198		

in untreated control seeds (31%). Among the storage periods, reduction in germination was observed with increase in storage period and significant reduction in germination was observed from fourth month (81%) onwards.

The interaction effect between low temperature treatment and storage period reveals that, loss of germination was rapid in untreated control seeds reaching 0% at fourth month followed by 2 h treated seeds. However, in 4 to 10 h exposed seeds, initial germination of 98% was reduced to 90% after 10 months of storage which was on par with chlorpyriphos @ 2 ml kg^{-1} treated seeds (Table 80).

4.5.2.4. Shoot length (cm)

Shoot length was significantly influenced by the low temperature treatment, storage period and their interaction effect. Among the treatments, longer shoot of 19.98 cm was registered in 4 h treated seeds which were on par with 6 to 10 h and chlorpyriphos @ 2 ml kg^{-1} treated seeds while the shorter shoot was recorded in untreated control seeds (6.99 cm). Among the storage periods, reduction in shoot length was observed with increase in storage period and significant reduction in shoot length was observed from fourth month (17.75 cm) onwards.

The interaction effect between low temperature treatment and storage period reveals that, reduction in shoot length was rapid in untreated control seeds reaching 0 cm at fourth month followed by 2 h treated seeds. However, in 4 h exposed seeds the shoot length was reduced only from 21.06 cm at the time of storage to 17.29 cm after 10 months of storage which was on par with 6 to 10 h exposed seeds and chlorpyriphos @ 2 ml kg^{-1} treated seeds (Table 81).

4.5.2.5. Root length (cm)

Root length was significantly influenced by the low temperature treatment, storage period and their interaction effect. Among the treatments, longer root of 14.42 cm was registered in 4 h exposed seeds which was on par with 6 to 10 h and chlorpyriphos @ 2 ml kg^{-1} treated seeds while the shorter root was recorded in untreated control seeds (5.10 cm). Among the storage periods, reduction in root length was observed with

Table 80. Effect of low temperature (-18°C) exposure duration on seed germination (%) in stored seeds of blackgram cv. TNAU Blackgram CO 6.

Low temperature treatment (T)	Storage period (Months) (M)						Mean
	Initial	2	4	6	8	10	
0 h	97 (80.03)	88 (69.73)	0 (0.29)	0 (0.29)	0 (0.29)	0 (0.29)	**31 (33.83)**
2 h	97 (80.03)	97 (80.03)	87 (68.87)	0 (0.29)	0 (0.29)	0 (0.29)	**47 (43.28)**
4 h	98 (81.87)	98 (81.87)	97 (80.03)	95 (77.08)	93 (74.66)	90 (71.57)	**95 (77.08)**
6 h	98 (81.87)	98 (81.87)	97 (80.03)	95 (77.08)	93 (74.66)	90 (71.57)	**95 (77.08)**
8 h	98 (81.87)	98 (81.87)	97 (80.03)	95 (77.08)	93 (74.66)	90 (71.57)	**95 (77.08)**
10 h	97 (80.03)	97 (80.03)	96 (78.47)	94 (75.82)	92 (73.57)	89 (70.63)	**94 (75.82)**
Chlorpyriphos @ 2 ml kg^{-1}	97 (80.03)	97 (80.03)	96 (78.47)	94 (75.82)	92 (73.57)	89 (70.63)	**94 (75.82)**
Mean	**97 (80.03)**	**96 (78.47)**	**81 (64.16)**	**68 (55.55)**	**66 (54.33)**	**64 (53.13)**	**79 (62.73)**
	M			T		M × T	
SEd	0.462			0.499		1.221	
CD (P = 0.05)	0.918			0.992		2.429	
CD (P = 0.01)	1.216			1.314		3.218	

Table 81. Effect of low temperature (-18°C) exposure duration on shoot length (cm) in stored seeds of blackgram cv. TNAU Blackgram CO 6.

Low temperature treatment (T)	Storage period (Months) (M)						Mean
	Initial	**2**	**4**	**6**	**8**	**10**	
0 h	21.04	20.92	0.00	0.00	0.00	0.00	**6.99**
2 h	21.04	20.99	19.87	0.00	0.00	0.00	**10.32**
4 h	21.06	21.01	20.88	20.51	19.13	17.29	**19.98**
6 h	21.05	21.01	20.88	20.51	19.13	17.29	**19.98**
8 h	21.05	21.00	20.87	20.50	19.12	17.28	**19.97**
10 h	21.04	20.99	20.86	20.49	19.12	17.27	**19.96**
Chlorpyriphos @ 2 ml kg^{-1}	21.05	21.00	20.87	20.50	19.12	17.28	**19.97**
Mean	**21.05**	**20.99**	**17.75**	**14.64**	**13.66**	**12.34**	**16.74**
	M			T		M × T	
SEd	0.015			0.017		0.041	
CD (P = 0.05)	0.031			0.033		0.081	
CD (P = 0.01)	0.041			0.044		0.107	

Table 82. Effect of low temperature (-18°C) exposure duration on root length (cm) in stored seeds of blackgram cv. TNAU Blackgram CO 6.

Low temperature treatment (T)	Storage period (Months) (M)						Mean
	Initial	**2**	**4**	**6**	**8**	**10**	
0 h	15.37	15.25	0.00	0.00	0.00	0.00	**5.10**
2 h	15.37	15.32	14.43	0.00	0.00	0.00	**7.52**
4 h	15.39	15.34	15.21	14.84	13.47	12.26	**14.42**
6 h	15.39	15.35	15.22	14.85	13.48	12.27	**14.43**
8 h	15.38	15.33	15.20	14.83	13.46	12.25	**14.41**
10 h	15.37	15.32	15.19	14.82	13.45	12.24	**14.40**
Chlorpyriphos @ 2 ml kg^{-1}	15.37	15.32	15.19	14.82	13.45	12.24	**14.40**
Mean	**15.38**	**15.32**	**12.92**	**10.59**	**9.62**	**8.75**	**12.10**
	M			T		M × T	
SEd	0.008			0.009		0.022	
CD (P = 0.05)	0.017			0.018		0.044	
CD (P = 0.01)	0.022			0.024		0.058	

increase in storage period and significant reduction in root length was observed from second month (15.32 cm) onwards.

The interaction effect between low temperature treatment and storage period reveals that, reduction in root length was rapid in untreated control seeds reaching 0 cm at fourth month followed by 2 h exposed seeds. However, in 4 h exposed seeds the root length was reduced only from 15.39 cm at the time of storage to 12.26 cm after 10 months of storage which was on par with 6 to 10 h exposed seeds and chlorpyriphos @ 2 ml kg^{-1} treated seeds (Table 82).

4.5.2.6. Dry matter production (g / 10 seedlings)

Significant influence on dry matter production was observed due to low temperature treatment, storage period and their interaction effect. Among the treatments, the maximum dry matter production of 0.204 g / 10 seedlings was registered in 4 h exposed seeds which was on par with 6 to 10 h and chlorpyriphos @ 2 ml kg^{-1} treated seeds while the minimum dry matter was recorded in untreated control seeds (0.069). Among the storage periods, reduction in dry matter production was observed with increase in storage period and significant reduction in dry matter was observed from fourth month (0.173) onwards.

The interaction effect between low temperature treatment and storage period reveals that, reduction in dry matter production was rapid in untreated control seeds reaching null at fourth month followed by 2 h exposed seeds. However, in 4 h exposed seeds the dry matter was reduced only from 0.214 g / 10 seedlings at the time of storage to 0.186 g / 10 seedlings after 10 months of storage which was on par with 6 to 10 h exposed seeds and chlorpyriphos @ 2 ml kg^{-1} treated seeds (Table 83).

4.5.2.7. Vigour index I

Significant influence on vigour index I was observed due to low temperature treatment, storage period and their interaction effect. Among the treatments, the maximum vigour index I of 3279 was registered in 8 h exposed seeds which was on par with 4 to 10 h and chlorpyriphos @ 2 ml kg^{-1} treated seeds while the lowest vigour index I was recorded in untreated control seeds (1119). Among the storage periods, reduction in

Table 83. Effect of low temperature (-18°C) exposure duration on dry matter production (g / 10 seedlings) in stored seeds of blackgram cv. TNAU Blackgram CO 6.

Low temperature treatment (T)	Storage period (Months) (M)						Mean
	Initial	2	4	6	8	10	
0 h	0.213	0.198	0.000	0.000	0.000	0.000	**0.069**
2 h	0.213	0.211	0.164	0.000	0.000	0.000	**0.098**
4 h	0.214	0.214	0.210	0.202	0.195	0.186	**0.204**
6 h	0.213	0.213	0.210	0.201	0.194	0.185	**0.203**
8 h	0.214	0.214	0.210	0.202	0.195	0.186	**0.204**
10 h	0.213	0.213	0.210	0.201	0.194	0.185	**0.203**
Chlorpyriphos @ 2 ml kg^{-1}	0.213	0.213	0.210	0.201	0.194	0.185	**0.203**
Mean	**0.213**	**0.211**	**0.173**	**0.144**	**0.139**	**0.132**	**0.169**
	M			T		M × T	
SEd	0.0013			0.0014		0.0035	
CD (P = 0.05)	0.0026			0.0028		0.0069	
CD (P = 0.01)	0.0035			0.0037		0.0092	

Table 84. Effect of low temperature (-18°C) exposure duration on vigour index I in stored seeds of blackgram cv. TNAU Blackgram CO 6.

Low temperature treatment (T)	Storage period (Months) (M)						Mean
	Initial	**2**	**4**	**6**	**8**	**10**	
0 h	3532	3183	0	0	0	0	**1119**
2 h	3532	3522	2984	0	0	0	**1673**
4 h	3553	3544	3482	3340	3015	2644	**3263**
6 h	3552	3563	3501	3359	3032	2660	**3278**
8 h	3570	3560	3498	3356	3030	2657	**3279**
10 h	3532	3522	3461	3319	2995	2626	**3243**
Chlorpyriphos @ 2 ml kg^{-1}	3532	3522	3461	3320	2996	2627	**3243**
Mean	**3543**	**3488**	**2912**	**2385**	**2153**	**1888**	**2728**
	M			T		M × T	
SEd	17.93			19.36		47.43	
CD (P = 0.05)	35.66			38.52		94.35	
CD (P = 0.01)	47.24			51.03		124.99	

Table 85. Effect of low temperature (-18°C) exposure duration on vigour index II in stored seeds of blackgram cv. TNAU Blackgram CO 6.

Low temperature treatment (T)	Storage period (Months) (M)						Mean
	Initial	**2**	**4**	**6**	**8**	**10**	
0 h	20.6	17.4	0.0	0.0	0.0	0.0	**6.3**
2 h	20.7	20.5	14.3	0.0	0.0	0.0	**9.3**
4 h	20.8	20.8	20.3	19.0	18.0	16.6	**19.3**
6 h	20.8	20.9	20.3	19.1	18.0	16.6	**19.3**
8 h	20.9	20.9	20.4	19.1	18.1	16.7	**19.4**
10 h	20.7	20.7	20.1	18.9	17.9	16.5	**19.1**
Chlorpyriphos @ 2 ml kg^{-1}	20.7	20.7	20.1	18.9	17.8	16.5	**19.1**
Mean	**20.7**	**20.3**	**16.5**	**13.6**	**12.8**	**11.8**	**16.0**
	M			T		M × T	
SEd	0.299			0.323		0.791	
CD (P = 0.05)	0.594			0.642		1.572	
CD (P = 0.01)	0.787			0.850		2.083	

vigour index I was observed with increase in storage period and significant reduction in vigour index I was observed from second month (3488) onwards.

The interaction effect between low temperature treatment and storage period reveals that, reduction in vigour index I was rapid in untreated control seeds reaching nil at fourth month followed by 2 h exposed seeds. However, in 4 h exposed seeds vigour index I was reduced only from 3553 at the time of storage to 2644 after 10 months of storage which was on par with 6 to 10 h exposed seeds and chlorpyriphos @ 2 ml kg^{-1} treated seeds (Table 84).

4.5.2.8. Vigour index II

Significant influence on vigour index II was observed due to low temperature treatment, storage period and their interaction effect. Among the treatments, the maximum vigour index II of 19.4 was registered in 8 h exposed seeds which was on par with 4 to 10 h and chlorpyriphos @ 2 ml kg^{-1} treated seeds while the lowest vigour index II was recorded in untreated control seeds (6.3). Among the storage periods, reduction in vigour index II was observed with increase in storage period and significant reduction in vigour index II was observed from fourth month (16.5) onwards.

The interaction effect between low temperature treatment and storage period reveals that, reduction in vigour index II was rapid in untreated control seeds reaching nil at fourth month followed by 2 h exposed seeds. However, in 4 h exposed seeds vigour index II was reduced only from 20.8 at the time of storage to 16.6 after 10 months of storage which was on par with 6 to 10 h exposed seeds and chlorpyriphos @ 2 ml kg^{-1} treated seeds (Table 85).

CHAPTER V

DISCUSSION

Studies were initiated using TNAU Blackgram CO 6 with a view to elicit information on standardization of vigour tests and their relation with field emergence, proteomics of seed deterioration, standardization of botanical seed treatment for maintenance of seed quality during storage, the probable mode of action of seed invigouration for viability maintenance and their effect on field performance and finally standardization of low temperature exposure duration to curb secondary infestation of pulse beetle during storage. The results obtained are discussed hereunder.

5.1. Standardization of vigour test

Vigour test is an analytical procedure to evaluate quality under standardized conditions. Identification of the right variable which significantly influences the test results and incorporation of additional measures for control of each variable except the one to be evaluated in the test conditions (McDonald, 1993) will add value to the test.

There is no universal test for assessing vigour and hence the evaluation of vigour test for predicting planting value nearer to field emergence is important than the germination test (Dahiya *et al.,* 2001; Wang *et al.,* 2004). Vigour test thus provides additional information on relative performance of seed lot under varied environmental conditions (Doornbos, 1995; TeKrony, 2003). In the present study accelerated ageing for different durations, controlled deterioration for varying moisture contents and durations, complex stressing vigour test using different durations at 40^{o}C followed by 5^{o}C, electrical conductivity test using different volume of distilled water and soaking durations at 20°C and mean germination time were attempted in seven different seed lots of TNAU Blackgram CO 6 for standardization of vigour test based on the grouping of seed lots similar to field emergence with higher correlation value.

Standard germination test grouped the seed lots only into two categories *viz*., lots L1, L2, L5, L6 and L7 having higher germination between 97 to 99% followed by lots L3 (95%) and L4 (96%). However, field emergence grouped the seed lots into three groups

viz., L7 (95%) having higher field emergence followed by L1, L2, L5 and L6 (90 – 92%) followed by L3 and L4 (86 - 87%) which recorded the lowest field emergence (Fig. 2).

Among the different vigour tests evaluated, accelerated ageing for 3 days, and electrical conductivity test incubated for 6 h in 75 ml distilled water at 20°C have statistically grouped seed lots similar to grouping derived based on its field emergence.

Correlation analysis of different vigour tests brought out that among the vigour tests evaluated, highest positive association with field emergence was found with accelerated ageing test (r = 0.993) (Fig. 3). The accelerated ageing test exposes seeds for short periods to the two environmental variables namely high temperature and high relative humidity which cause rapid deterioration. High vigour seed lots will withstand these extreme stress conditions and deteriorate at a slower rate than low vigour seed lots (Hampton and TeKrony, 1995).

The potential of accelerated ageing test in predicting the physiological performance, field emergence and classifying the seed lots into different vigour levels was also reported by Rodo and Filho (2003a) in onion, Mavi and Demir (2007) in melon, Havstad *et al.* (2011) in red clover and timothy and Lopes *et al.* (2012) in eggplant.

Standardization of electrical conductivity test conditions was done with different duration of soaking and volume of distilled water. Due to occurrence of split seeds and initiation of radicle protrusion after 8 h of soaking, electrical conductivity measurements after 8 h cannot be carried out. The electrical conductivity of seed leachates was decreased with the increase in volume of distilled water. The 75 ml of distilled water taken in 100 ml beaker which can accommodate the electrode cell was considered as optimum and used for soaking seeds. Therefore, 6 h soaking in 75 ml of distilled water at 20°C was taken as optimum test condition. Similar result was also reported by Demir *et al.* (2012) in radish. In the present study, the comparison of different vigour tests also brought out the highest negative association of electrical conductivity test (incubated for 6 h in 75 ml distilled water at 20°C) with field emergence (r = -0.962) (Fig. 3).

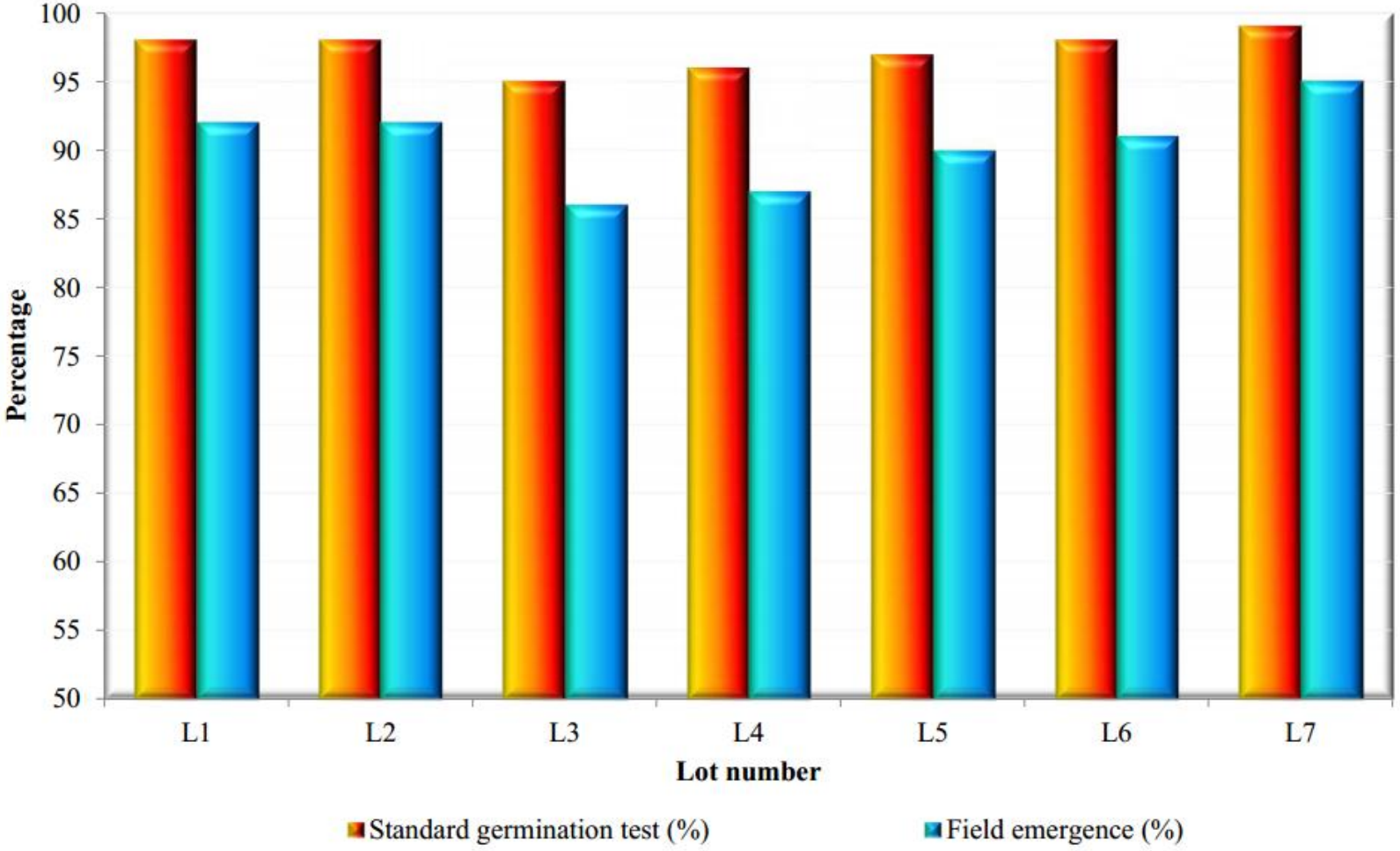


Fig. 2. Evaluation of initial seed quality parameters of seven seed lots of blackgram cv. TNAU Blackgram CO 6

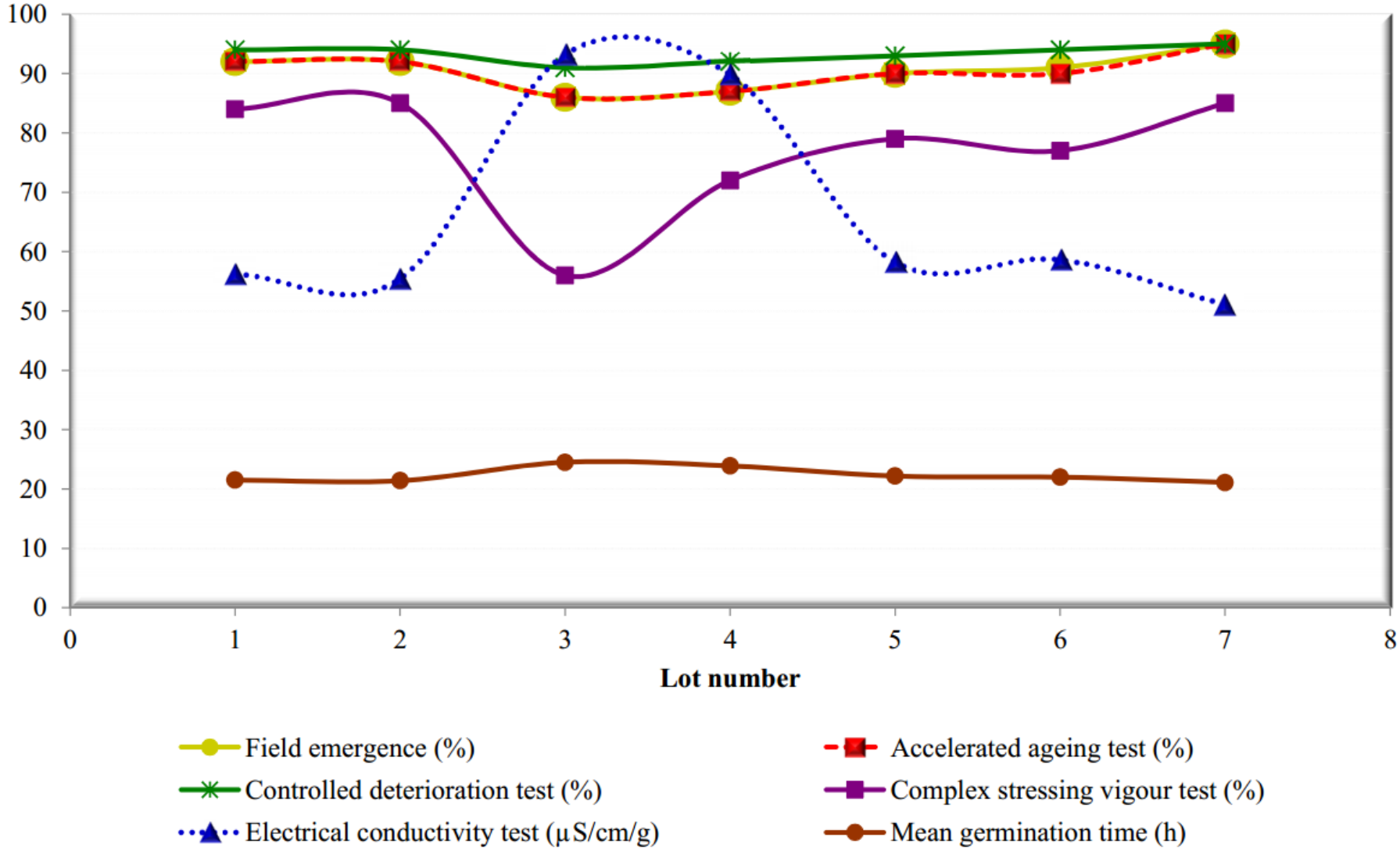


Fig. 3. Scatter diagram representing the relation between different vigour tests and field emergence

Changes in the organization of cell membrane occur during the development of seeds prior to physiological maturity, seed desiccation before harvest and during imbibition prior to germination (Abdul-Baki, 1980). The integrity of cell membranes, determined by deteriorative biochemical changes and/or physical disruption, can be considered the fundamental cause of differences in seed vigour which are indirectly determined as electrolyte leakage during the conductivity test (Powell, 1988).

As seed rehydrates during early imbibition, the ability of its cellular membranes to reorganize and repair any damage that may have occurred will influence the extent of electrolyte leakage from the seed. The greater the speed with which the seed is able to re-establish its membrane integrity the lower the electrolyte leakage. Higher vigour seeds are able to reorganize their membranes more rapidly and repair the damage to a greater extent, than low vigour seeds. Consequently, electrolyte leakage from high vigour seeds is less than compared to low vigour seeds (Matthews and Powell, 2006). The suitability of electrical conductivity test in predicting field emergence, determining seed quality and classifying seed lots in to different vigour status was confirmed by Tajbakhsh (2000) in wheat, Vieira *et al.* (2001) in soybean, Demir *et al.* (2008a) and Matthews *et al.* (2009) in cabbage, Powell (2010) in soybean and Demir *et al.* (2012) in radish.

In conclusion, accelerated ageing for 3 days using 40^{o}C and 98 ± 2% per cent relative humidity and electrical conductivity test by 6 h soaking in 75 ml of distilled water at 20°C can be considered as a suitable vigour test for discriminating TNAU Blackgram CO 6 seed lots based on electrical conductivity value and this formed the basis for further experimentation to study the pattern of seed deterioration in blackgram.

5.2. Pattern of seed deterioration in blackgram through proteomic approach

5.2.1. Physiological and biochemical basis of seed deterioration

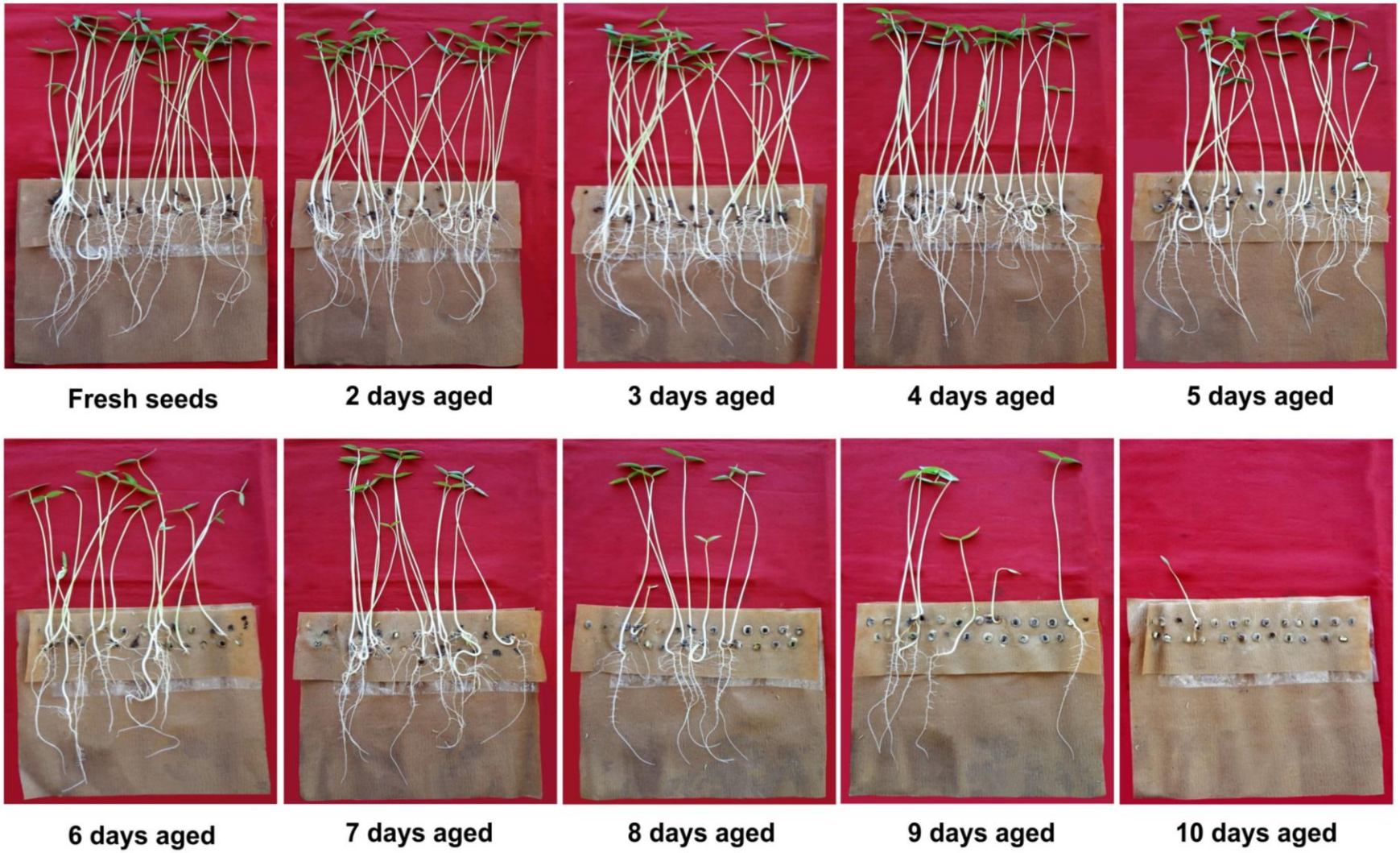
Though seed ageing starts immediately after development and maturation in plants (Anderson and Baker, 1983; McDonald, 2004), freshly harvested and well-processed blackgram seeds usually, have above 90% germination. But when subjected to prolonged storage, the rate of deterioration increases with passage of time and the market value of seed lot declines and ceases when viability percentage reaches below the

prescribed standard of 75% germination. To prevent or slowdown the rate of deterioration, a better understanding of the molecular changes that happens in the seeds having germination below Indian minimum seed certification standard is warranted. In the present investigation, physiological and biochemical basis of seed deterioration was studied by subjecting seeds to accelerated ageing. As expected, all the physiological parameters *viz*., germination, shoot and root length, dry matter and vigour started to decline with increase in ageing duration (Plate 6 and Fig. 4). Similar reduction in physiological performance concomitant with increase in ageing time was also reported by Jatoi *et al.* (2001) in pea, Kapoor *et al*. (2011) in rice and Godakahriz *et al. (*2012) in safflower.

In the present study, biochemical observations revealed that as ageing duration increased, there was a concomitant decrease in DPPH radical scavenging activity and an increase in solute leakages as evidenced from elevated electrical conductivity (Fig. 5). This substantiates the earlier findings that membranes are damaged by increased free radical attack during accelerated ageing of seeds (Bewley, 1986; Crowe *et al.*, 1992; Vertucci and Farrant, 1995; Leprince *et al.,* 1999; Bailly, 2004; Bailly *et al.,* 2008). Lee *et al.* (2010) reported that higher levels of ion leakage in aged non-transgenic tobacco seeds leading to decreased germination rate and suggested that higher expression of antioxidants like Cu/Zn-superoxide dismutase and ascorbate peroxidase genes in transgenic tobacco resulted in reduced ion leakage and maintained seed vigour and viability during ageing. In addition, the protease activity also increased with concomitant increase in free amino acid pool (Fig. 5). Changes in free radical scavenging enzymes, increased free radical production, degradation of protein and DNA and increase in free amino acid were shown as reasons for reduction in vigour and viability during ageing (McDonald, 2004).

Changes in protein pattern showed a reduction in intensity of protein bands in aged seeds and loss of a protein band of molecular weight 60.21 kDa from 6^{th} day of ageing onwards (Plate 3). Loss of protein bands may be due to post-translational modifications and degradation during ageing (Rajjou *et al.*, 2008). The result was in harmony with the findings of Machado *et al*. (2001). Similar loss of bands at 6 and 9 days accelerated aged seeds as compared to control was reported by Vasudevan *et al.* (2012).

Plate 6. Effect of accelerated ageing on germination and seedling growth of blackgram cv. TNAU Blackgram CO 6 seeds



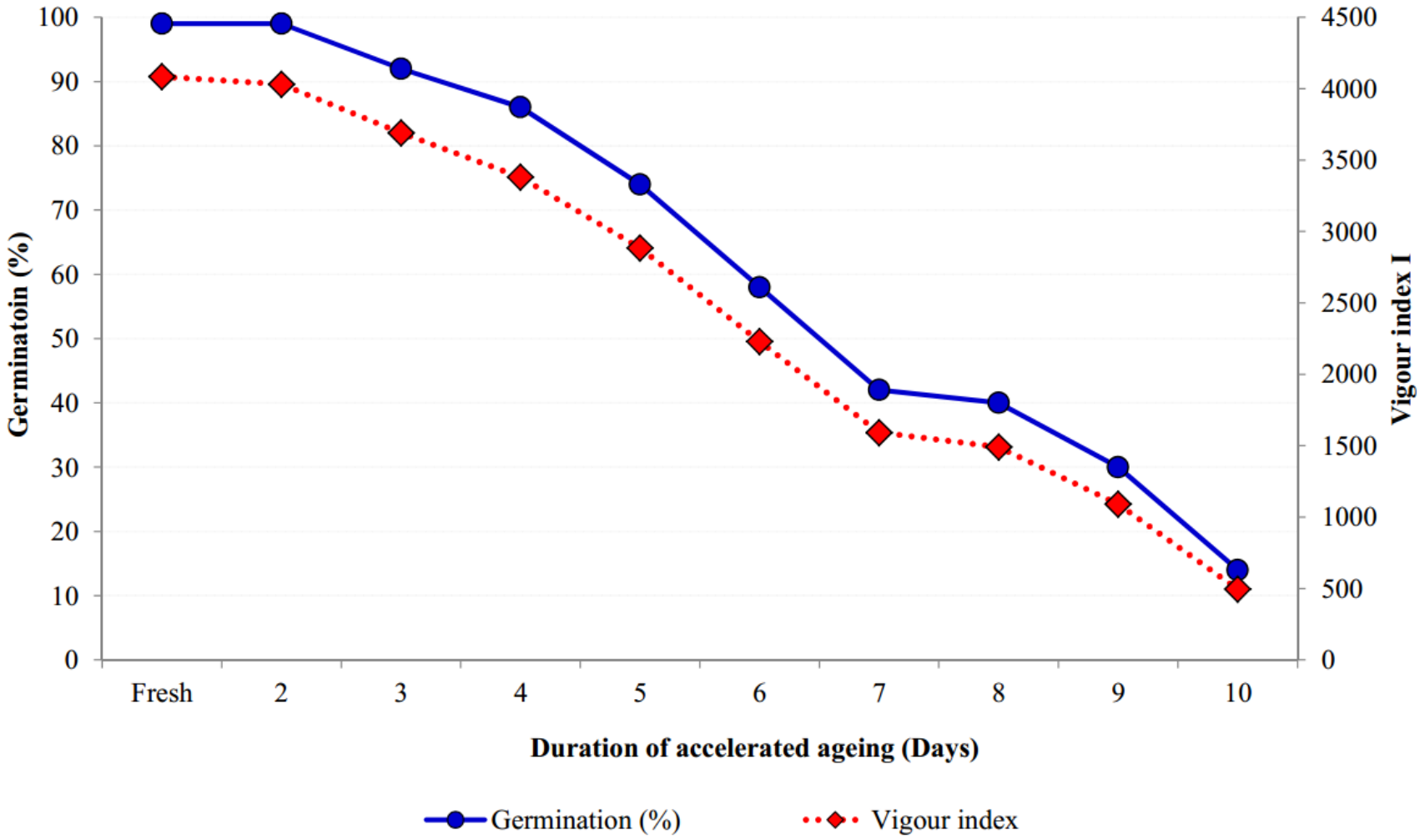


Fig. 4. Effect of duration of accelerated ageing on germination and vigor index I of blackgram seeds

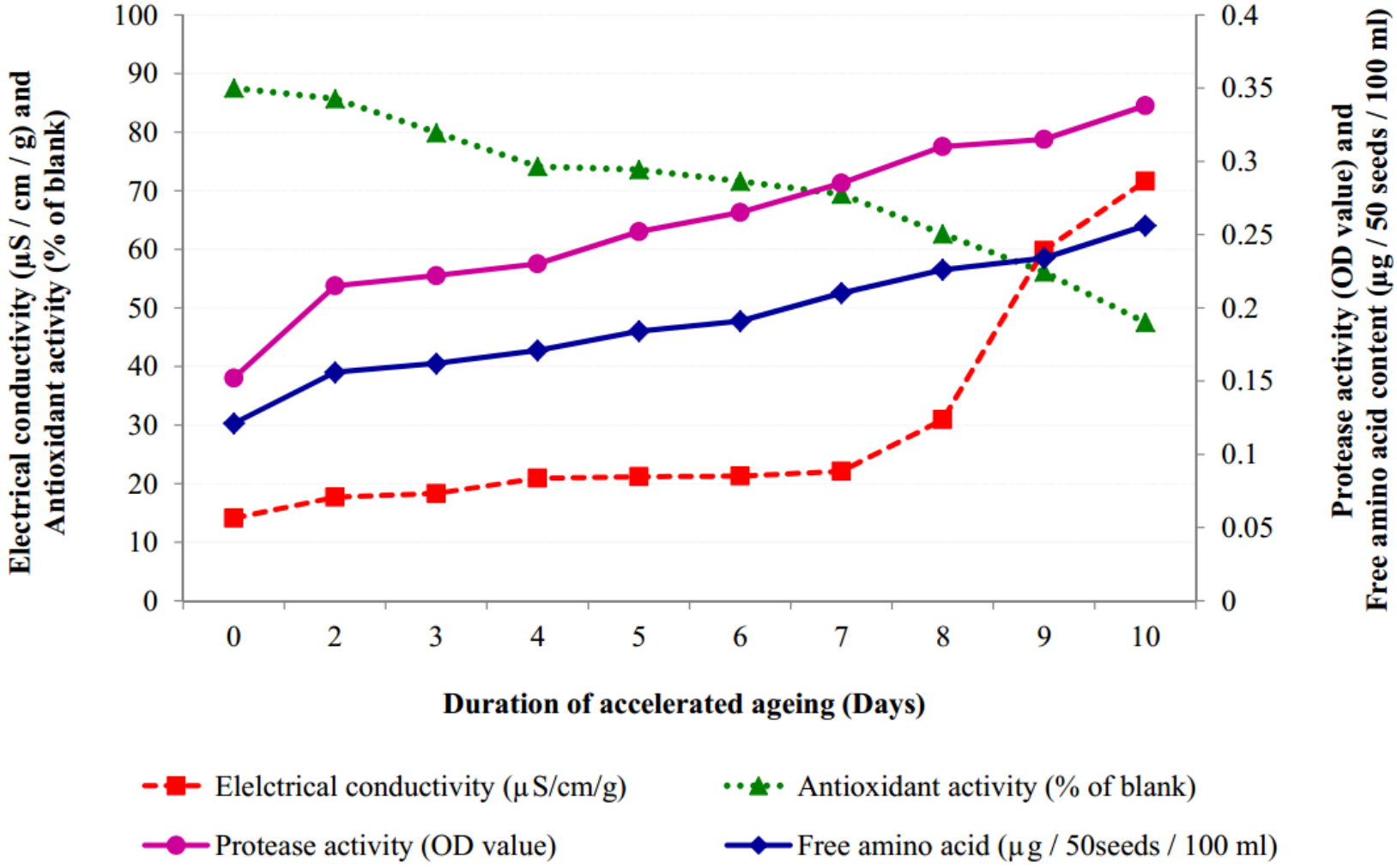


Fig. 5. Effect of accelerated ageing on biochemical characters of blackgram seeds

They also demonstrated that the biochemical changes during accelerated ageing were similar to natural ageing except the rate at which they occur.

These preliminary studies clearly indicated that prolonging ageing time increased the rate of viability loss with concomitant loss in antioxidant activity, cell membrane integrity and proteins, as described earlier. But the most interesting observation was both the viability reduction below germination standard and protein band loss coincided on 6 days of accelerated ageing of seeds, which raise our conjecture that the protein damage has a pivotal command on seed viability. Therefore, further comparative proteome studies with fresh and 6 days accelerated aged seeds were carried out.

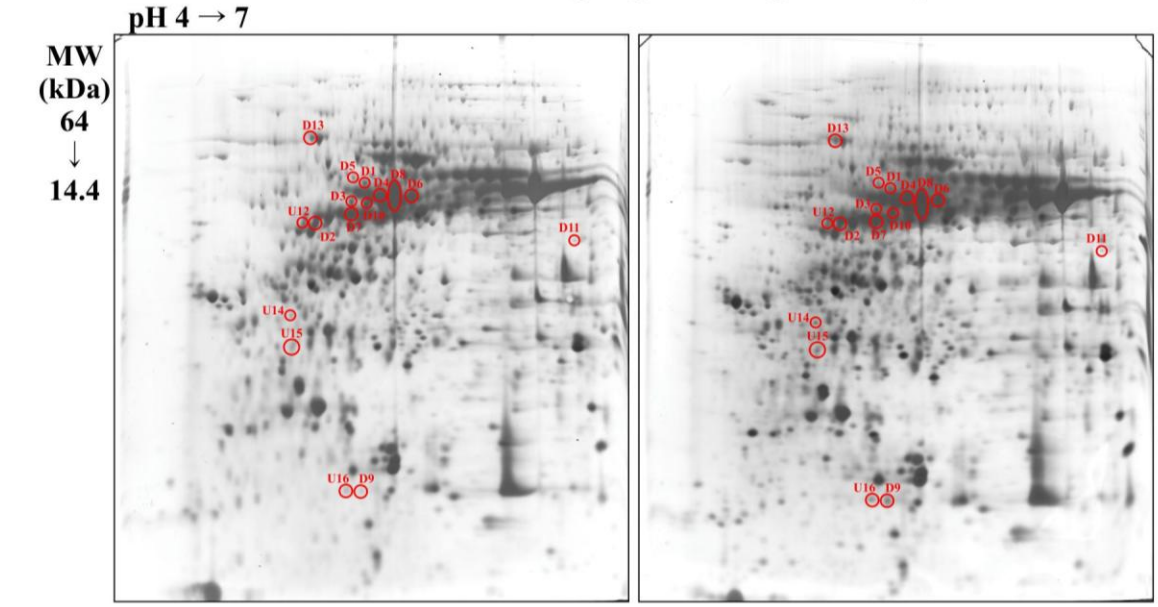
5.2.2. Proteome changes due to seed ageing

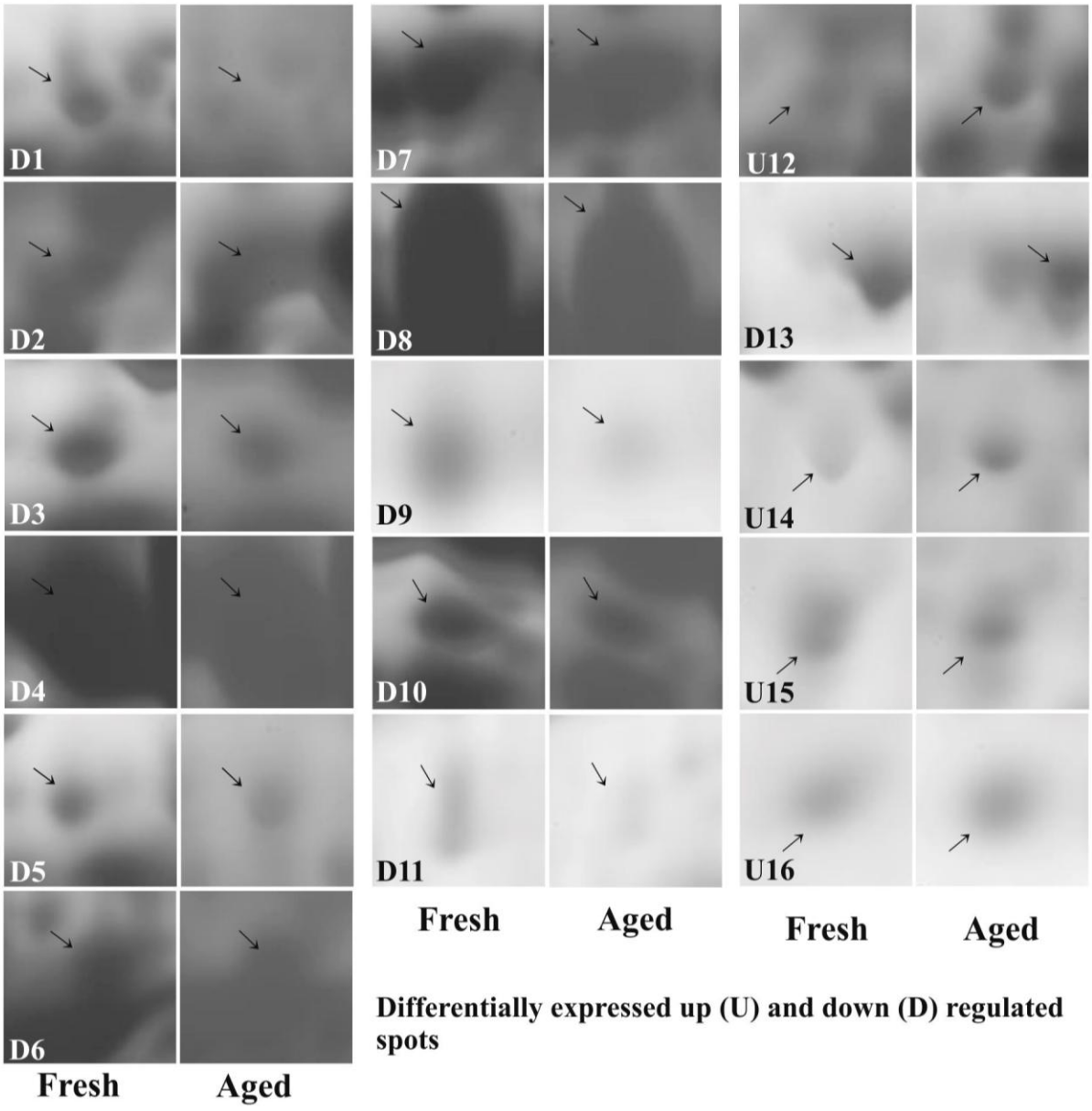
During seed ageing proteins are the major targets for free radical attack due to their abundance in biological systems and their high rate constants for reaction (Davies, 2005) and results in oxidation of protein and loss of their functions with increase in ageing duration (Rajjou *et al.,* 2008). In the present investigation with fresh and 6-days accelerated aged seeds, 4 up-regulated and 12 down-regulated totaling 16 spots were differentially expressed due to accelerated ageing (Plate 7). MALDI-TOF/MS analyses of these 16 differentially expressed spots revealed the identity of the proteins and were grouped into eight functional categories (Fig. 6) based on the classification of Bevan *et al*. (1998).

Down regulation of proteins involved in cell structure maintenance

Proteins involved in maintenance of cell structure are responsible for the intactness of the cell membranes. Many previous studies had positively correlated the loss of cell membrane integrity to loss of seed viability (Sacande *et al.,* 2001; Khan *et al.,* 2004; Ratajczak and Pukacka, 2005; Eliud *et al.,* 2010). In the present investigation, three identified spots (D03, D06 and D11) were related to cell structures, which were down-regulated when compared to fresh seeds (Plate 7). D03 and D11 are actin and actin-101 like proteins respectively, in which actin is a cytoplasmic protein that is capable of self-assembly into dynamic filamentous structures. In plants, actin filaments are presumed to play essential roles in many important processes including cell division, cell elongation, establishment of cytoplasmic organization, cytoplasmic streaming

Plate 7. Effect of accelerated ageing on blackgram seed proteome





Differentially expressed up (U) and down (D) regulated spots

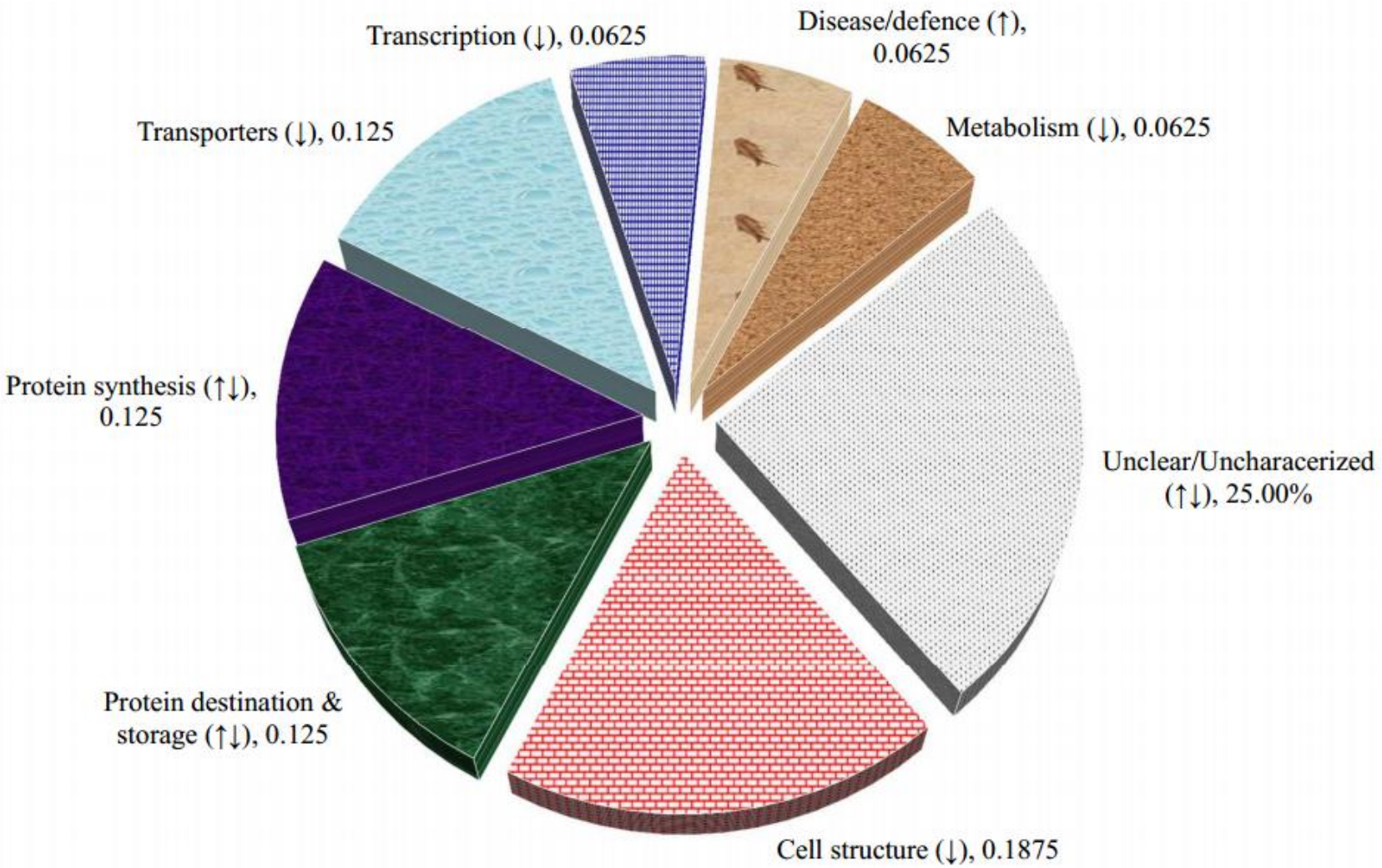


Fig. 6. The functional category distribution of the 16 differentially expressed proteins in blackgram seeds.
The downward arrows (↓) indicate down-regulated proteins and upward arrows (↑) indicate up-regulated proteins .

(Hussey *et al.,* 2006), and changes in response to bacterial signaling molecules arising from pathogens (Dantan-Gonzalez *et al.,* 2001). Several researchers have reported importance of actin in successful seed germination. Ghosh *et al*. (1989) suggested that in mung bean, actin like protein takes part directly or indirectly in cell division during seedling development. Lo *et al*. (2011) reported association of actin to mitochondrial membrane and mtDNA for structural maintenance and suggested that progressive import of actin into cotyledon mitochondria in concert with the conversion of quiescent mitochondria into active forms during seed germination is necessary for the persistent function of mitochondria until the very end of the orderly cell death process that occurs in mung bean seedling cotyledons during germination. A study in common bean by Villanueva *et al*. (1999) and in maize by Diaz-Camino *et al*. (2005) confirmed that concentration of actin in embryonic axis is higher than other part of seeds and concluded that its expression during post-imbibition both at the protein and mRNA levels is essential for morphological changes in developing seedling. This ascertains the role of actin in successful germination. Hence, the down-regulation of actin in aged seeds could result in severe damage to cell membrane leading to its disruption and further substantiate the eminent concept of increased solute leakage in aged seeds.

Apart from actin, another spot D06 related to structural maintenance was also down regulated. It was identified as LIM domain proteins, which act as the core of multiple protein complexes and function in various cellular processes. Cytoplasmic LIM-domain proteins take part in cytoskeletal organization through interaction with cytoskeleton and extracellular matrix (ECM) proteins. LIM Kinases (LIMKs) play an important role in regulating actin cytoskeleton organization in response to various extracellular stimuli (Zheng and Zhao, 2007). The majority of the identified cytoplasmic LIM-domain proteins are found to be ABLIM (actin-binding LIM protein) that binds with F-actin and actin-based cytoskeletons and mediate interactions between actin filaments and cytoplasmic targets, and consists of a C-terminal cytoskeletal domain and four N-terminal LIM motifs (Roof *et al.,* 1997; Barrientos *et al.,* 2007). This down- regulation of LIM domain proteins could add to further loss of actin and disruption of cell structure.

Protein storage and destination

Storage proteins are accumulated in high amounts during mid-maturation stage of seed development and will break down during imbibition to supply amino acids for seed germination and seedling development (Li *et al.,* 2007). Among the storage proteins, globulin comprises major composition in pulses (Bassuner *et al.,* 1983; Mandal and Mandal, 2000). It was well documented that the content of seed storage protein would decrease upon seed aging (Kapoor, 2010). Further loss in seed vigour and viability in aged seeds and inability of low vigour seeds to display a normal proteome during germination can be ascribed to protein changes in dry seeds during ageing (Rajjou *et al.,* 2008). In our investigation, the storage protein, 8S globulin alpha isoform precursor (D10) was down-regulated in aged seeds compared to fresh seeds (Plate 7). This result is in line with that of Xin *et al.* (2011) and Wu *et al.* (2011) who reported down-regulation of globulin in accelerated aged maize seeds. The breakdown of the storage proteins prior to germination might lead to inefficient amino acids supply for synthesis of new proteins essential for seed germination and seedling establishment.

During ageing, proteins might be degraded due to free radical attack and results in improper functioning of proteins. The proteolytic spot U16, identified as 26S proteasome subunit, was up-regulated during ageing. The 26S proteasome is an ATP dependent proteolytic complex that regulates protein turnover in all phases of plant development by degrading ubiquitylated protein (Sullivan *et al.,* 2003). It plays a myriad of roles during seed development (Ferreira *et al.,* 1995) and germination (Miyawaki, *et al.,* 1997; Soos *et al.,* 2010). 26S proteasome is made up of 31 different subunits arranged into two complexes, 20S core protease and two 19S regulatory particles. Later is responsible for identifying ubiquitylated proteins and the former exert catabolic reaction on it and releases amino acids (Moon *et al.,* 2004; Smalle and Vierstra, 2004). Up-regulation of 26S proteasome during ageing indicates the production of damaged proteins and substantiate breakdown of storage proteins. This result is in harmony with that of Delaplace *et al*. (2009) who reported up-regulation of proteasome complexes in aged potato tuber.

Transcription and Protein synthesis

Neo-synthesis of protein from long-lived mRNA during the initial phase of imbibition is essential for successful germination rather than *de novo* transcription. But, *de novo* transcription is essential for subsequent seedling growth and establishment (Rajjou *et al.,* 2004 in *Arabidopsis*; Sano *et al.,* 2012 in rice). In the present study, down-regulated spot D01 was identified as larger subunit of RNA polymerase II (RNAP II) enzyme which plays major role in DNA mediated transcription to synthesis mRNA and other micro-RNAs. Down-regulation of RNAP II could result in unfavorable modifications in the transcription process (Osborne *et al.,* 1974). The abnormality in transcription results in decline and loss of RNA synthesizing activity or the synthesized RNA molecules could be defective in the protein synthesizing system (Sen and Osborne, 1977). Zalewski (1989) related rye seed deterioration to reduced ability of RNA synthesis during germination. The reduced ability of RNA synthesis was due to reduction in polymerase activity of aged seeds (Grilli *et al.,* 1995). Recently, Rajjou *et al.* (2004) correlated the decline in germination rate and subsequent seedling growth of aged *Arabidopsis* seeds to blockage in transcription during later phase of germination.

It was well established in chick pea that cotyledons of dry seed contains preformed mRNA (Matilla and Nicolas, 1980) and translation of these long-lived mRNA is essential for radicle protrusion (Rajjou *et al.,* 2004). Valyl-tRNA synthetase (VARS) is a member of aminoacyl- tRNA synthetases family, which catalyzes the reaction of charging valine with its cognate tRNA during translational process (Tamura *et al.,* 1994). Substantial amount of valine was reportedly found in pulse seeds (Mandal and Mandal, 2000). In the present study, spot D05, which corresponds to VARS, was down-regulated in aged seeds. This down-regulation of VARS during ageing could impair translational addition of valine during imbibition and results in malformed protein. Zhang and Somerville (1997) reported loss of seed viability in transgenic *Arabidopsis*, due to alteration in the expression of VARS. Hence down-regulation of both RNAP II and VARS could be one of the causes for poor germination and reduced growth of seedlings in aged seeds.

In the present study, though translating enzyme VARS was down-regulated in aged seeds, intriguingly, another protein (spot D15) namely elongation factor Tu (EF-Tu), that participate in elongation of protein during translation (Slobin, 1980) was up-regulated in aged seeds. Dell' Aquila *et al.* (1976) reported presence of different forms of EF in wheat embryos and related its presence to viability. Many studies had reported up-regulation of EF-Tu during germination which correlated to increase in protein synthesis especially, proteolytic enzymes (Potokina *et al.,* 2002; Mak *et al.,* 2009; Sano *et al.,* 2012). Our conjecture is that the up-regulation of EF-Tu even in dry seeds might be responsible for up-regulation of other proteins such as proteolytic complex 26S proteasome, defense related protein, pheophorbide a oxygenase and other uncharacterized protein in aged seeds.

Transporters and signal transduction

In the present investigation, two spots D08 and D09 belong to the transportation group. D08 is a Sec14p-like lipid-binding domain, which is found in phosphatidyl inositol transfer family protein and is down-regulated in aged seeds of blackgram. It is a ubiquitous cytosolic domain involved in transport of phospholipids from their site of synthesis in the endoplasmic reticulum and golgi to other cell membranes. They are highly conserved proteins (Kader, 1996). It polarizes membrane growth of *Arabidopsis* root hairs (Vincent *et al.,* 2005). Another spot, D09 is SNARE (soluble N-ethylmaleimide-sensitive factor adaptor protein receptors) domain protein and it was also down-regulated in aged seeds. The SNARE complex is a key regulator of vesicular traffic, executing membrane fusion between transport vesicles or organelles and target membranes (Ebine *et al.,* 2008). It appears that reduction in Sec14p-like lipid-binding domain and SNARE during ageing would therefore impair the transport of cargos' from cotyledons to proliferative embryo during germination and hence might lead to malformed seedlings.

Metabolism

The spot D02, identified as 5' nucleotidase, deoxy (Pyrimidine), cytosolic type C protein, was down-regulated in aged blackgram seeds. Cytosolic 5' nucleotidase catalyzes hydrolysis of phosphate esterified at carbon 5' of the pyrimidine and release nucleoside

and phosphate (Zimmermann, 1992). It was also found to play a major role in cytokinin metabolism (Tomaz and Marina, 2010). Pyrimidine nucleotides are the building blocks for the direct synthesis of DNA and RNA and also participate in the metabolism of a large number of other cellular components from sugar interconversion to cellular polysaccharides to glycoproteins and phospholipids (Kafer *et al.,* 2004). In a study with *Arabidopsis,* Cornelius *et al.* (2011) reported that elevated pyrimidine degradation increased germination, seedling growth and also higher seed production. Therefore, down-regulation of 5' nucleotidase in aged seeds could alters the intracellular pool sizes of pyrimidine nucleotide and affects the vast areas of normal cellular metabolism during seed germination.

Defense / disease

Seeds are prone to microbial infection during ageing. Harman (1983) reviewed the mechanism of seed infection and pathogenesis during seed storage. Seeds usually develop their own natural defense mechanism against pathogen attack (Dalling *et al.,* 2011). In the present study, one of the up- regulated spot U12 has been identified as pheophorbide a oxygenase (PaO). In seeds, studies on role of PaO are scanty except its role in chlorophyll degradation during maturation of canola seeds (Chung *et al.,* 2006). However, in plants, apart from chlorophyll catabolism, PaO has been reported to be a cell death suppressor in *Arabidopsis* (Pruzinska *et al.,* 2003) and maize (Gray *et al.,* 1997). Recently its role in disease tolerance was also reported in wheat (Tang *et al.,* 2013). This study is first to report the up-regulation of PaO in aged seeds. It is proposed that the up-regulation of PaO might be accounted for defense against pathogenic attack during ageing.

Unclear and unknown proteins

In the present study, two spots, D04 and D13 were identified as retrotransposon protein and Ty3-gypsy subclass retrotransposon protein, respectively. Both were down-regulated in aged seeds. Retrotransposons are ubiquitous in plants and plays a major role in genome duplication (Amar Kumar and Bennetzen, 1999). However, their roles in seed are unclear.

Due to the incompleteness of the blackgram protein database and the limitation of MALDI-TOF mass spectrometry, two spots, D07 and U14 identified in the present study, did not match with any known sequence, and therefore the identity of them could not be verified and hence they represent novel candidate genes. Further studies are required to find the roles of these four proteins in seed aging.

Thus, our results revealed significant changes in proteome between fresh and six days accelerated aged seeds. Among the down-regulated proteins, cell structure related proteins were high in number. Maintaining integrity of cell membrane during storage is more important for its viability and also for successful cell multiplication during germination. Apart from this, down-regulation of enzymes like RNAP II, VARS and cytosolic 5'- nucleotidase (pyrimidine) could affect effective transcription, translation and other metabolic process essential for successful germination. Down-regulation of storage protein namely 8S globulin and transporters like Sec-14 lipid-binding domain protein and SNARE could desist from supplying adequate nutrients for prolific embryo during germination. These down-regulations could be mediated through up-regulation of proteolytic complex namely 26S proteasome. The down-regulated proteins in aged seeds might play important roles in the transition of seeds from quiescent to active state during germination and hence, the loss of these proteins may be responsible for the loss of vigour and viability in seeds having germination below Indian minimum seed certification standards. However, to understand the exact contribution of these proteins during germination, further studies are required.

5.3. Standardization of botanical seed treatments

The seeds with good physiological potential act as catalyst for all agricultural inputs. Invariably most crops require storage for one or more planting season, during which period the ageing is inevitable (Coolbear, 1995). Deterioration cannot be prevented completely, but can be delayed. Efforts are required to delay the process of deterioration in order to preserve the vigour and viability of seed until its fullest potential is exploited when sown in the field. In this aspect, chemicals occupy the cheapest mode of treating the seeds to preserve the vigour and viability. Nowadays, products of plant origin called botanicals are being effectively used for maintaining the vigour and viability.

To improve the seed quality and performance, seed invigouration treatments such as seed hardening (Vigneshwari, 2002 in ragi), pelleting (Hazarika *et al.,* 2000 in tomato) and priming (Ananthi, 2008 in maize) etc., have been developed. Several other strategies such as hydration and dehydration, halogenation and antioxidant treatments have been developed that could prevent deterioration and extend the shelf life of seeds as reported by Basu (1985) in lettuce and carrot, Mandal *et al.* (2000) in soybean, Kapri *et al.* (2003) in okra.

Since organic farming is gaining momentum in agriculture, seed treatment with botanicals assumes greater importance. Under these circumstances, an attempt has been made through the present investigation, to use botanicals to reduce the oxidative damage to improve the viability and vigour of fresh and aged seeds of blackgram.

5.3.1. Standardization of seed dry dressing treatment with botanicals

Dry dressing with crude plant materials such as neem leaf powder, red chilli powder, turmeric rhizome powder, *Vinca* leaf powder, etc., have been found to be very effective for the maintenance of vigour, viability and productivity of wheat, blackgram, soybean and okra seeds as reported by many researchers (De *et al.,* 1998, 2003; Mandal and De, 1999; Mandal *et al.,* 2000 and Kapri *et al.,* 2003).

Plant products are known to contain various antioxidants that would quench free radical attack during seed ageing and a loss in such components would lead to death of seeds. The antioxidants present in the plant products play a major role in improving the performance of the seeds (Ramya *et al.,* 2011). 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, present investigation was carried out with these botanicals.

Germination is the most important indicator of seed quality parameter and changes in seed germination may occur due to different treatments. In the present investigation, an attempt was made to improve the performance of both fresh and aged seeds resulted in astonishing effect. All the botanicals, its concentration and shaking

duration performed better than control both in the case of fresh and aged seeds. In fresh seeds, speed of germination and germination percentage was not significantly influenced by treatments but all the other parameters *viz*., shoot length, root length, dry matter production, vigour index I and II 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 fenugreek seed powder @ 3 g kg^{-1} of seed and custard apple leaf powder @ 4 g kg^{-1} of seed with 1 h shaking was found to be better than other treatments. Both the treatments increased germination percentage of aged seeds by 9% over control (81%) but no significant influence in germination of fresh seeds was observed (Fig. 7). Vigour index I was increased by 17% and 32% respectively for fresh and aged seeds over their respective control (Fig. 8). Similarly increase in vigour index II was also much pronounced in aged seeds than fresh seeds. Current results of botanicals were in harmony with Mythili (2012) in onion and Vijayalakshmi (2012) in tomato. The efficacy of dry treatments with botanicals in aged seeds than in fresh seeds was also reported by Rudrapal and Basu (2004) in french bean seeds. It is well proven that vigour tests can be used to study the efficacy of seed treatment (Schwember and Bradford, 2011). In the present investigation, the testing of efficacy of seed dry dressing with botanicals by subjecting to accelerated ageing test, also revealed the superiority of fenugreek seed powder @ 3 g kg^{-1} of seed and custard apple leaf powder @ 4 g kg^{-1} of seed with one hour shaking in terms of higher germination (Fig. 9), shoot and root length, dry matter production, vigour index I (Fig. 10) and II than other treatments and control.

It is possible that 1 h shaking could be sufficient to form uniform layer of botanicals over the seed coat and the active ingredients present in the powder may get enter through cracks and crevices in the seed coat as suggested by Sengupta *et al.* (2005), as the seeds are not totally sealed living unit there are cracks and crevices besides facilitating gaseous exchange, may serve as entry point of exogenously applied substances that may hither to invigourate the seed, through some unknown pathway. It is also possible that the surface application of dry powders on the outer surface of seed may facilitate a slow penetration of soluble materials through cracks and crevices during imbibition. However, it needs conformation by further studies. Corredor *et al.* (2009) and

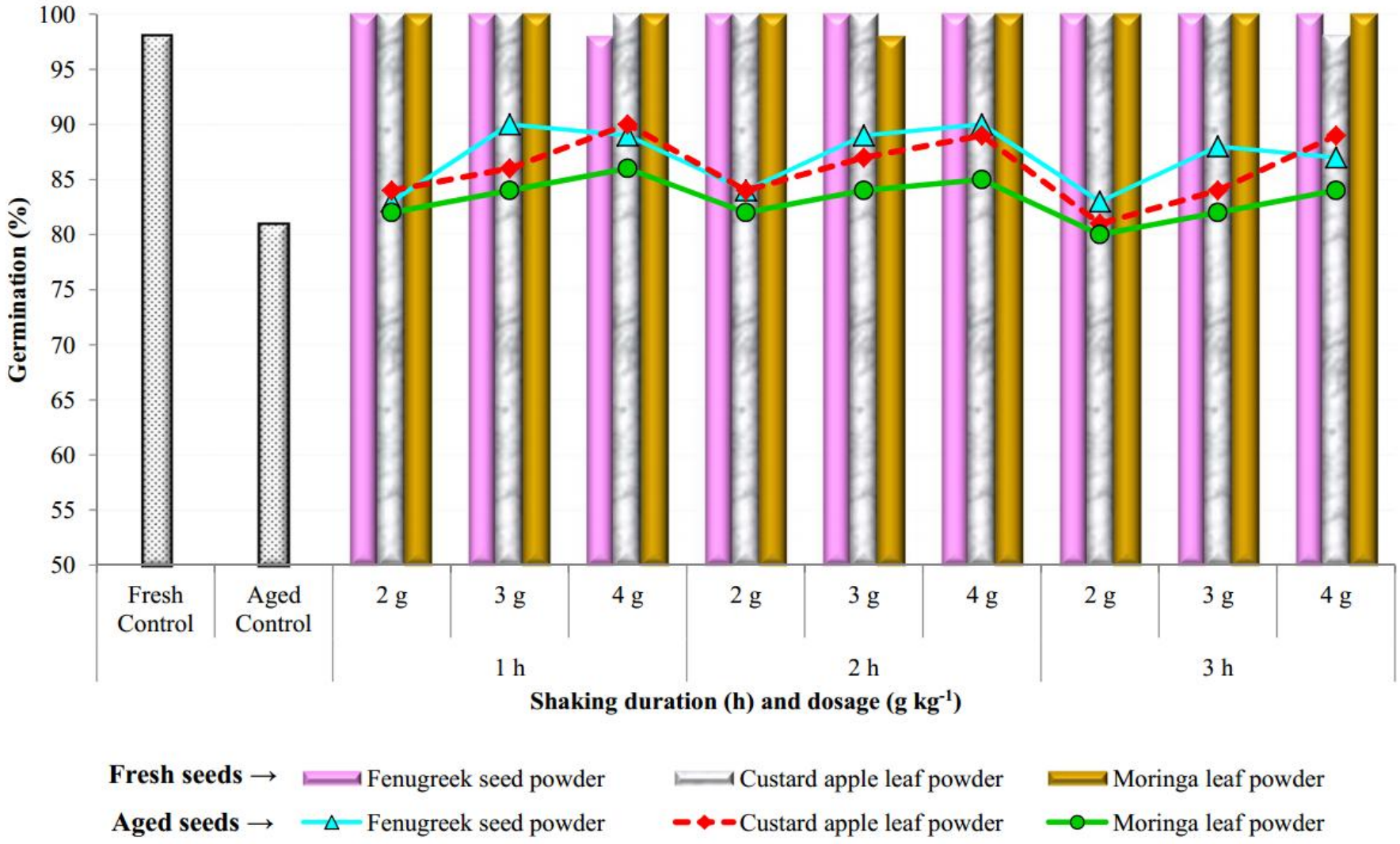


Fig. 7. Effect of dry dressing with botanicals on germination of fresh and aged seeds of blackgram

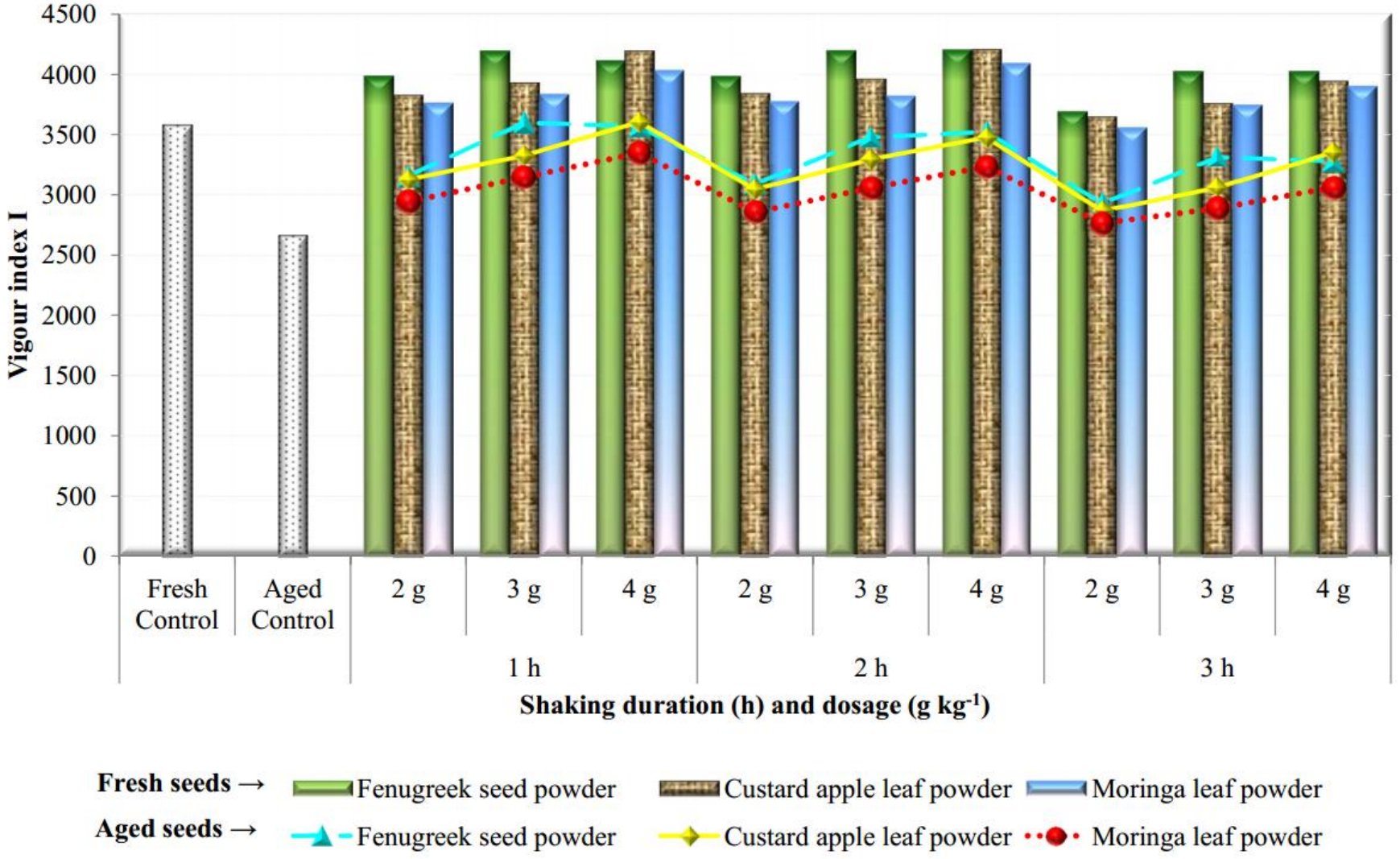


Fig. 8. Effect of dry dressing with botanicals on vigour index I of fresh and aged seeds of blackgram

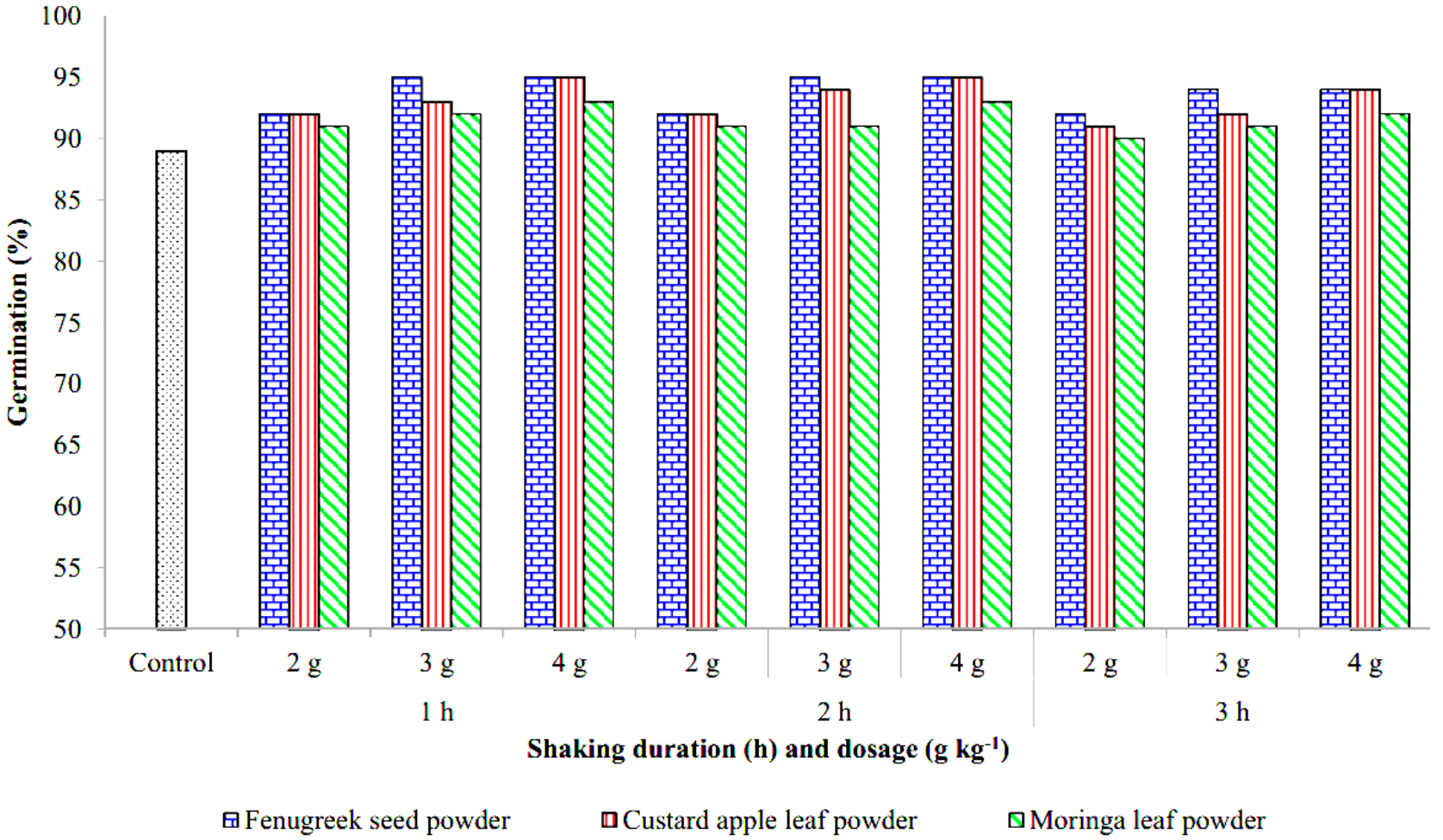


Fig. 9. Effect of dry dressing with botanicals on seed germination of blackgram seeds through accelerated ageing test

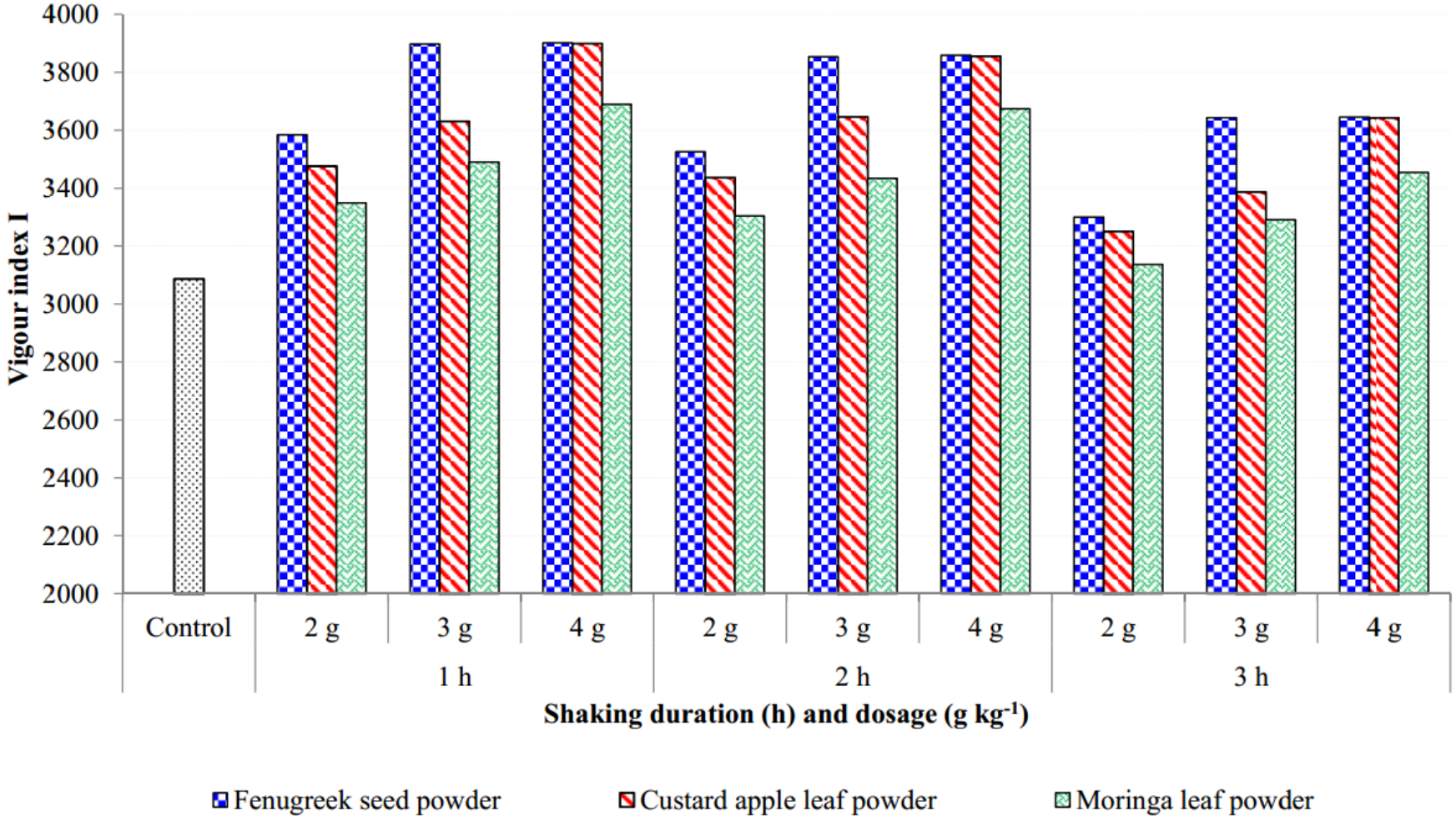


Fig. 10. Effect of dry dressing with botanicals on vigour index I of blackgram seeds through accelerated ageing test

Mushtag (2011) have demonstrated the entry of particles through seed coat in pumpkin and cucumber. The reduction in seed performance with increase in shaking duration might be due to increase in cracks causing damage to seeds. However, the results proved that these treatments certainly improved the seed performance, significantly.

Hence, seed dry dressing with fenugreek seed powder @ 3 g kg^{-1} of seed and custard apple leaf powder @ 4 g kg^{-1} of seed with one hour shaking could be imposed 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.

5.3.2. Standardization of wet treatment with botanicals

Good quality seed with rapid and uniform field emergence is an essential prerequisite for increased yield, quality and ultimately profit to the farmers. Uniformity and percentage seedling emergence of direct seeded crops have a major impact on final yield and quality of seed. Slow emergence results in weaker seedlings, which are prone to diseases (Osburn and Schroth, 1989). Various pre-sowing / pre-storage seed treatments have been practiced to reduce the time between sowing and seedling emergence. Seed invigouration by hydration and dehydration has become a common seed treatment method to increase the rate and uniformity of seedling emergence.

Nowadays organic based materials are used to invigourate the seeds. The fresh leaf extract of arappu (*Albizzia amara*) have been reported to be useful to harden the seeds with greater benefits (Angamuthu, 1991). In the present study, fresh and accelerated aged seeds were soaked for one, two and three hours in the solution of fenugreek seed powder, custard apple and moringa leaf powder of different concentrations *viz*., 0.5%, 1.0%, 1.5% and 2.0% along with water soaking for improving the performance of seeds.

Two possibilities of improving the vigour of seeds under wet treatments have been suggested - enzymatic repair of biochemical lesions during seed hydration (Villiers and Edgcumbe, 1975; Burgass and Powell, 1984) or control of free radical and cell organelle damage by hydration - dehydration treatments (Buchvarov and Gantcheff,

1984; Saha *et al.,* 1990). According to McDonald (1999), there are distinct possibilities of involvement of free radical induced cell organelle damage in seed deterioration.

The present study of evaluating the comparative effect of different botanicals in improving germination and vigour of both fresh and accelerated aged seeds showed great variations among invigouration treatments and concentrations. Mere water soaking itself was effective when compared to control. The beneficial effect of invigouration treatments, including water soaking in germination advancement is known to occur due to higher mitochondrial activity, formation of more high energy compounds and vital bio-molecules (Henckel, 1964; Heydecker and Coolbear, 1977) and rapid utilization of reserve materials during germination (Sathish *et al.,* 2012).

Among the different botanicals, significant difference in germination was not observed in fresh seeds. However, in aged seeds, 1% fenugreek seed powder (89%), 1.5% custard apple leaf powder (89%) and 1.5% moringa leaf powder solution (88%) recorded the maximum germination which was 8 and 7% respectively, higher than untreated seeds (81%) (Fig. 11). Rudrapal and Basu (2004) in french bean and Sathish *et al.* (2011) in maize reported that seed hydration and dehydration treatments are more effective in medium vigour lots than high vigour seed lots. Similar results were reported by Sasikala (1997) in cowpea and bhendi, Somasundaram (2003) and Sundaralingam (2005) in rice. Seed soaking might have helped in imbibing growth promoting substances present in the botanicals which activated hydrolysis of endospermic resources resulting in increased germination. The improvement in germination by organically treated seeds might also be due to activation of cells during soaking which resulted in enhancement of mitochondrial activity leading to the formation of more energy compounds and vital bio-molecules which were made available during the early phase of germination as reported by Renugadevi *et al.* (2001). Similar results were reported by Renugadevi and Vijayageetha (2007) in cluster bean.

Seeds soaked for 1h in 1.0% concentration for fenugreek seed powder and 1.5% for custard apple and moringa leaf powder recorded higher germination than the other durations of soaking and concentrations (Fig. 11). It may be due to toxic effect of higher concentration and prolonged soaking on the physiological and biochemical processes

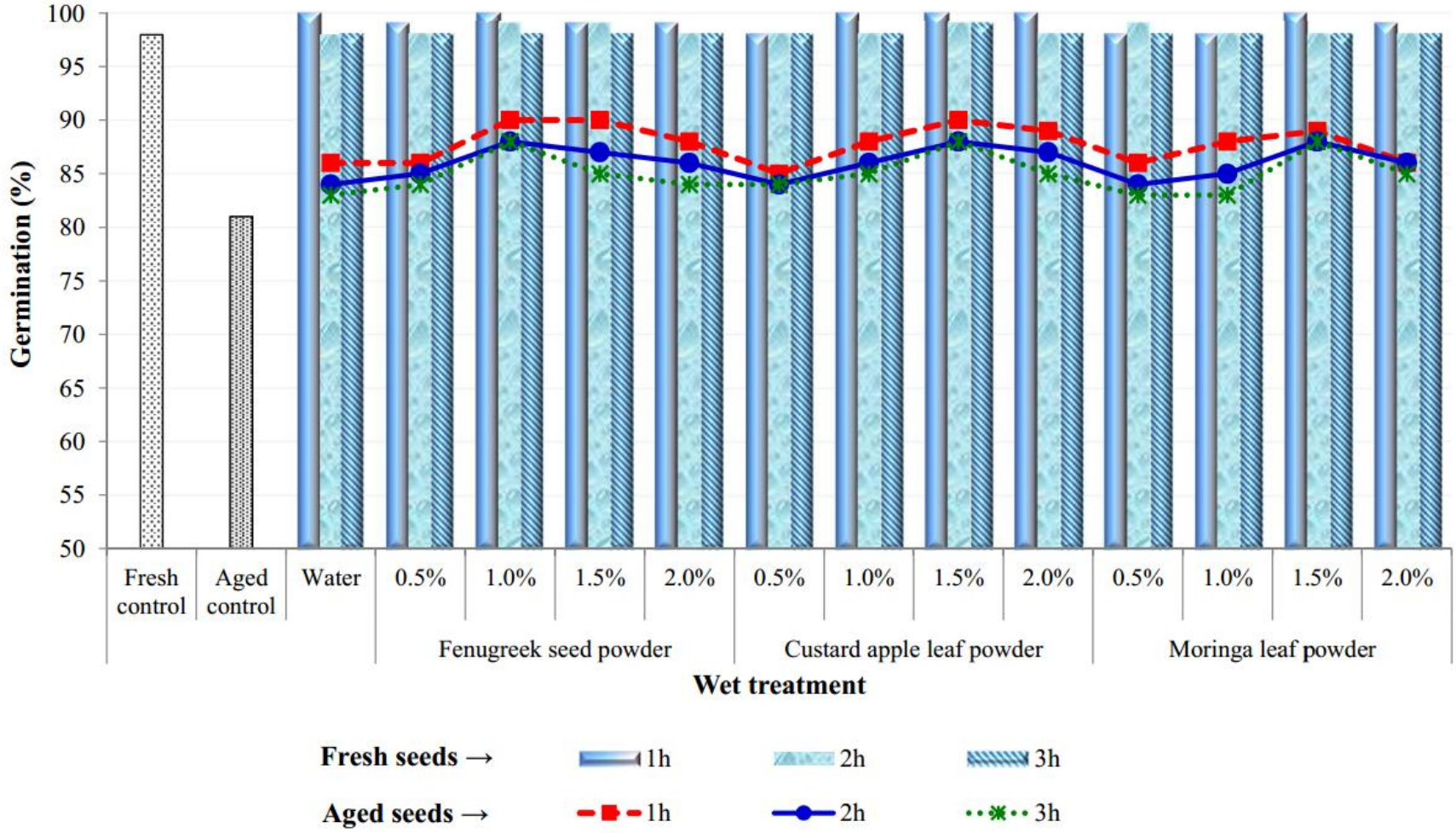


Fig. 11. Effect of wet treatment with botanicals on germination of fresh and aged seeds of blackgram

within the cell organelles and non-availability of oxygen for respiration which indulged with citric acid cycle (Bewley and Black, 1982). The beneficial effect of fenugreek seed powder and custard apple leaf powder might be due to the presence of antioxidants (Pandey and Barve, 2011) and phenols and flavonoids (Annegowda *et al.*, 2010). Similar results have been reported by Kareem *et al.* (1989), Lowell (2005) and Suma (2005) with botanical leaf extract treatments.

Seedling growth in terms of root and shoot has been regarded as a good index to measure the vigour of seeds (Abdul – Baki and Anderson, 1973). In the present study, shoot and root length were the maximum for 1.0% fenugreek seed powder and 1.5% custard apple leaf powder treatment followed by 1.5% moringa leaf powder solution over control.

The dry matter production is essentially the physiological manifestation of seed vigour largely influenced by the affluence of metabolites, growth regulating substances, enzyme activity etc. (Heydecker, 1972). In 1.0% fenugreek seed powder and 1.5% custard apple leaf powder treatment followed by 1.5% moringa leaf powder solution treated seeds recorded the high dry matter production in both fresh and aged seeds. Similar results have been reported by Layek *et al.* (2006) in gram.

In the present study, seeds soaked in 1.0% fenugreek seed powder and 1.5% custard apple leaf powder solution recorded the maximum vigour index I (Fig. 12) and vigour index II followed by the seeds soaked in moringa leaf powder solution. Similar results have been reported by Menaka *et al.* (2003) in sorghum, Vijayan (2005) in rice.

Accelerated ageing test also revealed the superiority of wet seed treatment with 1.0% fenugreek seed powder and 1.5% custard apple leaf powders solution soaked for one hour in terms of higher germination, shoot and root length, dry matter production, vigour index I and II over other treatments (Fig. 13 and 14).

The beneficial effect of seed invigouration with botanicals, in terms of increased seedling quality parameters have been discussed elsewhere in this chapter. The results of the laboratory studies on physiological seed quality parameters indicated that blackgram seeds wet treated by soaking for 1 h in 1.0% fenugreek seed powder solution and 1.5% custard apple leaf powder solution increased the germination and vigour of the seed.

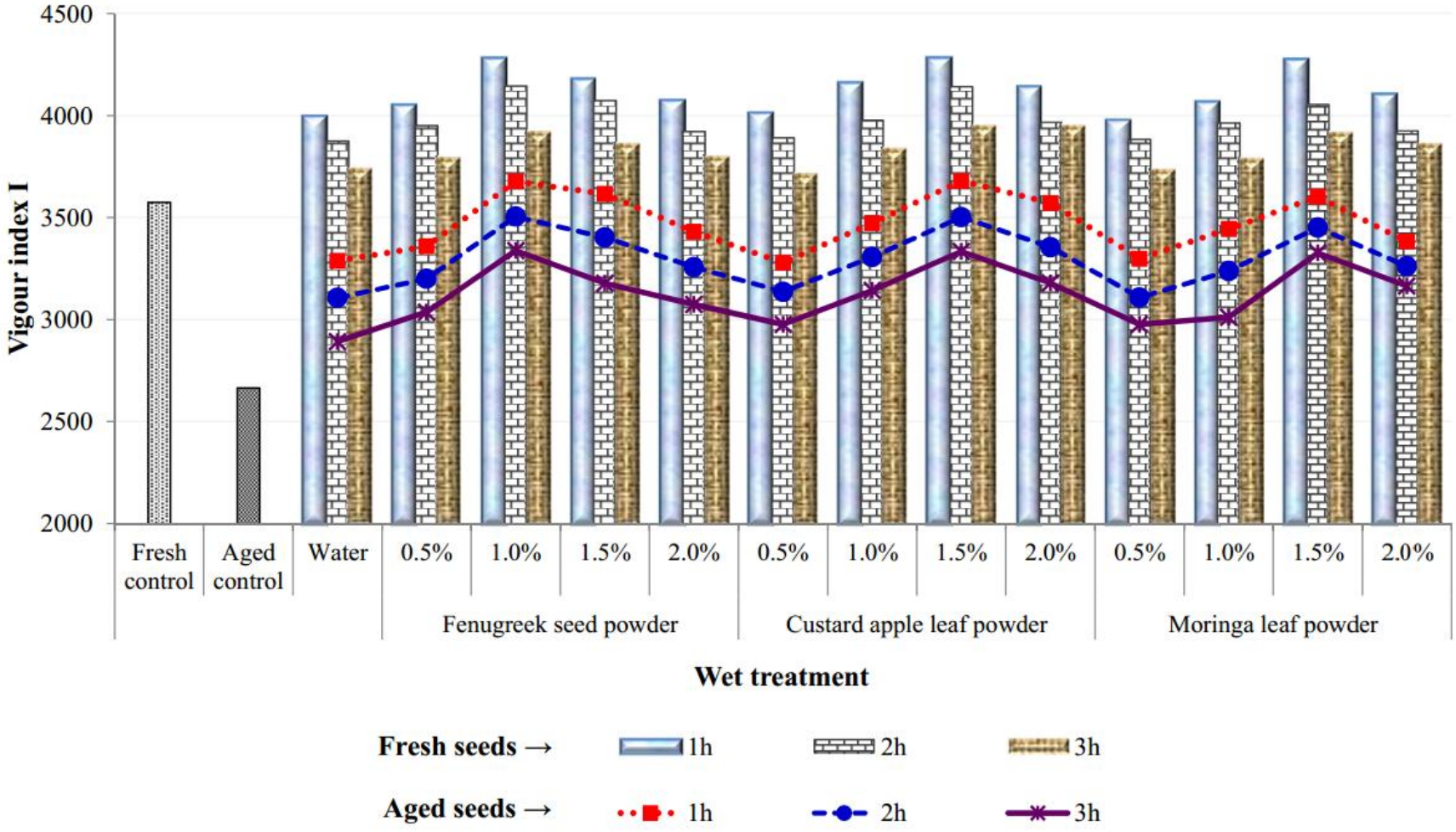


Fig. 12. Effect of wet treatment with botanicals on vigour index I of fresh and aged seeds of blackgram

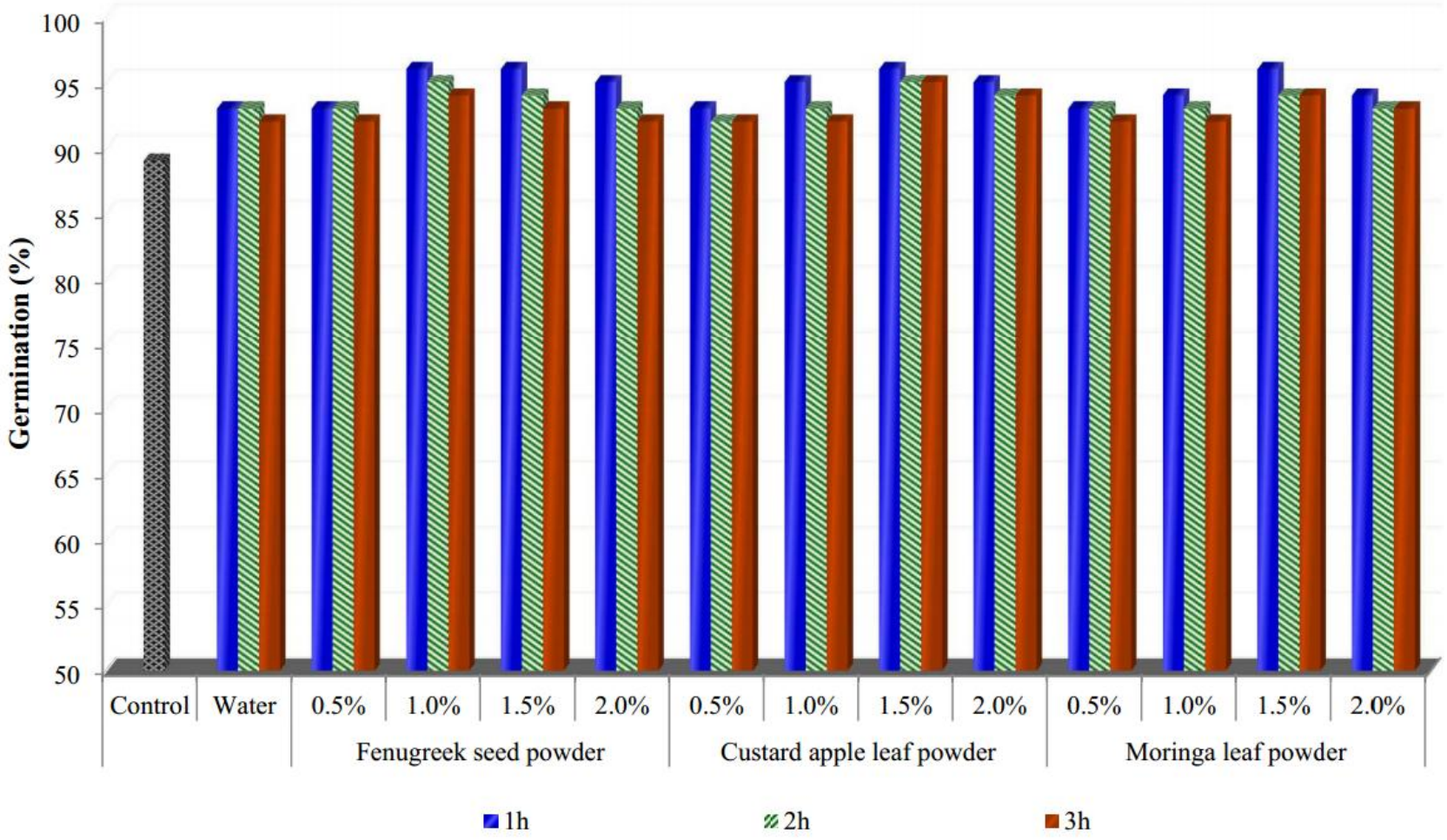


Fig. 13. Effect of wet treatment with botanicals on seed germination of blackgram seeds through accelerated ageing test

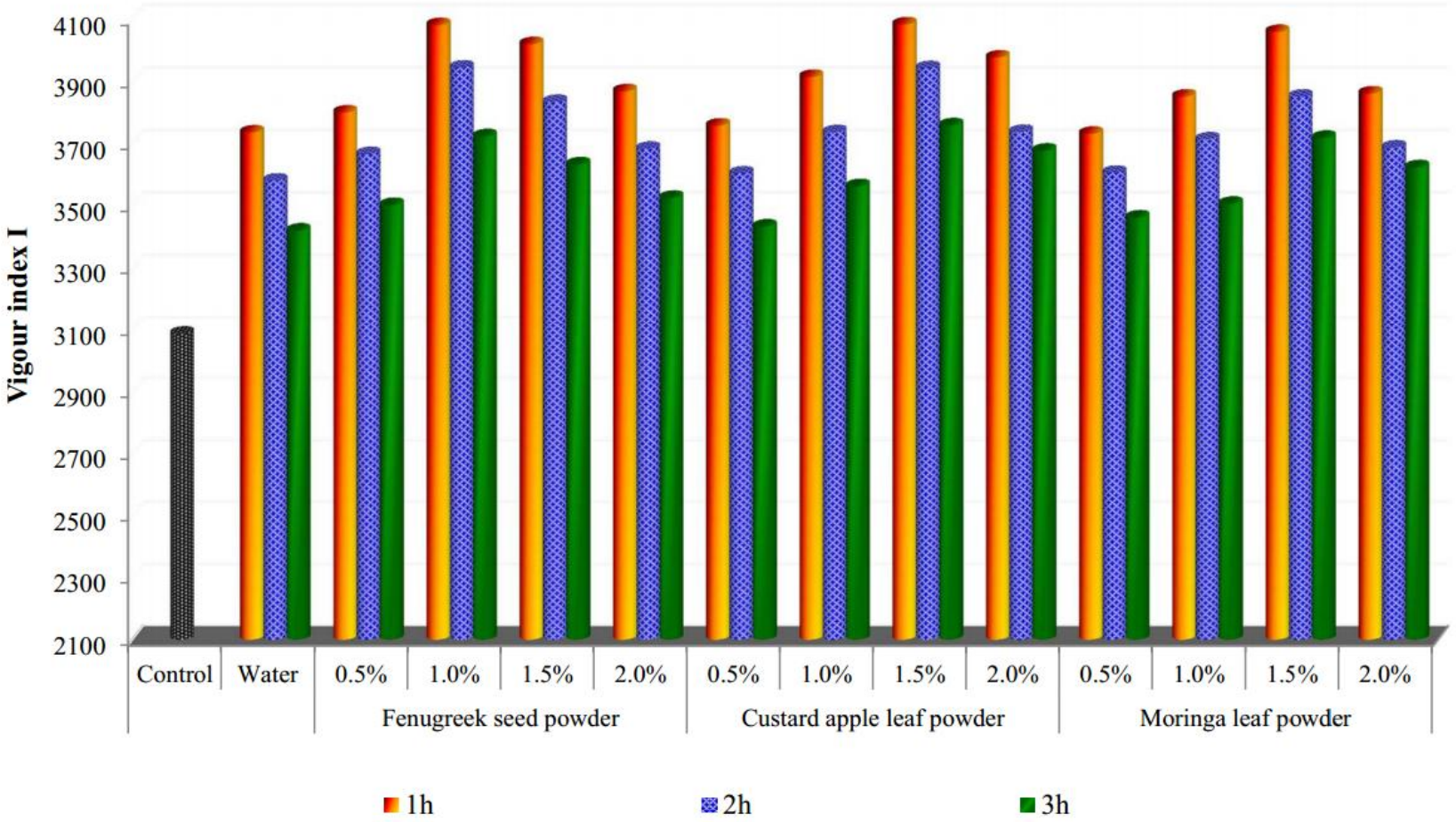


Fig. 14. Effect of wet treatment with botanicals on vigour index I of blackgram seeds through accelerated ageing test

5.4. Influence of botanicals seed treatment on biochemical characters

The changes in biochemistry of seeds at molecular level have been correlated with seed deterioration. Ageing induces progressive seed deterioration leading to lethal damage and inability to germinate (Bouteau *et al.,* 2011). Seed ageing associated with various alterations in macro and micro molecular structures resulted in decreased energy metabolism, impairment of RNA and protein synthesis and DNA degradation (Walters, 1998; McDonald, 1999; Corbineau *et al.,* 2002; Kibinza *et al.,* 2006) have been documented.

The beneficial effects of invigouration treatments on viability maintenance through formation of high energy compounds, increased DNA in the growing point, higher mitochondrial activity, respiration rate, protein synthesis and maintenance of cellular integrity and their advantage have been reported (Mandal *et al.,* 2000; Khan *et al.,* 2003; De *et al.,* 2005).

Damage to membranes is the suggested explanation for loss of viability during ageing (Roberts, 1972). Seeds with reduced activity for the repair system will be slower in germination compared to seeds which can undergo self-repair mechanism rapidly (Coolbear *et al.,* 1990). Electrical conductivity of the seed leachate is a measure of seed vigour (Grabe, 1965) and intensity of membrane damage (Matthews and Bradnock, 1968).

In the present study, difference in electrical conductivity of seed leachates was noticed due to different botanical treatments and control. Electrical conductivity of seed leachate was lesser in all the botanical treatments compared to control. However, fenugreek seed powder and custard apple leaf powder registered lower electrical conductivity both in dry dressing and wet treatment of fresh and aged seeds. The reduction in electrical conductivity of seed leachate was 16 and 13% higher in fresh seeds, wet treated with 1.0% fenugreek seed powder and 1.5% custard apple leaf powder solution, respectively and 14 and 12% higher in fresh seeds dry dressed with fenugreek seed powder @ 3 g kg^{-1} of seed and custard apple leaf powder @ 4 g kg^{-1} of seed, respectively over control. Whereas, in aged seeds the reduction was still wider registering 23% lower electrical conductivity in seeds wet treated with 1.0% fenugreek seed powder solution and 22.0% lower electrical conductivity in seeds wet treated with 1.5% custard

apple leaf powder solution, dry dressed with fenugreek seed powder @ 3 g kg^{-1} of seed and custard apple leaf powder @ 4 g kg^{-1} of seed, respectively over control (Fig. 15). The results were in conformity with the findings of Kavitha (2002) in blackgram and Sundaralingam (2005) in rice. Though cells are encoded with detoxifying enzymes and antioxidant compounds that could scavenge free radicals (Bernal-Lugo *et al.*, 2000; Shelar, 2007), while ageing proceeds these compounds also decreased or deactivated ultimately failing to maintain the physiological potential of seeds. Kavitha (2002) reported that the probable reason for low electrical conductivity in the invigourated seeds presumed to be the quenching of free radicals, which consequentially maintains the membrane integrity.

The present study gain support further through the assay for DPPH free radicals scavenging activity in treated seeds during ageing. It was evident that the seed treatment with fenugreek seed powder or custard apple leaf powder in dry or wet form possessed higher quenching property for free radicals. In fresh seeds, the quenching property was higher by 9 per cent for fenugreek seed powder and 8% for custard apple leaf powder and in aged seeds the difference was still wider (29 and 27%, respectively) over control clearly establishing the repair mechanism by the treatments (Fig. 15). Oxidants and antioxidants in any living organism are maintained in balance in a normal physiological state and over production of oxidants in certain adverse conditions can cause oxidative stress leading to damage to vital biomolecules and cells (Temple, 2000). These damages can be rectified through different treatments which may lead to leaching of toxic compounds generated during ageing and counteraction of free radicals and lipid peroxidation products with the antioxidants (Burguieres *et al.*, 2007).

α - Amylase activity could be used as a measure of vigour in seeds. In the present study, both in fresh and accelerated aged seeds, all the treatments recorded maximum α - amylase activity over control. However, it was highly pronounced in seeds wet treated with 1.0% fenugreek seed powder (22.0 mm in fresh seeds and 15.8 mm in aged seeds) and 1.5% custard apple leaf powder solution treated seeds (21.2 mm and 14.7 mm, respectively) (Plate 8 and 9). α - amylase is an important pre-requisite enzyme in germinating seeds which degrades the complex starch, maltose and release energy in the

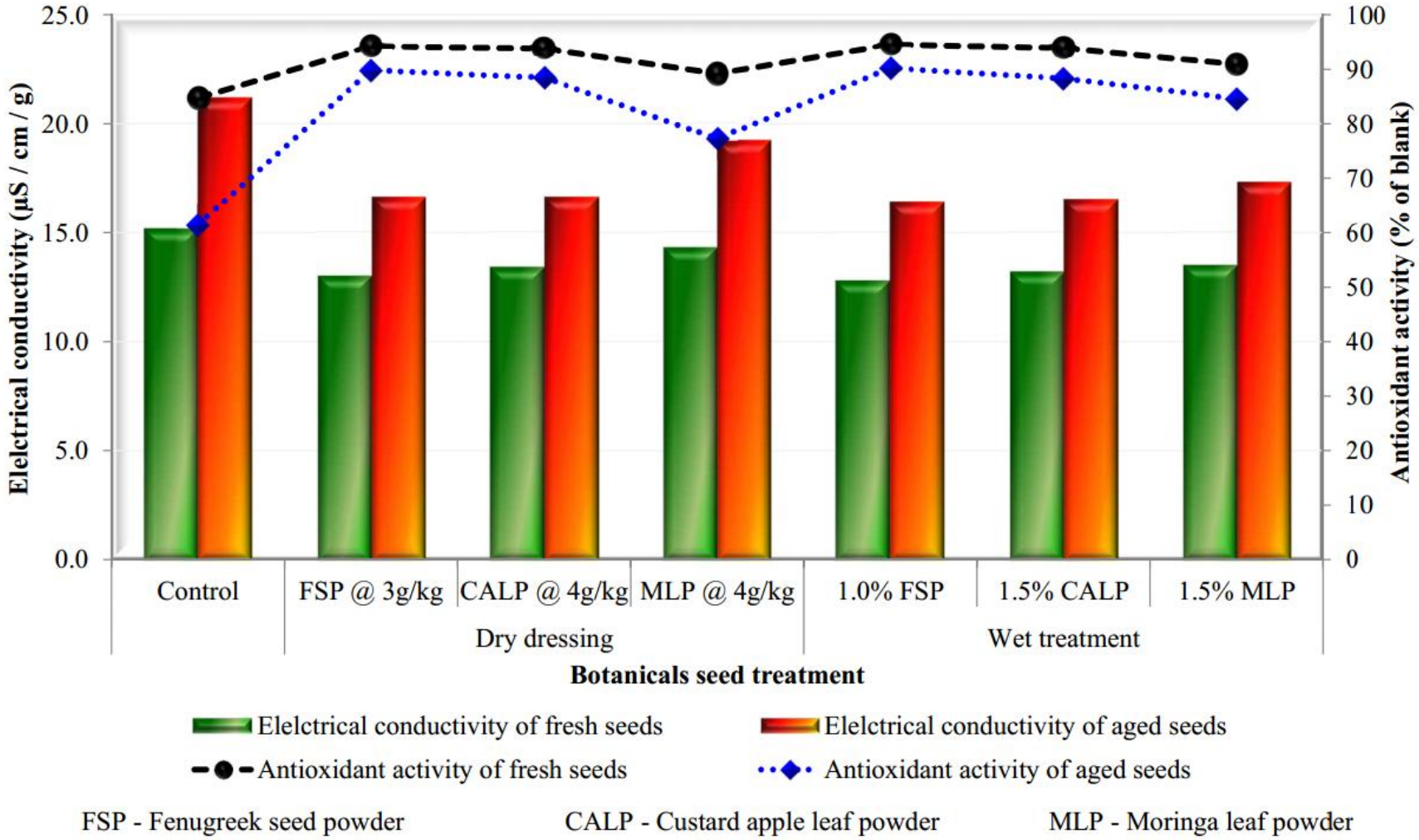


Fig. 15. Effect of botanical seed treatment on electrical conductivity and antioxidant activity of fresh and aged seeds of blackgram

Plate 8. Effect of botanical seed treatment on α-amylase activity in fresh seeds of blackgram cv. TNAU Blackgram CO 6

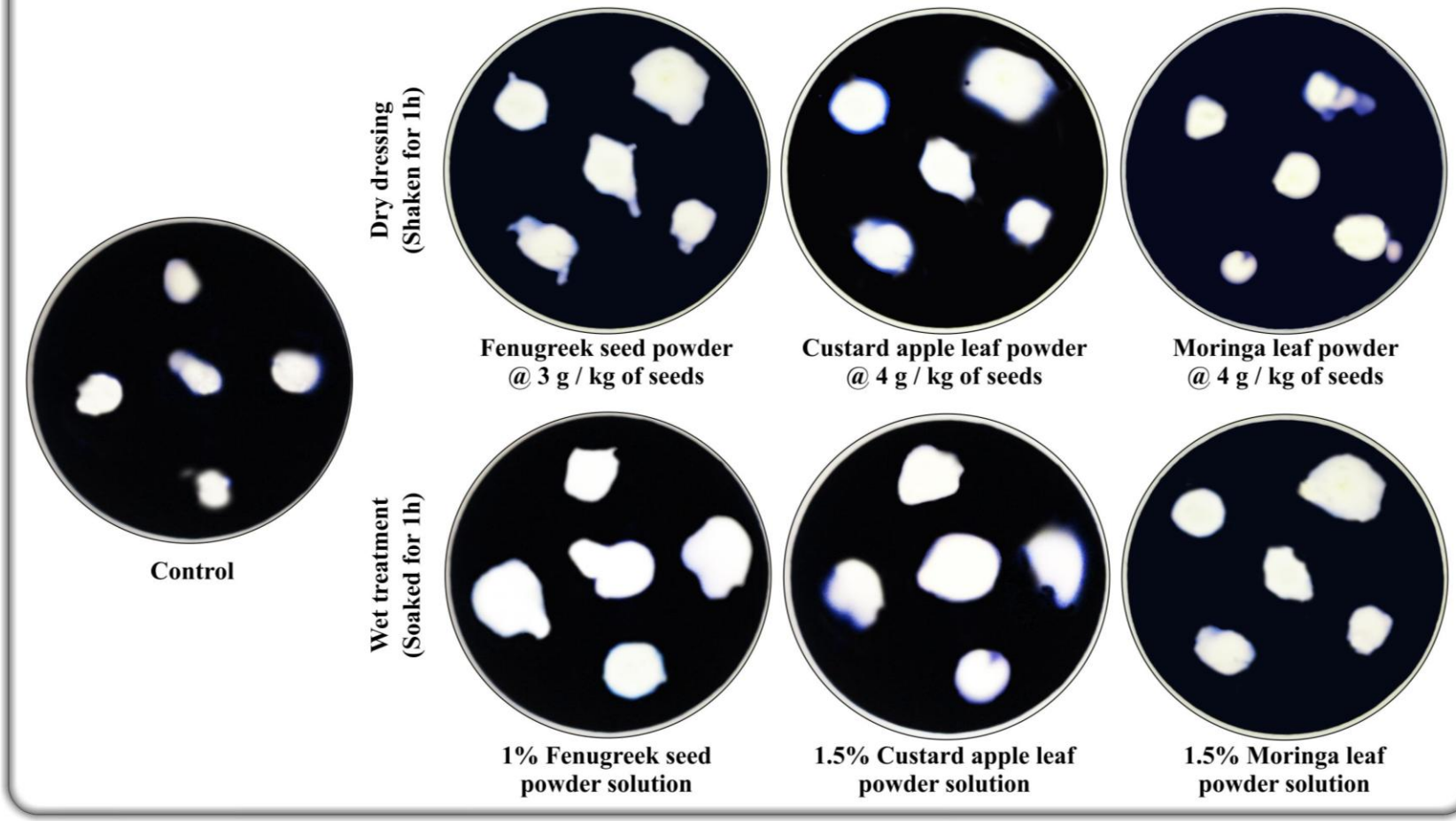
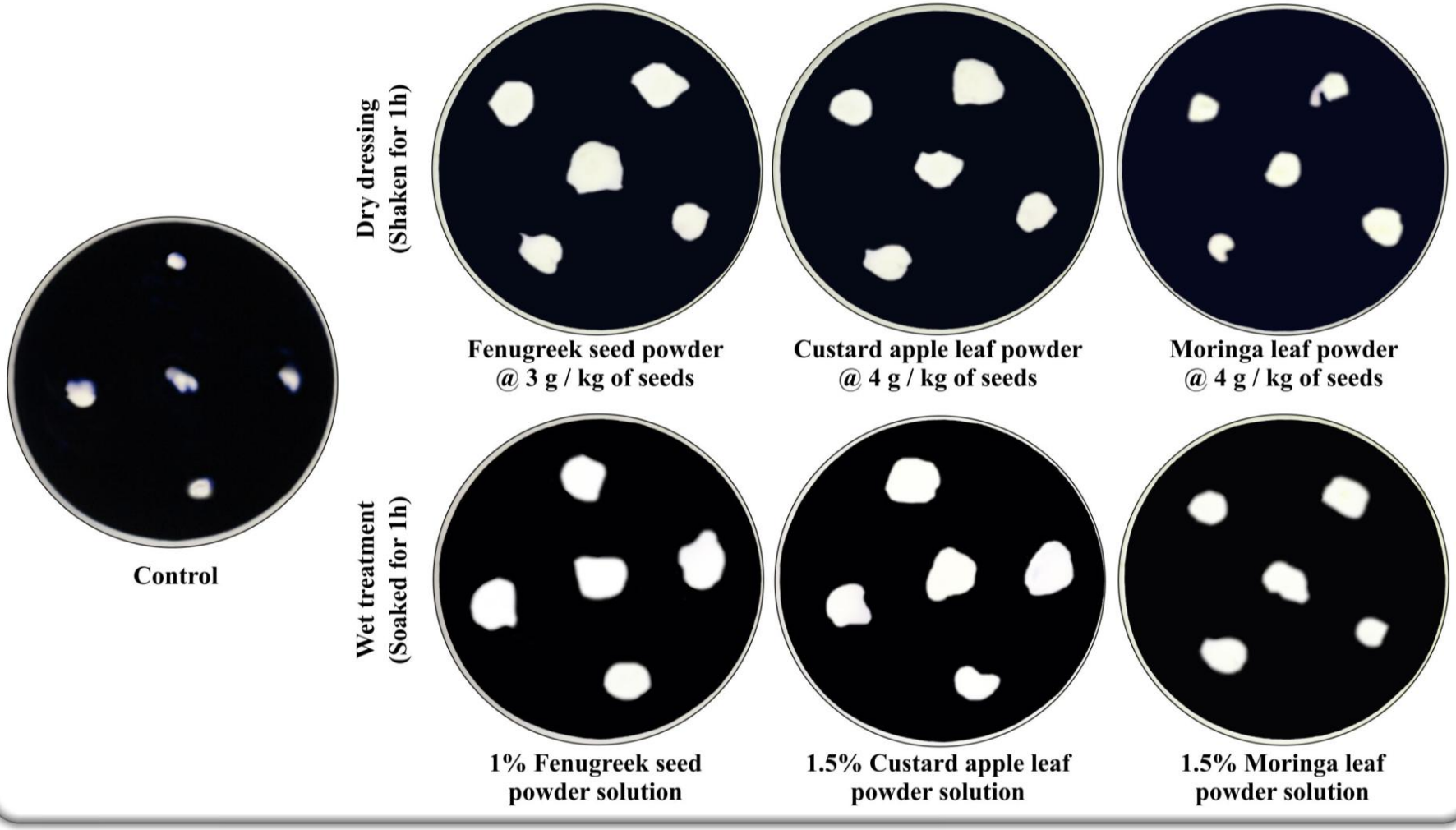


Plate 9. Effect of botanical seed treatment on α-amylase activity in aged seeds of blackgram cv. TNAU Blackgram CO 6



form of ATP, which is utilized by emerging seedlings (Bewley and Black, 1978). Similar results were reported by Malarkodi (2003) in greengram.

Viability and vigour of seeds also affected by increase in protease activity and free amino acid pool (Lee *et al.,* 1995). The variation in protease activity and amino acids are directly proportional to loss of seed vigour and viability (McDonald, 2004). In the present study, the lower protease activity and free amino acids were recorded by the seeds treated with fenugreek seed powder and custard apple leaf powder followed by moringa leaf powder compared to control, in both fresh and accelerated aged seeds. The results were in accordance with Raja (2000) in greengram. Lower level of protease activity and free amino acid by the botanical seed treatment which was evident in the present study might be due to controlled biochemical changes in the treated seeds (Jeeva, 2003).

Any repair of biochemical lesions require protein synthesis which depends on the pre-existing enzymes. Activation of pre-existing enzymes are useful in breakdown of toxic substances accumulated in the seeds during ageing. Both dry dressing and wet treatment influenced the total protein profile of fresh and aged seeds. The intensity of protein bands of seeds dry dressed with fenugreek seed powder @ 3 g kg^{-1} of seed, custard apple leaf powder @ 4 g kg^{-1} of seed and moringa leaf powder @ 4 g kg^{-1} of seed were slightly higher than untreated control. However, the intensity of protein bands of seeds wet treated with 1.0% fenugreek seed powder, 1.5% custard apple and moringa leaf powder solution were predominantly higher than dry dressed seeds and untreated control (Plate 4 and 5). This indicates synthesis of new proteins during wet treatment. Increase in protein intensity due to wet treatment might be due to synthesis of new polypeptides after imbibition (Sung and chang, 1993; Job *et al.,* 1997). Capron *et al.* (2000) found that, a hydration treatment of sugarbeet seeds to be the most efficient, an increase in the level of soluble 11-S globulin B-subunit per seed is necessary. Gallardo *et al.* (2001) proved that synthesis of new proteins is responsible for improved performance in wet treated seeds.

Lower biochemical activity in terms of free radical scavenging potential, α - amylase activity and higher values of electrical conductivity, protease activity and free amino acids in untreated control and vice versa in fenugreek seed powder and custard apple leaf powder treated seeds might indicate their capacity to maintain the cell

endowments triggering the germination events as suggested by Bailly (2004). A comparison of fresh and accelerated aged seeds for the responsiveness of the seed treatments either as dry dressing or wet treatments using botanicals brought out the advantageous effect mostly in aged seeds is an indication of damage already received through ageing and subsequent repair by the treatments.

5.4.1. Biochemical properties of botanicals

Analysis of DPPH free radical scavenging activity of botanicals revealed higher antioxidant property in all the botanicals among which fenugreek seed powder and custard apple leaf powder topped with 95.9 and 96.0% free radical scavenging activity followed by moringa leaf powder (91.7%). The results were in strong conformity with the findings of earlier researchers in fenugreek seed powder (Bukhari *et al.,* 2008; Toppo *et al.,* 2009), custard apple leaf powder (Baskar *et al.,* 2007; Pandey and Brave, 2011; Bose *et al.,* 2011) and moringa leaf powder (Fahey, 2005; Ferreira *et al.,* 2008). Fenugreek seed powder and custard apple leaf powder was 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). This reduce the process of deterioration caused by free radicals mediated lipid peroxidation as observed through concurrent increase in germination percentage, seedling length, dry matter production and vigour index.

Apart from antioxidant property, botanicals also acted as a good source for minerals which evidenced from ICP analysis. Fenugreek seed powder contain higher amount of Mo (40.24 ppm), Ti (38.34 ppm), Fe (20.51 ppm), Al (3.20 ppm), Zn (1.35 ppm) and traces of Mn, B, Cu, Sr, Ba. While custard apple leaf powder was rich in Ti (107.1 ppm), Mo (24.86 ppm), Fe (11.68 ppm), Al (6.10 ppm), Sr (3.19 ppm), B (2.36 ppm) and traces of Ba, Zn, Cu, Mn. Moringa leaf powder was rich in Ti (47.71 ppm), Mo (13.73 ppm), Fe (7.59 ppm), Al (2.79 ppm), Sr (2.78 ppm) and traces of Zn, B, Mn, Ba, Cu. However, moringa leaf powder contains fewer minerals than fenugreek seed powder and custard apple leaf powder. Among all these minerals the availability of titanium, molybdenum and iron were higher in all the three botanicals (Fig. 16). These may contribute to further improvement in performance of treated seeds as observed through significant increase in vigourous seedling formation. Ti plays a

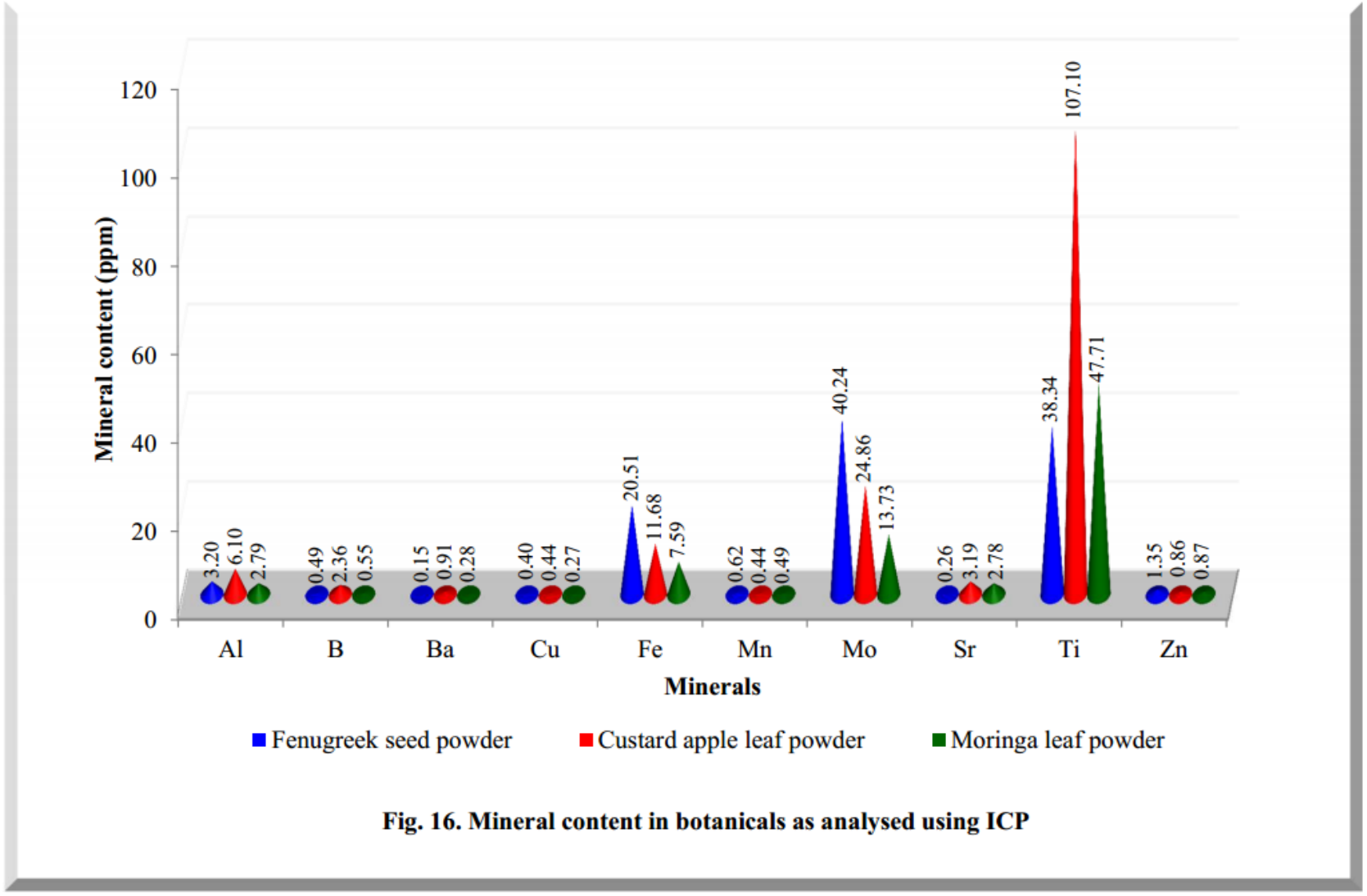


Fig. 16. Mineral content in botanicals as analysed using ICP

major role in biomass production and participates in cell metabolism as redox catalyst (Tlustos *et al.,* 2005). Molybdenum is utilized by selected enzymes to carry out redox reactions and helps in vigourous 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 fenugreek seed powder and custard apple leaf powder could be attributed to excellent proton radical scavenging property as described earlier and subsequent alleviation of deteriorative effect (Bhatia *et al.,* 2002). Seed soaking might have helped in imbibing growth promoting substances present in the botanicals which activated hydrolysis of endospermic resources resulting in increased germination. The improvement in germination by organically treated seeds might also be due to activation of cells during soaking which resulted in enhancement of mitochondrial activity leading to the formation of more energy compounds and provides substantial mineral supplement required for seed germination and vigourous seedling growth during the early phase of germination (Chandrashekar and Kulkarani, 2011).

5.5. Effect of botanical seed treatments on crop productivity

Based on laboratory experimental results for enhancement of germination and seedling vigour, the efficacy of botanical seed treatment could be arranged in descending order as wet treatment by soaking seeds for 1h in 1.0% fenugreek seed powder > 1.5% custard apple leaf powder solutions > dry dressing with fenugreek seed powder @ 3g kg^{-1} of seed > dry dressing with custard apple leaf powder @ 4g kg^{-1} of seed > 1.5% moringa leaf powder solution > dry dressing with moringa leaf powder @ 4g kg^{-1} of seed.

In view of the advantages realized in laboratory experiments by using botanicals on seed vigour and viability maintenance, studies were carried out to evaluate the field performance of above mentioned treated seeds. The crop was raised with constant plant population per unit area during *kharif* and *rabi* season 2012- 13 at Agricultural Research Station, Bhavanisagar, using TNAU Blackgram CO 6 seeds.

In the present study, growth attributes such as plant height, leaf area and leaf area index were significantly higher in all the botanical treatment over untreated fresh and aged seeds at vegetative stage and dry matter production was higher in all the stages *viz*.,

vegetative, flowering and harvesting stage. Higher dry matter also contributed to the increased crop growth rate in fresh and aged treated seeds over their respective control. The pronounced effect of treatment was observed in both seasons. However, all the treatments including control recorded higher values for growth and yield parameters in the crop raised during *rabi* season than *kharif* season. It has been suggested that C-3 plants such as pulses usually perform better in cool climates and results in higher production when grown in *rabi* season (Reddy, 2009).

When the edaphic and agronomic variables are almost constant, the factors that cause variation could only be pre sowing seed management practices. Plant height is very important criterion for a crop in providing more place for flower production leading to higher pod production. The maximum plant height was recorded in seeds treated with wet and dry form of fenugreek seed powder and custard apple leaf powder. The increase in plant height by botanicals might be due to availability of micro nutrients such as titanium, molybdenum and iron in botanicals which plays major role in biomass production and participate in cell metabolism as redox catalyst ultimately enhancing cell division and thereby increase the crop growth rate (Guerinot and Yi, 1994; Kaiser *et al.,* 2005; Tlustos *et al.,* 2005). Per cent increase in plant height due to botanical treated seeds over control is as given below

Parameters	**Best treatment**	***Kharif***		***Rabi***	
		% increase over control		**% increase over control**	
		Fresh seeds	**Aged seeds**	**Fresh seeds**	**Aged seeds**
Plant height (Vegetative stage)	1.0% fenugreek seed powder solution	22	24	23	25
	1.5% custard apple leaf powder solution	21	21	21	21
	Fenugreek seed powder @ 3 g kg^{-1}				
	Custard apple leaf powder@ 4 g kg^{-1}	19	17	19	18

The photosynthetic rate depends on leaf area, leaf area index and canopy structure which in turn is related to dry matter production and hence crop yield. Therefore, early attainment of an optimum LAI is considered to be very essential for better bio-productivity. One of the principle factors influencing the canopy net photosynthesis is leaf area index (Hansen, 1972). In the present study, all the treatments were found to increase leaf area and leaf area index over control among which seeds treated with wet and dry form of fenugreek seed powder and custard apple leaf powder registered higher leaf area and leaf area index. Larger leaf area development aids in the effective interception of light leading to higher dry matter production (Shibles and Weber, 1966). There was an increase in leaf area up to vegetative stage and then the difference was nullified towards harvesting stage. The cease of further increase in leaf area might be due to the transport of assimilates from the leaves to the developing sink which later caused senescence of leaves. Similar result was reported by Major and Daynard (1972), Sinclair (1986) and Sinclair *et al*. (1987) in blackgram and soybean.

The data pertaining to dry weight per plant indicated that, botanical seed treatment increased the dry matter and maintained its superiority till harvest stage in *kharif* (Fig. 17) and *rabi* season (Fig. 18). The increase in total dry matter up to harvest might be due to higher rate of CO_2 fixation and Ribulose bisphosphate carboxylase (RUBISCO) enzyme activity as observed by Karthikeyan and Shukla (2008) in mustard and sunflower.

The higher crop growth rate was observed in all the treatments. Crop growth rate is related to light interception by the crop canopy and LAI determines 95% light interception (Shibles and Weber, 1966). In the present study, all the treatments registered higher crop growth rate than control among which wet and dry form of fenugreek seed powder and custard apple leaf powder recorded higher crop growth rate. Increased LAI, dry weight in fenugreek seed powder and custard apple leaf powder treated seeds might have contributed to greater CGR values. These findings are in conformity with the reports of Siddiqui *et al.* (2009). The salient findings for these growth parameters are depicted in the following table.

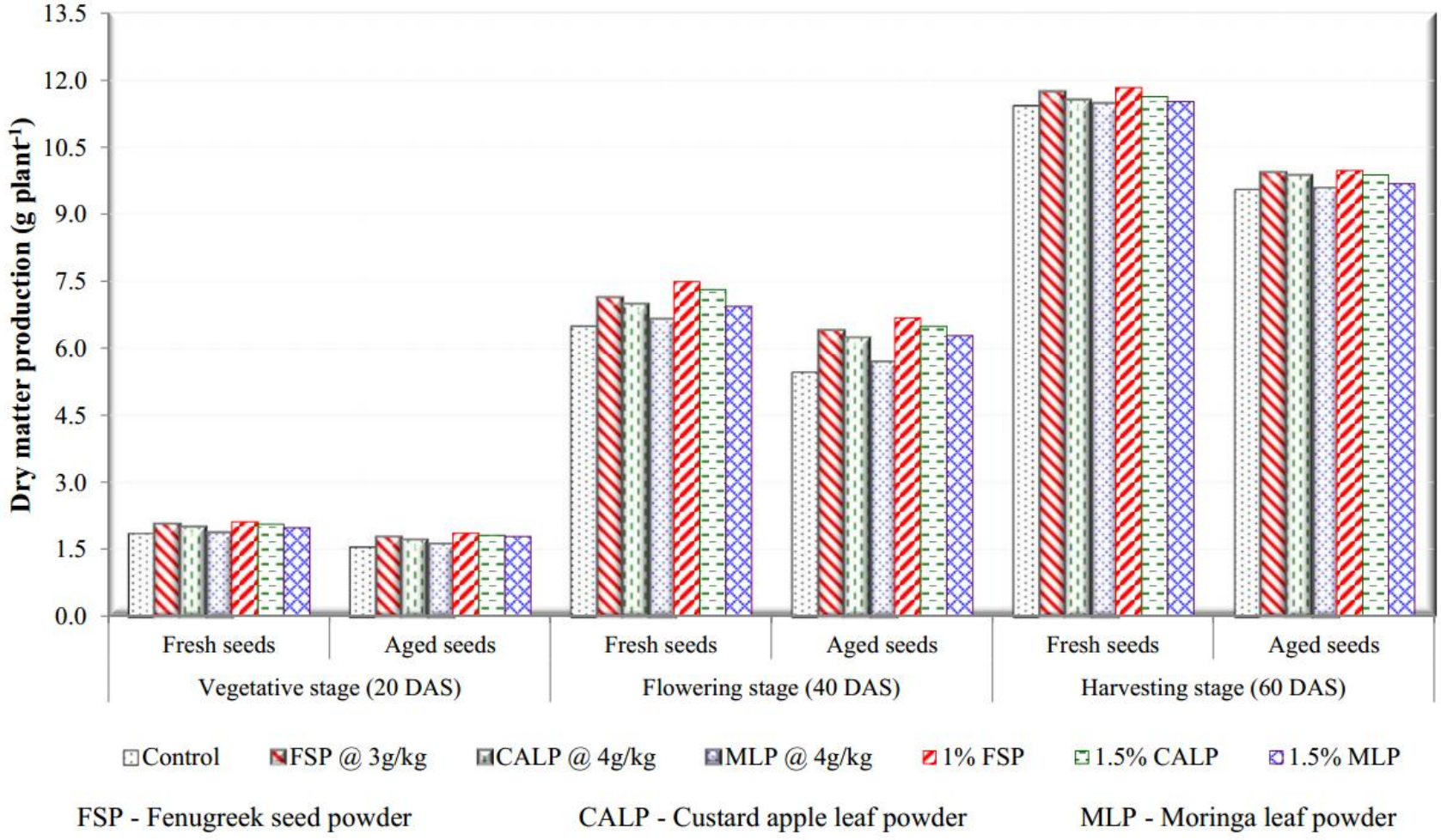


Fig. 17. Effect of botanical seed treatments on dry matter production (g plant^{-1}) of blackgram in *kharif* 2012

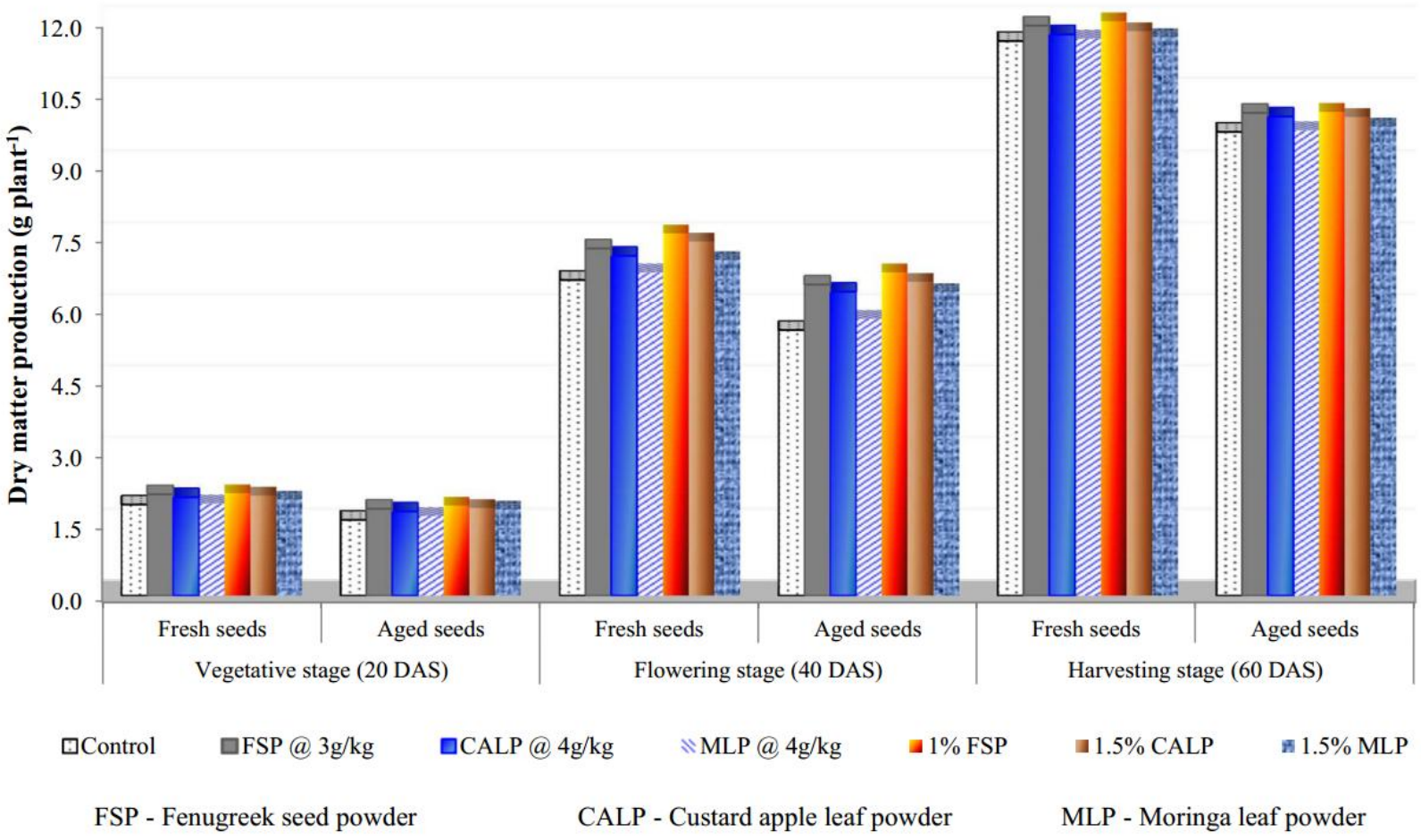


Fig. 18. Effect of botanical seed treatments on dry matter production (g plant^{-1}) of blackgram in *rabi* 2012

Parameters	Best treatment	*Kharif*		*Rabi*	
		% increase over control		% increase over control	
		Fresh seeds	Aged seeds	Fresh seeds	Aged seeds
Dry matter production					
Vegetative stage	1.0% fenugreek seed powder solution	13	18	13	18
	1.5% custard apple leaf powder solution	10	15	10	15
	Fenugreek seed powder @ 3 g kg^{-1}	12	15	12	14
	Custard apple leaf powder@ 4 g kg^{-1}	9	11	8	11
Flowering stage	1.0% fenugreek seed powder solution	15	22	15	22
	1.5% custard apple leaf powder solution	12	19	12	19
	Fenugreek seed powder @ 3 g kg^{-1}	10	18	10	17
	Custard apple leaf powder@ 4 g kg^{-1}	8	15	8	15
Harvesting stage	1.0% fenugreek seed powder solution	4	4	4	4
	1.5% custard apple leaf powder solution	2	3	2	3
	Fenugreek seed powder @ 3 g kg^{-1}	3	4	3	4
	Custard apple leaf powder@ 4 g kg^{-1}	1	3	1	3
Crop growth rate	1.0% fenugreek seed powder solution	15	24	15	23
	1.5% custard apple leaf powder solution	13	20	13	20
	Fenugreek seed powder @ 3 g kg^{-1}	9	19	9	18
	Custard apple leaf powder@ 4 g kg^{-1}	7	16	7	16

Generally the seedlings with high initial vigour performed better and utilize all the available resources for better growth. The high initial vigour of the fenugreek seed

powder and custard apple leaf powder treated seeds might have induced the early seedling growth and enabled better nutrient absorption by the foliage, encouraging quick growth and increased plant height with increased leaf area and leaf area index and this might be resulted in the higher dry matter production throughout the growth period. Similar results were reported by Sridhar *et al*. (2001) in chilli, Somasundaram (2003) in maize, sunflower and greengram and Sundaralingam (2005) in rice due to different organic invigouration treatments.

At constant plant density, the increase in dry matter and crop growth rate in treated seeds could contributed to the higher number of pods and pod and seed yield plant^{-1} than untreated control. Among the treatments wet and dry treatment of fenugreek seed powder and custard apple leaf powder registered its superiority both in fresh and aged seeds during both *kharif* and *rabi* season (Fig. 19). The increase in seed yield per hectare was 9 and 17 per cent over control of fresh and aged seeds respectively.

Parameters	**Best treatment**	***Kharif***		***Rabi***	
		% increase over control		**% increase over control**	
		Fresh seeds	**Aged seeds**	**Fresh seeds**	**Aged seeds**
Pod yield plant^{-1}, pod yield plot^{-1}, pod yield hectare^{-1}, seed yield plant^{-1}, seed yield plot^{-1}, seed yield hectare^{-1}	1.0% fenugreek seed powder solution	9	18	11	19
	1.5% custard apple leaf powder solution	8	15	8	18
	Fenugreek seed powder @ 3 g kg^{-1} of seed	9	16	9	17
	Custard apple leaf powder@ 4 g kg^{-1} of seed	6	15	6	16

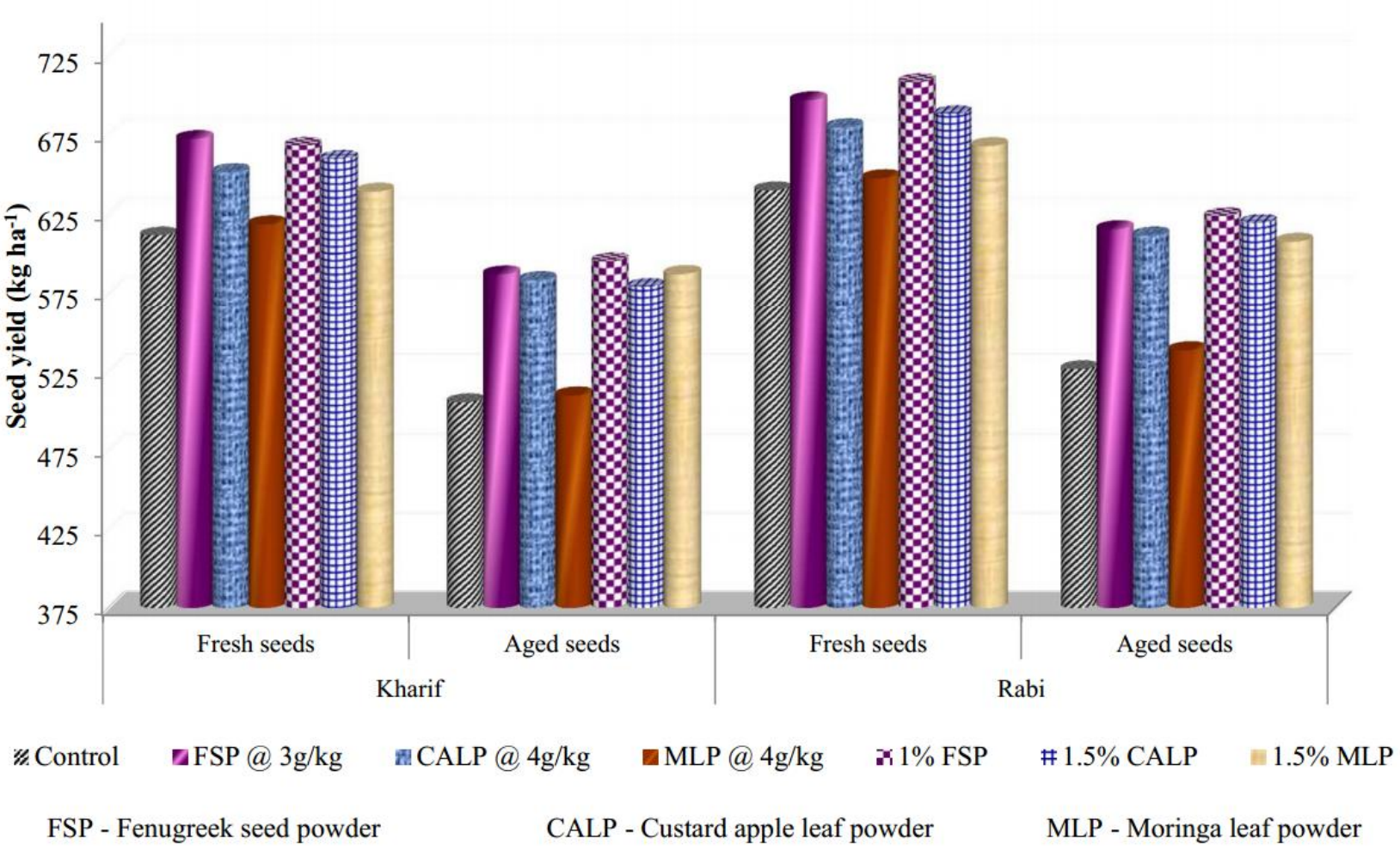


Fig. 19. Effect of botanical seed treatments on seed yield (kg ha^{-1}) of blackgram in *kharif* and *rabi* 2012

The positive effect of botanical seed treatments for improved yield was also reported by Sabir-Ahamed (1999a) in blackgram, Sasikala (1997) in cowpea and bhendi, Jegathambal (1996) in sorghum, Kavitha (2002) in blackgram, Somasundaram (2003) in maize, sunflower and greengram, Albert (2004) in tomato, Sundaralingam (2005), Vijayan (2005) in rice, Manimekalai (2006) in blackgram and Vijayalakshmi (2012) in tomato. The pronounced yield increase in aged seeds could be assigned to seed enhancement imposed by botanical treatments and loss of seed vigour in untreated seeds due to ageing as reported by Harrison (1966) and Perry (1977) in barley; TeKrony and Egli (1977) in soybean; Ramamoorthy and Basu (1997) in groundnut.

The botanical treatment with fenugreek seed powder and custard apple leaf powder either in dry or wet form, proved to be beneficial in laboratory experiments, have confirmed their efficacy under field condition also and the effect was always higher in aged seeds compared to fresh seeds.

The resultant seeds of crop raised from botanical treated seeds did not show any significant difference for germination, root length, dry matter production and vigour index over control seeds. The non-significant difference in seed quality characters in the resultant seeds were also reported by Angamuthu (1991) in small millets, Vijaya (1996) in blackgram and cowpea, Sabir-Ahamed (1999a; 1999b) in blackgram, greengram and cowpea and Manimekalai (2006) in blackgram.

5.6. Standardization of low temperature treatments to curb secondary infestation of pulse beetle (*Callosobruchus maculatus*) in blackgram

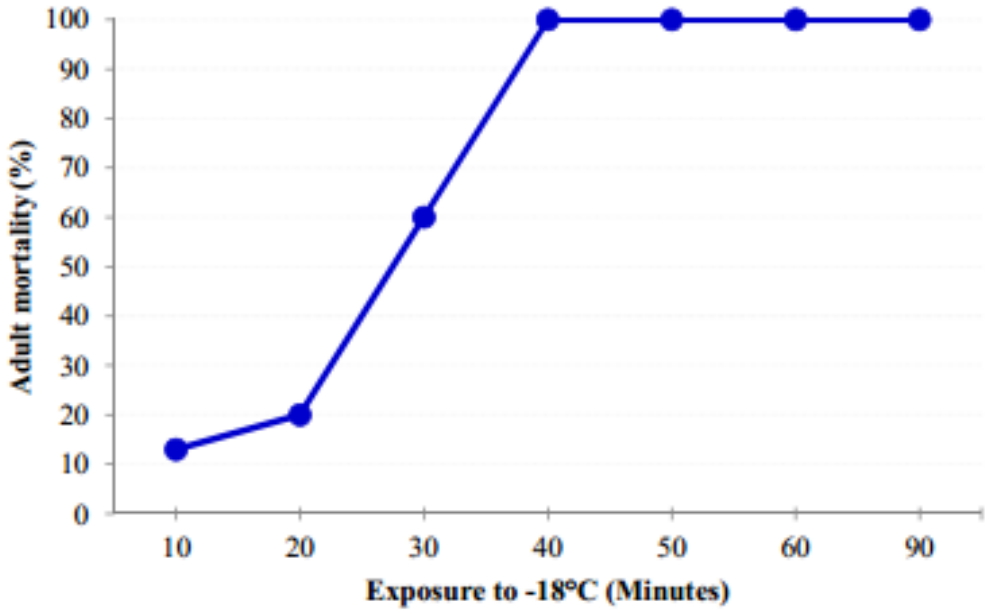
Pulse beetle start attacking the seed during the crop growth period in the field and the young larvae already started infesting dry bean seeds at the time of harvest. If disinfestation is not carried out immediately after harvest, the juvenile instars of pulse beetle will emerge and grow rapidly during the storage period and cause a serious damage to the seeds. Damaged seeds with emergence holes do not meet quality standard for sowing in subsequent season. Application of low temperature is known to be effective in controlling a wide variety of stored product insect pest (Fields, 1992). Therefore, current study was carried out with low temperature of -18°C, a temperature found in freezer of commercial refrigerator to curb the secondary infestation of pulse beetles.

5.6.1. Cold tolerant stage of pulse beetle

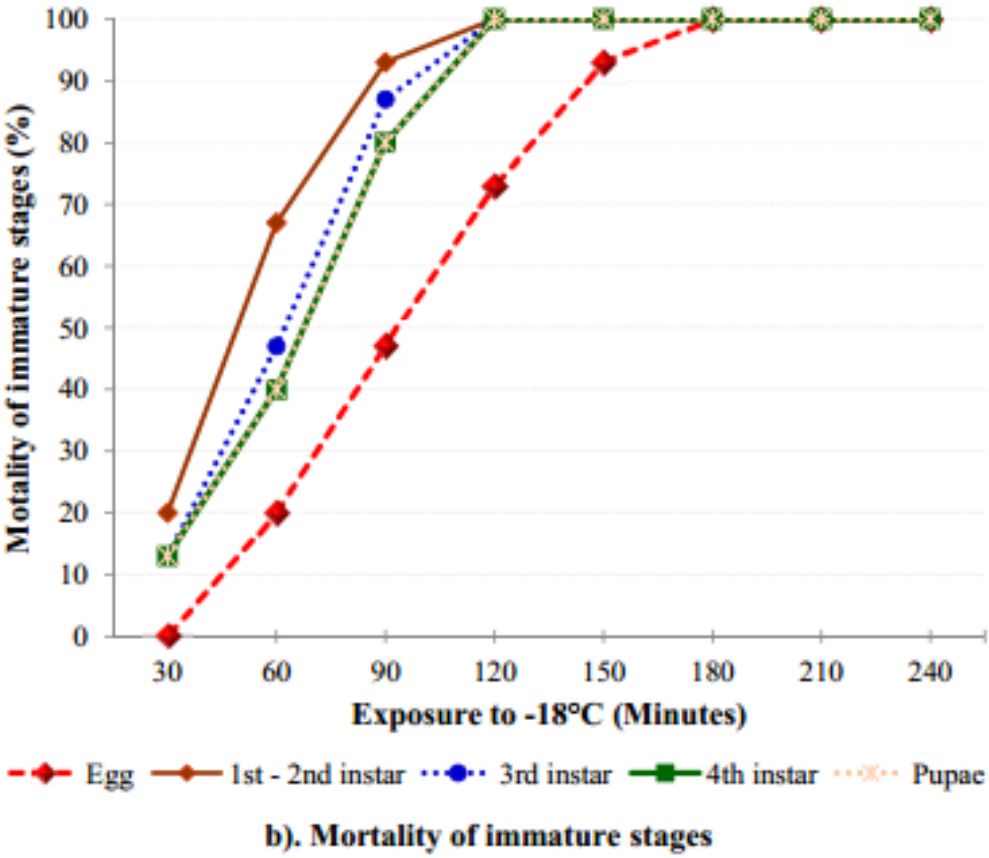
Early work with cold storage disinfestation of cowpea weevil dealt with treatment temperatures of -7 to 4°C (Duvel, 1905; Larson and Simmons, 1924). Very little work was done with relative cold tolerance of different life stages. Results of the present investigation shows that, among the different stages of pulse beetle *viz.*, egg, $1^{st} – 2^{nd}$, 3^{rd} and 4^{th} instar larva, pupa and adult, egg stage was more tolerant to low temperature of -18°C and adult stage was the most susceptible than the other immature stages. Forty minutes exposure is sufficient for 100% mortality in adults (Fig. 20a) while 120 min exposure is required for 100% mortality in $1^{st} – 2^{nd}$, 3^{rd} and 4^{th} instar larva and pupa. But egg stages require 180 min of exposure to low temperature for 100% mortality (Fig. 20b).

Although, Larson and Simmons (1924) indicated that eggs were the most susceptible stage when exposed to 7°C. In contrast, Mullen and Arbogast (1979) compared the effect of low temperatures on the eggs of five species of stored product insects and found *C. maculatus* eggs to be among the most cold tolerant. Results of present investigation were in harmony with Johnson and Valero (2003) and confirm that *C. maculatus* eggs are the most tolerant stage to freezing temperatures and that adults were highly susceptible. The reason for this difference was attributed to the fact that the larval stages of the pulse beetle feed and pupate within host seeds, larvae and pupae may experience some degree of insulation from cold temperatures when compared with free living adults. This may account for some of the difference in cold tolerance between these stages. However, cowpea weevil eggs are laid on the seed surface and are not insulated from freezing temperatures, suggesting that cold tolerance of cowpea weevil eggs is due to a physiological mechanism and not placement within a protected microhabitat.

Another line of reasoning is that mechanisms evolved for drought tolerance also impart cold tolerance (Ring and Danks, 1994). Appel *et al*. (1999) found that the cuticular permeability values of cowpea weevil adults were similar to xerically adapted arthropods, most probably an adaptation to the dry environments of stored beans. A relatively high proportion (67.7%) of cowpea weevil eggs were shown to hatch at humidity levels as low as 20% (Utida, 1972). The structure of *Callosobruchus* eggs may be an adaptation to xeric storage environments; the chorion not in immediate contact with the seed is thick



a). Adult mortality



b). Mortality of immature stages

Fig. 20. Effect of low temperature (-18°C) exposure on mortality of

(10 – 20 µm), with a posterior funnel that seems to be the single route for water loss (Credland, 1992). The cold tolerance of eggs of cowpea weevil may be due to mechanisms developed to prevent dehydration under dry conditions.

The reason for the mortality of other stages is due to the immobilization of rapidly cooled insects and then, they are physiologically injured when their temperature is reaching the super cooling point, at which crystallization of body fluid is starting (Fleurat-Lessard and Le Torch, 2001).

5.6.2. Effect of low temperature treatment (-18°C) on level of pulse beetle infestation and seed quality parameters during storage

Though exposure to low temperature of -18°C for 180 min can create 100% mortality in all the life stages of pulse beetle, the duration of exposure for controlling secondary infestation in packed seeds could be higher (Johnson and Valero, 2003) and the effect of low temperature exposure on seed quality during ambient storage needs further elucidation. Therefore, 500 g of fresh untreated seeds of blackgram cv. TNAU Blackgram CO 6 containing 10 seeds infested with pulse beetle eggs were packed air tight in 700 gauge polyethylene bag and subjected to low temperature treatments in the freezer of commercial refrigerator (-18°C) for 0, 2, 4, 6, 8 and 10 hours.

Among the different exposure durations, 6 h and higher durations resulted in 0% seed damage after 10 months of storage and also maintained slow rate of deterioration during storage which was evidenced from maintenance of moisture content at 8.1% without any further increase till the end of 10 months of storage and slow reduction in germination from 98% at the time of storage to 90% at the end of 10 months of storage. Similar slow reduction was also found for other seed quality parameters such as shoot and root length, dry matter and vigour index during ambient storage. This result was on par with the chemical insecticide (chlorpyriphos @ 2 ml kg^{-1}) treated seeds (Fig. 21 and 22). The maximum seed damage was observed in untreated control seeds where 100% seed damage was observed after 4 months of storage followed by 2 h exposed seeds in which 100% seed damage was observed after 6 months of storage (Plate 10; Fig. 21) with concomitant increase in seed moisture content which reached 10.0% in infested seeds at the end of 10 months of storage and higher reduction of seed

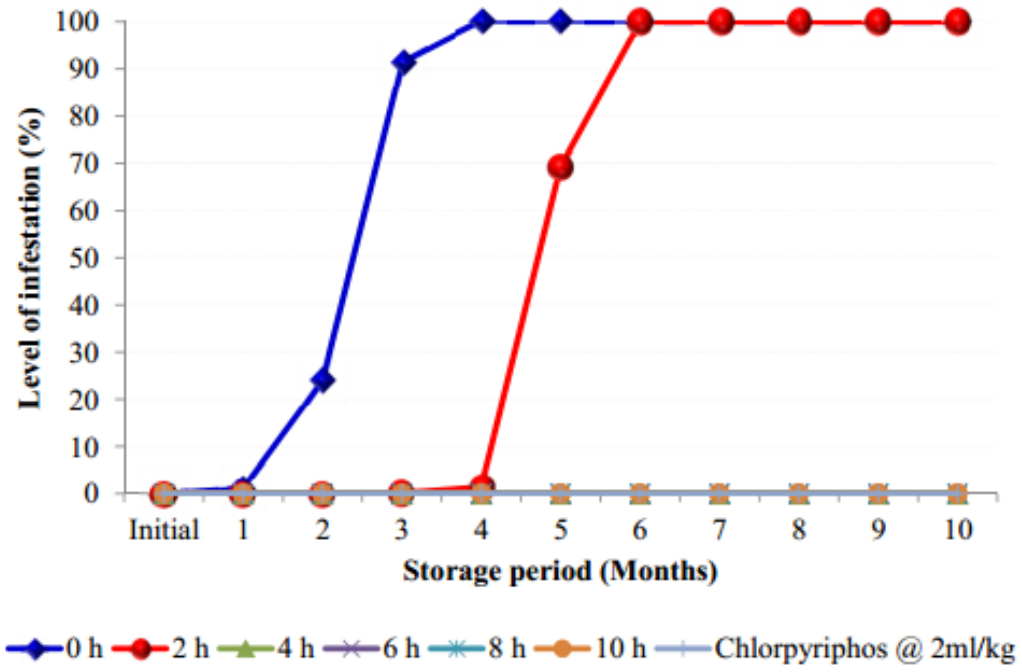


Fig. 21. Effect of low temperature (-18°C) exposure on pulse beetle infestation in stored seeds of blackgram

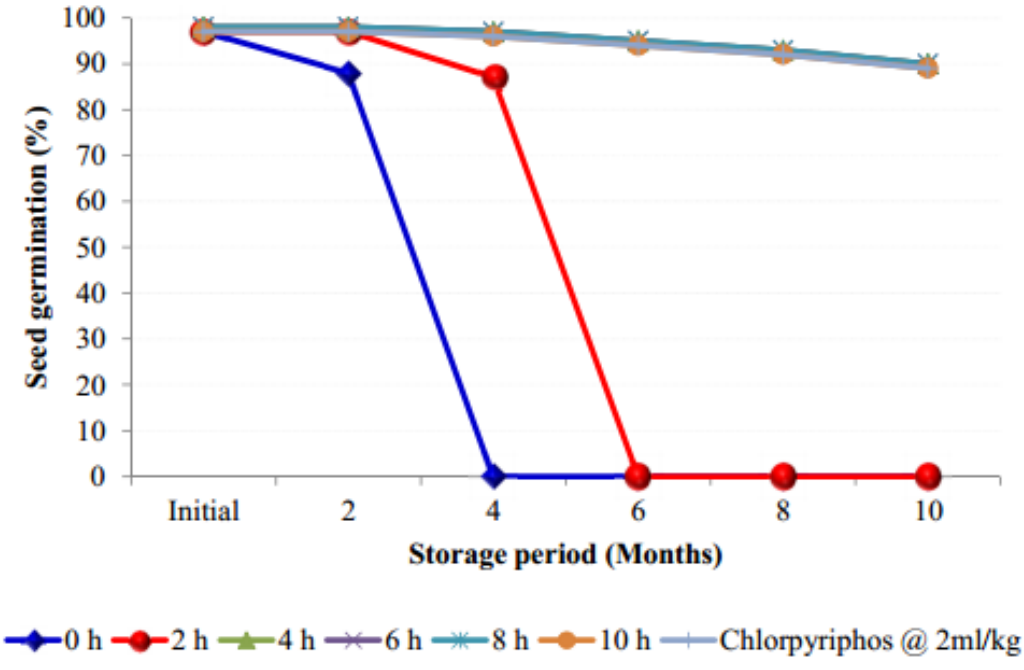


Fig. 22. Effect of low temperature (-18°C) exposure on seed germination in stored seeds of blackgram

Plate 10. Effect of low temperature (-18°C) exposure of blackgram seeds on pulse beetle infestation during storage

quality parameters such as germination, shoot and root length, dry matter and vigour index (Fig. 22). The result is` in harmony with Patro *et al.* (2007) who reported that population count and per cent seed infestation were directly proportional to the level of pulse beetle infestation and also proved that moisture content of seeds increased to the tune of 15 – 22% with increase in level of infestation. The increase in moisture content in infested seeds might be due to excretions produced by the different life stages of pulse beetle.

In 4 h exposed seeds, trace amount (0.01%) of seed damage was observed after 3 months of storage without any further increase in the level of infestation. Appearance of adults in traces without any further increase might be due to absence of opposite sex in the meager population that ceases further multiplication.

Therefore, low temperature (-18°C) exposure of blackgram seeds for 6 h could be optimum and sufficient to curb pulse beetle infestation of seeds packed in 500 g packets and could be used to control secondary infestation of pulse beetle during storage of blackgram seeds.

CHAPTER VI

SUMMARY

In order to bring out information on standardization of suitable vigour tests to delineate field emergence potential of seed lots, proteomics of seed deterioration, standardization of botanical seed treatment for maintenance of seed quality during storage, the probable mode of action of seed invigouration for viability maintenance and its effect on crop productivity and standardization of low temperature exposure duration to curb secondary infestation of pulse beetle during storage, studies were initiated using blackgram cv. TNAU Blackgram CO 6 seeds. The findings of the study are summarized and concluded hereunder.

6.1. Standardization of seed vigour test

To standardize suitable seed vigour test, seven seed lots of blackgram cv. TNAU Blackgram CO 6 were exposed to different vigour tests *viz*., accelerated ageing for different duration, controlled deterioration for varying moisture content and duration, complex stressing vigour test using different duration at 40^{o}C followed by 5^{o}C and electrical conductivity test using different volume of soaking and soaking duration at 20°C and mean germination time, which correlate well with field emergence.

Standard germination test grouped the seed lots only into two categories *viz*., seed lots having high germination of 97 to 99% (L1, L2, L5, L6 and L7) and low germination of 95 to 96% (L3 and L4). However, field emergence grouped the seed lots into three groups *viz*., seed lots with high germination of 95% (L7), medium germination of 90-92% (L1, L2, L5 and L6) and low germination of 86 to 87% (L3 and L4).

Among the different vigour tests evaluated, accelerated ageing for 3 days and electrical conductivity test with incubation of 6h in 75 ml distilled water at 20°C grouped seed lots similar to grouping based on field emergence.

Correlation analysis showed a significantly highest positive association between accelerated ageing test (r=0.993) and field emergence, and a significantly highest negative association between electrical conductivity test (r= -0.962) and field emergence. Hence,

these two vigour tests could be considered as suitable vigour test for discriminating blackgram seed lots based on vigour status.

6.2. Pattern of seed deterioration in blackgram through proteomic approach

Physiological observations on fresh and 2-10 days accelerated aged seeds revealed that germination and other vigor parameters (i.e., shoot and root length, dry matter production, vigor index I and II) declined gradually during accelerated ageing. Fresh seeds showed high initial germination (99%) and then started to decline as ageing progresses and reached 14% after 10 days of accelerated ageing. Importantly, on 6^{th} day of ageing, germinability of the seeds deteriorated to 58%, which is less than Indian Minimum Seed Certification Standard for germination (75%).

As ageing progresses, decrease in DPPH radical scavenging activity and increase in solute leakages was observed. The protease activity also increased with concomitant increase in free amino acid level. Changes in protein pattern showed a reduction in intensity of protein bands in aged seeds and loss of a protein with molecular weight 60.21 kDa in the aged seeds from 6^{th} day of ageing onwards

Prolonged ageing increased the rate of viability loss with concomitant loss in antioxidant activity, cell membrane integrity and proteins. The most interesting observation was both the viability reduction below germination standard and loss of protein band coincided on 6 days of accelerated ageing of seeds, which raise our conjecture that the protein damage has a pivotal command on seed viability. Therefore, further comparative proteome studies with fresh and 6 days accelerated aged seeds were carried out.

Comparative analysis of fresh and 6 days accelerated aged seeds using two dimensional electrophoresis and MALDI-TOF-MS revealed a total of 16 differentialy expressed proteins that couldmay be classified into 8 functional groups. Out of 16, 12 proteins were down-regulated and 4 proteins were up-regulated, indicating that artificial ageing affected the proteome of the dry seeds. Among the down-regulated proteins, cell structure related proteins were high in number. Down-regulation of enzymes like RNAP II, VARS, cytosolic 5'- nucleotidase (pyrimidine) that could affect effective transcription, translation and other metabolic process essential for successful germination and down-

regulation of storage protein namely 8S globulin and transporters like Sec-14 lipid-binding domain protein and SNARE that could desist from supplying adequate nutrients for prolific embryo during germination were observed. Apart from this 4 unclassified proteins were also down-regulated. Up-regulated proteins were related to proteolytic complex namely 26S proteasome, defense related protein namely PaO and elongation factor Tu. The down-regulated proteins in aged seeds might play important roles in transition of seeds from quiescent to active state of germination and hence, the loss of these proteins might be responsible for the loss of vigor and viability in seeds having below standard germination. However, to understand the exact contribution of these proteins during germination, further studies are required.

6.3. Standardization of botanical seed treatment

6.3.1. Standardization of seed dry dressing treatment with botanicals

To find out a suitable dry dressing treatment to improve the performance of blackgram seeds, fresh seeds and four days accelerated aged seeds were dry dressed with finely ground botanicals *viz*., fenugreek seed powder, custard apple leaf powder and moringa leaf powder @ 2, 3 and 4 g kg^{-1} of seeds and shaken for 1, 2 and 3h to find effective duration of shaking for uniform seed dressing.

All the botanicals, irrespective of its dosage and shaking duration, performed better than control both in the case of fresh and aged seeds. In fresh seeds, speed of germination and germination percentage was not significantly influenced by treatments, but all the other parameters *viz*., shoot length, root length, dry matter production, vigour index I and II 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 fenugreek seed powder @ 3 g kg^{-1} of seeds and custard apple leaf powder @ 4 g kg^{-1} of seeds with 1h shaking was found to be better in enhancing seed germination and vigour. Both the treatments increased germination percentage of aged seeds by 9% over control (81%) but no significant influence in germination of fresh seeds was observed. Vigour index I was increased by 17% and 32%, respectively for fresh and aged seeds over their respective

control. Similarly, increase in vigour index II was also much pronounced in aged seeds than fresh seeds.

To test the efficacy of seed dry dressing of fresh seeds with botanicals, treated seeds were subjected to accelerated ageing for 3 days and evaluated for seed quality. The result revealed the superiority of fenugreek seed powder @ 3 g kg^{-1} of seeds or custard apple leaf powder @ 4 g kg^{-1} of seeds with 1h shaking over control in terms of higher germination, shoot and root length, dry matter production, vigour index I and II.

Hence, seed dry dressing with fenugreek seed powder @ 3 g kg^{-1} of seeds and custard apple leaf powder @ 4 g kg^{-1} of seeds with 1h shaking could be imposed to improve the physiological performance of blackgram seeds. It is highly effective in case of aged seeds to alleviate the deleterious effect of ageing and subsequently, to improve its physiological performance during germination.

6.3.2. Standardization of wet treatment with botanicals

In order to standardize the wet seed treatment with botanicals to improve the performance of blackgram seeds, fresh and four days accelerated aged seeds were invigourated with 0.5, 1.0 1.5 and 2.0% solutions of botanicals *viz*., fenugreek seed powder, custard apple leaf powder and moringa leaf powder for 1, 2 and 3h to find effective botanical concentration and duration of soaking.

No significant difference in germination was observed in fresh seeds among the botanicals. However, in aged seeds, 1h soaking in 1% fenugreek seed powder (89%), 1.5% custard apple leaf powder (89%) and 1.5% moringa leaf powder solution (88%) recorded the maximum germination than untreated seeds (81%). Other physiological parameters *viz*. shoot and root length, dry matter production, vigour index I and II were the maximum for 1% fenugreek seed powder and 1.5% custard apple leaf powder treatment followed by 1.5% moringa leaf powder solution over control in both fresh and aged seeds.

Fresh treated seeds subjected to vigour test *viz*., accelerated ageing for 3 days also revealed the superiority of wet seed treatment with 1% fenugreek seed powder and 1.5%

custard apple leaf powder solution soaked for 1h in terms of higher germination, shoot and root length, dry matter production, vigour index I and II over other treatments.

Hence, the blackgram seeds wet treated by soaking for 1h in 1% fenugreek seed powder solution and 1.5% custard apple leaf powder solution increased the seed germination and vigour.

6.4. Influence of botanical seed treatment on biochemical characteristics

Electrical conductivity of seed leachate was lesser in all the botanical treatments compared to control. However, fenugreek seed powder and custard apple leaf powder registered lowest electrical conductivity both in dry dressing and wet treatment of fresh and aged seeds. The reduction in electrical conductivity of seed leachate in fresh seeds were 16 and 13% in wet treatment with 1% fenugreek seed powder and 1.5% custard apple leaf powder solution, respectively and 14 and 12% in dry dressing with fenugreek seed powder @ 3 g kg^{-1} of seeds and custard apple leaf powder @ 4 g kg^{-1} of seeds, respectively over control. In aged seeds, the reduction was still higher registering 23% lower electrical conductivity in seeds wet treated with 1% fenugreek seed powder solution over control.

The seeds treated with fenugreek seed powder or custard apple leaf powder in dry or wet form possessed high free radical scavenging property. In fresh seeds, the DPPH scavenging activity was higher by 9% in seeds treated with fenugreek seed powder and 8% in custard apple leaf powder treated seeds compared to control. In aged seeds, the difference in activity was still higher (29 and 27%, respectively) clearly establishing the repair mechanism initiated by the treatments.

All the treatments recorded maximum α - amylase activity over fresh and aged control. However, it was highly pronounced in seeds wet treated with 1% fenugreek seed powder (22.0 mm in fresh seeds and 15.8 mm in aged seeds) and 1.5% custard apple leaf powder solution (21.2 mm and 14.7 mm, respectively). Protease activity and free amino acid content were lower in seeds treated with fenugreek seed powder and custard apple leaf powder followed by moringa leaf powder in both fresh and accelerated aged seeds.

6.4.1. Biochemical properties of botanicals

Analysis of DPPH free radical scavenging activity of botanicals revealed higher antioxidant property in all the botanicals among which fenugreek seed powder and custard apple leaf powder topped with 95.9 and 96.0% free radical scavenging activity followed by moringa leaf powder (91.7%).

Apart from antioxidant property, botanicals also acted as a good source of minerals which was evidenced from ICP analysis. Fenugreek seed powder contain higher amount of Mo (40.24 ppm), Ti (38.34 ppm), Fe (20.51 ppm), Al (3.20 ppm), Zn (1.35 ppm) and traces of Mn, B, Cu, Sr, Ba. While custard apple leaf powder was rich in Ti (107.1 ppm), Mo (24.86 ppm), Fe (11.68 ppm), Al (6.10 ppm), Sr (3.19 ppm), B (2.36 ppm) and traces of Ba, Zn, Cu, Mn. Moringa leaf powder contains Ti (47.71 ppm), Mo (13.73 ppm), Fe (7.59 ppm), Al (2.79 ppm), Sr (2.78 ppm) and traces of Zn, B, Mn, Ba, Cu. However, moringa leaf powder contains less minerals than fenugreek seed powder and custard apple leaf powder. Among all these minerals, the availability of titanium, molybdenum and iron were higher in all the three botanicals. These might contribute to further improvement in performance of treated seeds as observed through significant increase in vigourous seedling formation.

6.5. Effect of seed treatments on field performance

The studies were carried out to evaluate the field performance of blackgram seeds treated with various botanicals. The crop was raised with constant plant population per unit area during *kharif* and *rabi* season 2012-13 at Agricultural Research Station, Bhavanisagar, using TNAU Blackgram CO 6 seeds.

The growth attributes such as plant height, leaf area and leaf area index were significantly higher in all the treated seeds over fresh and aged control at vegetative stage, while dry matter production was higher in all the stages *viz*., vegetative, flowering and harvesting stage. All the botanical treatments recorded higher dry matter compared to control which might be contributed to the increased crop growth rate in fresh and aged treated seeds. All the treatments including control recorded higher growth and yield during *rabi* season than *kharif* season.

At constant plant density, the increase in dry matter and crop growth rate in treated seeds could contributed to the higher number of pods and pod and seed yield per plant. Among all the treatments, wet and dry treatment of fenugreek seed powder and custard apple leaf powder registered its superiority both in fresh and aged seeds in both *kharif* and *rabi* season. The increase in seed yield per hectare was to the tune of 9 and 17 per cent over control in fresh and aged seeds, respectively.

6.6. Standardization of low temperature treatment to curb secondary infestation of pulse beetles (*Callosobruchus maculatus*) in blackgram

A study was carried out with low temperature of -18°C, a temperature found in freezer of commercial refrigerator, to curb the secondary infestation of pulse beetles. The most cold tolerant stage of pulse beetle was identified by exposing all the stages of pulse beetle to low temperature (-18°C) in different durations. Among the different stages of pulse beetle, egg stage was more tolerant to low temperature of -18°C and adult was the most susceptible stage than other immature stages. Exposure of adults for 40 min was sufficient to cause 100% mortality. While 120 min exposure was required for 100% mortality in 1^{st}, 2^{nd}, 3^{rd} and 4^{th} instar larva and pupa, but egg stage requires 180 min of exposure to cause 100% mortality

In order to find out the exposure duration required to control the secondary infestation in seed packets, 500 g of fresh untreated seeds of blackgram cv. TNAU Blackgram CO 6 containing 10 seeds infested with pulse beetle eggs were packed air tight in 700 gauge polyethylene bag and subjected to low temperature treatments in the freezer of commercial refrigerator (-18°C) for 0, 2, 4, 6, 8 and 10h.

The blackgram seeds exposed to -18°C temperature for 6h or more resulted in 0% seed damage after 10 months of storage and it reduced the rate of deterioration during storage which was evidenced from maintenance of moisture content at 8.1% without any further increase till the end of 10 months of storage and slow down the rate of reduction in germination from 98% at the time of storage to 90% at the end of 10 months storage. Similarly, slow down in reduction of other seed quality parameters such as shoot and root length, dry matter and vigour index was observed during ambient storage. The result was on par with the chemical insecticide chlorpyriphos @ 2 ml kg^{-1} treated seeds.

In seeds exposed for 4h at -18°C recorded trace amount (0.01%) of seed damage after 3 months of storage without any further increase in the level of infestation. Appearance of adults in traces without any further increase might be due to absence of opposite sex in the meager population that ceases further multiplication.

The maximum seed damage was observed in untreated control seeds where 100% seed damage was observed after 4 months of storage followed by 2h exposed seeds in which 100% seed damage was observed after 6 months of storage with concomitant increase in seed moisture content which reached 10.0% in infested seeds at the end of 10 months of storage.

Therefore, low temperature (-18°C) exposure for 6h could be optimum and sufficient to curb pulse beetle infestation in blackgram seeds packed in 500 g packets and it could be very effectively used to control secondary infestation of pulse beetle during storage of blackgram seeds.

REFERENCES

Abdul-Baki, A. A. 1980. Biochemical aspects of seed vigour. **HortScience, 15:** 765 – 771.

Abdul-Baki, A. A. and J. D. Anderson. 1973. Vigour determination of soybean seed by multiple criteria. **Crop Sci., 13:** 630-633.

Agrawal, G. K., M. Yonekura, Y. Iwahashi, H. Iwahashi and R. Rakwal. 2005a. Systems, trends and perspectives of proteomics in dicot plants Part I: Technologies in proteome establishment. **J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci., 815:** 109 – 123.

Agrawal, G. K., M. Yonekura, Y. Iwahashi, H. Iwahashi and R. Rakwal. 2005b. Systems, trends and perspectives of proteomics in dicot plants Part II: Proteomes of the complex developmental stages. **J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci., 815:** 125 – 136.

Agrawal, G. K., M. Yonekura, Y. Iwahashi, H. Iwahashi and R. Rakwal. 2005c. Systems, trends and perspectives of proteomics in dicot plants Part III: Unraveling the proteomes influenced by the environment and at the levels of functions and genetic relationships. **J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci., 815:** 137 – 145.

Albert, V. A. 2004. Organic Seed production in Tomato. **M.Sc. (Ag.). Thesis,** Tamil Nadu Agricultural University, Coimbatore.

Al-Maskri, A. Y., M. M. Khan, I. A. Khan and K. Al. Habisi. 2003. Effect of accelerated ageing on viability, vigour (PGR), lipid peroxidation and leakage in carrot (*Daucus carrota* L.) seeds. **Intl. J. Agri. Biol., 5(4):** 580-584.

Al-Maskri, A., M. M. Khan, O. Al-Manthery and K. Al-Habsi. 2002. Effect of accelerated aging on lipid peroxidation, leakage and seedling vigor (RGR) in cucumber (*Cucumis sativus* L.) seeds. **Pakistan J. Agri. Sci., 39:** 330 - 337.

Amar Kumar and J. L. Bennetzen. 1999. Plant retrotransposons. **Annu. Rev. Genet., 33:** 479 – 532.

Ananthi, E. C. 2008. Response of COH(M) 5 maize hybrid and its parents to seed priming. **M.Sc (Ag.). Thesis**, Tamil Nadu Agricultural University, Coimbatore.

Anderson, A. D. and J. E. Baker. 1983. Deterioration of seeds during aging. **Phytopathology, 73(2):** 321 – 325.

Anderson, J. D. 1970. Physiological and biochemical differences in deteriorating barley seed. **Crop Sci., 10:** 36-39.

Angamuthu, K. 1991. Studies on pelleting, physiological maturity, sowing quality and storagae in small millets. **M.Sc (Ag.). Thesis,** Tamil Nadu Agricultural University, Coimbatore.

Annegowda, H. V., W. Nee, M. N. Mordi, S. Ramanathan and S. M. Mansor. 2010. Evaluation of phenolic content and Antioxidant property of hydrolysed extracts of (*Terminalia catappa* L.) Leaf. **Asian J. Pl. Sci., 9:** 479 - 485.

Anuja, G. and K. R. Aneja. 2004. Seed deterioration in soybean varieties during storage and physiological attributes. **Seed Res., 32 (7):** 26-32.

AOSA. 1983. Seed vigour testing handbook, contribution no. 32. To the handbook on seed testing. Association of Official Seed Analysts, NE, USA, pp 88.

Appel, A. G., W. J. Moar, and M. J. Tanley. 1999. Water loss and mortality of adult cowpea weevils (Coleoptera: Bruchidae) exposed to desiccants and desiccating environments. **Environ. Entomol., 28:** 979 - 982.

Arati, P. 2000. Influence of containers and seed treatment on storability of chickpea. **M.Sc. (Ag.). Thesis.** University of Agricultural Science, Dharwad.

ASSOCHAM. 2012. Study paper on "Emerging pulses scenerio in 2015 - productivity, awareness and affordability to drive pulses economy in India". The Associated Chambers of Commerce and Industry of India, New Delhi, India. pp 1 – 18.

Atici, O., G. Agar and P. Battal. 2007. Influence of long term storage on plant growth substance levels, germination and seedling growth in legume seeds stored for 37 years. **Indian J. Agric. Sci., 12:** 1-5.

Bailly, C. 2004. Active oxygen species and antioxidants in seed biology. **Seed Sci. Res., 14 (2):** 93-107.

Bailly, C., H. E. M. Bouteau and F. Corbineau. 2008. From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology. **C. R. Biologies, 331:** 806–814.

Bailly, C., L. Bogatek, D. Come and F. Corbinean. 2002. Changes in activities of antioxidant enzymes and lipoxygenase during growth of sunflower seedlings from seeds of different vigour. **Seed Sci. Res., 12:** 47-55.

Barla-Szabo, G., B. Dolinka and M. Odiemah. 1990. Diallel analysis of seed vigour in maize. **Seed Sci. & Technol., 18:** 721-729.

Barla-Szabo, G. and B. Dolika. 1988. Complex stress vigour test: a new method for wheat and maize. **Seed Sci. & Technol., 16**: 63-73.

Barla-Szabo, G. and B. Dolinka. 1984. Relations between biological quality and size of seed in maize hybrids. **Novemytermeles, 33:** 501 – 506.

Barrientos, T., D. Frank, K. Kuwahara, S. Bezprozvannaya, R. Bassel-Duby, J. A. Richardson, H. A. Katus, E. N. Olson and N. Frey. 2007. Two novel members of the ABLIM protein family, ABLIM-2 and -3, associate with STARS and directly bind F-actin. **J. Biol. Chem., 282:** 8393 – 8403.

Barton, L. V. 1961. Seed preservation and longevity. Leonard Hill (Books) Ltd., London. p. 216.

Basak, O., I. Demir, K. Mavi and S. Matthews. 2006. Controlled deterioration test for predicting seedling emergence and longevity of pepper (*Capsicum annuum* L.) seed lots. **Seed Sci. & Technol., 34:** 723 – 734.

Baskar, R., V. Rajeswari and T. Sathish Kumar. 2007. *In vitro* antioxidant studies in leaves of *Annona* species. **Indian Journal of Experimental Biology, 45:** 480 – 485.

Basra, S. M. A., K. U. Rehman and S. Iqbal. 2000. Cotton seed deterioration: Assessment of some physiological and biochemical aspects. **Int. J. Agric. Biol., 2(3):** 195-198.

Bassuner, R., R. Manteuffel, K. Muntz, M. Puchel, P. Schmidt and E. Weber. 1983. Analysis of in vivo and in vitro globulin formation during cotyledon development of field beans (*Vicia faba* L. var. minor). **Biochem. Physiol. Pflanz., 178:** 665–684.

Basu, R. N. 1985. Mid-storage and pre-sowing seed treatments for lettuce and carrot. **Scientia Horticulturae**, **25(1):** 11–19

Bedi, S., R. Kaur, J. S. Sital and J. Kaur. 2006. Artificial ageing of Brassica seeds of different maturity levels. **Seed Sci. & Technol., 34:** 287-296.

Bellard, S. B., M. N. Merwade and L. Kukanoor. 2006. Seed quality as influenced by accelerated and natural ageing in bitter gourd (*Momordica charantia* L.). **Seed Res., 34 (1):** 66 – 69.

Berlett, B. S. and E. R. Stadtman. 1997. Protein oxidation in aging, disease and oxidative stress. **J. Biol. Chem., 272:** 20313 – 20316.

Bernal-Lugo, I., A. Camacho and A. Carballo. 2000. Effects of seed ageing on the enzymic antioxidant system of maize cultivars. **In:** Seed biology, Advances and applications, (eds. Black, M., K. J. Bradford and J. Vazquez - Ramos), Wallingford, CABI Publishing. pp. 151 - 160.

Bevan, M., I. Bancroft, E. Bent, K. Love, H. Goodman, C. Dean, R. Bergkamp, W. Dirkse, M. Van Staveren, W. Stiekema, L. Drost, P. Ridley, S. A. Hudson, K. Patel, G. Murphy, P. Piffanelli, H. Wedler, E. Wedler, R. Wambutt, T. Weitzenegger, T. M. Pohl, N. Terryn, J. Gielen, R. Villarroel, R. De Clerck, M. Van Montagu, A. Lecharny, S. Auborg, I. Gy, M. Kreis, N. Lao, T. Kavanagh, S. Hempel, P. Kotter, K. D. Entian, M. Rieger, M. Schaeffer, B. Funk, S. Mueller-Auer, M. Silvey, R. James, A. Montfort, A. Pons, P. Puigdomenech, A. Douka, E. Voukelatou, D. Milioni, P. Hatzopoulos, E. Piravandi, B. Obermaier, H. Hilbert, A. Düsterhöft, T. Moores, J. D. Jones, T. Eneva, K. Palme, V. Benes, S.

Rechman, W. Ansorge, R. Cooke, C. Berger, M. Delseny, M. Voet, G. Volckaert, H. W. Mewes, S. Klosterman, C. Schueller and N. Chalwatzis. 1998. Analysis of 1.9 Mb of contiguous sequence from chromosome 4 of *Arabidopsis thaliana*. **Nature, 391(6666):** 485 - 488.

Bewley, J. D. 1986. Membrane changes in seeds as related to germination and the perturbation resulting from deterioration in storage. **In:** McDonald MB, Nelson CJ, eds.Physiology of seed deterioration. CSSA Special Publication No 11. Crop Science Society of America, Madison, WI: 27–45.

Bewley, J. D. and M. Black. 1978. Physiology and biochemistry of seeds in relation to germination. Brelin springer-verlag. pp.306.

Bewley, J. D. and M. Black. 1982. Viability, Dormancy and Environment control. **In:** Physiology and biochemistry of seed. Vol. II, (eds. Bewley, J. D. and M. Black), Berlin, Springer verlag. pp. 1-59.

Bhalla, S., K. Gupta, B. Lal, M. L. Kapur and R. K. Khetarpal. 2008. Efficacy of various non-chemical methods against pulse beetle, *Callosobruchus maculatus* (Fab.). **In:** Endure International Conference on Diversifying Crop Protection, 12-15 October 2008, La Grande-Motte, France. pp. 1 – 4.

Bhatia, V. S., S. P. Tiwari and S. Pandey. 2002. Soybean seed quality scenario in India - A Review. **Seed Res., 30 (2):** 171 – 185.

Billaud, C. 2001. Composition, nutritional value and physiological properties. **Adrian J. fenugreek. Sciecnes-des-ailments, 21(1):** 3 - 26.

Blum, H., H. Beier and H. J. Gross. 1987. Improved silver staining of plant proteins,RNA and DNA in polyacrylamide gels. **Electrophoresis, 8:** 93-99.

Bose, A., S. Bhattacharya, J. Pandey and M. Biswas. 2011. Thin layer chromatographic studies and in vitro free radical scavenging activity of *Annoa squamosa* leaf extracts: **Pharmacology online, 1:** 1223- 1229.

Bouteau, H. E. M., C. Mazuy, F. Corbineau and C. Baily. 2011. DNA alteration and programmed cell death during ageing of sunflower seed. **J. Exp. Bot.,** doi:10.1093/jxb/err198

Brever, O. 1986. Medicinal plants in tropical West Africa, Cambridge University Press, Cambridge, UK.

Buchvarov, P. and T. S. Gantcheff. 1984. Physiological influence of accelerated and natural ageing on free radical levels in soybean seeds**. Physiologia Plantarum, 60:** 53-56.

Bukhari, S. B., M. I. Bhanger and S. Memon. 2008. Antioxidative activity of extracts from fenugreek seeds (*Trigonella foenum-graecum*). **Pak. J. Anal. Environ. Chem., 9(2):** 78 – 83.

Burgass, R. W. and A. A. Powell. 1984. Evidence of repair processes in the invigouration of seeds by hydration. **Ann. Bot., 53:** 753 - 757.

Burguieres. E., P. McCue, Y. I. Kwon and K. Shetty. 2007. Effect of vitamin C and folic acid on seed vigour response and phenolic-linked antioxidant activity**. Bioresource Technol., 98:** 1393 -1404.

Burris, J. S., O. T. Edje and A. H. Wahaab. 1969. Evaluation of various indices of seed and seedling vigour in soybeans (*Glycine max* L. Merr.). **Proceedings of the Association of Official Seed Analysts, 59:** 73 – 81.

Bustamante, L., M. G. Seddon, R. Don and W. J. Rennie. 1984. Pea seed quality and seedling emergence in the field. **Seed Sci. & Technol., 12:** 551-558.

Cakmak, T., A. Okkes, A. Guleray and S. Serap. 2010. Natural aging-related biochemical changes in alfalfa (*Medicago Sativa* L.) seeds stored for 42 years**. Int. Research J. Plant Sci., 1(1):** 001-006.

Cakmak, T., O. Atici and G. Agar. 2009. The natural aging related biochemical changes in the seeds of two legume varieties stored for 40 years. **Acta Agriculturae Scandinavica, Section B - Soil and Plant Science, 60(4):** 353 – 360.

Canovas, F. M., E. Dumas-Gaudot, G. Recorbet, J. Jorrin, H. P. Mock and M. Rossignol. 2004. Plant proteome analysis. **Proteomics, 4:** 285 – 298.

Capron, I., F. Corbineau, F. Dacher, C. Job, D. Come and D. Job. 2000. Sugarbeet seed priming: effects of priming conditions on germination, solubilisation of 11-S globulin and accumulation of LEA proteins. **Seed Sci. Res., 10:** 243-254.

Cash, T. P., Y. Pan and M. C. Simon. 2007. Reactive oxygen species and cellular oxygen sensing. **Free Radical Biology and Medicine, 43:** 1219–1225.

Chandrashekar, C. and V. R. Kulkarni. 2011. Isolation, characterizations and Free radical scavenging activity of *Annona squamosa* leaf. **J. Pharmacy Res., 4(3):** 610-611.

Channabasanagowda, N., K. Biradar Patil, B. T. Ninganur, B. N. Patil, Ravi Hunje and J. S. Awaknavar. 2008. Effect of Botanical Seed Treatment on storability of wheat. **Karnataka J. Agric. Sci., 21(3):** 361 – 365.

Chhabra, D. 1984. Seed deterioration studies in wheat and mung bean. **M. Phil., Dissertation.** Gujarat University, Ahmedabad, India.

Ching, T. M. and K. K. Ching. 1964. Freeze drying of pine pollen. **Plant Physiol., 39:** 705-709.

Chipungahelo, M. S., R. N. Misangu and S. O. W. M. Reuben. 2001. The effect of sowing bruchid damaged bean (*Phaseolus vulgaris* L.) seeds on germination, plant development and yield. Proceedings: Bean Seed Workshop

Chung, D. W., A. Pruzinska, S. Hortensteiner and D. R. Ort. 2006. The role of pheophorbide a oxygenase expression and activity in the canola green seed problem. **Plant Physiol., 142:** 88–97.

Cookson, W. R., J. S. Rowarth and J. R. Sedcole. 2001. Seed vigour in perennial ryegrass (*Lolium perenne* L.): effect and cause. **Seed Sci. & Technol., 29:** 255 – 270.

Coolbear, P. 1995. Mechanisms of seed deterioration, **In:** Seed Quality: Basic mechanisms and agricultural implications (Eds., Basra, A. S.). Food Products Press, New York pp. 223 - 277.

Coolbear, P., R. J. Slater and J. A. Bryant. 1990. Changes in nucleic acid levels associated with improved germination performance of tomato seeds after low temperature pre- sowing treatment. **Ann. Bot., 65:** 187-195.

Corbineau, F., C. G. Mathieu, D. Vinel and D. Come. 2002. Decrease in sunflower (*Helianthus annuus*) seed viability caused by high temperature as related to energy metabolism, membrane damage and lipid composition. **Physiologia Plantarum, 116:** 489 - 496.

Corbineau, F., M. A. Picard, J. A. Fougereux, F. Ladonne and D. Come. 2000. Effects of dehydration conditions on desiccation tolerance of developing pea seeds as related to oligosaccharide content and cell membrane properties. **Seed Sci. Res., 10:** 329 - 339.

Cornelius, S., S. Witz, H. Rolletschek and T. Mohlmann. 2011. Pyrimidine degradation influences germination seedling growth and production of *Arabidopsis* seeds. **J. Exp. Bot., 62(15):** 5623 – 5632.

Corredor, E., P. S. Testillano, M .J. Coronado, P.G. Melendi, R. F. Pacheco and C.Marquina. 2009. Nanoparticle penetration in living pumpkin plants: in situ subcellular identification. **BMC Plant Biology, 9:** 45 - 56.

Credland, P. 1992. The structure of bruchid eggs may explain the ovicidal effect of oils. **J. Stored Prod. Res., 28:** 1 - 9.

Crocker, W. and L. V. Barton. 1953. Physiology of seeds. **Chronica Botanica,** Waltham, Mass: 140-151.

Crowe, J. H., F. A. Hoekstra and L. M. Crowe. 1992. Anhydrobiosis. **Annual Review of Physiology, 54:** 570–599.

Dahiya, O. S., S. Verma and R. P. S. Tomer. 2001. Efficacy of vigour parameters for prediction of field potential in chickpea (*Cicer arietinum* L.). **Crop Res., 22(3)**: 363-366.

Dal, A. 1984. Seed deterioration studies in maize and pigeon pea. **M. Phil., Dissertation.** Gujarat University, Ahmedabad, India.

Dalling, J. W., A. S. Davis, B. J. Schutte and A. E. Arnold. 2011. Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community. **Journal of Ecology, (6):** 1 – 7.

Dantan-Gonzalez, E., Y. Rosenstein, C. Quinto and F. Sanchez. 2001. Actin monoubiquitylaton is induced in plants in response to pathogens and symbionts. **Molecular Plant–Microbe Interactions, 14:** 1267–1273.

Das, G. and S. Sen-Mandi. 1992. Scutellar amylase activity in naturally aged and accelerated aged wheat seeds. **Ann. Bot., 69:** 497-501.

Davies, M. J. 2005. The oxidative environment and protein damage. **Biochim Biophys Acta, 1703:** 93 – 109.

Dawale. R. P., G. S. K. Swamy, V. C. Kanamadi, P. Kumar, N. Thammaiah, T. Shantappa and R. C. Jagadeesha. 2011. **Karnataka J. Agric. Sci., 24 (4)**: 606 - 607.

De, B. K., A. K. Mandal and R. N. Basu. 1998. Effect of dry physiological seed treatments for improved vigour, viability and productivity of black gram (*Phaseolus mungo* Roxb.). **Indian Agric., 42:** 13-20.

De, B. K., A. K. Mandal and R. N. Basu. 2003. Seed invigouration treatments on different seed sizes of wheat (*Triticum aestivum* L.**)** for improved storability and field performance. **Seed Sci. & Technol., 31(2)**: 206 - 213.

De, B. K., A. K. Mandal and R. N. Basu. 2004. Compatibility of fungicides with pre-storage dry and mid-storage wet physiological seed treatments for storability and field performance of wheat (*Triticum aestivum* L.). **Tropical Agric., 81(2):** 117-129.

De, B. K., A. K. Mandal and R. N. Basu. 2005. Pre-sowing aerated and non-aerated hydration treatments for improved field performance of wheat. **Indian J. Plant Physiol., 10(2):** 133 - 138.

Delaplace, P., M. L. Fauconnier, K. Sergeant, J. F. Dierick, M. Oufir, F. Van der Wal, A. H. P. America, J. Renaut, J. F. Hausman and P. du Jardin. 2009. Potato (*Solanum tuberosum* L.) tuber ageing induces changes in the proteome and antioxidants associated with the sprouting pattern. **J. Exp. Bot., 60(4):** 1273–1288.

Dell'Aquila, A., G. Zocchi, G. A. Lanzani and P. De Leo. 1976. Different forms of EF1 and viability in wheat embryos Different forms of EF1 and viability in wheat embryos. **Phytochemistry, 15(11):** 1607–1610.

Delouche, J. C. and C. C. Baskin. 1973. Accelerated ageing techniques for predicting the relative storability of seed lots. **Seed Sci. & Technol., 12:** 427 - 452.

Demir, I. and K. Mavi. 2008. Controlled deterioration and accelerated aging tests to estimate the relative storage potential of cucurbit seed lots. **HortScience, 43(5):** 1544 – 1548.

Demir, I., C. Cebbeci and T. Guloksuz. 2012. Electrical conductivity measurement to predict germination of commercially available radish seed lots. **Seed Sci. & Technol., 40:** 229 – 237.

Demir, I., K. Mavi, B. B. Kenanoglu and S. Matthews. 2008a. Prediction of germination and vigour in naturally aged commercially available seed lots of cabbage (*Brassica oleracea* var. *capitata*) using the bulk conductivity method. **Seed Sci. & Technol., 36:** 509 – 523.

Demir, I., S. Ermis, K. Mavi and S. Matthews. 2008b. Mean germination time of pepper seed lots (*Capsicum annuum* L.) predicts size and uniformity of seedlings in germination tests and transplant modules. **Seed Sci. & Technol., 36:** 21 – 30.

Demirkaya, M., K. J. Dietz and H. O. Sivritepe. 2010. Changes in Antioxidant Enzymes during Ageing of Onion Seeds. **Not. Bot. Hort. Agrobot. Cluj., 38 (1) :** 49 – 52.

Diaz-Camino, C., R. Conde, N. Ovsenek and M. A. Villanueva. 2005. Actin expression is induced and three isoforms are differentially expressed during germination in *Zea mays*. **J. Exp. Bot., 56(412):** 557–565.

Doornbos, D. L. 1995. Seed vigor, **In:** Seed quality, basic mechanisms and agricultural implications (Eds., Basra, A. S.). Food Products Press, Binghamton, NY. pp. 45 - 80.

Dutra, A. S., and R. D. Vieira. 2006. Accelerated ageing test to evaluate seed vigour in pumpkin and zucchini. **Seed Sci. & Technol., 34(1):** 209-214.

Duvel, J. W. T. 1905. Cold storage for cowpeas. **U.S. Dep. Agr. Bur. Entomol. Bull., 54:** 49 -54.

Ebine, K., Y. Okatani, T. Uemura, T. Goh, K. Shoda, M. Niihama, M. T. Morita, C. Spitzer, M. S. Otegui, A. Nakano and T. Ueda. 2008. A SNARE complex unique to seed plants is required for protein storage vacuole biogenesis and seed development of *Arabidopsis thaliana*. **The Plant Cell, 20:** 3006–3021.

Edje, O. T. and J. S. Burris. 1970. Seedling vigour in soybeans. **Proceedings of the Association of Official Seed Analysts, 60:** 149 – 157.

Egli, D. B. and D. M. TeKrony. 1979. Relationship between seed vigour and the storability of soybean seeds. **J. Seed Technol., 3(2):** 1-11.

Eksi, C. and I. Demir. 2011. The use of a shortened controlled deterioration vigour test in predicting field emergence and longevity of onion seed lots. **Seed Sci. & Technol., 39:** 190 – 198.

Eliud, R., M. Reuben and G. Linnet. 2010. Longevity of bean (*Phaseolus vulgaris*) seeds stored at locations varying in temperature and relative humidity. **J. agric. pure appl. sci. technol., 5:** 60-70.

Ellis, R. H. 1989. The effects of differences in seed quality resulting from priming or deterioration in the relative growth rate of onion seedlings. **Acta Hort., 253:** 203-211.

Ellis, R. H. and E. H. Roberts. 1980. Towards a rational basis for testing seed quality. **In:** Seed production (ed. P. D. Hebblethwaite). Butterworths, London: pp 605 – 645.

Fahey, J. W. 2005. *Moringa oleifera*: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part 1. Trees for Life Journal: http://www.tfljournal.org/images/articles/20051201124931586_3.pdf

Ferguson, J. M. 1993. AOSA perspective of seed vigor testing. **J. Seed Technol., 17(2)**: 101-104.

Ferreira, P. M. P., D. F. Farias, J. T. A. Oliveira and A. F. U. Carvalho. 2008. *Moringa oleifera*: bioactive compounds and nutritional potential. **Revista de Nutricao, 21(4):** 431 – 437.

Ferreira, R. M. B., P. C. Rodrigues-Ramos, E. Franco, C. P. P. Ricardo and A. R. N. Teixeira. 1995. Changes in ubiquitin and ubiquitin-protein conjugates during seed formation and germination. **J. Exp. Bot., 46:** 211-219.

Fields, P. G. 1992. The control of stored product insects and mites with extreme temperatures. **J. Stored Prod. Res., 28:** 89 - 118.

Fleurat-Lessard, F. and J. M. Le Torch. 2001. Control of insects in post-harvest: high temperature and inert atmospheres. **In:** Physical control methods in plant protection, (Eds., Vincent, C., B. Panneton, F. Fleurat-Lessard). INRA-Editions / Springer, Paris / Berlin, pp. 74-94.

Freitas, R. A., D. C. F. Dos, M. G. A. Oliveira, L. A. Dos and P.C. Hilst. 2009. Physiological and biochemical changes in cotton seeds during storage. **Revista Brasileira de Armazenamento, 34(1):** 64-69.

Gallardo, K., C. Job, S. P. C. Groot, M. Puype, H. Demol, J. Vandekerckhove, and D. Job. 2001. Proteomic analysis of *Arabidopsis* seed germination and priming. **Plant Physiol., 126:** 835 – 848.

Germ, H. 1949. Die Feststellung der physiologisch bedingten triebkraft von samen. **Proceedings of the International Seed Testing Association, 15:** 1 – 23.

Ghosh, G., A. Pal and S. Biswas. 1989. Immunochemical identification of mung bean actin like protein and its cellular involvement during germination. **J. Biosci., 14(3):** 209-219.

Godakahriz, S. J., Z. Rastegar and M. Aghighi Shahverdikandi. 2012. Effect of seed aging on safflower (*Carthamus tinctorius* L.) seed vigor and germination parameters. **International Research Journal of Applied and Basic Sciences, 3(3):** 445 – 449.

Goel, A. and I. S. Sheoran. 2003. Lipid peroxidation and peroxide-scavenging enzymes in cotton seeds under natural ageing. **Biologia Plantarum, 46(3):** 429 - 434.

Goel, A., A. K. Coel and I. S. Sheoran. 2003. Changes in oxidative stress enzymes during artificial ageing in cotton (*Gossypium hirsutum* L.) seeds. **J. Plant Physiol., 160:** 1093 - 1100.

Grabe, D.F. 1965. Prediction of relative storability of corn seed lots. **Proc. Asso. Off. Seed Anal., 55:** 92-96.

Gray, J., P. S. Close, S. P. Briggs and G. S. Johal. 1997. A novel suppressor of cell death in plants encoded by the Lls1gene of maize. **Cell, 89:** 25 - 31.

Grilli, I., E. Bacci, T. Lombardi, C. Spano and C. Floris. 1995. Natural ageing: Poly (A) polymerase in germinating embryos of *Triticum durum* wheat. **Ann. Bot., 76:** 15 – 21.

Guerinot, M. L. and Y. Yi. 1994. Iron: nutritious, noxious, and not readily available. **Plant Physiol., 104:** 815 – 820.

Gujar, G. T. and T. D. Yadav. 1978. Feeding of *Callosobruchus maculatus* F. (Colepotera: Bruchidae) reared on different food and temperature. **Journal of Stored Products Research, 22(2):** 71 - 75.

Hampton, J. G. 1991. Herbage seed lot vigour – do problems start with seed production?. **Journal of Applied Seed Production, 9:** 87 – 93.

Hampton, J. G. 1998. Report of the vigour test committee 1995 – 1998. **Supplement to Seed Sci. & Technol., 26:** 265 – 269.

Hampton, J. G. and D. M. TeKrony. 1995. Handbook of vigour test methods. International Seed Testing Association, Switzerland.

Hampton, J. G. and P. Coolbear. 1990. Potential versus actual seeds performance – can vigour testing provide an answer?. **Seed Sci. & Technol., 18:** 215-228.

Hansen, W. R. 1972. Net photosynthesis and evapo-transpiration of field grown soyabean canopies. **Ph.D. Thesis,** IOWA State University Library**,** Ames.

Harman, G. E. 1983. Mechanisms of seed infection and pathogenesis. **Phytopathology, 73(2):** 326 – 329.

Harrison, B. J. 1966. Seed deterioration in relation to seed storage conditions and its influence upon seed germination, chromosomal damage and plant performance. **J. Natl. Instt. Agric. Bot., 10:** 644-663.

Havstad, L. T., T. S. Aamlid and A. Lomholt. 2011. Evaluation of vigour tests for determination of seed storage potential in red clover (*Trifolium pratensis* L.) and timothy (*Phleum pretense* L.). **Seed Sci. & Technol., 39:** 637 - 648.

Hazarika, D. K., S. Sarmah, T. Paramanick, K. Hazarika and A. K. Phookan. 2000. Biological management of tomato damping off caused by *Pythium aphanidermatum*. **Indian J. Plant Pathol., 18(1&2):** 36-39.

Henckel, P. A. 1964. Physiology of plant under drought**. Ann. Rev. Pl. Physiol., 15**: 365 – 386.

Hendry, G. A. F. 1993. Oxygen, free radical processes and seed longevity. **Seed Sci. Res., 3:** 141-153.

Heydecker, W. 1972. Vigour. **In:** Viability of Seeds (ed. Roberts, E.H.)., Chapman and Hall, London. pp. 209 - 286.

Heydecker, W. and P. Coolbear. 1977. Seed treatment for improved performance survey and attempted progress. **Seed Sci. & Technol., 5:** 354-425.

Hibbard, R. P. and E. V. Miller. 1928. Biochemical studies on seed viability. 1. Measurements of conductance and reduction. **Plant Physiol., 3:** 335 – 352.

Hsu, C. C., J. J. Chen and J. M. Sung. 2003. Accelerated aging-enhanced lipid peroxidation in bitter gourd seeds and effects of priming and hot water soaking treatments. **Scientia Horticulturae, 98:** 201-212.

Hussaini, S. H., Z. A. Ahmed and A. Dhanraj. 1988. The effect of accelerated ageing on germination, vigour and yield of maize. **Seed Res., 16**: 68-74.

Hussey, P. J., T. Ketelaar and M.J. Deeks. 2006. Control of the actin cytoskeleton in plant cell growth. **Annu. Rev. Plant Biol., 57:**109–25.

ISTA. 1993. International rules for seed testing. Supplement to **Seed Sci. & Technol., 21:** 1 – 288.

ISTA. 2007. International rules for seed testing. International Seed Testing Association, Bassersdorf, Switzerland.

Jatoi, S. A., M. Afzal, S. Nasim and R. Anwar. 2001. Seed deterioration study in pea, using accelerated ageing techniques. **Pakistan Journal of Biological Sciences, 4(12):** 1490 – 1494.

Jayanthi, M. 2008. Organic fortification of seed and seed crop of rice with pulse sprout extract (*Oryza sativa* L.). **M. Sc. (Ag.) Thesis**, Tamilnadu Agricultural University, Coimbatore.

Jeeva, B. 2003. Seed development and maturation, picking and storage studies in *Mucuna pruriens* (L.) **M. Sc. (Ag.) Thesis,** Tamil Nadu Agricultural University, Coimbatore.

Jegathambal, R. 1996. Pre-sowing seed treatment to augment productivity of sorghum cv. CO 26 under rainfed agriculture. **Ph.D. Thesis,** Tamil Nadu Agricultural University, Coimbatore – 3.

Job, C., A. Kersulec, L. Ravasio, S. Chareyre, R. Pepin and D. Job. 1997. The solubilisation of the basic subunit of sugarbeet seed 11-S globulin during priming and early germination. **Seed Sci. Res., 7:** 225-243.

Johnson, J. A. and K. A. Valero. 2003. Use of commercial freezers to control cowpea weevil, *Callosobruchus maculatus* (coleoptera: bruchidae), in organic garbanzo beans. **Journal of Economic Entomology, 96(6):** 1952 – 1957.

Jorrin, J. V., A. M. Maldonado and M. A. Castillejo. 2007. Plant proteome analysis: a 2006 update. **Proteomics, 7:** 2947 – 2962.

Kader, J. C. 1996. Lipid-transfer proteins in plants. **Annu. Rev. Plant Physiol. Plant Mol. Biol., 47:** 627–654.

Kaewnaree, P., V. Sukanda, K. Preekamol, S. Boonmee and V. Kanit. 2011. Electrolyte leakage and of fatty acid changing associated with seed germination in accelerated aging sweet pepper seeds. **J. Biotechnol., 136:** 147–169.

Kafer, C., L. Zhou, D. Santoso, A. Guirgis, B. Weers, S. Park and R. Thornburg. 2004. Regulation of pyrimidine metabolism in plants. **Frontiers in Bioscience, 9:** 1611 – 1625.

Kaiser, B. N., K. L. Gridley, J. N. Brady. 2005. The Role of Molybdenum in Agricultural Plant Production. **Ann. Bot., 96:** 745 - 754.

Kapoor, N., A. Arya, M. A. Siddiqui, A. Amir and H. Kumar. 2010. Seed deterioration in chickpea (*Cicer arietinum* L.) under accelerated ageing. **Asian Journal of Plant Sciences, 9(3):** 158 – 162.

Kapoor, N., A. Arya, M. A. Siddiqui, H. Kumar and A. Amir. 2011. Physiological and biochemical changes during seed deterioration in aged seeds of rice (*Oryza sativa* L.). **American Journal of Plant Physiology, 6(1):** 28 – 35.

Kapri, B., A. K. Sengupta, B. K. De, A. K. Mandal and R. N. Basu. 2003. Pre-storage seed invigoration treatments for improved germinability and field performance of okra (*Hibiscus esculentus*). **Indian J. Agric. Sci., 73 (5):** 276-279.

Kareem, A. A., R. C. Sexena, M. E. M. Boncodin, V. Krishnasamy and D. V. Seshu. 1989. Neem as seed treatment for rice before sowing: effects on two homopterous for insects and seedling vigour. **J. Eco. Ent., 82(4):** 1219-1223.

Karthikeyan, K. and L. M. Shukla. 2008. Effect of Boron-sulphur Interaction on their uptake and quality parameters of mustard (*Brassica juncea* L) and sunflower (*Helianthus annuses* L.) **J. Ind. Soci. Soil. Sci., 56(2):** 225-230.

Kavak, S., H. Ilbi and B. Eser. 2008. Controlled deterioration test determines vigour and predicts field emergence in pepper seed lots. **Seed Sci. & Technol., 36:** 456 – 461.

Kaviarasan, S., G. H. Naik, R. Gangabhagirathi, C. V. Anuradha and K. I. Priyadarsini. 2007. *In vitro* studies on antiradical and antioxidant activities of fenugreek (T*rigonella foenum graecum*) seeds: **Food Chemistry, 103:** 31- 37.

Kavitha, S. 2002. Seed hardening and pelleting for maximizing the productivity of black gram (*Vigna mungo* (L.) Hepper). Cv. Vamban 3 under rainfed condition. **M.Sc. (Ag.) Thesis**, Tamil Nadu Agricultural University, Coimbatore.

Kavitha, S., K. Vanangamudi and A. Sabir-Ahamed. 2003. Vigour and viability of hardened and pelleted seeds of black gram (*Vigna mungo L.*). **Madras Agric. J., 90(4-6):** 304-309.

Khan, M. M., M. J. Iqbal, M. Abbas, H. Raza, R. Waseem and A. Ali. 2004. Loss of vigour and viability in aged onion (*Allium cepa* L.) seeds. **Int. J. Agri. Biol., 6(4):** 708 – 711.

Khan, W., B. Prithiviraj and D. L. Smith. 2003. Photosynthetic responses of corn and soybean to foliar application of salicylates. **J. Plant Physiol., 160:** 485-492.

Khanahmadi, M., S. Hezazadeh and M. Taran. 2010. In vitro antimicrobial and antioxidant properties of *Smyrnium cordifolium* Boiss.(Umbelliferae) extract. **Asian J. Plant Sci., 9**: 99-103.

Kharab, R. P. S. and B. S. Dahiya. 2000. Influence of natural ageing of seeds on field performance in pigeon pea. **Seed Res., 28 (2):** 149-152.

Kibinza, S., D. Vinel, C. Daniel, C. Bailly and F. Corbineau. 2006. Sunflower seed deterioration as related to moisture content during ageing, energy metabolism and active oxygen species scavenging. **Physiol. Plant., 128:** 496 - 506.

Koleckar, V., D. Jun, L. Opletal, L. Jahodar and L. Kuca. 2007. Assay of radical scavenging activity of antidotes against chemical warfare against by DPPH test using sequential injection technique. **J. Appl. Biomed., 5:** 81-84.

Krishnan, P., N. Shantha and A. V. Moharir. 2004. Thermodynamic characterization of Seed deterioration during storage under accelerated ageing conditions. **Biosystems Engineering, 89 (4):** 425 - 433.

Krishnaveni, K. 2003. Field performance of differentially aged seeds using seed and plant leaf extracts on seed yield and quality of paddy variety IR 20. **Madras Agric. J**., **90 (10-12):** 686-690.

Kumar, P. and S. Bhalla. 2007. Effect of low temperatures on growth of pulse beetle (*Callosobruchus maculatus* Fab.) in mungbean (*Vigna radiata* L. Wilczek). **Indian Journal of Plant Protection, 35:** 39 - 42.

Kummar, V., A. K. Abbas, N. Fausto, S. L. Robbins and R. S. Cotran. 2004. Pathology basis of disease. New York: WB Saunders.

Kundagrami, S., B. K. De and A. K. Mandal. 2008. Post-harvest seed invigouration treatments for improved storability and field performance of rice (*Oryza sativa* L.). **Crop Res., 34(3):** 317 -322.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. **Nature, 227(5259):** 680 - 685.

Larson, A. O. and P. Simmons. 1924. Insecticidal effect of cold storage on bean weevils. **J. Agric. Res., 27:** 99 -105.

Layek, N., B. K. De, S. K. Mishra and A. K. Mandal. 2006. Seed invigouration treatments for improved germinability and field performance of gram *(Cicer arietinum* L.). **Legume Res., 29 (4):** 257 – 261.

Lee, S. S., A. G. Taylor, M. B. Beresniewicz and D. H. Paine. 1995. Sugar leakage from aged leek, onion and cabbage seeds. **Plants Var. Seeds, 8:** 81 - 86.

Lee, Y. P., K. H. Baek, H. S. Lee, S. S. Kwak, J. W. Bang and S. Y. Kwon. 2010. Tobacco seeds simultaneously over-expressing Cu/Zn-superoxide dismutase and ascorbate peroxidase display enhanced seed longevity and germination rates under stress conditions. **J. Exp. Bot., 61(9):** 2499–2506.

Leprince, O., J. Buitink and F. A. Hoekstra. 1999. Axes and cotyledons of recalcitrant seeds of *Castanea sativa* Mill. exhibit contrasting responses of respiration to drying in relation to desiccation sensitivity. **J. Exp. Bot., 50(338):** 1515–1524.

Levine, R. L. and E. R. Stadtman. 2001. Oxidative modification of proteins during aging. **Exp. Gerontol., 36:** 1495 - 1502.

Levine, R. L., D. Garland, C. N. Oliver, A. Amici, I. Climent, A. G. Lenz, B. W. Ahn, S. Shaltiel and E. R. Stadtman. 1990. Determination of carbonyl content in oxidatively modified proteins. **Methods Enzymol., 186:** 464 – 478.

Li, Q., B. C. Wang, Y. Xu and Y. X. Zhu. 2007. Systematic studies of 12S seed storage protein accumulation and degradation patterns during Arabidopsis seed

maturation and early seedling germination stages. **J. Biochem. Mol. Biol., 40:** 373–381.

Lo, Y. S., N. Cheng, L. J. Hsiao, A. Annamalai, G. Y. Jauh, T. N. Wen, H. Dai and K. S. Chiang. 2011. Actin in mung bean mitochondria and implications for its function. **The Plant Cell, 23:** 3727–3744.

Loganathan, M., D. S. Jayas, P. G. Fields and N. D. G. White. 2011. Low and high temperatures for the control of cowpea beetle, *Callosobruchus maculatus* (F.) (coleoptera: Bruchidae) in chickpeas. **Journal of Stored Products Research, 47:** 244 – 248.

Lopes, M. M., R. M. Barbosa and R. D. Vieira. 2012. Methods for evaluating the physiological potential of scarlet eggplant (*Solanum aethiopicum*) seeds. **Seed Sci. & Technol., 40:** 86 – 94.

Lovato, A. and N. Balboni. 1997. Seed vigour in maize (*Zea mays* L.): two year laboratory and field test compared. **Italian. J. Agron., 1(1):** 1-6.

Lowell, G. 2005. Moringa leaf extract as a plant growth hormone. Paper presented at ECHO's conference church world service, Daker.

Lyon, W. F. 1997. Seed weevils. The Ohio State University Extension fact sheet HYG-2085-97 (http://www.ag.ohiostate.edu/~ohioline/hyg-fact/2000/2085.html).

Machado, N. B., C. Custodio and M. Takai. 2001. Evaluation of naturally and artificially aged seeds of *Phaseolus vulgaris*. **Seed Sci. & Technol., 29:** 137-149.

Maeda, H., Y. Sakuragi, D. A. Bryant and D. Della Penna. 2005. Tocopherols Protect *Synechocystis* sp. Strain PCC 6803 from Lipid Peroxidation. **Plant Physiol., 138:** 1422–1435.

Maguire, I. D. 1962. Speed of germination aid in selection and evaluation for seedling emergence and vigour. **Crop Sci., 2**: 176 – 177.

Major, D. J. and T. B. Daynard. 1972. Hyperpolic realtion between leaf area index and plant population in corn (*Zea mays*). **Canadian J. Plant Sci., 52:** 112-115.

Mak, Y., R. D. Willows, T. H. Roberts, C. W. Wrigley, P. J. Sharp and L. Copeland. 2009. Germination of Wheat: A Functional Proteomics Analysis of the Embryo. **Cereal Chem., 86(3):** 281–289.

Makkar, H. P. S. and K. Becker. 1997. Nutrients and antiquality factors in different morphological parts of the *Moringa oleifera* tree. **J. Agric. Sci., 128(3):** 311 - 322.

Malarkodi, K. 2003. Integrated management techniques for seed storage in green gram (*Vigna radiata* L. Wilczek). **Ph.D. Thesis,** Tamil Nadu Agriculture University, Coimbatore.

Mandal, A. K. and B. K. De. 1999. Prestorage seed invigouration for maintenance of vigour and viability in onion seeds. **Indian J. Agric. Sci., 69:** 627- 630.

Mandal, A. K., B. K. De, R. Saha and R. N. Basu. 2000. Seed invigoration treatments for improved storability, field emergence and productivity of soybean (*Glycine max* (L.). Merrill). **Seed Sci. &Technol., 28:** 349-355.

Mandal, S. and R. K. Mandal. 2000. Seed storage proteins and approaches for improvement of their nutritional quality by genetic engineering. **Current Science, 79(5):** 576- 589.

Manimekalai, C. 2006. Organic seed invigouration in black gram (*Vigna mungo* (L.) Hepper) cv. APK 1. **M.Sc. Thesis,** Tamil Nadu Agricultural University, Coimbatore.

Manjunathaswamy, K. K. and R. Narayanaswamy. 1996. Effect of natural ageing and seed rate compensation on crop growth performance and grain yield in hybrid maize (*Zea mays L*.). **Seed Res., 24(2):** 93-96.

Maraddi, B. 2002. Influence of growth regulators on seed yield and quality and seed treatments on storability of cowpea cv. C-252. **M.Sc (Ag.). Thesis,** University of Agricultural Sciences, Dharwad.

Marcos Filho, J. 2005. Fisiologia de sementes de plantas cultivadas. (Seed physiology of cropped species). Piracicaba: FEALQ.

Marcu, M. G. 2005. Miracle Tree. **KOS Health Publications**, Canada, pp. 108-115.

Maree, P. H., D. Marais and C. F. Recnhardt. 2007. Prediction of field emergence of maize (*Zea mays* L.) hybrids exposed to cold wet conditions. **African Crop Sci. Proc., 8:** 23-26.

Marshall, A. H. and R. E. L. Naylor. 1985. Seed vigour and field establishment in Italian ryegrass (*Lolium multiforum* Lam.). **Seed Sci. & Technol., 13:** 781 – 794.

Matilla, A. and G. Nicolas. 1980. Preformed mRNA in cotyledons of ungerminated seeds of *Cicer arietinum* L. **Plant Physiol., 65:** 1128-1132.

Matthews, S. 1980. Controlled deterioration: a new vigour test for crop seeds. **In:** Seed Production, P. D. H ebblethwaite (ed) Butterworths, London : 647-660.

Matthews, S. and A. A. Powell. 1987. Controlled deterioration test. **In:** Handbook of Vigour Test Methods, 2nd edition, F. Fiala (ed), ISTA, Zurich. pp. 49-56.

Matthews, S. and A. A. Powell. 2006. Electrical conductivity test: Physiological basis and use. **Seed Testing International, 131:** 32 – 35.

Matthews, S. and W. T. Bradnock. 1968. Relationship between seed exudation and field emergence in peas and French beans. **Hort. Res., 8:** 89-93.

Matthews, S., I. Demir, T. Celikkol, B. B. Kenanoglu and K. Mavi. 2009. Vigour tests for cabbage seeds using electrical conductivity and controlled deterioration to estimate relative emergence in transplant modules. **Seed Sci. & Technol., 37:** 736 – 746.

Matthews, S., M. H. Wagner, A. Ratzenboeck, M. Khajeh-Hosseini, E. Casarini, R. El-Khadem, M. El Yakhlifi and A. A. Powell. 2011. Early counts of radicle emergence during germination as a repeatable and reproducible vigour test for maize. **Seed Testing International, 141:** 39 – 45.

Matthews, S., R. El-Khadem, E. Casarini, M. Khajeh-Hosseini, M. Nasehzadeh and M. H. Wagner. 2010. Rate of physiological germination compared with the cold test and accelerated ageing as a repeatable vigour test for maize. **Seed Sci. & Technol., 36:** 379 – 389.

Mavi, K. and I. Demir. 2005. Controlled deterioration for vigour assessment and predicting seedling growth of winter squash (*Cucurbita maxima*) seed lots under salt stress. **New Zealand Journal of Crop and Horticultural Science, 33:** 193 – 197.

Mavi, K. and I. Demir. 2007. Controlled deterioration and accelerated ageing tests predict relative seedling emergence potential of melon seed lots. **HortScience, 42(6):** 1431 – 1435.

Mavi, K., I. Demir and S. Matthews. 2010. Mean germination time estimates the relative emergence of seed lots of three cucurbit crops under stress conditions. **Seed Sci. & Technol., 38:** 14 – 25.

McDonald, M. B. 1993. The history of seed vigour testing. **J. Seed Technol., 17(2):** 93-104.

McDonald, M. B. 1999. Seed deterioration: Physiology, repair and assessment. **Seed Sci. & Technol., 27:** 177-237.

McDonald, M. B. 2004. Orthodox seed deterioration and its repair. In Handbook of seed physiology: applications to agriculture (Eds R.L. Benech-Arnold and R.A. Sanchez). Food Products Press and The Haworth Reference Press, imprints of The Haworth Press, Inc, New York, London. pp 273 – 304.

McDonough, C. M., C. D. Floyd, R. D. Waniska and L. W. Rooney. 2004. Effect of accelerated aging on maize, sorghum and sorghum meal. **J. Cereal Sci., 39:** 351-361.

McQuaker N. R., D. F. Brown and P. D. Fluckner. 1979. Digestion of environmental materials for analysis by inductively coupled plasma atomic emission spectrometry. **Anal. Chem., 51:** 1082–1084.

Menaka, C., K. Vanangamudi, R. Prabhakar, A. Bharathi and P. Natesan. 2003. Management of seed borne grain mould disease of sorghum with botanicals. **Madras Agric. J., 90 (7-9):** 553-557.

Mendonca, E., N. Ramos, S. A. Fessel and R. Sader. 2000. Controlled deterioration test used to evaluate. The seed quality of broccoli (*Brassica oleraceae* L.) var italica. **Revista Brasiliera de sementes, 22(1):** 280-287.

Miyawaki, M., M. Arro, N. Ito, Y. Yanagawa, R. E. Kendrick, K. Tanaka, T. Sato and H. Nakagawa. 1997. Changes in proteasome levels in spinach (*Spinacia oleracea*) seeds during imbibition and germination. **Biosci. Biotech. Biochem., 61(6):** 998 – 1001.

Moller, I. M., P. E. Jensen and A. Hansson. 2007. Oxidative modifications to cellular components in plants. **Annu. Rev. Plant Biol., 58:** 459 – 481.

Montgomery, E. G. 1911. Correlation in corn. **Nebraska Agric. Experimental station Annual Report, 24:** 108-159.

Moon, J., G. Parry and M. Estelle. 2004. The Ubiquitin-Proteasome Pathway and Plant Development. **The Plant Cell, 16:** 3181–3195.

Mullen, M. A. and R. T. Arbogast. 1979. Time-temperature mortality relationships for various stored-product insect eggs and chilling times for selected commodities. **J. Econ. Entomol., 72:** 476 – 478.

Murali, M. R., S. D. Shashidhara and B. S. Vyakatanahal. 2002. Investigation on seed viability in blackgram and greengram. **J. Res. Angrau., 30(1):** 34-39

Murthy, U. M. N., P. P. Kumar and W. Q. Sun. 2003. Mechanisms of seed ageing under different storage conditions for (*Vigna radiata* L.) Wilczek: Lipid peroxidation, sugar hydrolysis, maillard reactions and their relationship to glass state transition. **J. Exp. Bot., 54:** 1057-1067.

Mushtag, Y. K. 2011. Effect of nano size $Fe3O_4$, TiO_2 and carbon particles on cucumber seeds germination. **J. Environ. Sci. Health**, **46:** 1732 – 1735.

Mythili, V. 2012. Seed invigouration with botanicals in onion cv. CO (On) 5 (*Allium cepa* var. *aggregatum* L.). **M.Sc. Thesis,** Tamil Nadu Agricultural University, Coimbatore.

Nagel, M. and A. Borner. 2010. The longevity of crop seeds stored under ambient conditions. **Seed Sci. Res., 20:** 1- 12.

Niakan, M. and K. Sabari. 2009. Effects of eucalyptus allelopathy on growth characters and antioxidant enzymes activity in phalaris weed. **Asian J. Plant Sci., 8:** 440 - 446.

Niedzielski, M., C. Walters, W. Luczak, L. M. Hill, L. J. Wheeler and J. Puchalski. 2009. Assessment of variation in seed longevity with in rye, wheat and the intergeneric hybrid triticale. **Seed Sci. Res., 19:** 213 – 224.

Norton, C. R. 1986. Seed germination under environmental stress. **HortScience, 21(5):** 11 - 1105.

Nouman, W., M. T. Siddiqui and S. M. A. Basra. 2012. Moringa oleifera leaf extract: An innovative priming tool for rangeland grasses. **Turk. J. Agric. For., 36:** 65 – 75.

Nutile, G. E. 1964. Effect of desiccation on viability of seeds. **Crop Sci., 4:** 325-328.

Nystrom, T. 2005. Role of oxidative carbonylation in protein quality control and senescence. **Embo. J., 24:** 1311 – 1317.

Osborne, D. J., B. E. Roberts, P. I. Payne, S. Sens. 1974. Protein synthesis and viability in rye embryos. **In:** R. L. Bieleski, A. R. Ferguson, M. M. Cresswell, eds. Mechanism of regulation of plant growth, Bulletin 12. Wellington, New Zealand: The Royal Society of New Zealand: 805 – 812.

Osburn, R. M. and M. N. Schroth. 1989. Effect of osmopriming sugarbeet seed on germination rate and incidence of *Pythium ultimum* damping-off. **Plant Disease, 73:** 21-24.

Pallavi, M., S. S. Kumar, K. S. Dangi and A. V. Reddy. 2003. Effect of seed ageing on physiological, biochemical and yield attributes in sunflower cv. Morden. **Seed Res., 31(2):** 161-168.

Pandey, N. and D. Barve. 2011. Phytochemical and pharmacological review on *Annona squamosalinn.* **Int. J. Res. Pharma. Bio. Sci., 2(4):** 1404-1412.

Paramasivam, V. and P. Balamurugan. 2007. Physiological and Biochemical changes in rainfed and irrigated groundnut seeds during storage. **Seed Res., 34:** 73-79.

Park, O. K. 2004. Proteomic studies in plants. **J. Biochem. Mol. Biol., 37:** 133 – 138.

Patro, B., K. C. Sahu and P. Sahoo. 2007. Effect of different levels of *Callosobruchus chinensis* (Linn.) infestation on deterioration of some pulse seeds. **Legume Res., 30 (4):** 301 – 304.

Perry, D. A. 1977. A vigour test for seeds of barley (*Hordeum vulgare*) based on measurement of plumule growth. **Seed Sci. & Technol., 5:** 709 – 719.

Perry, D. A. 1987. Seedling growth and evaluation tests. **In:** Handbook of vigour test methods, (Ed., F. Fiala). International Seed Testing Association, Zurich: pp 10 – 20.

Perry, D. A. and J. G. Harrison. 1977. Effect of seed deterioration and seed bed environment on emergence and yield of spring sown barley. **Ann. Appl. Biol., 36:** 291-300.

Phiri, C. and N. D. Mbewe. 2010. Influence of *Moringa oleifera* leaf extracts on germination and seedling survival of three common legumes. **Int. J. Agric. Bio., 12:** 315- 317.

Poonguzhali, S. 2012. Standardization of vigour tests in TNAU blackgram (*Vigna mungo* L. hepper) cv. CO 6 and their relative performance under field condition. **Ph. D. Thesis**, Tamil Nadu Agricultural University, Coimbatore.

Potokina, E., N. Sreenivasulu, L. Altschmied, W. Michalek and A. Graner. 2002. Differential gene expression during seed germination in barley (*Hordeum vulgare* L.). **Funct. Integr. Genomics, 2:** 28–39.

Powell, A. A. 1988. Seed vigour and field establishment. **Advances in Research and Technology of Seeds, 11: 29-80.**

Powell, A. A. 2009. Evaluation of the controlled deterioration test as a repeatable and reproducible vigour test for *Brassica* species. Method validation report 2009, International Seed Testing Association.

Powell, A. A. 2010. Seed vigour conductivity test for *Glycine max*. **Seed Testing International, 139:** 37 – 39.

Powell, A. A. and S. Matthews. 1981. Evaluation of controlled deterioration, a new vigour test for small seeded vegetables. **Seed Sci. & Technol.,** 9**:** 633-640.

Powell, A. A. and S. Matthews. 1984. Prediction of the storage potential of onion seed under commercial storage conditions. **Seed Sci. & Technol., 12:** 641-647.

Pruzinska, A., G. Tanner, I. Anders, M. Roca and S. Hortensteiner. 2003. Chlorophyll breakdown: pheophorbide aoxygenase is a Riesketype iron–sulfur protein, encoded by the accelerated cell death 1 gene. Proceedings of the National Academy of Sciences, USA, 100: 15259 – 15264.

Pukacka, S. and E. Ratajczak. 2005. Production and scavenging of reactive oxygen species in *Fagus sylvatica* seeds during storage at varied temperature and humidity. **Journal of Plant Physiology, 162:** 873 - 885.

Raja, K. 2000. Storage studies for bruchid management and quality characteristics in green gram cv. CO 6 (*Vigna radiata* (L.) Wilczek). **M.Sc. (Ag.) Thesis,** Tamil Nadu Agricultural University, Coimbatore.

Rajjou, L. and I. Debeaujon. 2008. Seed longevity: Survival and maintenance of high germination ability of dry seeds. **C. R. Biologies, 331:** 796 - 805.

Rajjou, L., K. Gallardo, I. Debeaujon, J. Vandekerckhove, C. Job and D. Job. 2004. The effect of α-amanitin on the *Arabidopsis* seed proteome highlights the distinct roles of stored and neosynthesized mrnas during germination. **Plant Physiol., 134:** 1598–1613.

Rajjou, L., Y. Lovigny, S. P. C. Groot, M. Belghazi, C. Job and D. Job. 2008. Proteome-wide characterization of seed aging in *Arabidopsis*: a comparison between artificial and natural aging protocols. **Plant Physiol., 148:** 620 – 641.

Rajkumar, S., N. Nagarajan and S. C. Rana. 2004. Effect of natural ageing under controlled storage on seed quality and yield performance of field pea cv. DMRT. **Seed Res., 32(1):** 96-97.

Ramakrishna, V. and P. R. Rao. 2005. Purification of acidic protease from the cotyledons of germinating Indian bean (*Dolichos lablab* L. var lignosus) seeds. **African Journal of Biotechnology, 4(7):** 703 – 707.

Ramamoorthy, K. and R. N. Basu. 1997. Seed invigouration for improved vigour, viability and productivity of peanut (*Arachis hypogagea*). **East African J. of Agric. and Forestry., 61 (3):** 151 – 154.

Ramamoorthy, K., D. Kalavathi and M. Paramasivam. 1992. Maintenance of vigour and viability of stored pea (*Pisum sativum* L.) seeds. **J. Agron. Crop Sci., 169:** 228-233.

Ramya, P., J. Sudisha, N. Lakshmi devi and S. M. Aradhya. 2011. Antibacterial and anti-oxidant activities of fenugreek (*Trigonella foenum graceum* L.) leaves. **Res. J. Med. Pl., 5(6):** 695 - 705.

Rangaswamy, R. 2002. A text book of agricultural statistics. New Age International Pvt., Ltd. New Delhi.

Rao, R. G. S., P. M. Singh and M. Rai. 2006. Storability of onion seeds and effects of packaging and storage conditions on viability and vigour. **Scientia Horticulturae, 110:** 1- 6.

Ratajczak, E. and S. Pukacka. 2005. Decrease in beech (*Fagus sylvatica*) seed viability caused by temperature and humidity conditions as related to membrane damage and lipid composition. **Acta Physiologiae Plantarum, 27(1):** 3-12.

Ravichandran, G. 1991. Studies on the pattern of seed deterioration, the performance of progenies of differentially aged seeds in the field and the quality of their resultant seeds in Maize, Groundnut and Soybean. **M.Sc. Thesis**, Tamil Nadu Agricultural University, Coimbatore.

Reddy, A. A. 2009. Pulses production technology: status and way forward. **Economic & Political Weekly, 52:** 73 – 80.

Reddy, Y. T. N. and M. M. Khan. 2001. Effect of osmopriming on germination, seedling growth and vigour of khirni (*Mimusops hexandra*) seeds. **Seed Res., 29(1):** 24-27.

Renugadevi, J. and V. Vijayageetha. 2007. Organic seed fortification in cluster Bean (*Cyamopsis tetragonoloba* L.) Taub. **Acta Horticulturae,** : 752.

Renugadevi, J., N. Natarajan and P. Srimathi. 2008. Efficacy of botanicals in improving the seeds and seedling quality charactristics of cluster bean. **Legume Res., 31(3):** 164 -168.

Renugadevi, J., P. Sumathi, A. Nirmalakumari, K. Ramamoorthy and P. Gnanamurthy. 2001. Combination of organic and inorganic ingredients of seed hardening of blackgram. **National symposium of pulses and oil seeds for sustainable agriculture.** Tamil Nadu Agricultural University, Coimbatore, 29 - 31 July.

Ring, R. A. and H. V. Danks. 1994. Desiccation and cryoprotection: overlapping adaptations. **Cryo-letters, 15:** 181 - 190.

Roberts, E. H. 1972. Viability of seed. (ed. Roberts, E. H., Chapman and Hall, London), pp. 14 - 58.

Roberts, E. H. 1983. Loss of viability, ultra structure and physiological aspects. **Seed Sci. & Technol., 1:** 529-545.

Rodo, A. B. and J. M. Filho. 2003a. Accelerated aging and controlled deterioration for the determination of the physiological potential of onion seeds. **Scientia Agricola, 60(3):** 465 - 469.

Rodo, A. B. and J. M. Filho. 2003b. Onion seed vigor in relation to plant growth and yield. **Horticultura Brasileira Brasília, 21:** 220-226.

Roof, D. J., A. Hayes, M. Adamian, A. H. Chishti and T. Li. 1997. Molecular characterization of ABLIM, a novel actin-binding and double zinc finger protein. **J. Cell. Biol., 138:** 575–588.

Rossignol, M., J. B. Peltier, H. P. Mock, A. Matros, A. M. Maldonado and J. V. Jorrin. 2006. Plant proteome analysis: a 2004-2006 update. **Proteomics, 6:** 5529 – 5548.

Rudrapal, D. and R. N. Basu. 2002. Seed invigouration of mustard (*Brassica juncea* L.) as influenced by seed vigour status at the time of treatment. **Indian Agriculturist, 46 (1/2)**: 312 - 318.

Rudrapal, D. and R. N. Basu. 2004. The role of initial seed vigour status in the expression of invigouration effect of wet and dry seed treatments in French bean (*Phaseolus vulgaris* L.). **Seed Sci. & Technol., 32(1):** 205-212.

Rutzke, C. F. J., A. G. Taylor and R. L. Obendorf. 2008. Influence of Aging, Oxygen and Moisture on Ethanol Production from Cabbage Seeds. **J. Amer. Soc. Hort. Sci.**, **133(1):** 158 - 164.

Sabir-Ahamed, A. 1999a. Effect of seed pelleting on field performance of black gram. **Legume Res., 22(2):**109-112.

Sabir-Ahamed, A. 1999b. Field performance of hardened green gram seeds. **Legume Res., 22(3):** 207-208.

Sacande, M., E. A. Golovina, A. C. Van Aelst and F. A. Hoekstra. 2001. Viability loss of neem (*Azadirachta indica*) seeds associated with membrane phase behavior. **J. Exp. Bot., 52(358):** 919 – 931.

Saha, R., A. K. Mandal and R. N. Basu. 1990. Physiology of seed invigoration treatments in soybean (*Glycine max L.*). **Seed Sci. & Technol., 28:** 201-207.

Sano, N., H. Permana, R. Kumada, Y. Shinozaki, T. Tanabata, T. Yamada, T. Hirasawa and M. Kanekatsu. 2012. Proteomic analysis of embryonic proteins synthesized from long-lived mrnas during germination of rice seeds. **Plant Cell Physiol., 53(4):** 687–698.

Sasikala, K. 1997. Management of aged seeds and their progenies in bhendi cv. (Parbanikranti) and cowpea (*Vigna unguiculata* (L.) savi) cv. CO 6. **Ph.D., Thesis,** Tamil Nadu Agricultural University, Coimbatore.

Sathish, S. and S. Sundareswaran. 2010. Biochemical evaluation of seed priming in fresh and aged seeds of maize hybrid [COH (M) 5] and its parental lines. **Current Biotica, 4(2):** 162-170.

Sathish, S., S. Sundareswaran and N. Ganesan. 2011. Influence of seed priming on physiological performance of fresh and aged seeds of maize hybrid [COH (M) 5] and its parental lines. **ARPN Journal of Agricultural and Biological Science, 6(3):** 12-17.

Sathish, S., S. Sundareswaran, N. Senthil and K. N. Ganesan. 2012. Biochemical changes due to seed priming in maize hybrid COH(M) 5. **Research Journal of Seed Science, 5(3):** 71-83.

Sattler, S. E., L. Mene-Saffrane, E. E. Farmer, M. Krischke, M. J. Mueller and D. DellaPenna. 2006. Nonenzymatic lipid peroxidation reprograms gene expression and activates defense markers in *Arabidopsis* tocopherol-deficient mutants. **The Plant Cell, 18:** 3706 – 3720.

Sauvaire, Y., G. Ribes, J. C. Baccou and M. M. Loubatieres-Mariani. 1991. Implication of steroid saponins and sapogenins in the hypocholesterolemic effect of fenugreek**. Lipids, 26:** 191 – 197.

Schwember, A. R. and K. J. Bradford. 2011. Oxygen interacts with priming, moisture content and temperature to affect the longevity of lettuce and onion seeds. **Seed Sci. Res., 21:** 175- 185.

Sen, S. and D. J. Osborne. 1977. Decline in ribonucleic acid and protein synthesis with loss of viability during the early hours of imbibition of rye (*Secale cereal*) embryos. **Biochemical Journal, 166**: 33 – 38.

Sengupta, A. K., B. K. De and A. K. Mandal. 2005. Pre-storage seed invigoration treatments for the maintenance of vigour, viability and field performance of high-vigour onion seed (*Allium cepa* L.). **J. Intl. Seed Testing Assoc., 33(3):** 753-760.

Shelar, V. R. 2007. Chemical and biochemical means of soybean seed deterioration - A Review. **Agric. Rev., 28(1):** 10 - 17.

Shevchenko, A., M. Wilm, O. Vorm and M. Mann. 1996. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. **Annals of Chemistry, 68:** 850-858.

Shibles, R. M. and C. R. Weber. 1966. Interception of solar radiation and dry matter production by various soybean planting patterns. **Crop sci., 6:** 55-59.

Siddiqui, M. H., F. C. Oad, M. K. Abbasi and A. W. Gandahi. 2009. Zinc and Boron fertility to optimize physiological parameters, nutrient uptake and seed yield of Sunflower. **Sarhad J. Agric., 25(1):** 53-57.

Simpson, G. M. and J. M. Naylor. 1962. Dormancy studies in seeds of *Avena testa*. III. A relationship between maltase, amylase and gibberellins. **Can. J. Bot., 40:** 1659 - 1673.

Sinclair, T. R. 1986. Water and nitrogen limitations in soybean grain production in model development. **Field Crop. Res., 15:** 125-141.

Sinclair, T. R., R. C. Muchow, M. M. Ludlow, G. J. Leach, R. J. Lawn and M. A. Foale. 1987. Field and model analysis of the effect of water deficits on carbon and nitrogen accumulation by soybean, cowpea and blackgram. **Field Crop. Res., 17:** 121- 140.

Singh, S. R. and L. E. N. Jackai. 1985. Insect pests of cowpea in Africa: their life cycle, economic importance and potential for control. **In:** S. R. Singh and O. Rachiek (Eds.), Cowpea Research, Production and Utilisation. Wiley Interscience Publications/John Wiley and Sons Ltd, Chichester. pp. 217 - 231.

Sital, J. S., A. S. Sandhu and S. Sharma. 2008. Biochemical changes associated with accelerated ageing of sunflower (*Helianthus annus* L.) seeds. **Indian J. of Agric. Biochem., 21(1&2):** 16-20.

Slobin, I. L. 1980. The role of eukaryotic elongation factor Tu in protein synthesis – The measurement of the elongation factor Tu content of rabbit reticulocytes and other mammalian cells by a sensitive radioimmuno assay. **Eur. J. Biochem., 110:** 555 – 563.

Smalle, J. and R. D. Vierstra. 2004. The ubiquitin 26S proteasome proteolytic pathway. **Annu. Rev. Plant Biol., 55:** 555–90.

Smith, D. E., N. N. Weich and O. D. Mc Coy. 1973. Studies on lettuce seed quality II. Relationship of seed vigour to emergence seedling weight and yield. **J. Amer. Soc. Hort. Sci., 98:** 552-556.

Somasundaram, E. 2003. Evaluation of organic sources of nutrient and panchagavya spray on the growth and productivity of maize, sunflower and green gram system. **Ph.D. Thesis,** Tamil Nadu Agricultural University, Coimbatore.

Soos. V., E. Sebestyen**,** A. Juhasz**,** M. E. Light**,** L. Kohout**,** G. Szalai**,** J. Tandori**,** J. Van Staden and E. Balazs. 2010. Transcriptome analysis of germinating maize kernels exposed to smoke-water and the active compound KAR. **BMC Plant Biology, 10:** 236-251.

Sorensen. K. A. 1994. Control of weevils in stored peas and beans. North Carolina State University insect note 10 (http://www.ces.ncsu.edu/depts/ent/notes/Vegetables/veg10.html).

Southgate, B. J. 1979. Biology of the Bruchidae. **Ann. Rev. Entomol., 24:** 449 - 473.

Sridhar, S., A. Arunsugasamy, K. Vijayalakshmi and A. V. Balasubramanian. 2001. Vrikshayurveda – Ayurveda for plants centers for Indian Knowledge systems, Chennai, Tamil Nadu, p.47.

Stadtman, E. R. 2001. Protein oxidation in aging and age-related diseases. **Ann. N Y Acad. Sci., 928:** 22 – 38.

Stadtman, E. R. 2004. Role of oxidant species in aging. **Curr. Med. Chem., 11:** 1105 – 1112.

Strehler, B. L. 1962. Time, cells and ageing. Academic Press, N.Y. p. 4-32.

Subhashini, N., A. Thangathirupathi and N. Lavanya. 2011. Antioxidant activity of *Trigonella foenum graecum* using various *in vitro* and *ex vivo* models. **International Journal of Pharmacy and Pharmaceutical Sciences, 3(2):** 96 – 102.

Sujatha, K. and P. Srimathi. 2006. Chemical priming for improved seed yield and quality in blackgram (*Vigna mungo* (L.) Hepper) var. Co 5. **Plant Arch., 6(1):** 177 - 180.

Sullivan, J. A., K. Shirasu and X. W. Deng. 2003. The diverse roles of ubiquitin and the 26s proteasome in the life of plants. **Nature Reviews - Genetics, 4:** 948 – 958.

Suma, N. 2005. Studies on seed quality enhancement techniques in sesamum (*sesamum indicum*) cv. CO 1. **M.Sc (Ag.). Thesis,** Tamil Nadu Agriculture University, Coimbatore.

Sundaralingam, K. 2005. Organic seed production in hybrid rice ADTRH 1, **Ph.D. Thesis**, Tamil Nadu Agricultural University, Coimbatore.

Sung, F. J. and Y. H. Chang. 1993. Biochemical activities associated with priming of sweet corn seeds to improve vigour. **Seed Sci. & Technol., 21:** 97-105.

Szirtes, J. and G. Barla-Szabo. 1981. A methos for the determination of vigour in winter wheat seeds. **Norentyermeles, 6:** 493-500.

Tajbakhsh, M. 2000. Relationships between electrical conductivity of imbibed seeds leachate and subsequent seedling growth (viability and vigour) in omid wheat. **J. Agr. Set. Tech., 2:** 67 – 71.

Tamura, K., N. Nameki, T. Hasegawa, M. Shimizu and H. Himenon. 1994. Role of the CCA Terminal Sequence of tRNA(Val) in Aminoacylation with Valyl-tRNA Synthetase. **The Journal of Biological Chemistry, 269(35):** 22173 – 22177.

Tang, C., X. Wang, X. Duan, X. Wang, L. Huang and Z. Kang. 2013. Functions of the lethal leaf-spot 1 gene in wheat cell death and disease tolerance to *Puccinia striiformis*. **J. Exp. Bot., 64(10):** 2955 – 2969.

TeKrony, D. M. 2003. Precision is an essential component in seed vigour testing. **Seed Sci. & Technol., 31:** 435 - 447.

TeKrony, D. M. and D. B. Egli. 1977. Relationship between laboratory indices of soy bean seed vigour and field emergence. **Crop Sci., 17**: 573-577.

TeKrony, D. M. and D. B. Egli. 1991. Relationship of seed vigour to crop yield: A review. **Crop Sci., 31**: 816-822.

Temple, N. J. 2000. Antioxdants and disease. More questions than answers. **Nutrition Res., 20:** 449–459.

Thangaraj, M., T. V. Karivaratharaju, V. Ramarkrishnan and C. Dharmalingam. 1973. Glutamic acid decarboxylase activity as an index to cotton seed quality. **Madras J. Agric., 60:** 1596-1600.

Thornberry, G. O. and F. Smith. 1995. Relation of respiratory and enzymatic activity on corn seed viability. **Plant Physiol., 30:** 337-343.

Tlustos, P., P. Cigler, M. Hruby, S. Kuzel, J. Szakova and J. Balík. 2005. The role of titanium in biomass production and its influence on essential elements contents in field growing crops. **Plant Soil Environ., 51(1):** 19 - 25.

Tomaz, R. and D. Marina. 2010. Cytokinins and their Function in Developing Seeds. **Acta Chim. Slov., 57:** 617–629.

Tommasi, F., C. Paciolla, M. C. D. Pinto and L. Degara. 2006. A comparative study of glutathione and ascorbate metabolism during germination of *Pinus pinea* L. Seeds. **J. Exp. Bot., 52:** 1647- 1654.

Toppo, F. A., R. Akhand and A. K. Pathak. 2009. Pharmacological actions and potential uses of *Trigonella foenum-graecum*: a review. **Asian Journal of Pharmaceutical and Clinical Research, 2(4):** 29 – 38.

Trawatha, S. E., D. M. TeKrony and D. F. Hildebrand. 1995. Relationship of soybean seed quality to fatty acid and C_6-aldehyde levels during storage. **Crop Sci., 35:** 1415-1422.

Umarani, R. 1999. Studies on the physiological and biochemical basis of seed germination and deterioration on *Casuarina equisetifolia.* **Ph.D. Thesis**, Tamil Nadu Agricultural University, Coimbatore.

Upadhyay, R. K. and S. Ahmad. 2011. Management strategies for control of stored grain insect pests in farmer stores and public ware houses. **World Journal of Agricultural Sciences, 7(5):** 527 - 549.

Utida, S. 1972. Density dependent polymorphism in the adult of *Callosobruchus maculatus* (Coleoptera, Bruchidae). **J. Stored Prod. Res., 8:** 111 - 126.

Vadivelu, K. K., K. Vanangamudi and R. Umarani. 2001. Recent Techniques and Participatory Approaches on Quality Seed Production. (Ed. Vanangamudi K., *et al.*,), Department of Seed Sci. & Technol., Tamil Nadu Agricultural University, Coimbatore.

Vadivelu, K. K., T. V. Karivaratharaju and V. Ramakrishnan. 1985. Influence of seed treatments, seed moisture content, containers on viability of CO 1 bengalgram. **Seed Res., 13(1):** 39-49.

Van de Venter, H. A., G. Barla-Szabo and S. G. Ybema. 1993. A study of single and multiple stress vigour test for undeteriorated seed lot of wheat. **Seed Sci. & Technol., 29:** 117- 125.

Vanderlip, R. C., M. E. Mockeh and H. Jain. 1973. Evaluation of vigour tests for sorghum seeds. **Agron. J., 64**: 13-18.

Vanitha, C., K. Ramamoorthy, A. Vijayakumar and K. Sivasubramaniam. 2005. Mist sand conditioning to minimize loss of viability in cocoa (*Theobroma cacao* l.) seed. **Green Page**, **4(6):** 487-489.

Vasudevan, S. N., N. M. Shakuntala, S. R. Doddagoudar, R. C. Mathad and S. I. Macha. 2012. Biochemical and molecular changes in aged peanut (*Arachis hypogaea* L.) seeds. **The Ecoscan: Special issue, 1:** 347 – 352.

Verma, S. S., R. P. S Tomer, U. Verma and S. L. Saini. 2001. Electrical conductivity and accelerated ageing techniques for evaluating deterioration in Brassica species. **Crop Res., 21:** 148-152.

Vertucci, C. W. and J. M. Farrant.1995. Acquisition and loss of desiccation tolerance. **In:** Kigel J, Galili G, eds. Seed development and germination. New York, NY: Marcel Dekker, 237–271.

Vieira, R. D., D. M. TeKrony, D. B. Egli and M. Rucker. 2001. Electrical conductivity of soybean seeds after storage in several environments. **Seed Sci. & Technol., 29:** 599 – 608.

Vigneshwari, R. 2002. Seed hardening and pelleting in ragi cv. CO 13 to induce drought resistance and to improve productivity under rainfed condition. **M.Sc (Ag.) Thesis,** Tamil Nadu Agricultural University, Coimbatore.

Vijaya, J. 1996. Standardization of pre-sowing seed management techniques for pulses **M.Sc. (Ag.) Thesis,** Tamil Nadu Agricultural University, Coimbatore – 3.

Vijayalakshmi, V. 2009. Effect of organic seed fortification and foliar spray with pulse sprout extract in rice seed crop (*Oryza sativa* L.). **M.Sc. Thesis**, Tamil Nadu Agricultural University, Coimbatore.

Vijayalakshmi, V. 2012. Seed vigour and viability studies in TNAU tomato hybrid CO 3 (*Lycopersicon esculentum* Mill.). **Ph.D. Thesis**, Tamil Nadu Agricultural University, Coimbatore.

Vijayan, R. 2005. Organic seed production in rice cv. ADT 43. **Ph.D. Thesis,** Tamil Nadu Agricultural University, Coimbatore.

Villanueva, M. A., F. Campos, C. Diaz, J. M. Colmenero-Flores, E. Dantan, F. Sanchez and A. A. Covarrubias. 1999. Actin expression in germinating seeds of *Phaseolus vulgaris* L. **Planta, 207:** 582–589.

Villiers, T. A. and E. J. Edgcumbe. 1975. On the cause of seed deterioration in dry storage. **Seed Sci. & Technol., 3:** 761- 774.

Vincent, P., M. Chua, F. Nogue, A. Fairbrother, H. Mekeel, Yue Xu, N. Allen, T. N. Bibikova, S. Gilroy and V. A. Bankaitis. 2005. A Sec14p-nodulin domain phosphatidylinositol transfer protein polarizes membrane growth of *Arabidopsis thaliana* root hairs. **The Journal of Cell Biology, 168(5):** 801–812.

Vyakaranahal, B. S., M. Sherkhargouda, A. S. Prabhakar and S. A. Patil. 2000. Efficacy of halogens, plant products and fungicides on storage potentiality of sunflower restorer lines. **Karnataka J. Agric. Sci., 13 (1):** 36-45.

Walters, C. 1998. Understanding the mechanisms and kinetics of seed aging**. Seed Sci. Res., 8:** 223 - 244.

Walters, C., P. Landre, L. Hill, F. Corbineau and C. Bailly, 2005. Organization of lipid reserves in cotyledons of primed and aged sunflower seeds. **Planta, 222:** 397-407.

Wang, Y. R., L. Yu, Z. B. Nan and Y. L. Liu. 2004. Vigor Tests Used to Rank Seed Lot Quality and Predict Field Emergence in Four Forage Species. **Crop Sci., 44**: 535-541.

Watson, D. T. 1958. The physiological basis of the effect of potassium on crop yield. Proc. 4th Cong. International Potash Institute, Berne (Switzerland): 101 – 119.

Wilson, D. O. and M. B. McDonald. 1986. The lipid peroxidation model of seed ageing. **Seed Sci. & Technol., 16:** 115-121.

Woodstock, L. W. 1969. Seedling growth as a measure of seed vigour. **Proceedings of the International Seed Testing Association, 34:** 273 – 280.

Wu, X., H. Liu, W. Wang, S. Chen, X. Hu and C. Li. 2011. Proteomic analysis of seed viability in maize. **Acta Physiol. Plant., 33:** 181–191.

Xin, X., X. H. Lina, Y. C. Zhoub, X. L. Chena, X. Liua and X. X. Lua. 2011. Proteome analysis of maize seeds: the effect of artificial ageing. **Physiologia Plantarum, 143:** 126-138.

Zalewski, K. 1989. The metabolism of aged seeds. The effects of actinomycin D and cordycepin on RNA synthesis in germinating rye grains of different ages. **Acta Societatis Botanicorum Poloniae, 58(1):** 85 – 95.

Zhang, J. Z. and C. R. Somerville. 1997. Suspensor-derived polyembryony caused by altered expression of valyl-tRNA synthetase in the twn2 mutant of *Arabidopsis*. **Proc. Natl. Acad. Sci., 94:** 7349 – 7355.

Zheng, Q. and Y. Zhao. 2007. The diverse biofunctions of LIM domain proteins: determined by subcellular localization and protein–protein interaction. **Biol. Cell, 99:** 489 – 502.

Zimmermann, H. 1992. 5'-Nucleotidase: molecular structure and functional aspects. **Biochem. J., 285:** 345 – 365.