SEED INVIGORATION FOR IMPROVED FIELD PERFORMANCE AND STORABILITY IN ASH GOURD (*Benincasa hispida* (Thunb.) Cogn.)

By

BENNETT THOMAS, K.

(2016-11-125)

DEPARTMENT SEED SCIENCE AND TECHNOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR -680 656

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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Faculty of Agriculture

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DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

COLLEGE OF HORTICULTURE

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KERALA, INDIA

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DECLARATION

I, hereby declare that the thesis entitled **'Seed invigoration for improved field performance and storability in ash gourd (*Benincasa hispida* (Thunb.) Cogn.),'** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellanikkara **Bennett Thomas. K**

(2016-11-125)

CERTIFICATE

Certified that the thesis entitled **'Seed invigoration for improved field performance and storability in ash gourd (*Benincasa hispida* (Thunb.) Cogn.),'** is a record of research work done independently by **Bennett Thomas, K. (2016-11-125)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to his.

Vellanikkara

Dr. Rose Mary Francies
(Chairman)
Professor and Head
Department of Seed Science and Technology
College of Horticulture
Vellanikkara

CERTIFICATE

We, the undersigned members of the advisory committee of **Bennett Thomas, K. (2016-11-125)** a candidate for the degree of **Master of Science in Agriculture**, with major field in Seed Science and Technology, agree that the thesis entitled **'Seed invigoration for improved field performance and storability in ash gourd (*Benincasa hispida* (Thunb.) Cogn.),'** may be submitted by **Bennett Thomas, K. (2016-11-125)**, in partial fulfilment of the requirement for the degree.

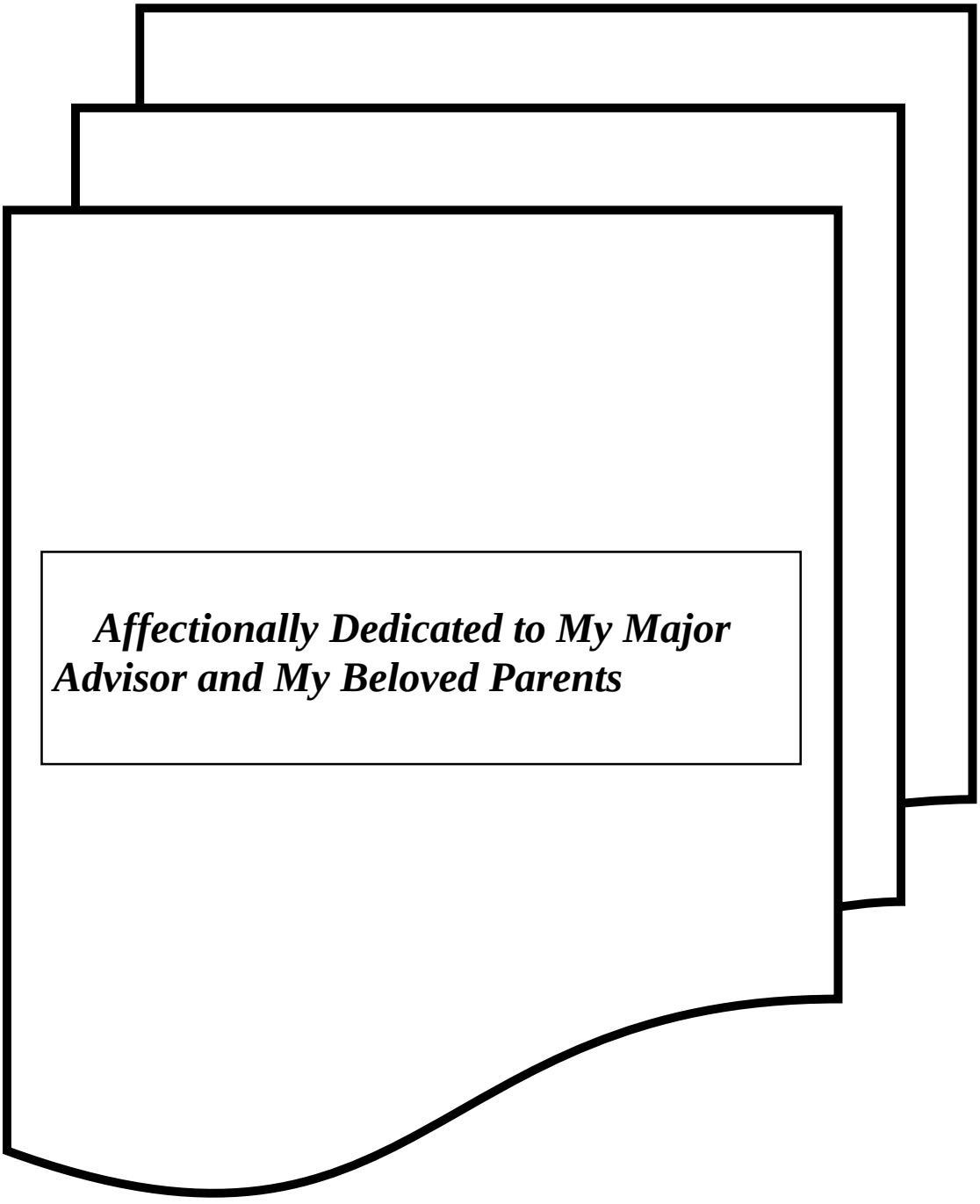
Dr. Rose Mary Francies
(Chairman)
Professor (Pl. Br. & Gen.) and Head
Dept. of Seed Science and Technology
College of Horticulture, Vellanikkara

Dr. Dijee Bastian
Professor (Pl. Br. & Gen.)
Dept. of Seed Science and Technology
College of Horticulture, Vellanikkara

Dr. Jiji Joseph
Professor and Head
Dept. of Plant Breeding and Genetics
College of Horticulture, Vellanikkara

Dr. C. Beena
Professor and Head
AICRP on MAP & B
College of Horticulture, Vellanikkara

External examiner
Dr. R. Jerlin
Professor (Seed Science and Technology)
Dept. of Seed Science and Technology
TNAU, Coimbatore


Affectionally Dedicated to My Major Advisor and My Beloved Parents

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Bennett Thomas. K

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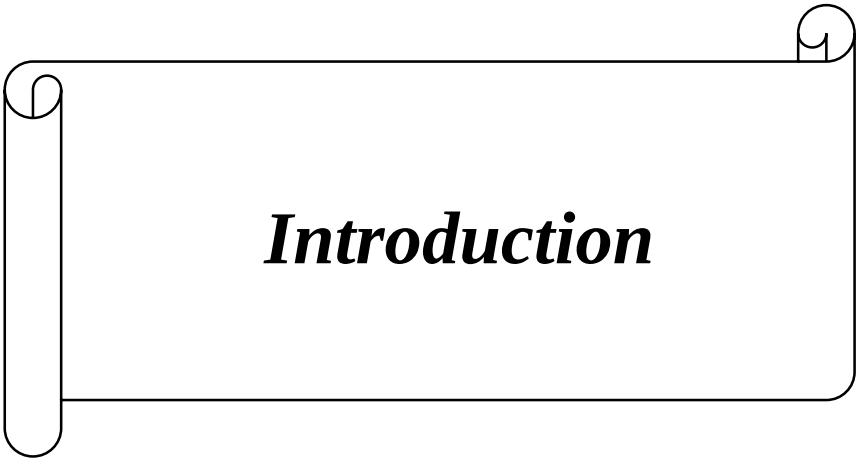
List of abbreviation

C.D	- Critical Difference
cm	- centimetre
^{o}C	- degree Celsius
EC	- Electrical conductivity
g	- gram
GA_3	- Gibberellic acid
h	- hour
KAU	- Kerala Agricultural University
kg	- kilogram
m	- metre
MAS	- Months after storage
mg	- milligram
ml	- millilitre
μSm^{-1}	- micro Siemens per metre
mm	- millimetre
%	- per cent
$SE_{(m)}$	- Standard Error Mean
t/ha	- tonnes/hectare
VI-I	- Seedling vigour index- I
VI-II	- Seedling vigour index- II


Introduction

1. INTRODUCTION

Ash gourd (*Benincasa hispida* (Thunb.) Cogn.); syn ash gourd, wax gourd, white pumpkin), a native of South Asia and South-east Asia belonging to the family Cucurbitaceae, is a widely cultivated crop of India. The immature fruits are an integral part of the Indian tradition, culture, and cuisine, and it also offers ample scope for value-addition (candy, peda, pickle etc). Apart from the fruit (rind, pulp and seeds), the leaves and roots of ash gourd are attributed to have anthelmintic, anti-inflammatory, demulcent, diuretic, expectorant, febrifuge, laxative and tonic properties. Every 100 g edible portion of the fruit is composed of 96.10 g water, 3 g carbohydrates, 0.4 g protein, 0.2 g fat, 160 mg minerals, 13.71 mg vitamins and 13 kcal energy (USDA, 2016).

Ash gourd is a popular cucurbitaceous vegetable crop of Kerala and is locally known as '*Kumbalanga*'. The cultivation of ash gourd in the state is mainly confined to January - March and September - December (winter) and most districts produce marketable surplus during the winter season (KAU, 2016). Total production of ash gourd in Kerala during 2017-18 was about 34 tons from an area of 1,056 ha with an average productivity of about 0.032 t/ha (DES, 2018).

Lack of quality seeds in sufficient quantities at the right time and location, is a setback to ash gourd cultivation in the state. Owing to the recent vagaries of weather and the hot humid climate prevalent in the state, a rapid deterioration of seed, leading to quick loss of viability is also a matter of concern. The poor life span of seeds is attributed to environment factors like relative humidity, temperature, and internal factors, mainly the initial moisture content of seed. Thus, it is imperative that the rate of seed deterioration is to be slowed down.

Several earlier reports have indicated that storing seeds in moisture impervious containers are advantageous to prolong seed longevity under ambient storage in high humid regions. Tripathi and Lawande (2014) had studied the effect of seed moisture and packing material on viability of onion seed. The onion seeds were packed in various packing materials *i.e.*, cloth bags, polyethylene bags, laminated aluminum bags and laminated aluminum bags with vacuum packing and stored in ambient condition. Seeds packed in aluminum laminated bags remained viable for 27 months while those packed in cloth bags lost their complete viability and within 18 months of storage.

In addition to faster pace of deterioration under high humid conditions prevailing, seed germination in ash gourd is found to be generally low in Kerala. Poor germination immediately after extraction is attributed to dormancy. Seed invigouration with chemicals and various osmotica has been found to enhance germination. Such treatments are also found to be effective in cucurbits. An increase in germination was observed when seeds of bitter gourd were treated with KH_2PO_4 10^{-1} M for 24 hours. Similar results were also obtained on treating seeds of bottle gourd seeds with KH_2PO_4 10^{-3} M for 48 hours (AICRP (VC), 2014).

Priming of ash gourd seeds immediately after harvest for 24 hours with kinetin (10 ppm) or KH_2P0_4 (10^{-1} M) resulted in early breaking of dormancy and uniform germination. Over the period of storage, seed priming with $CaCl_2$ (50 mM) for 12 hours or 24 hours, *Psuedomonas fluorescens* (1×10^6 cfu.ml^{-1}) or kinetin (10 ppm) for 12 hours proved to be more beneficial in extending seed viability (Shobha, 2016). However, despite the immediate improvements in seed performance following invigouration treatments, there have been contrasting reports of seed storage potential and crop performance following the treatment (Liu *et al*., 1996).

Considering the above, the present study was formulated to:

- Elucidate the effect of seed invigouration on field performance in ash gourd
- Assess the impact of packing material on seed quality and longevity.

Review of Literature

2. REVIEW OF LITERATURE

Seed viability and vigour are dynamic and deterioration of these seed qualities occur with time. Over the period of storage, several changes at cytological, physical, physiological and biochemical levels occur within the seed leading to loss of viability and ultimately death (Jyothi and Malik, 2013). Poor field performance owing to reduction in seed vigour and loss of seed viability are two major factors that determine the establishment of crops in the field and its subsequent performance. Seed deterioration due to ageing is an irreversible phenomenon and the best way to control is to lower its rate (Coolbear, 1995). According to Farhadi *et al.* (2012), unless special precautions are taken, the annual losses due to seed deterioration alone can be as much as 25 per cent of the harvested crop.

Regulating environmental factors causing seed deteriorating is the basis for longer seed storage. Exposing seeds to various priming treatments can minimise or nullify the detrimental effects of ageing (Tilden *et al.*, 1985)**.** Seed invigouration methods are used to enhance its germination and field establishment. Adverse effects in performance of seeds following invigouration treatments has also been reported. According to Liu *et al*. (1996), after the priming treatment, the storage potential of primed seeds get reduced if a large number of cells in the radical tip have entered the G2 phase of cell cycle.

A study conducted by Shobha (2016) at Kerala Agricultural University revealed that seed invigouration with either calcium chloride or cytokinin or KH_2PO_4 or *Pseudomonas fluorescens* was highly effective in breaking the dormancy and prolonging viability of seeds of ash gourd variety KAU Local stored under ambient conditions. However, the impact of these treatments on field performance of the crop as well as the effect of packing materials on quality and longevity of treated seed is little known.

Considering the above, the present study was formulated to elucidate the effect of seed invigouration on field performance in ash gourd and to assess the impact of packing material on seed quality and longevity of primed seeds.

The literature related to the study is detailed below in brief under the following headings.

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2.1. Effect of invigouration treatments on seed indices

Seed invigouration or seed enhancements are beneficial techniques performed on seeds after harvest but prior to sowing which helps to enhance germination, emergence and seedling growth by altering the physiological state of the seed (Black and Peter, 2006). The main purpose of these invigouration treatments is to shorten the time between planting and emergence (Basra *et al*., 2005a; Farooq *et al*., 2008). Generally seed invigouration are administered as: i) pre-sowing hydration treatments (priming), ii) seed coating technologies and iii) integration of these methods to enhance seed quality. Of these, the most widely used technique is seed priming. Seed priming involves controlled hydration of seeds. The seeds are soaked either in water or low osmotic potential solution to a point where germination related metabolic activities begin in the seeds but radicle emergence does not occur (McDonald, 2000; Farooq *et al.*, 2007a). Through this process of priming, metabolic processes necessary for germination to occur is initiated without the actual germination taking place.

Seeds priming with chemicals is assumed to cover the pores in the seed coat and prevents the entry of both moisture as well as insect infestation and provide protection from physical damage during storage (West *et al.*, 1985). Similar findings have been reported by Shanmugavel *et al*. (1995) and Maurya *et al*. (2002) in soybean. According to Saracco *et al.* (1995), the primed seeds showed an increase in tolerance to deteriorative factors that appear during storage as a consequence of advanced germinative events. In many species (e.g., *Allium cepa* L. *Capsicum annum* L., *Pisum sativum*, *Daucus carota*), osmotic-priming improved the seed storability. Mid-storage treatment of seeds with growth substances or hydration followed by drying or seed priming with chemicals are reported to improve seed quality (Savino *et al*., 1979).

A brief review of literature pertaining to the effect of seed priming on seed parameters are detailed below.

2.1.1 Effect of halopriming with $CaCl_2$

Sl. No.	Crop	Experiment details	Reference
1.	Chilli	Calcium was found to have a positive correlation with germination rate by playing a significant role in membrane stabilization by acting as an enzyme cofactor.	Hecht-Buchholz (1979); Chrystiansen and Foy (2008)
2.	Sunflower	Emergence and growth of seedlings was enhanced after priming the seeds with $CaCl_2$ (1%).	Kathiresan *et al.* (1984)
3.	Marigold	Seeds priming with $CaCl_2$ (50 mM) resulted in enhanced germination through repair mechanism, mobilization of storage reserves for utilization during germination and dormancy breakdown.	Burgass and Powell (1984); Hocart *et al.* (1990a).
4.	Wheat	Seed treatment with $CaCl_2$ (2%) significantly enhanced the number of seedlings per unit area, dry matter accumulation and seedling height.	Bhati and Rathore (1988)
5.	Maize	Treating seeds with $CaCl_2$ (1%) resulted in significant increase in germination, speed of germination, emergence and seedling vigour over control.	Kulkarni and Eshanna (1988)
6.	Maize	Osmopriming with $CaCl_2$ (50 mM) was found to enhance germination owing to reduction in mean time to germination and higher germination index, energy of germination, final germination per cent.	Hocart *et al.* (1990b)
7.	Pigeon pea and cowpea	An increase in germination (97.8%) and vigour index (5007) was exhibited by seeds primed with 0.4% $CaCl_2$.	Rangaswamy *et al.* (1993)
8.	Rice	Seeds primed with $CaCl_2$ exhibited increased germination energy and germination index, while, it slightly reduced the mean germination time (MGT).	Ruan *et al.* (2002a)

9.	Jatropha	Seed priming with $CaCl_2$ (1%) resulted in increased germination and higher seedling survival.	Kathiravan (2004)
10.	Rice	Osmo-hardening with $CaCl_2$ (-1.25 MPa) for 24 h resulted in early and synchronized germination and higher germination per cent accompanied by enhanced amylase activity and total sugars compare to the traditional soaking.	Farooq *et al.* (2006a)
11.	Wheat	Salt tolerance of wheat cultivars was improved when seeds were primed with $CaCl_2$ (100 mmol/L) due to improved seedling vigour.	Iqbal *et al.* (2006) Afzal *et al.* (2008a)
12.	Melon	Priming melon seeds with 1% $CaCl_2$ resulted in improved germination (%) and energy of germination. They also reported that osmo-priming the seeds with KNO_3 and 3% $CaCl_2$ resulted in improved root length compared to control. However, priming with 1% $CaCl_2$ reduced root length. In addition, priming with 2% $CaCl_2$ resulted in increased shoot length.	Farooq *et al.* (2007b)
13.	Wheat	Seeds primed with $CaCl_2$ exhibited higher speed of germination and enhanced salt tolerance capacity.	Gupta *et al.* (2008)
14.	Marigold	Halopriming the seeds with $CaCl_2$ (50 mM for 24 h) had resulted in maximum germination and germination index in comparison with other treated seeds (KNO_3 and NaCl) and the control.	Afzal *et al.* (2008b)
15.	Tomato	Halopriming with 150 mM $CaCl_2$ had triggered higher germination and reduced mean germination time (MGT) in tomato cv. Roma.	Afzal *et al.* (2011)
16.	Lettuce	Seeds primed with $CaCl_2$ showed maximum germination index and reduced mean time to emergence	Ahmed and Farag (2011)

17.	Rice	Improved crop stand and higher values for emergence index, crop growth rate, and harvest index were recorded from seeds osmo-hardened with $CaCl_2$ (–1.25 MPa)	Rehman *et al.* (2011)
18.	Rice	Osmopriming with $CaCl_2$ (-1.00 MPa) increased energy of germination, coefficient of velocity of germination and reduced mean time to germination.	Yousof (2013)
19.	Chilli	Seeds treated with KH_2PO_4 (0.5%) and $CaCl_2$ (1%) exhibited an increased germination per cent.	Vishwanath *et al.* (2014)
20.	Castor	Reduced mean germination time and higher daily germination index and coefficient of velocity of germination, was exhibited by seeds treated with $CaCl_2$ (2%).	Jamadar and Chandrashekar (2015)
21.	Maize	Under optimal as well as in late sown conditions, maize seeds osmoprimed with $CaCl_2$ (–1.25 MPa) resulted in minimum mean time for germination over control.	Mahboob *et al.* (2015)
22.	Ash gourd	Seeds invigourated with $CaCl_2$ (50 Mm) recorded the highest germination. The treated seeds retained their germination above minimum standards for seed certification (MSCS) for 7 months after storage (MAS).	Shobha (2016)
23.	Maize	Seeds primed with 1% $CaCl_2$ for 12 h at 25^0C exhibited increased germination, germination index, energy of emergence, seedling root length, shoot length, seedling fresh weight, dry weight and vigour index I	Kumari *et al.* (2017)
24.	Chickpea	Immediate sowing of seeds primed with $CaCl_2$ (ψs -1.25 MPa) triggered germination metabolism more than the other priming treatments. It also resulted in higher stand establishment and crop growth.	Farooq *et al.* (2018)

25.	Chick pea	Seeds primed with $CaCl_2$ (1%) recorded significantly high values for seed germination (95.67%), speed of germination (33.75), root length (12.22), shoot length (15.03), seedling length (27.25), seedling fresh weight (1.08), seedling dry weight (0.23), seed vigour index-I (2606.89), seed vigour index-II (21.36), compared to other treatments including untreated control.	James *et al*. (2018)
26.	Groundnut	Higher germination, vigour index and lower electrical conductivity (0.411 dSm^{-1}) was recorded in seeds hydrated with $CaCl_2$ (1% and 2%). The lower electrical conductivity of leachate in seeds treated with $CaCl_2$ was due to the beneficial effect of $CaCl_2$ in strengthening the cell membrane integrity and permeability.	Bahu *et al.* (2018)
27.	Lentil	Treating the seeds with $CaCl_2$ (2%) increased the field performance in comparison with other seed treatments	Meena *et al.* (2018)

2.1.2 Effect of hormonal priming with cytokinin

Sl. No.	Crop	Experimental details	References
1	Egg plant and radish	Seed leachate with a lower EC obtained when seeds were primed with cytokinin was attributed to improved membrane repair in treated seeds.	Rudrapal and Nakamura (1988)
2	Cabbage, cotton, sunflower	In seeds of cabbage, cotton, sunflower, red clover and alfalfa, priming with kinetin (0.5 mM) increased emergence of shoot and seedling growth under saline conditions. However, in members of F: Poaceae, seeds treatment with GA_3 was more effective.	Kabar (1990)
3	Green gram and black gram	Increase in fresh and dry weight of seedlings were noted in seeds treated with kinetin and GA_3 as compared to untreated seeds.	Patel and Saxena (1994)
4	Corn and soybean	At 10^0C, seedling emergence and development was stimulated in corn and soybean by GA_3 and kinetin (0.1mM). However, GA_3 was more effective than kinetin.	Wang *et al*. (1996)
5	Wheat grass	Seed priming with cytokinin (50 ppm) was helpful in improving the seed performance by increasing mean germination time under drought conditions respectively.	Eisvand *et al*. (2008)
6	Spring maize	Maximum germination index and germination energy was observed on seed priming with kinetin, followed by priming with moringa leaf extract. However, higher increase in seedling fresh weight was observed on priming with kinetin.	Afzal *et al.* (2012a)

7	Wheat	Seed priming with cytokinin (50 ppm for 12 h) resulted in significantly high germination, mean germination rate, shoot and root length, speed of germination and dry weight compared to other treatments.	Ghobadi *et al*. (2012)
8	Onion	Increased root growth and time taken for germination was detected when seeds were primed with GA_3 unlike priming with IAA and kinetin. Similarly, a decrease in shoot length but maximum dry weight was achieved through seed priming with kinetin (10^{-10} M for 10 h).	Yarnia *et al*. (2012)
9	Tomato	Seed priming with kinetin (10 ppm for 24 h) increased final germination per cent, germination index, shoot length and seedling fresh weight. Maximum decrease in electrolyte leakage and increase in seed indices including seedling dry weight was induced by cytokinin at 10 ppm followed by priming with 50 ppm cytokinin or 10 ppm BAP, or 10 ppm kinetin or 50 ppm kinetin.	Nawaz *et al*. (2013)
10	Maize	Seed priming with kinetin (10 mM) stimulated an increase in germination, radicle and hypocotyl length. However, an increase in concentration of kinetin and abscisic acid treatment was detrimental.	Bahrani and Pourreza (2015)
11	Green gram	Enhanced germination, root length, shoot length, fresh and dry weight of seedling was observed in seeds primed with 10^{-4} M of kinetin.	Marutirao (2016)
12	Tomato	Improved germination and seedling vigour owing to breakdown of dormancy was observed in seeds osmoprimed with 10 ppm, 50 ppm and 100 ppm of kinetin for 24 h at 25 ^{0}C in comparison with untreated seeds. Highest vigour was observed in seeds treated with 10 ppm kinetin followed by seed treatment at 100 ppm or 50 ppm.	Zeb *et al.* (2018)

2.1.3. Effect of priming with KH_2PO_4

Sl. No.	Crop	Experimental details	Reference
1.	Sunflower	Seed priming with KH_2PO_4 stimulated seedling emergence and growth.	Kathiresan *et al.* (1984)
2.	Muskmelon	Seed treatment with 2% to 3% KH_2PO_4 + KNO_3 (1:1), for one to five days significantly increased emergence rate and synchronization of emergence.	Nerson and Govers (1986)
3.	Wheat	Seeds primed with KH_2PO_4 stimulated the number of seedlings per unit area, dry weight and seedling height.	Bhati and Rathore (1988)
4.	Onion	Seedling size of tomato, capsicum and onion seeds, was improved when primed with KH_2PO_4.	Jagadish *et al*. (1994)
5.	Bitter gourd	Increased germination and seedling vigour index was observed in the seeds primed with 1.5% KH_2PO_4.	Renugadevi and Selvaraj (1994)
6.	Maize hybrid COH(M) 5	A concomitant increase in potassium and a parallel reduction in cell phytate content and stored minerals *viz.*, boron, copper, magnesium, manganese, iron and zinc was found when seeds were osmoprimed with 1% KH_2PO_4.	Sathish (2009)
7.	Sunflower	Priming of normal as well as low-vigour seeds with KH_2PO_4 (-1.25 MPa) for 24h was effective in improving the vigour of seedling in term of radicle length, plumule length and their root/shoot fresh weight.	Kauser *et al*. (2009)
8.	Wheat	Seeds primed with wheat seeds KH_2PO_4 (0.5 % for 12 h) at the temperature of 20ºC resulted in higher germination and vigour compared to other treatments.	Yari *et al*. (2010)

9.	Maize	Increased germination, shoot length, root length, dry matter production and vigour index and earlier germination in terms of days to 50% germination and days to maximum germination, was observed in the seeds primed with KH_2PO_4 (1%) for 6 h. The effect of seed priming with KH_2PO_4 was more pronounced in aged seeds than in fresh seeds.	Sathish *et al.*(2011)
10.	Bitter gourd	Priming the seeds with KH_2PO_4 (10^{-1}M for 24 h) followed by packing them in moist gunny bags for 48 h resulted in maximum, germination per cent and seedling vigour index-II over other treatments.	Kumar and Singh (2013)
11.	Okra	Okra seeds primed with KH_2PO_4 (3%) at 25^0C or 30^0C, registered the highest speed index and seedling length. An increase in dry weight was also evident.	Sahib (2014)
12.	Chilli	Germination per cent increased when seeds were primed with KH_2PO_4 (0.5%) and $CaCl_2$ (1%).	Vishwanath *et al.* (2014)
13.	Cabbage	Germination and vigour index of cabbage cultivars were enhanced by priming with (1%) KNO_3 or KH_2PO_4 (2%). Seed priming with KH_2PO_4 (1%, 2% and 3%) and KCl (1%, 2% and 3%) substantially increased the root length of the cabbage cultivars.	Batool *et al.* (2015)
14.	Chilli	Seed priming with KH_2PO_4 (2% and 6%) increased seed germination to 37.50 per cent compared to 24.46 per cent in control.	Dutta and Singh (2015)
15.	Cucumber	Priming treatment resulted in an improvement in germination per cent over the untreated seed. The speed of germination increased from 3.7 to 105.6 per cent while mean time of germination decreased.	Krainart *et al.* (2015)

16.	Bitter gourd	Priming the seeds with KH_2PO_4 (10^{-1} M for 24 h) followed by packing them in moist gunny bags for 48 h resulted in higher germination per cent over other treatments.	Singh *et al.* (2016)
17.	Sorghum	Best results were obtained for speed of germination, root length and vigour index when the seeds were primed with KH_2PO_4 (2%).	Chauhan *et al.* (2016)
18.	*Zea mays*	The seeds treated with KH_2PO_4 (2%) or $ZnSO_4$ (0.5%) had increased seed germination, germination speed index (GSI), seedling vigour index and dry weight compared to control.	Hussein (2016)
19.	Lentil	Priming seeds with KH_2PO_4 (2%) significantly increased the germination, vigour index and other yield parameters of the crop.	Meena *et al.* (2018)

2.1.4. Effect of bio-priming with *Psuedomonas fluorescens*

Sl. No.	Crop	Experimental details	References
1	Sweet corn	Bio-priming seeds with *Pseudomonas fluorescens* resulted in early seedling growth. This may have resulted from the combined effects of accelerated germination due to pre-plant seed hydration, alleviation of imbibitional chilling injury, and reduction of detrimental effects of *Pseudomonas ultimum* on the seed and seedling root system.	Callan *et al*. (1991)
2	Bitter gourd	Bio-priming of seeds resulted in an increased levels of malondialdehyde and total peroxides in accelerated-aged seeds	Hsu *et al.* (2003)
3	Sunflower	Bio-priming of sunflower seeds with *Pseudomonas fluorescens* (1×10^6 cfu.ml^{-1}) suspension for 3 hours was found to be effective in enhancing the seed indices and improvement of seedling growth.	Moeinzadeh *et al*. (2010)
4	Common bean	The per cent of germination, shoot length, root length, dry weight, vigour index I and vigour index II, was observed in seeds bio-primed with *T. harzianum* (4 h) was found to be on par with the performance of seeds bio-primed with *P. fluorescens*.	Monasila (2014)
5	*Abies hickelii* *Abies religiosa*	A combination of hydropriming and bio-priming (*Pseudomonas fluorescens, Pseudomonas putida* and *Bacillus subtilis)* improved the germination.	Zulueta -Rodríguez *et al*. (2015)
6	Chilli	Seeds bio-primed with *T. viride* (60% w/v) for 3 h followed by priming with *Pseudomonas fluorescens* (60% w/v) for 12 h exhibited an increased rate of germination, germination per cent, root length, shoot length, biomass production and seedling vigour index	Ananthi *et al.* (2017)
7	Chickpea	Chickpea seed bio-primed with *Pseudomonas fluorescens* (0.8%) + *Trichoderma harzianum* (0.8%) as well as vermiculite, registered the highest seed germination and vigour index.	Jainapur *et al.* (2018)

2.2. Effect of invigouration treatments on crop growth and performance

Seed priming includes a single cycle of alternate wetting and drying (Lee and Kim, 1999). Seed priming techniques was effective in reducing the emergence time, achieve uniform emergence, better allometric factors and a high potential for plant characters in many horticultural and field crops (Ashraf and Foolad, 2005; Farooq *et al*., 2005a).

Various seed priming techniques have been successfully developed (Taylor *et al*., 1998; Basra *et al*., 2004; Farooq *et al*., 2006b). These techniques include hormonal priming or soaking prior to sowing, hydro-priming, hardening, osmo-hardening and osmo-conditioning (Basra *et al*., 2005a; Ashraf and Foolad, 2005). Osmo-hardening for rice seed invigouration in which both hardening and osmo-conditioning were integrated was introduced by Farooq *et al*. (2006b). In this technique instead of tap or distilled water, rice seeds were hardened in various salt solutions. Osmo-hardening with $CaCl_2$ (–1.25 MPa) solution was more effective for enhancing vigour than simple hardening.

Seed priming has been reported to result in better crop stand and higher yields in several crops (Khan, 1992; Farooq *et al*., 2009; Kaymak *et al*., 2009). Primed seeds when raised, usually emerge faster with better, uniform, and high potential for vigourous crop stand persistent under less than optimum field conditions. Farooq *et al*. (2008) and Ruan *et al*. (2002b) also observed that priming reduced mean emergence time, enhanced seedling emergence and produced better crop stand establishment. Harris *et al*. (2001) reported that crops raised from primed seeds lead to earlier flowering and higher grain yield than non-primed seeds. On-farm priming resulted in faster rate of germination and emergence, more uniform and vigourous seedling growth, and a wide range of phenological and yield associated benefits (Harris *et al*., 2002).

Priming is reported to improve productivity through improved crop establishment, crop stand and growth, under varying climatic condition. Priming or pre-treatment of seeds were effective under sub-optimum field conditions, such as low or high temperature (Bradford *et al*., 1990; Pill and Finch-Savage, 1988; Wahid and Shabbir, 2005), salinity (Muhyaddin and Weibe 1989; Wahid *et al*., 2006) and under low soil moisture availability (Lee *et al*., 1998; Du and Tuong 2002).

Given below is a brief insight into the effect of various priming treatments embraced in the study, on crop growth and performance.

2.2.1. Effect of halopriming with $CaCl_2$

Sl. No.	Crop	Experimental details	Reference
1.	Rice	Kernel quality was improved in direct seeded rice seeds after they were osmo-primed with KCl as well as $CaCl_2$. These also proved to be very effective under flooded conditions.	Zheng *et al*. (2002)
2.	Wheat	Pre-sowing treatment of wheat seeds with $CaCl_2$ (100 $molm^{-3}$) improved the emergence per cent by stimulating pre-emergence metabolic activities during priming and resulted in higher crop performance under salinity.	Basra *et al*. (2005b)
3.	Wheat	The adverse effects of salt stress was alleviated on wheat cultivars in terms of shoot fresh and dry weights and grain yield, when seeds were primed with $CaCl_2$.	Iqbal *et al.* (2006)
4.	Rice	The seeds osmo-hardened with KCl followed by priming with $CaCl_2$ for 24 h registered higher kernel and straw yield and harvest index. The priming enhanced the starch hydrolysis and made available more sugars for embryo growth and resulted in production of vigourous seedlings, further leading to improved allometric traits and kernel yield and quality attributes.	Farooq *et al*. (2006c)
5.	Wheat	$CaCl_2$ (100 mol m^{-3} for 12 h) priming was found to be very effective in promoting shoot growth and grain yield under both saline and non-saline conditions. The treatment improved net CO_2 assimilation rate in cultivars.	Iqbal and Ashraf (2007)
6.	Rice	Rice seeds osmo-hardened with $CaCl_2$ resulted in better crop performance through improved germination speed, seedling vigour and starch metabolism. It also stimulated maximum straw and kernel yield and harvest index.	Farooq *et al.* (2007c)

7.	Marigold	Seeds of marigold primed with 50 mM $CaCl_2$ for 24 h recorded reduced mean emergence time, days to 50% emergence, increased seedling emergence uniformity, final seedling emergence per cent as well as seedling and crop growth compared to the other treatments including untreated control. This was attributed to the effect of lower electrical conductivity of seed leachates, higher reducing and total sugars as well as higher α -amylase activity in haloprimed seeds.	Afzal *et al*. (2008b)
8.	Rice	Osmohardening with $CaCl_2$ resulted in improved crop stand as indicated by less time to emergence and higher values for emergence index and final emergence, higher crop growth rate, improved plant height, tiller numbers, straw and kernel yield and high harvest index. In addition, seed priming treatments also improved the kernel quality. Priming with $CaCl_2$ reduced the number of sterile spikelets, abortive and chalky kernels and improved the kernel length, kernels per branch, 1000-kernel weight, kernel width, and kernel water absorption ratio.	Rehman *et al*. (2010)
9.	Rice	Osmo-hardening with $CaCl_2$ was found to be an effective seed priming technique for enhancing the crop stand, growth, yield, and quality of direct seeded rice.	Rehman (2011)
10.	Rice	Improved crop stand was observed on osmopriming the seeds with $CaCl_2$ followed by re-drying. The treatment reduced emergence time, enhanced high seedling vigour and yield attributes such as higher panicle bearing tillers, number of kernels per panicle, 1000-kernel weight, straw and kernel yield, and high harvest index (HI).	Rehman *et al.* (2011)
11.	Rice	Priming the seeds $CaCl_2$ (-1.25 MPa) for 36 h was effective in inducing salt tolerance in rice cultivars owing to enhanced germination capacity, speed of germination, seedling length	Afzal *et al*. (2012b)

		and dry weight in saline medium. The primed seeds registered high Na+/K+ exclusion resulting in higher saline tolerance.	
12.	Wheat	Seeds osmo-primed with $CaCl_2$ (50 mgl^{-1} for 12 h) resulted in maximum fertile tillers, grains per spike, 1000grain weight, grain yield and harvest index compared to untreated control under saline growth condition.	Jafar *et al*. (2012)
13.	Sorghum	Priming sorghum seeds with $CaCl_2$ resulted in significantly high root growth (length) and higher vigour index compared to untreated control.	Shehzad *et al.* (2012)
14.	Safflower	Priming with calcium chloride increased the Ca^{++} levels leading to a decreased fresh weight and dry weight of cotyledonary leaves	Jam *et al*. (2012)
15.	Wheat	The seeds treated with $CaCl_2$ (2%) recorded highest germination, shoot length, root length, seed vigour index as well as the seedling dry weight. In addition, these seeds hardened also registered higher field emergence, plant growth as well as seed quality parameters.	Abasaheb (2014)
16.	Soybean	Seeds primed with $CaCl_2$.$2H_2O$ (0.5%) reached 50 per cent flowering earlier compared to other priming treatments. This was attributed to the fast emergence of the seeds at the beginning. The treatment also resulted in the highest number of pods per plant, number of seeds per pod and yield per hectare compared to other treatments including control	Chavan *et al*. (2014a)
17.	Linola	Osmopriming with $CaCl_2$ reduced the emergence time and produced seedlings with high fresh and dry weights as well as Chlorophyll a content. Priming with $CaCl_2$ reduced crop branching, flowering and maturity time. However, it induced maximum plant height, number of branches, tillers, pods and seeds per pod. The $CaCl_2$ osmopriming also resulted	Rehman *et al*. (2014a)

		an increase in seed weight, biological and seed yield, harvest index (4.12%) and oil contents (13.39%).	
18.	Okra	Seeds treated with 1% $CaCl_2$ showed lesser number of fruits, number of seeds per fruit, 100 seed weight, dry weight and leachate of electrical conductivity compared to control	Sharma *et al*. (2014)
19.	Wheat	Osmopriming with $CaCl_2$ (ψs -1.25 MPa) resulted in improved crop allometry and productivity, with a significant increase in all yield parameters under both well-watered and drought stress conditions	Hussain *et al.* (2018)
20.	Maize	Seeds primed with $CaCl_2$ recorded enhanced Ca^{2+} content in seeds leading to an improved carbohydrate metabolism that helped the crop to withstand adverse environmental conditions.	Mehboob *et al.* (2018)
21.	Soybean	Crop raised from seeds primed with $CaCl_2$ (2%) recorded earlier flowering and days to maturity, greater plant height, number of branches per plant, number of pods per plant, number of seeds per pod, 100-seed weight and seed yield per plant followed. Seed priming with KH_2PO4 (2%) was found to be the next best treatment.	Narendrabhai (2018)
22.	Rice	Seeds primed with $CaCl_2$ (1%) recorded higher seed quality characters such as germination, speed of germination, root length, shoot length, seedling length, dry matter production, vigour index I and vigour index II. The crop raised from the $CaCl_2$ primed seeds recorded higher values of growth and yield characters.	Prabhu *et al*. (2018)
23.	Groundnut	The germination (94.17%), root length (12.28 cm), shoot length (18.41 cm), total seedling length (29.10 cm) and vigour index I (2739.41) were enhanced by priming seeds with $CaCl_2$ (2%) followed by the seeds treated with $CaCl_2$ (1%). The crop raised from the hardened	Venkateshbabu (2018)

		seeds also recorded higher values for field emergence (89.67 %), plant height (39.87 cm), number of pods per plant (27) and 100 seed weight (38 g) followed by those treated with $CaCl_2$ (1%). The seeds hydrated with $CaCl_2$ (2%) had also registered earlier flowering and maturity and lower electrical conductivity (0.411 dSm^{-1}) compared to control.	

2.2.2. Effect of hormonal priming with cytokinin

Sl. No.	Crop	Experimental details	Reference
1.	Corn	No significant improvement was observed with respect to seedling emergence, seedling vigour, nitrogen response, and grain yield of corn when primed with cytokinin.	Subedi and Ma (2005)
2.	Wheat	Treating seeds with kinetin (150 mg/l) consistently resulted in improvement of crop growth and grain yield in wheat cultivars. This was attributed to the beneficial effects of kinetin priming on water use efficiency and photosynthetic rate under conditions of salt stress.	Iqbal and Ashraf (2005)
3.	Wheat	Priming the seeds of saline tolerant wheat cultivar with mild concentration (100 mgl^{-1}) of kinetin increased leaf free salicylic acid under saline conditions. Benzylaminopurine (150 mgl^{-1}) priming also resulted in increased leaf free polyamines (Spermidine and Spermine) in leading to increased growth and grain yield under salt stress condition.	Iqbal and Ashraf (2006)
4.	Bromegrass	Cyokinin primed seeds registered a positive effect on chlorophyll content as well as on leaf area	Eiswand *et al*. (2010)
5.	*Silybum marianum* L.	Under drought stress, kinetin (10 ppm) reverted the decrease of germination, germination rate, seedling length, seed vigour and seedling dry weight. However, it decreased the proline content and catalase activity.	Zavariyan *et al*. (2015)
6.	Green gram	Seeds primed with kinetin showed earlier germination and higher values of shoot length and root length. The stem and root dry weight were maximum at 30 days after germination. Maximum stem dry weight was also recorded in this treatment at 60 days as well as 75 days.	Marutirao (2016)

7.	*Eryngium*	Priming the seeds with growth regulator GA_3 (500 ppm) or kinetin (50 ppm) for 72 h resulted in the highest seed germination and biomass yield.	Mozumder *et al*. (2016)
8.	Cucumber	Seeds primed with 50 mg salicylic acid (SA) L^{-1} and 25 mg kinetin (Kin) L^{-1} increased shoot and root dry biomass and also reduced the salt injury in the crop under saline soil conditions	Gurmani *et al.* (2018)
9.	Rice	Seed treatment with kinetin (5 ppm) improved the plant growth and development of late sown rice variety HUR 105.	Joshi *et al*. (2018)

2.2.3. Effect of Osmo-priming with KH_2PO_4

Sl. No.	Crop	Experimental details	Reference
1.	Wheat	Seeds primed with KH_2PO_4 (0.5%) resulted in higher vigour index (VI) under field conditions.	Yari *et al*. (2010)
2.	Onion	Priming seeds with KH_2PO_4 reduced the days to emergence without any change in emergence ratio. In field experiments, seeds primed with KH_2PO_4 emerged approximately four days earlier than the control seeds, regardless of the date of sowing.	Arin *et al*. (2011)
3.	Maize	Germination of seeds primed with KH_2PO_4 were on par with those of unprimed seeds. Invigouration of maize seeds with KH_2PO_4 resulted in reduction of mean seedling emergence, higher seedling emergence and establishment in the field, compared to other priming treatments including control.	Mir-Mahmoodi *et al*. (2011)
4.	Mung bean	Osmopriming with KH_2PO_4 significantly improved the Superoxide Dismutase (SOD), Peroxidase (POD), and Catalase (CAT) activity which lead to enhanced seedlings vigour in terms of germination and vigour index in field conditions.	Umair *et al*. (2012)
5.	Maize	Seeds primed with KH_2PO_4 showed higher vigour than unprimed seeds in terms of increased fresh and dry shoot weights, shoot height and shoot P content. The nutrient uptake of seedling was increased four times due to priming with KH_2PO_4 (1%) solution. Yield of maize increased in response to	Miraj *et al*. (2013)

		KH_2PO_4 priming reflected by significant increase in cobs yield, grain and straw yields	
6.	Soybean	Seeds primed with KH_2PO_4 (50 ppm) showed increased number of seeds per plant, 100 seed weight compared to other treatments including control	Chavan *et al.* (2014b)
7.	Okra	Seeds treated with KH_2PO_4 (5000 ppm) showed higher values for number of fruits, 100 seed weight and dry weight and lower for number of seeds per fruit and leachate of electrical conductivity compared to control	Sharma *et al.* (2014)
8.	Green gram	Osmopriming the seeds with KH_2PO_4 (2%) enhanced seed germination and yield attributes, and induced early canopy development, flower initiation, compared to control and other treatments.	Krishnaprabhu (2018)
9.	Lentil	Priming seeds with KH_2PO_4 (2%) significantly increased the germination per cent, vigour index and other yield parameters of the crop.	Meena *et al.* (2018)
10.	*Zea mays*	Early tassel initiation (73.7 days) and maturity (100.7 days) was observed when seeds were primed with KH_2PO_4 (0.5%) for 12 h. These parameters were delayed in the untreated control.	Kotambari *et al.* (2018)

2.2.4. Effect of biopriming with *Psuedomonas fluorescens*

Sl. No.	Crop	Experimental details	Reference
1.	Pearl millet	Biopriming pearl millet seeds with *Pseudomonas fluorescens* resulted in improved growth of the plants and also induced resistance against downy mildew disease caused by the fungus *Sclerospora graminicola*. The treatment enhanced germination and resulted in higher levels of vegetative and reproductive growth accompanied by an increase in grain yield (22%) under greenhouse and field conditions.	Raj *et al*. (2004)
2.	Sunflower	Biopriming with *Pseudomonas fluorescens* strains UTPf76 and UTPf86, enhanced seed characters such as germination index, germination, germination rate and vigour index and also seedling growth indices including root length, shoot height, dry and wet weight of seedlings and numbers of lateral roots in comparison with other treatments and the control.	Moeinzadeh *et al*. (2010)
3.	*Cicer arietinum* and *Phaseolus vulgaris*	In both chickpea and rajma, germination, root length and biomass accumulation was highest in the seeds bioprimed with *Pseudomonas fluorescens* OKC (GenBank accession JN128891).	Yadav *et al*. (2013)
4.	Common bean	Seeds bio-primed with *Pseudomonas fluorescens* (40%) for 4 h resulted in enhanced plant growth characters, reduction in disease incidence and increased seed yield.	Monalisa (2014)

5.	Pepper	Biopriming with *Pseudomonas fluorescens* PG01 reduced anthracnose disease incidence from 81 per cent down to 9 per cent in the infected seeds. The priming also improved plant growth, fruit yield, and seed quality of harvested seeds.	Ilyas *et al*. (2014)
6.	Chilli	Seeds bio-primed with *P. fluorescens* (60% for 12 h) followed by a spray of neem seed kernel extract (5%) controlled the pest, increased the seed yield and quality in chilli in both kharif and rabi seasons.	Ananthi *et al.* (2017)
7.	Pumpkin	Seeds bio-primed with *Azospirillum* 10% + Phosphobacteria 20% + *Pseudomonas fluorescens* 20% for 12h was found to enhance plant growth and development as well as seed characteristics.	Sivakalai and Krishnaveni (2017)
8.	Soybean	A significant higher yield was observed in *Pseudomonas fluorescens* 13 bioprimed seeds. This was attributed to the cumulative effect of phosphate solubilization, plant growth promotion, nodulation promotion and disease suppression activities	Sharma *et al.* (2018)
9.	Okra	Priming okra seeds with *Pseudomonas fluorescens* (10^7 cfu) was found to effectively mitigate drought stress in okra plants.	Pravisya *et al*. (2019)

2.3 Effect of packing materials on seed indices

The seed parameters are maximum when the seed completes its structural and functional development on the plant itself; thereafter, it deteriorates irreversibly at varying rates (Pavithravani *et al*. 2008; Lakshmi *et al*. 2009). The seed viability loss due to seed deterioration is inexorable, irreversible and inevitable, but the rate of deterioration could be slowed down to a greater extent. Delayed germination, reduced seedling growth rates, decreased tolerance to adverse conditions and loss of germinability are the changes which occur during storage (Abdul-Baki and Anderson, 1973). These phenomena are some of the physiological manifestations of seed deterioration.

The rate of seed deterioration during storage can be slowed down by controlling the storage conditions or by applying certain seed treatments before or during mid-storage (Mandal and Basu, 1983; Basu, 1994). Apart from priming, storing seeds in suitable packing material at appropriate moisture content is also crucial in determining the seed longevity during storage. A brief review of literature on the effect of packing material on seed indices is enumerated below.

2.3.1. Effect of Polyethylene 700 G pouches

Sl. No.	Crop	Experimental details	Reference
1.	Field bean	The germination of seeds packed in polyethylene 700 gauge pouches was significantly higher than that stored in cloth bag due to its moisture impervious nature	Vanangamudi *et al.* (1986)
2.	Barnyard millet	Seeds treated with Thiram (2 g/kg of seed) and stored in 700 G polythene bags was found to the best at maintaining the viability, vigour and other seed qualities for more than 15 months of storage.	Kalavathi *et al*. (2000)
3.	Rice	Seeds treated with Thiram + Bavistin (1 g/kg of seed) and packed in polythene bag (700G) maintained high germination up to 10 months under ambient condition	Choudhury *et al*. (2001)
4.	Cowpea	The seeds treated with Captan (2 g/kg of seed) and stored in polyethylene bag (700 G) maintained higher germination and vigour during storage than those stored in cloth bag.	Srimathi *et al*. (2003)
5.	*Cassia siamea* Lamk.	The seeds scarified with commercial sulphuric acid (200 ml/kg of seeds for 25 minutes) + 2% KNO_3 + thiram 2 g + 5% carbaryl 200 mg and stored in polyethylene bags recorded the highest germination and vigour index after 10 months of storage than those stored in cloth bag.	Masilamani *et al*. (2004)
6.	Onion	Better seed quality was assured in the seeds treated with bavistin (2 g/kg of seed) and packed in polythene bag or aluminium foil and stored under cold storage over a period of 12 months of storage.	Nagaveni (2005)

7.	Bitter gourd	The seeds subjected to *arappu* (*Albizia amara*) leaf powder pelleting @ 200 gkg^{-1} of seed and stored in 700 gauge polyethylene bag, showed less electrolyte loss due to the water proof nature of the packing material. The seed indices (germination, vigour and dry matter production) were superior to those stored in cloth bags.	Thirusenduraselvi and Jerlin (2007)
8.	Tomato	During the storage period of 10 months, the seeds packed in polyethylene bags (700 G) after pelleting with carbendazim (2 g/kg) maintained high germination and vigour index compared to the unpelletted seeds packed in cloth bags, when stored under ambient environment.	Shashibhaskar *et al*. (2009)
9.	Soyabean	The moisture content increased with advancement of storage period. But, the rate of moisture absorbance was higher in cloth bag than polythene bag. The rate of decrease in germination and the increase in rate of deterioration was higher in seeds stored in cloth bag compared to polyethylene bag. At the end of storage period, the shoot and root length of seedling, and seedling vigour was the least in cloth compared to that in polyethylene bag.	Monira *et al*. (2012)
10.	Bitter gourd	The germination was higher in seeds treated with captan (2 g/kg) and stored in polyethylene 700 G. At the end of 10^{th} month of cold storage, germination of treated seeds stored in aluminium pouches was found to be on par with seeds stored in Polyethylene 700 G bag .	Tirakannanavar *et al*. (2012)
11.	Cucumber	Seeds treated with thiram and packed in polythene bag (700 gauge) recorded higher germination per cent and seedling vigour index I and lowest electrical conductivity	Gaurav (2013)

		at the end of nine months of storage. It was found to be on par with untreated seeds stored in polythene bag (200 gauge).	
12.	Field pea	Seeds stored in polyethylene 700 gauge was able to retain maximum germination per cent at three, six and nine months after storage, compared to those packed in other materials.	Kishore *et al*. (2014)
13.	Pigeon pea	The moisture content of seeds stored in polythene bag 700 G did not vary over storage while, it retained higher germination (%), seedling vigour index and also registered the least seed infection and seed infestation compared to that stored in cloth bag.	Hareesh *et al*. (2014)
14.	Groundnut	The indices of seed kernels vacuum packed in 700 G polyethylene bag was higher compared to those stored in 400 G polyethylene bags.	Vasudevan *et al.* (2014a)
15.	Jatropha	Packing seeds of *J. curcas* in polyethylene bag at a moisture of 6 per cent, ensured better germination, seedling length and seedling vigour index up to eight months under ambient storage.	Vasudevan *et al*. (2014b)
16.	Sunhemp	The sunhemp seeds stored under ambient condition in polyethylene bag of 700G after treating with Malathion (2.5 g/kg of seed), was found to be superior to those stored in cloth bags and HDPE bags after 10 months of storage with respect to germination and vigour index.	Thimmanna *et al*. (2014)
17.	Brinjal	Seeds pelleted with bavistin and stored in polyethylene bag (700 G) showed minimum loss of quantitative parameters throughout the storage period of 10 months under ambient condition, when compared to those stored in cloth bags.	Kumar *et al*. (2016)

18.	Alfalfa	Higher dehydrogenase enzyme activity leading to higher germination was recorded in seeds treated with diflubenzuron @ 2 ppm (8 mg WP per kg seeds) and stored in high density polythene bag (700 G) throughout the storage period of sixteen months under ambient environment.	Kumar *et al*. (2017a)
19.	Onion	Seeds packed in polythelene bag (500 gauge) and stored in cold storage ($7 \pm 2\ ^{0}C$) condition without seed treatment exhibited a significant high germination and seed indices for a period of 2 years.	Patel *et al*. (2017)
20.	Chickpea	Seeds treated with neem oil and stored in polythene bag showed high germination per cent (84.83%) and the least electrical conductivity (1.54 dSm^{-1}). It also recorded superiority in other parameters like root length (14.83 cm), shoot length (11.19 cm), seedling length (26.00 cm), fresh weight (3.40 g), dry weight (1.27 g), vigour index -I (1969) and vigour index- II (94.24).	Gupta *et al*. (2018)

2.3.2 Effect of aluminium laminated pouches

Sl. No.	Crop	Experimental details	Reference
1.	Onion	The life of seeds stored in aluminium foil pouch at 5.3 per cent moisture increased by seven months relative to the seeds stored in a cloth or paper bags.	Padma *et al.* (2000)
2.	Groundnut	Tri-layered aluminium foil pouches (TLP) registered the highest germination (%) up to 30 months of storage compared to those stored in other containers.	Rajgopal and Chandran (2000)
3.	Groundnut	Seeds in tri-layered aluminium pouches retained viability for a longer period (30 months) due to reduced rate of deterioration under ambient storage.	Chandran (2002)
4.	Onion	Seeds pelleted with a combination of binding agent polyvinyl acetate and filler materials and stored in aluminium foil pouch recorded higher germination throughout the storage period of ten months compared to those stored in other packing materials.	Gouda (2007)
5.	Wheat	Seeds packed in aluminium bags and stored for 18 months had registered low electrical conductivity value (33.5 dSm^{-1}) and high germination values (beginning and end germination: 68.3% and 88.0% respectively). The seeds recorded high values for seed viability and vigour parameters until 12 months of storage, producing strong and tall seedling with heavier seedling dry weights.	Naguib *et al*. (2011)
6.	Onion	Throughout the period of storage, the radicle protrusion (%) and germination was higher in seeds stored in aluminium foil pouches.	Selvarani *et al.* (2011)

7.	Cabbage, carrot, lettuce, onion, muskmelon and tomato seeds	Under varying environmental conditions, seeds packed in aluminium foil pouches stored better than those packed in other packing materials owing to lesser rate of deterioration.	Kuchi. *et al.* (2014)
8.	Onion	Seeds stored in aluminum laminated bags at a moisture content less than 5 per cent recorded highest value of germination percentage (78.12%) followed by seed kept in polyethylene bags (76.08%). The germination of seeds stored in aluminium laminated bags was 61.27 per cent even after 27 months of storage.	Tripathi and Lawande (2014)
9.	Radish	Seeds stored in laminated aluminium pouches at 5°C was cost effective as well as advantageous to prolong seed longevity.	Doijode (2016)
10.	Indian bean	After 12 months of storage, vigour index of seeds stored in aluminium foil was superior to those stored in other containers. The results indicated that aluminium foil could be utilized for long term storage.	Moharana *et al*. (2017)
11.	Corn and soybean	Germination in seeds dried to a moisture content of 5 per cent and packed in aluminum pouch, was above 70 per cent upto 16 months of storage, while, it was lower in seeds packed in other packing materials. The seeds also registered decreased values for lipid peroxidation and electrical conductivity during storage period compared to cloth bags.	Kartoori and Patil (2018)
12.	Dolichos bean	Seeds stored in aluminium foil bag after drying to 10 per cent moisture showed maximum seed germination (86.75%), vigour index (2115.50), speed of	Khadtar *et al.* (2018)

		germination (45.50) and low moisture content (11.75%) and electrical conductivity (0.191), even at 11th month of storage, compared to other packaging materials	
13.	Onion	The germination of seeds packed in aluminium foil bags and polyethylene bags decreased within 140 days of storage. At 140 DAS the highest germination (74%) was recorded in the seeds stored in aluminium foil bags. However, the germination was on par with the seeds stored polythene bag.	Khan *et al*. (2018)

Materials and Methods

3. MATERIALS AND METHODS

The study 'Seed invigouration for improved field performance and storability in ashgourd (*Benincasa hispida* (Thunb.) Cogn.),' was carried out at Dept. of Seed Science and Technology, in Kerala Agricultural University (KAU) during 2016-19. The study was undertaken with the objective of elucidating the effect of seed invigouration on field performance in ash gourd as well as to assess the impact of packing material on seed quality and longevity during storage in ash gourd variety KAU Local. The details of the materials used and techniques applied for the research work are described below.

3.1. Location and climate

The present study was conducted at the Department of Seed Science and Technology, College of Horticulture, Kerala Agricultural University (KAU), Vellanikkara P. O., Thrissur 680656, located at 40 m MSL between 10054' North latitude and 76028' East longitude, Vellanikkara experiences humid tropical climate. During the study period, relative humidity varied between 69.00 per cent (April, 2018) and 76.00 per cent (October, 2018), while, rainfall ranged from 28.9 mm (April, 2018) to 393.0 mm (October, 2018). The monthly mean maximum temperatures ranged from 36.1^{0}C in April, 2018 to 32.8^{0}C in October, 2018, while, the mean minimum temperature varied between 24.8^{0}C in April, 2018 and 22.9^{0}C during October, 2018. The soil temperature ranged from 26.03^{0}C (July, 2018) and 26.5^{0}C (October, 2018).

3.2 Experimental material

The seeds of ash gourd variety KAU Local were procured from VFPCK Alathur, Palakkad, immediately after the harvest of the winter crop. The procured seeds were further cleaned, primed and dried before storage.

3.3. Experiment 1: Seed storage studies

3.3.1 Experimental method

3.3.1.1 Treatment details

The experiment was conducted as a completely randomized design, with seven treatments and three replications. Priming of seeds was done as per the invigouration treatments

specified in Table 1. The performance of the primed seeds was compared to that of unprimed seeds that served as the control.

Table 1: Details of treatment

Treatment	Details
I_1	$CaCl_2$ (50 mM for 12 h)
I_2	$CaCl_2$ (50 mM for 24 h)
I_3	Kinetin (Cytokinin) (10 ppm for 12 h)
I_4	Kinetin (Cytokinin) (10 ppm for 24 h)
I_5	KH_2PO_4 (10^{-1} M for 24 h)
I_6	*Pseudomonas fluorescens* (1×10^6 cfu.ml^{-1} for 12 h)
I_7	Absolute control

3.3.1.2 Preparation of stock solutions and seed treatment procedure

To prepare the stock solution of 50 mM Calcium chloride, 14.701 g of $CaCl_2.2H_2O$ is dissolved in 100 ml of distilled water.

Similarly, 1000 ml of 10 ppm of Kinetin stock solution is prepared by adding 10 mg of Kinetin in 1000 ml of distilled water. A few drops of (3-5 mL) of 1 N NaOH is added to dissolve the powder.

In order to prepare 100 ml of 10^{-1} M KH_2PO_4 stock solution 1.36 g of $CaCl_2.2H_2O$ is dissolved in 100 ml of distilled water.

To prepare broth solution of *P. fluorescens*, inoculate purified KAU reference culture of *P. fluorescens* for incubation in Kings Broth medium for 24 hrs.

The seeds were primed with the respective priming agents (Table 1) for the specified time, in the ratio 1:2 on volume basis. Prior to packing, the invigourated seeds were shade dried to $\leq$ 8 per cent moisture.

3.3.1.3 Method of storage

Three replicates each of the treated and untreated seeds were packed individually in polyethylene pouches (700G) and also in aluminium laminated pouches (Plate 1). In each replication, care was taken to pack the seeds (15.0 g) required for the monthly assessment (up to 8 months) of seed quality in separate pouches. The seeds thus packed were stored under

ambient condition (72% RH and 32 ^{0}C). In addition, sufficient quantity of treated and untreated seeds required for initiating the field studies after the fourth month of storage *i.e.*, Experiment 2, were set aside.

3.3.1.4 Observations recorded

The seed quality parameters of the invigourated seeds stored in different packing materials stored under ambient condition were recorded at the start of the storage as well as at monthly intervals up to eight months of storage. Quantification of lipid peroxidation, sugar and amino acids leached out from the seeds was undertaken at bimonthly intervals.

3.3.1.4.1. Germination (%)

Seed germination was assessed in sand medium (ISTA, 2010). Four replicates each consisting of 100 seeds were germinated in a germination room at a temperature of 25±2°C and RH 90±3%. On the 14th day, *i.e.*, at the end of germination period, the number of normal seedlings emerged in each replication were counted and expressed as per cent.

3.3.1.4.2. Root length of seedling (cm)

The distance between the collar region and tip of the root of ten randomly selected normal seedlings were measured on the 14th day of germination and the average expressed in centimetre.

3.3.1.4.3. Shoot length of seedling (cm)

The distance between the tip of the leaf and the collar region of the ten seedlings used to assess the shoot length, was measured and the average expressed in centimetre.

3.3.1.4.4. Seedling dry weight (mg)

Ten normal seedlings were dried in hot air oven at 85ºC for 24 hours. The dried seedlings were cooled further in desiccators for 45 minutes, weighed and the average expressed in milligram.

3.3.1.4.5. Vigour index - I (VI-I)

The formula suggested by Abdul - Baki and Anderson (1972) was used to calculate Vigour index - I.

Vigour index - I (VI-I) = Germination (%) × Seedling length (cm)

3.3.1.4.6. Vigour index-II (VI-II)

Vigour index - II was calculated using the formula developed by Bewly and Black, (1994).

Vigour index - II (VI-II) = Germination (%) × Seedling dry weight (g)

3.3.1.4.7. Electrical conductivity of seed leachate (μSm^{-1})

Three replicates of 25 seeds from each treatment were pre-washed thrice with the distilled water to remove the adhering chemicals before soaking in 25 ml of distilled water for 24 hours. The soaked seeds were maintained at room temperature and occasionally stirred. The beakers were covered in order to reduce evaporation and other contaminants. The seed leachate was collected in 50 ml beaker from which EC was measured and expressed in microSeimens per meter (μSm^{-1}) (Jackson, 1973).

3.3.1.4.8. Seed moisture content (%)

Five gram of ground seed was placed in a moisture weighing bottle and kept for drying at 103±2°C for 16±1h in a hot air oven. The seeds were cooled by placing inside a desiccator for 30 minutes. The weight of the seeds before and after drying was recorded (in grams). The moisture content (%) of the seed was calculated using the formula detailed below and expressed (ISTA, 2010).

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

Where,

M1 – Weight of moisture bottle alone

M2 – Weight of bottle + Seed sample before drying

M3 – Weight of bottle + Seed sample after drying.

3.3.1.4.9. Leakage of amino acid (µg leucine eqiv.ml^{-1})

The method advocated by Moore *et al.* (1948) and Misra *et al.* (1975) with slight modifications was used for conducting amino acid estimation. Twenty-five seeds from each replication of each treatment were soaked in 30ml distilled water for 24 h at room temperature (26±1^{0}C) and then 0.1 ml of leachate was taken in the test tube and 1 ml of 2% Ninhydrin

solution was added and mixed thoroughly. The volume was made up to 2 ml with distilled water and the mixture heated in boiling water bath for 20 minutes. Five millilitre of the diluent (water: propanol 1:1) was added and the intensity of colour was measured in spectrophotometer at wavelength of 570 nm.

3.3.1.4.10. Leaching of sugar (µg glucose eqiv.ml^{-1})

For estimating the amount of sugar leached out from the seed, the method described by McCready *et al*. (1950) with minor modifications was used. The pre-cooled 2 ml leachate of ash gourd seeds from each replication of all treatments were taken separately in the test tube and 4 ml of freshly prepared ice cold anthrone reagent (0.2% anthrone in 95% H_2SO_4) was added. The mixture cooled for 30 minutes for development of bluish green colour and the intensity of colour was measured in a spectrophotometer at 580nm wavelength.

3.3.1.4.11. Lipid peroxidation (OD)

The magnitude of lipid peroxidation that occurred in ash gourd seeds over storage was estimated using the protocol by Health and Packer (1968). Seed tissue (0.1 g) after the removal of seed coat was homogenised with 0.5 ml of 0.1% Trichloroacetic acid. The homogenate was centrifuged at 15000 rpm at 4^0C for10 minutes. The supernatant (0.5 ml) was collected and to this 1.5 ml of 0.5% TBA diluted in 20% TCA was added and incubated in water-bath at 95^0C for 25 minutes. The reaction was stopped by incubating in ice and the solution was centrifuged for 5 minutes (15000 x g, 4^0C) and absorbance read at 532nm.

3.4 Experiment 2: Field performance of primed seeds

The experiment was laid out in the field facility of Department of Seed Science and Technology, College of Horticulture, as Randomized Block Design (RBD) using seeds subjected to priming treatments as enumerated under Experiment 1 (Treatments: 7) at two different times *viz.*, pre-sowing treatment (just prior to sowing) and pre-storage treatment (prior to 4 months of storage) and three replications. Ridges of 60 cm width were aligned along the plot area. The field was divided into 42 sub-plots for the randomized application of treatments. A spacing of 3 m between rows and 60 cm between plants was ensured in each sub-plot of size 3 m x 3 m to accommodate 10 plants. The seeds were sown on ridges in the last fortnight of July, 2018. Appropriate agronomic practices as per the package of practices of KAU (2016) were followed during crop growth period to raise a good crop.

3.4.1. Observations recorded

Five plants in each replication of each treatment were tagged to record observations.

3.4.1.1 Days to first flowering (female flower)

The days taken from sowing to anthesis of the first female flower in all the tagged plants were recorded.

3.4.1.2 Fruit length (cm)

Length of fruits from the tagged plants was measured at physiological maturity. Fruit length was measured as the distance between the proximal end (stalk end) and distal end and the average fruit length expressed in centimetres.

3.4.1.3 Fruit weight (g)

Fruits from each tagged plants were harvested separately at physiological maturity and the fruit weight was recorded and the average expressed in grams.

3.4.1.4 Fruit diameter (cm)

Fruits harvested from the tagged plants were taken and the diameter measured using a thread and further measured in a measuring scale. The average fruit diameter was expressed in centimetres.

3.4.1.5. Seeds per fruit

The seeds from the harvested fruits were extracted through wet extraction and the total number of seeds per fruit was counted and the average worked out.

3.4.1.6. Fresh weight of seed per fruit (g)

The seeds collected from each fruit immediately after extraction were weighed and the average expressed in grams.

3.4.1.7. Dry weight of seeds per fruit (g)

The seeds collected from each fruit were dried at room temperature to $\leq$ 8 per cent moisture weighed and the average expressed in grams.

3.4.1.8. 100 seed weight (g)

The weight of 100 randomly picked seeds after drying were recorded in each replication and the average expressed in grams.

3.4.1.9. Fruit yield per vine (kg)

The weight of fruits per vine were recorded and expressed in kilograms.

3.5 Statistical analysis

3.5.1 Statistical analysis for Experiment I

3.5.1.1 Analysis of data

Statistical analysis of the data on various seed quality parameters was performed following the factorial completely randomized design (CRD) with three replications, seven invigouration treatments (Factor 1), two packing materials (storage condition; Factor 2) and period of storage (Factor 3), as per Fisher's method of analysis of variance (Gomez and Gomez 1976). Arc sine transformation of data was done wherever applicable (Snedecor and Cochran, 1967).

3.5.1.2. ANOVA for factorial design

The data recorded in each of the experiment was analysed using three factorial ANOVA (CRD) so as to estimate the effect of invigouration treatment storage condition and period of storage on dependent variables. It helps us to distinguish whether there are interactions between the different factors considered. The mean squares due to different sources of variation were worked out using the following analysis of variance (Gomez and Gomez, 1976).

Source	**df**	**Mean square**	**Expected mean squares**
Replication	(r-1)	M_r	M_r/M_e
Main effect (A)	(a-1)	M_A	M_A/M_e
Main effect (B)	(b-1)	M_B	M_B/M_e
Factor (AB)	(a-1) (b-1)	M_{AB}	M_{AB}/M_e
Main effect (C)	(c-1)	M_B	M_B/M_e
Factor (AC)	(a-1) (c-1)	M_{AC}	M_{AC}/M_e
Factor (BC)	(b-1) (c-1)	M_{BC}	M_{BC}/M_e
Factor (ABC)	(a-1) (b-1) (c-1)	M_{ABC}	M_{ABC}/M_e
Error	ab (r-1)	M_e	

The treatments were compared using the critical difference (C.D) estimate at P = 0.05

3.5.2 Statistical analysis for Experiment II

3.5.2.1 Analysis of data

Statistical analysis of the data on various seed quality parameters was performed following the factorial randomized blocks design (RBD) with three replications, seven invigouration treatments (Factor 1) and different periods of seed invigouration (Factor 2) as per Fisher's method of analysis of variance (Gomez and Gomez, 1976). Arc sine transformation of data in per cent was done wherever applicable (Snedecor and Cochran, 1967).

3.5.2.2. ANOVA for Factorial design

The data recorded in each of the experiment was analysed using two factorial ANOVA so as to estimate the effect of invigouration treatments and the time of seed invigouration on dependent variables. It helps us to distinguish whether there are interactions between the different factors considered. The mean squares due to different sources of variation were worked out using the following analysis of variance (Gomez and Gomez, 1976).

Source	**df**	**Mean square**	**Expected mean squares**
Replication	(r-1)	M_r	M_r/M_e
Treatment	(2^n-1)	M_t	M_t / M_e
Main effect (A)	1	M_A	M_A/M_e
Main effect (B)	1	M_B	M_B/M_e
Factor (AB)	1	M_{AB}	M_{AB}/M_e
Error	(r-1) (2^n-1)	M_e	

The treatments were compared using the critical difference (C.D) estimate at P = 0.05.

Results and Discussion

4. RESULTS AND DISCUSSION

The requirement of quality seeds, prolonging its longevity to obtain seedlings that exhibit high vigour and superior is essential for good crop growth to ensure high economic returns. Ageing of seeds is an inevitable natural phenomenon subsequently resulting in loss of vigour and viability. As ageing process accelerates, the seed deterioration sets in at a faster pace. By adopting appropriate packaging, ensuring optimum storage environment apart from seed treatment methods, the deteriorative process can be slowed down and the longevity of the seed extended. Apart from maintenance of seed quality, these methods have proven to be advantageous in ensuring high field performance of the crop raised.

Specific information on the storage and field performance of treated seeds of popular ash gourd cultivar in Kerala (KAU Local) is unavailable. Information on the techniques and its time of application to enhance the performance of seeds in lab as well as in field trials is presently very limited. A study undertaken by Shobha (2016) in KAU pointed out that priming of ash gourd seeds immediately after harvest for 24 hours with kinetin (10 ppm) or KH_2P0_4 (10^{-1} M) resulted in early breaking of dormancy and uniform germination. Seeds of KAU Local remain dormant for a period of month from harvest. Over the period of storage, seed priming with $CaCl_2$ (50 mM) for 12 or 24 hours, *Psuedomonas fluorescens* (1×10^6 cfu.ml^{-1}) or kinetin (10 ppm) for 12 hours proved to be more beneficial in extending seed viability.

In the backdrop of the above findings, the present study was formulated and conducted in the Department of Seed Science and Technology, Kerala Agricultural University (KAU) during 2016 - 2018, aiming to elucidate the effect of various pre-sowing and pre-storage seed invigouration techniques on germination and field performance of ash gourd and to assess the impact of packing material on seed quality and longevity. The results obtained are enumerated and discussed below.

4.1 Experiment I: Seed storage studies

4.1.1 Seed quality before storage

Before storage (Table 2) germination varied between 77.30 per cent in I_1 ($CaCl_2$ 50 mM 12 h) and 88.00 per cent in I_7 (Untreated control). The germination in all treatments was above the minimum standards for seed certification for the crop *i.e.*, above 60 per cent. The per cent

Table 2. Seed quality of primed seeds before storage

Parameter	Invigoration treatments						
	I_1 $CaCl_2$ 12 h	I_2 $CaCl_2$ 24 h	I_3 Kinetin 12 h	I_4 Kinetin 24 h	I_5 KH_2PO_4 24 h	I_6 *Pf* 12 h	I_7 Control
Germination (%)	77.30	84.66	88.00	82.00	80.66	86.00	86.66
Vigour index I	1698	1835	1979	1873	1756	2036	1901
Vigour index II	1.21	1.21	1.26	1.23	1.75	1.63	1.36
Seed moisture content (%)	5.60	4.10	6.00	4.80	4.80	4.50	4.60
Electrical conductivity (EC) of seed leachate (μSm^{-1})	286.00	259.67	155.83	154.30	321.00	138.27	64.93
Leakage of amino acid (µg leucine eqiv.ml^{-1})	9.07	9.37	9.12	9.20	9.98	9.08	9.39
Lipid peroxidation (OD)	0.076	0.052	0.049	0.064	0.051	0.043	0.023
Leaching of sugar (µg glucose eqiv.ml^{-1})	1.09	1.19	1.21	1.45	1.44	1.42	1.74

germination of the seeds invigourated with Kinetin 10 ppm 12 h (I_3) was found to be higher than all the treatments including the untreated control.

Vigour index - I (VI-I) varied between 1698 (I_1: $CaCl_2$ 50 mM 12 h) and 2036 (I_6: *Psuedomonas fluorescens* 1×10^6 cfu.ml^{-1} 12 h) while, Vigour index - II (VI-II) ranged from 1.21 (I_1: $CaCl_2$ 50 mM 12 h and I_2: $CaCl_2$ 50 mM 24 h) to 1.75 (I_5: KH_2P0_4 10^{-1} M for 24 h).

The moisture content of the seeds before storage varied between 4.10 (I_2: $CaCl_2$ 50 mM 12 h) and 6.00 (I_3: Kinetin 10 ppm 12 h). The electrical conductivity (EC) of seed leachate ranged between 64.93 µSm^{-1} in I_7 (Untreated control) and 321.00 µSm^{-1} in I_5 (KH_2P0_4 10^{-1} M for 24 h). Leakage of amino acid of the seeds ranged between 9.07 µg leucine eqiv.ml $^{-1}$ (I_1: $CaCl_2$ 50 mM 12 h) and 9.98 µg leucine eqiv.ml $^{-1}$ (I_5: KH_2P0_4 10^{-1} M for 24 h) while, lipid peroxidation (OD) of the seeds ranged between 0.023 (I_7: Untreated control) and 0.076 (I_1: $CaCl_2$ 50 mM 12 h). Leaching of sugar of the seeds 1.09 µg glucose eqiv.ml $^{-1}$ (I_1: $CaCl_2$ 50 mM 12 h) and 1.74 µg glucose eqiv.ml $^{-1}$ (I_7: Untreated control).

It was evident that before the initiation of storage, the quality parameters of the seeds varied among themselves. The increased per cent germination observed in seeds treated with Kinetin 10 ppm 12 h (I_3) confirmed the findings of Shobha (2016). It was reported that priming of ash gourd seeds immediately after harvest for 24 hours with kinetin (10 ppm) resulted in early breaking of dormancy and uniform germination.

Treatment I_1 ($CaCl_2$ 50 mM 12 h) had registered poor germination, VI-I, VI-II in spite of registering the least estimates for leakage of amino acid of the seeds and leaching of sugar from seeds. This is in contrast with the findings of Styer and Cantliffe (1983). According to them, rapid water uptake during germination interferes with cell membrane reorganization and seed coat integrity of dried seed and allows increased metabolite leakage from seeds resulting in poor germination. The leakage of sugars during imbibition is also reported to encourage pre-emergence mortality (Kull, 1992).

4.1.2 Analysis of variance

Results indicated existence of wide variability in the impact of packing materials, invigouration treatments, storage period and their interaction on most of the seed indices studied during the period of seed storage.

4.1.3.1 GERMINATION (%)

The results on germination (%) as influenced by packing materials, invigouration treatments, the storage period and their interaction are presented in Table 3, 4 and 5 and Plate 1.

4.1.3.1.1 Effect of packing material (P)

Irrespective of the invigouration treatments and the storage period, germination of seeds stored in the polyethylene 700 G pouches (Poly. 700 G, P_1: 55.84%) and aluminium laminated pouches (Al. pouches, P_2: 55.19%) did not vary significantly from each other (Table 3).

Storing seeds in suitable packing material at appropriate moisture content is crucial in determining the seed longevity during storage. The air tight environment inside the moisture impervious packing material prevent the loss of moisture and retains the membrane integrity of the stored seeds is the reason for the high germination observed (Tang and Ngome, 2015).

4.1.3.1.2 Effect of invigouration treatments (I)

Irrespective of the packing materials and storage period, invigouration treatments exerted significant influence on germination (Table 3).

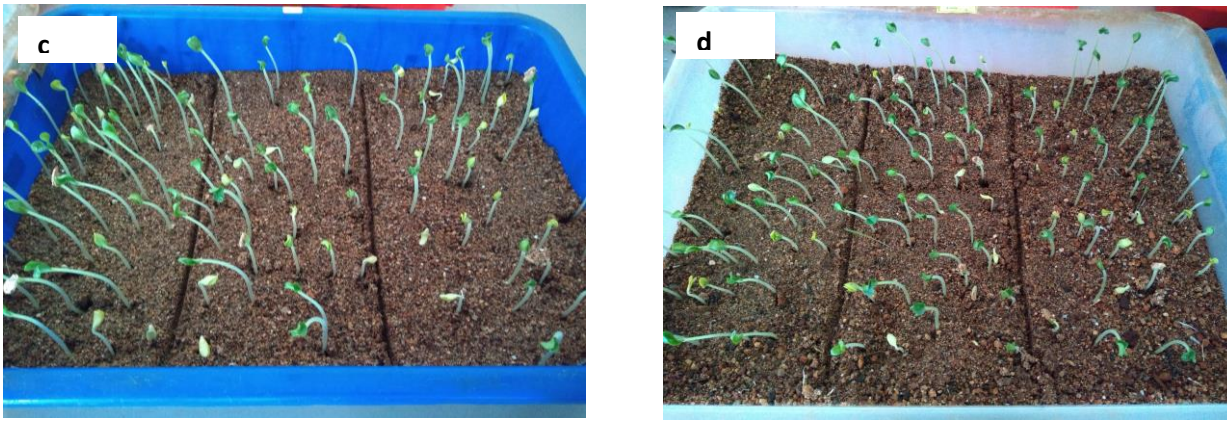
The mean germination varied between 62.44 per cent (I_3: Kinetin 10 ppm for 12 h) and 40.44 per cent (I_5: KH_2PO_4 10^{-1} M 24 h). Germination in I_3 (Kinetin 10 ppm for 12 h) was found to be on par with that observed in untreated control (I_7: 60.67%), but, differed significantly from all other treatments.

Results thus pointed out that seeds invigouration with Kinetin 10 ppm for 12 h (I_3) was on par with untreated control irrespective of the storage period and packing material. It was also evident that seed longevity was the least when seeds were treated with KH_2PO_4 10^{-1}M 24 h (I_5). The germination was not retained above MSCS beyond 1 MAS in this case.

Kinetin was found to be effective in breaking dormancy (Shobha, 2016) and subsequently enhances the ethylene biosynthesis resulting in faster germination (Saini *et al*., 1989; Matilla, 2000). Kepczynski (1997) had reported that kinetin is very effective in mobilisation of storage reserve for effective utilization during germination. From the studies of Misiha and EL-Ashry (1991) on *Magnolia grandiflora* L., Kang-Bing *et al*. (2001) on *Toona sinensis* and Singh *et al*. (2002) on *Artocarpus heterophyllus* Lam., it was elucidated that exogenously applied cytokinins could enhance protein synthesis and thereby increase germination.

Plate 1: Packed seeds and performance of stored seeds

Packing materials used: Polyethylene 700G (a) and Aluminium laminated bags (b)



Germination of seeds packed in Polyethylene 700G (c) and Aluminium laminated bags (d) at 1 MAS

Seedling length of seeds packed in Polyethylene 700G (e) and Aluminium laminated bags (f) at 1 MAS

Table 3. Impact of packing material, invigoration treatment and their interaction on germination (%)

Packing material (P)	Invigoration (I)							Mean P (Packing only)
	I_1 $CaCl_2$ 12 h	I_2 $CaCl_2$ 24 h	I_3 Kinetin 12 h	I_4 Kinetin 24 h	I_5 KH_2PO_4 24 h	I_6 *Pf* 12 h	I_7 Control	
P_1 (Poly. 700G)	50.33	60.22	62.22	59.78	37.11	57.22	64.00	**55.84**
P_2 (Al. bags)	52.44	59.44	62.67	60.67	43.78	58.33	57.33	**56.38**
Mean I (Invigoration only)	**51.39**	**59.83**	**62.44**	**60.22**	**40.44**	**57.78**	**60.67**	

Factors	C.D. (5%)	SE(m) ±
Factor P	NS	0.36
Factor I	1.86	0.67
Interaction P × I	2.63	0.94

Table 4. Impact of invigoration treatment, period of storage and their interaction on germination (%)

Invigoration (I)	Storage period (P)						Mean I (Invigoration only)
	S_1 (1 MAS)	S_2 (2 MAS)	S_3 (3 MAS)	S_4 (4 MAS)	S_5 (5 MAS)	S_6 (6 MAS)	
I_1	80.67	74.67	57.00	48.00	27.67	20.33	**51.39**
I_2	80.00	77.33	61.00	58.33	54.33	28.00	**59.83**
I_3	81.67	77.33	67.67	54.33	53.67	40.00	**62.44**
I_4	78.33	75.67	71.33	57.67	43.67	34.67	**60.22**
I_5	76.00	58.67	39.00	28.00	25.67	15.33	**40.44**
I_6	70.33	67.00	65.67	65.67	54.00	24.00	**57.78**
I_7	85.00	67.00	62.33	58.00	52.00	39.67	**60.67**
Mean S (Storage only)	**78.86**	**71.10**	**60.57**	**52.86**	**44.43**	**28.86**	

Factors	C.D. (5%)	SE(m)±
Factor I	1.86	0.67
Factor S	1.72	0.62
Interaction I × S	4.56	1.63

P_1: Polyethylene 700 G pouches, P_2: Aluminium laminated pouches, I: Invigoration treatments and MAS: Months after storage

4.1.3.1.3 Effect of storage period (S)

Irrespective of the packing materials and invigouration treatments, storage period exerted significant influence on germination (Table 4).

The mean germination per cent decreased over the storage period irrespective of the packing materials and invigouration treatments observed. It decreased from 78.86 per cent at 1 MAS (S_1) to 28.86 per cent at 6 MAS (S_6).

Seed deterioration is inevitable over storage. However, the rate of deterioration can be slowed down by subjecting them to suitable seed treatment measures as well as effectively choosing the packing material. In line with this, the results indicated that germination of seeds declined over the storage period of six months, irrespective of invigouration treatment and packing material. The seed parameters are maximum when the seed completes its structural and functional development on the plant itself; thereafter, it deteriorates irreversibly at varying rates (Pavithravani *et al*. 2008; Lakshmi *et al*. 2009). Delayed germination, reduced seedling growth rates, decreased tolerance to adverse conditions and loss of germinability are the changes which occur during storage (Abdul-Baki and Anderson, 1973).

4.1.3.1.4 Effect to interaction

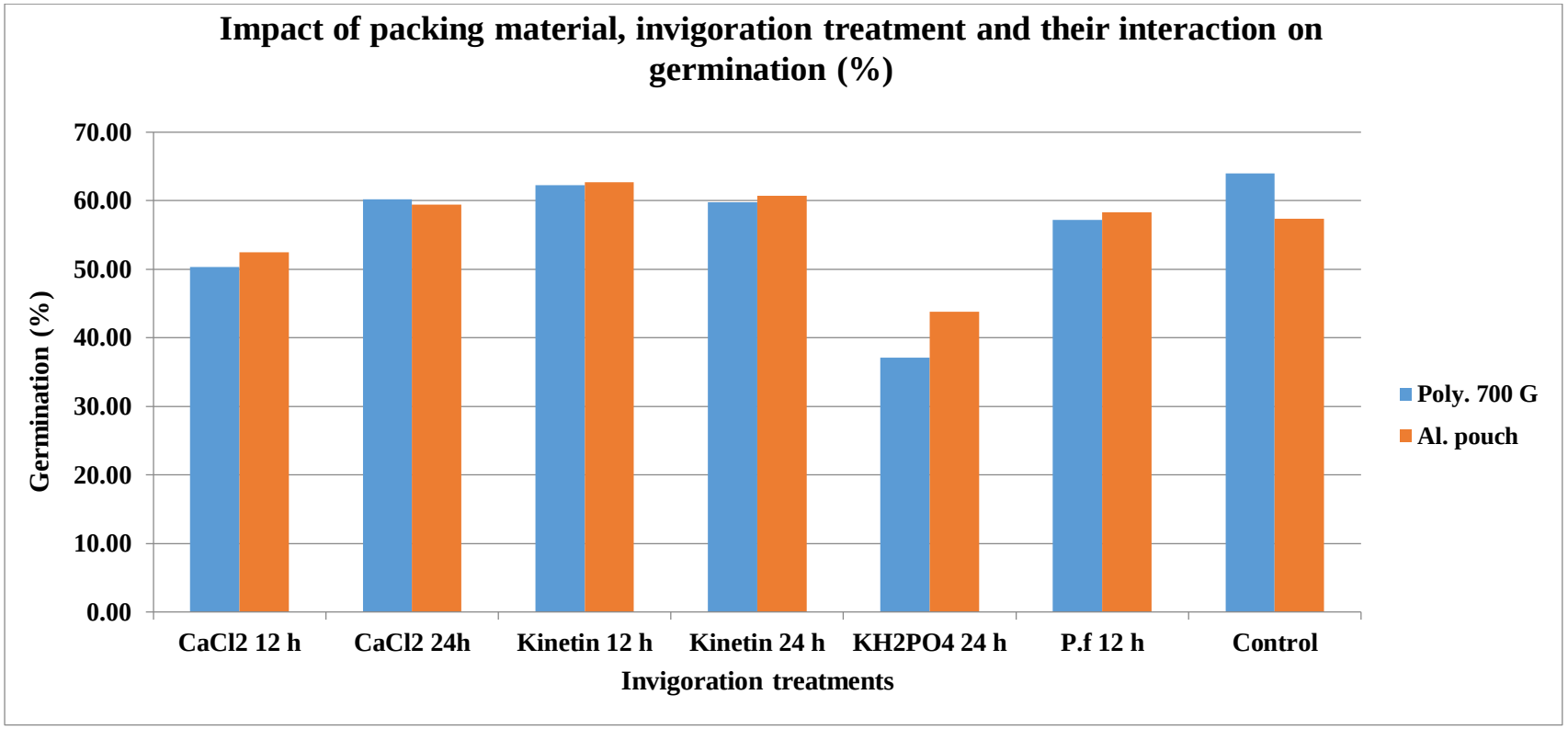
4.1.3.1.4.1 Packing materials × Invigouration treatments (P × I)

Significant difference in germination (Table 3 and Fig. 1) was observed due to the interaction of packing materials and invigouration treatments (P × I), irrespective of the period of storage.

Germination varied between 37.11 per cent (P_1I_5: Poly. 700 G - KH_2PO_4 10^{-1}M 24 h) and 64.00 per cent (P_1I_7: Poly. 700 G pouches - Untreated: 64.00%). Germination in P_1I_7 was found to be on par with P_2I_3 (Al. pouches - Kinetin 10 ppm 12 h: 62.67%) and P_1I_3 (Poly. 700 G pouches - Kinetin 10 ppm 12 h: 62.22%), but was significantly different from all other treatments.

Germination in P_2I_4 (Al. pouches - Kinetin 10 ppm 24 h: 60.67%), P_1I_2 (Poly. 700 G - $CaCl_2$ 50 mM 24 h: 60.22%), P_1I_4 (Poly. 700 G - Kinetin 10 ppm 24 h: 59.78%), P_2I_2 (Al. pouches- $CaCl_2$ 50 mM 24 h: 59.44%) and P_2I_6 (Al. pouches - *P. fluorescens* 1×10^6 cfu ml^{-1} 12 h: 58.33%), were found to be the next best to P_1I_3 and were also on par with each other.

Fig. 1


Impact of packing material, invigoration treatment and their interaction on germination (%)
Germination (%)
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
CaCl2 12 h
CaCl2 24h
Kinetin 12 h
Kinetin 24 h
KH2PO4 24 h
P.f 12 h
Control
Invigoration treatments
Poly. 700 G
Al. pouch

Seeds treated with I_5 (KH_2PO_4 10^{-1} M 24 h) and packed in either Poly 700 G pouches or Al. pouches registered the least germination.

4.1.3.1.4.2 Invigouration treatments × Storage period (I × S)

Interaction between invigouration treatments and storage period (I × S) significantly influenced germination (Table 4), irrespective of the packing material used.

The mean germination ranged from 20.33 per cent (I_1S_6: $CaCl_2$ 50 mM 24 h - 6 MAS) to 85.00 per cent (I_7S_1: Untreated control - 1 MAS). Germination in I_7S_1 was found to be on par with I_3S_1 (Kinetin 10 ppm 12 h: 81.67%) and I_1S_1 ($CaCl_2$ 50 mM 12 h: 80.67%) but significantly differed from all other treatments.

At 3 MAS, except in case of I_1S_3 ($CaCl_2$ 50 mM 12 h: 57.00%) and I_5S_3 (KH_2PO_4 10^{-1}M 24 h: 39.00%), germination of seeds treated with Kinetin 10 ppm 24 h (I_4S_3: 71.33 %), *P. fluorescens* 1 × 10^6 cfu ml^{-1} 12 h (I_6S_3: 67.67%), Kinetin 10 ppm 12 h (I_3S_3: 65.67%), I_7S_3 (Untreated: 62.66 %), I_2S_3 ($CaCl_2$ 50 mM 24 h: 61.00%), was retained above the MSCS. Seeds treated with *P. fluorescens* 1 × 10^6 cfu ml^{-1} 12 h (I_6S_4: 65.67%) exhibited germination per cent above MSCS at 4 MAS also.

4.1.3.1.4.3 Packing materials × Storage period (P × S)

Irrespective of the invigouration treatments, the germination was significantly influenced (Table 5) by the interaction between packing materials and storage period (P × S).

The mean germination varied from 28.48 per cent (P_1S_6: Poly 700 G - 6 MAS) to 80.19 per cent (P_2S_1: Al. pouches - 1 MAS). Germination of seeds tended to be higher in both packing materials during initial months of storage. Germination of seeds packed in Al. pouches during the first two months of storage- P_2S_1 (80.19 %) and P_2S_2 (72.57 %) was significantly superior to seeds packed in Poly. 700 G, at one and two month of storage (P_1S_1: 77.52% and P_1S_2: 69.62%). Germination of seeds packed in Al. pouches from 3 MAS onwards upto the end of storage period *i.e.*, at 6 MAS [P_2S_3 (59.81 %), P_2S_4 (52.48 %), P_2S_5 (44.00 %), P_2S_6 (29.24 %)] were on par with the germination estimates of seeds packed in Poly. 700 G during the corresponding period [P_1S_3 (61.33 %), P_1S_4 (53.24 %), P_1S_5 (44.86 %), P_1S_6 (28.41 %)].

In general, seeds treated with I_5 (KH_2PO_4 10^{-1} M 24 h) and packed in Poly. 700 G or Al. pouches has registered the least germination over the storage period.

Table 5. Impact of packing materials, invigoration treatments, storage period and their interaction on germination (%)

Storage period (S)	Packing material (P) × Invigoration (I)															
	P_1I_1	P_1I_2	P_1I_3	P_1I_4	P_1I_5	P_1I_6	P_1I_7	Mean ($P_1 \times S_n$)	P_2I_1	P_2I_2	P_2I_3	P_2I_4	P_2I_5	P_2I_6	P_2I_7	Mean ($P_2 \times S_n$)
S_1 (1 MAS)	74.67	78.00	80.67	74.67	76.00	72.00	86.67	**77.52**	86.67	82.00	82.67	82.00	76.00	68.67	83.33	**80.19**
S_2 (2 MAS)	72.67	74.67	72.67	76.00	50.67	70.00	70.67	**69.62**	76.67	80.00	82.00	75.33	66.67	64.00	63.33	**72.57**
S_3 (3 MAS)	50.67	63.33	72.00	72.67	38.00	68.67	64.00	**61.33**	63.33	58.67	63.33	70.00	40.00	62.67	60.67	**59.81**
S_4 (4 MAS)	49.33	61.33	52.00	58.00	23.33	66.67	62.00	**53.24**	46.67	55.33	56.67	57.33	32.67	64.67	54.00	**52.48**
S_5 (5 MAS)	42.67	57.33	52.00	42.00	22.67	44.00	53.33	**44.86**	12.67	51.33	55.33	45.33	28.67	64.00	50.67	**44.00**
S_6 (6 MAS)	12.00	26.67	44.00	35.33	12.00	22.00	47.33	**28.48**	28.67	29.33	36.00	34.00	18.67	26.00	32.00	**29.24**
Mean (P×I×S)	**50.33**	**60.22**	**62.22**	**59.78**	**37.11**	**57.22**	**64.00**	**55.84**	**52.44**	**59.44**	**62.67**	**60.67**	**43.78**	**58.33**	**57.33**	**56.38**

Factors	C.D. (5%)	SE(m)±
Interaction P × S	2.44	0.87
Interaction P × I × S	6.45	2.31

P_1: Polyethylene 700 G pouches, P_2: Aluminium laminated pouches, I: Invigoration treatments and MAS: Months after storage

4.1.3.1.4.4 Packing materials × Invigouration treatments × Storage period (P × I × S)

Germination (Table 5) was found to be significantly affected by the interaction between packing material, invigouration treatments and the storage period (P × I × S).

Germination of both treated and untreated seeds packed in Poly 700 G and Al. pouches decreased as storage period increased. It ranged from 12.00 per cent ($P_1I_1S_6$: Poly. 700 G - $CaCl_2$ 50 mM 12 h - 6 MAS and $P_1I_5S_6$: Poly. 700 G - KH_2PO_4 10^{-1} M 24 h - 6 MAS) to 86.67 per cent ($P_1I_7S_1$: Poly. 700 G - Untreated control - 1 MAS and $P_2I_1S_1$: Al. pouches - $CaCl_2$ 50 mM 12 h - 1 MAS).

At 1 MAS, germination of seeds packed in Al. pouches after treatment with $CaCl_2$ 50 mM 12 h ($P_2I_1S_1$: 86.67%) was the highest was found to be on par with untreated seeds packed in Poly. 700 G ($P_1I_7S_1$: 86.67%), $P_2I_7S_1$ (Al. pouches - Untreated - 1 MAS: 83.33%), $P_2I_3S_1$ (Al. pouches - Kinetin 10 ppm 12 h - 1 MAS: 82.67%), $P_2I_2S_1$ (Al. pouches - $CaCl_2$ 50 mM 24 h - 1 MAS: 82.00%), $P_2I_4S_1$ (Al. pouches - Kinetin 10 ppm 24 h - 1 MAS: 82.00 %), $P_2I_3S_2$ (Al. pouches - Kinetin 10 ppm 12 h - 2 MAS: 82.00%), as well as $P_1I_3S_1$ (Poly. 700 G pouches - Kinetin 10 ppm 12 h - 1 MAS: 80.67%).

At 2 MAS, both treated and untreated seeds retained germination above MSCS except for $P_1I_5S_2$ (Poly. 700 G - KH_2PO_4 10^{-1}M 24 h - 2 MAS: 50.67%).

At 3 MAS, all seeds retained germination above MSCS except for $P_1I_1S_3$ (Poly. 700 G - $CaCl_2$ 50 mM 12 h - 3 MAS: 50.67%), $P_1I_5S_3$ (Poly. 700 G - KH_2PO_4 10^{-1}M 24 h - 3 MAS: 38.00%), $P_2I_2S_3$ (Al. pouches - $CaCl_2$ 50 mM 24 h - 3 MAS: 58.67%) and $P_2I_5S_3$ (Al. pouches - KH_2PO_4 10^{-1} M 24 h - 3 MAS: 40.00%).

At 4 MAS, only $P_1I_6S_4$ (Poly. 700 G - *P. fluorescens* 1×10^6 cfu ml^{-1} 12 h - 4 MAS: 66.67 %), $P_1I_7S_4$ (Poly. 700 G - Untreated control - 4 MAS: 62.00 %) and $P_1I_2S_4$ (Poly. 700 G - $CaCl_2$ 50 mM 24 h - 4MAS: 61.33%) retained viability above MSCS. They were also found to be on par with each other.

Although in the present study, considering the influence of invigouration treatment alone on seed germination, priming with kinetin proved beneficial, on delineating the interaction between packing material and invigouration, it was evident that seeds bio-primed with *P. fluorescens* 1×10^6 cfu ml^{-1} 12 h and stored in Aluminium laminated pouches had retained viability above MSCS for the longest period *i.e.,* for 5 MAS ($P_2I_6S_5$: 64.00%). But, storing bioprimed seeds in Poly. 700 G pouches (*P. fluorescens* (*P. fluorescens* 1×10^6 cfu ml^{-1} 12 h: $P_1I_6S_4$) or with $CaCl_2$ 50 mM 24 h ($P_1I_2S_4$) or as untreated ($P_1I_7S_4$), helped retain germination

above MSCS only for 4 MAS. Seed invigouration with Kinetin 10 ppm either for 12 h (I_3) or for 24 h (I_4) followed by storage in either of the packing materials had retained viability above MSCS up to 3 MAS only.

Shobha (2016) had also reported that over the period of storage, seed priming with *Psuedomonas fluorescens* (1×10^6 cfu.ml^{-1}) or Kinetin (10 ppm) for 12 hours, proved to be more beneficial in extending seed viability. Similarly, Athmaja (2018) had found that bio-priming of ash gourd seeds with *Pf* (I_6: *P. fluorescens* 1×10^6 cfu.ml^{-1} for 12 h) can be recommended if the ambient storage period anticipated is less than 6 MAS. It was also reported that germination above MSCS was retained for 13 MAS in ash gourd seeds under refrigerated storage compared to only 5 MAS in ambient stored seeds.

Hsu *et al.* (2003) found that bio-priming of seeds resulted in an increased levels of malondialdehyde and total peroxides in accelerated-aged seeds. The better performance of seeds bio-primed with *P. fluorescens* (9 cfu s^{-1}) may have resulted from the combined effects of accelerated germination due to pre-plant seed hydration, alleviation of imbibitional chilling injury, and reduction of the effects of seed borne pathogen on the seed and seedling root system (Callan *et al*., 1991).

Evidence pointed to the existence of strong influence of the interaction between packing material, invigouration treatment and storage period on seed germination, rather than their individual effect. Under ambient storage environment it would be best to pack seeds in aluminium laminated pouches after invigouration with *P. fluorescens* 1×10^6 cfu ml^{-1} for 12 h, as this was found to prolong seed longevity the longest *i.e.,* 5 MAS. Bioprimed seeds stored in polyethylene 700 G pouches had retained viability above MSCS for 4 MAS only. However, it was observed that it would be apt to store untreated seeds in polyethylene 700G as well as seeds treated with $CaCl_2$ 50 mM for 24 h in polyethylene 700 G pouches rather than in aluminium laminated pouches as the seeds in aluminium laminated pouches registered germination above MSCS for a longer period (4 MAS) than those packed in the latter *i.e.,* upto 3 MAS and 2 MAS respectively in case of untreated seeds and $CaCl_2$ treated seeds. Seed invigouration with kinetin 10 ppm either for 12 h (I_3) or for 24 h (I_4) followed by storage in either of the packing materials had retained viability above MSCS up to 3 MAS.

The seed viability loss due to seed deterioration is inexorable, irreversible and inevitable. However, it was evident that the rate of deterioration could be slowed down to a greater extent

by controlling the storage conditions or by applying certain seed treatments before or during mid-storage (Mandal and Basu 1983; Basu 1994, Dhatt, 2018).

4.1.3.2 VIGOUR INDEX - I

The results on vigour index - I (VI-I) as influenced by packing materials, invigouration treatments, the storage period and their interaction effects are presented in Table 6, 7 and 8 and Plate 1.

4.1.3.2.1 Effect of packing material (P)

Irrespective of the invigouration treatments and the storage period, vigour index - I of seeds stored in the Al. pouches (P_2: 1374) and Poly. 700 G (P_1: 1346) did not vary significantly from each other (Table 6).

Results thus indicated that packing seeds in neither aluminium laminated pouches nor Polyethylene 700 G pouches influenced vigour (VI-I) of the seedlings. Similar to the results obtained in the present study, Tirakannanavar *et al*. (2012) and Naguib *et al.* (2011) also observed that the seedling characteristics of primed seeds packed either in Polyethylene 700 G or in aluminium laminated pouches were on par with each other over the storage period. The findings of Ruan *et al*. (2002b) in rice was in agreement to the present study. They attributed the improved seed vigour to the retention of moisture by the tight environment inside the packing materials. Raikar *et al*. (2011) reported that the seeds stored in polyethylene exhibited higher vigour index compared to those stored in moisture pervious packing materials like cloth pouches. The effectiveness and suitability of Polyethylene 700 G for packing seed have also been established by several earlier workers (Choudhary *et al*., 2011; Owolade *et al*., 2011; Kamara *et al*., 2014; and Wani *et al*., 2014).

4.1.3.2.2 Effect of invigouration treatments (I)

Irrespective of the packing materials and storage period, invigouration treatments exerted significant influence on vigour index - I.

Vigour index - I (Table 6) varied between 976 (I_5: KH_2PO_4 10^{-1}M 24 h) and 1521 (I_3: Kinetin 10 ppm 12 h). VI-I in I_3 was found to be on par with that observed in I_2 ($CaCl_2$ 50 mM 24 h: 1490), but significantly differed from all other treatments. I_2 however, was on par with Kinetin 10 ppm 24 h (I_4: 1443.96) and untreated control (I_7: 1441.87).

Table 6. Impact of packing material invigoration treatments and their interaction on Vigour index I

Packing material (P)	Invigoration (I)							Mean P (Packing only)
	I_1 $CaCl_2$ 12 h	I_2 $CaCl_2$ 24 h	I_3 Kinetin 12 h	I_4 Kinetin 24 h	I_5 KH_2PO_4 24 h	I_6 *Pf* 12 h	I_7 Control	
P_1 (Poly. 700G)	1221	1506	1516	1451	849	1360	1519	**1346**
P_2 (Al. bags)	1247	1474	1526	1437	1103	1465	1365	**1374**
Mean I (Invigoration only)	**1234**	**1490**	**1521**	**1444**	**976**	**1412**	**1442**	

Factors	C.D. (5%)	SE(m)±
Factor P	NS	11.08
Factor I	57.89	20.73
Interaction P × I	81.86	29.32

Table 7. Impact of invigoration treatments, period of storage and their interaction on Vigour index I

Invigoration (I)	Storage period (P)						Mean I (Invigoration only)
	S_1 (1 MAS)	S_2 (2 MAS)	S_3 (3 MAS)	S_4 (4 MAS)	S_5 (5 MAS)	S_6 (6 MAS)	
I_1	2014	1817	1381	1149	631	413	**1234**
I_2	2093	1932	1518	1423	1313	660	**1490**
I_3	2145	1935	1622	1282	1245	897	**1521**
I_4	1977	1838	1708	1346	1016	779	**1444**
I_5	1944	1403	938	666	584	321	**976**
I_6	1839	1695	1605	1596	1260	479	**1412**
I_7	2154	1636	1476	1341	1191	852	**1442**
Mean S (Storage only)	**2024**	**1751**	**1464**	**1258**	**1034**	**629**	

Factors	C.D. (5%)	SE(m)±
Factor I	57.89	20.73
Factor S	53.59	19.19
Interaction I × S	141.79	50.78

P_1: Polyethylene 700 G pouches, P_2: Aluminium laminated pouches, I: Invigoration treatments and MAS: Months after storage

Invigouration with Kinetin 10 ppm 12 h (I_3: 1521) was found to be as effective as halopriming with $CaCl_2$ 50 mM 24 h (I_2: 1490). Farooq *et al.* (2007d) found that priming boosts the physiological activities of the seeds which in turn increases the germination and vigour of seeds. They reported that osmo-priming the seeds with KNO_3 and $CaCl_2$ (3%) resulted in improved root length compared to control. Shobha (2016), Singh *et al*. (2017) and Athmaja (2018), had pointed out that it was advantageous to haloprime seeds with $CaCl_2$ for improved vigour during storage. Similarly, Kumari *et al.* (2017) found that seed priming in maize with the treatment $CaCl_2$ (1%) for 12 h at 25^0C increased germination, germination index, energy of emergence, seedling root length, shoot length, seedling fresh weight and dry weight and vigour index. Jie *et al*. (2002) found that halopriming induces release of enzymes in seed thereby accelerating seed metabolism.

Ghobadi *et al.* (2012) found that wheat seeds primed with cytokinin recorded higher vigour index compared to other priming treatments. Similarly, the high performance of cytokinin treated seeds was reported by several earlier workers (Afzal *et al.*, 2012 in spring maize; Nawaz *et al.*, 2013 in tomato; Bahrani and Pourreza, 2015 in maize; Marutirao, 2016 in green gram; Zeb *et. al.*, 2018 in tomato).

4.1.3.2.3 Effect of storage period (S)

Vigour index - I (Table 7) differed significantly as well as decreased over the storage period irrespective of packing materials and invigouration treatments. It ranged from 2024 at 1 MAS (S_1) to 629 at 6 MAS (S_6).

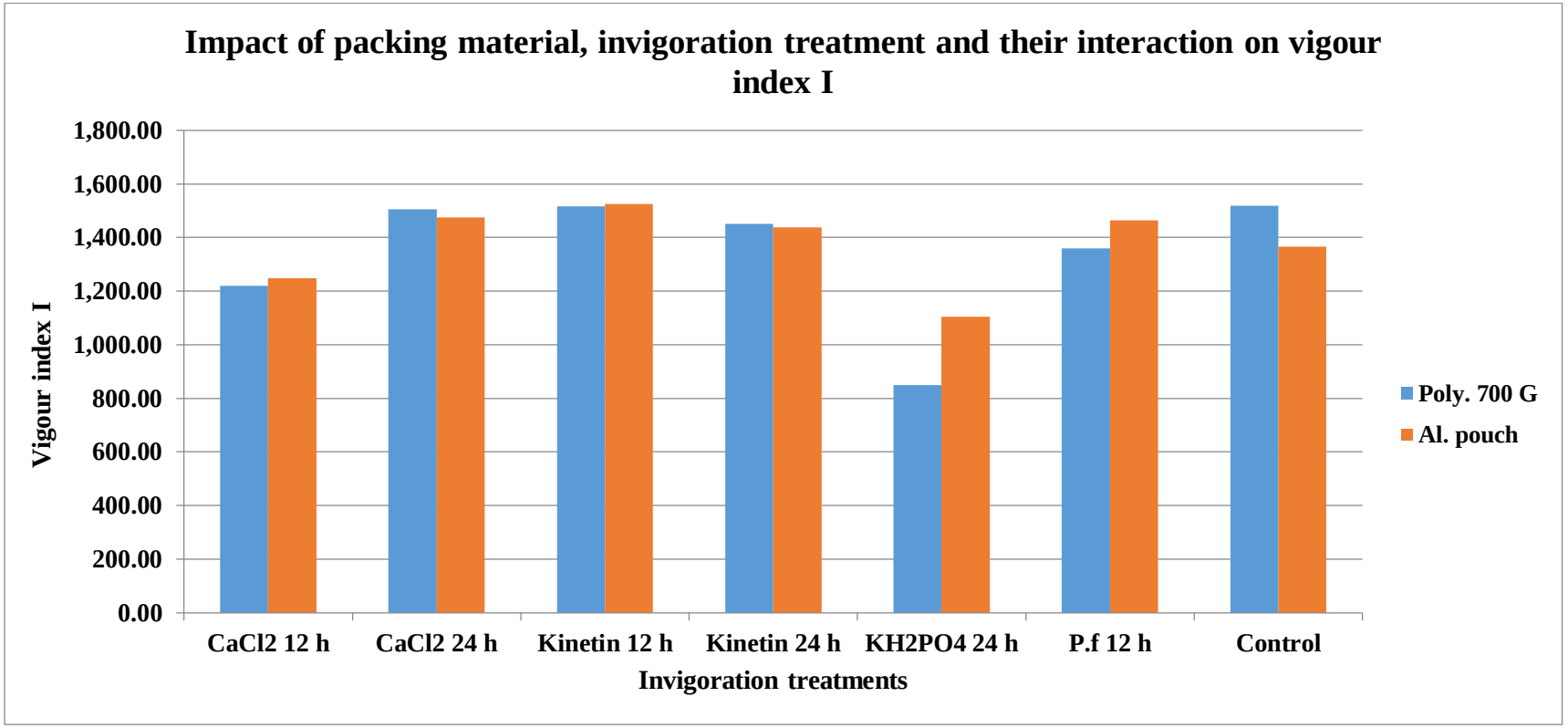
It is universally agreed upon that the loss of seed viability and vigour during storage is a natural phenomenon occurring due to deterioration process. According to Fessel *et al.* (2006), decrease in seed vigour was directly proportional to the increased leakage of Ca, Mg and K ions, confirming a close relationship of membrane integrity and loss of vigour which occurred during the storage period.

4.1.3.2.4 Effect of interaction

4.1.3.2.4.1 Packing materials × Invigouration treatments (P × I)

Significant difference in VI-I was observed (Table 6 and Fig. 2) due to the interaction of Packing materials and Invigouration treatments (P × I), irrespective of the period of storage.

Fig. 2


Impact of packing material, invigoration treatment and their interaction on vigour index I
Vigour index I
1,800.00
1,600.00
1,400.00
1,200.00
1,000.00
800.00
600.00
400.00
200.00
0.00
CaCl2 12 h
CaCl2 24 h
Kinetin 12 h
Kinetin 24 h
KH2PO4 24 h
P.f 12 h
Control
Invigoration treatments
Poly. 700 G
Al. pouch

Vigour index - I varied between 849 (P_1I_5: Poly. 700 G - KH_2PO_4 10^{-1}M 24 h) and 1525 (P_2I_3: Al. pouches - Kinetin 10 ppm 12 h).

Vigour index - I in P_2I_3 was found to be on par with P_1I_7 (Poly. 700 G - Untreated control: 1519), P_1I_3 (Poly. 700 G - Kinetin 10 ppm 12 h: 1516), P_1I_2 (Poly. 700 G - $CaCl_2$ 50 mM 24 h: 1506), P_2I_2 (Al. pouches - $CaCl_2$ 50 mM 24 h: 1474), P_2I_6 (Al. pouches - *P. fluorescens* 1×10^6 cfu ml^{-1} 12 h: 1464) and P_1I_4 (Poly. 700 G - Kinetin 10 ppm 24 h: 1451) and also significantly differed from all other P × I interactions.

Seeds treated with KH_2PO_4 10^{-1}M and stored in either of the packing materials (P_1I_5: 849 and P_1I_4: 1103) registered the least VI-I during storage.

4.1.3.2.4.2 Invigouration treatments × Storage period (I × S)

Interaction between invigouration treatments and storage period (I × S) significantly influenced VI-I, irrespective of the packing material used.

Vigour index - I (Table 7) ranged from 321 (I_5S_6: KH_2PO_4 10^{-1}M 24 h - 6 MAS) to 2154 (I_7S_1: Untreated control - 1 MAS). Vigour index - I in I_7S_1 at I MAS was found to be on par with I_3S_1 (Kinetin 10 ppm 12 h: 2145), I_2S_1 ($CaCl_2$ 50 mM 24 h: 2093) and I_1S_1 ($CaCl_2$ 50 mM 12 h: 2,013).

In general, VI-I of seeds treated with Kinetin 10 ppm 12 h (I_3), $CaCl_2$ 50 mM 24 h (I_2) and Kinetin 10 ppm 24 h (I_4) was found to be high during storage. Although VI-I of seeds invigourated with *P. fluorescens* 1×10^6 cfu ml^{-1} 12 h (I_6S_1: 1838) was the least at 1 MAS, it had registered high VI-I in later months of storage.

4.1.3.2.4.3 Packing materials × Storage period (P × S)

Irrespective of the invigouration treatments, vigour index - I (Table 8) was significantly influenced by the interaction between packing materials and storage period (P × S).

It varied from 626 in P_2S_6 (Al. pouches - 6 MAS) to 2086 in P_2S_1 (Al. pouches - 1 MAS). Vigour index - I of seeds packed in Al. pouches was higher at 1 MAS (P_2S_1: 2086) and 2 MAS (P_2S_2:1805) and was found to be significantly superior to that of seeds stored in Poly. 700 G pouches in the corresponding months *i.e.*, 1 MAS (P_1S_1: 1962) and 2 MAS, (P_1S_2:1697). However, after 2 MAS no such significant difference was evident.

Table 8. Impact of packing materials, invigoration treatments, storage period and their interaction on Vigour index I

Storage period (S)	Packing material (P) × Invigoration (I)															
	P_1I_1	P_1I_2	P_1I_3	P_1I_4	P_1I_5	P_1I_6	P_1I_7	**Mean ($P_1 \times S_n$)**	P_2I_1	P_2I_2	P_2I_3	P_2I_4	P_2I_5	P_2I_6	P_2I_7	**Mean ($P_2 \times S_n$)**
S_1 (1 MAS)	1893	2044	2067	1890	1808	1833	2196	**1962**	2134	2142	2224	2064	2080	1844	2112	**2086**
S_2 (2 MAS)	1752	1873	1830	1867	1108	1705	1746	**1697**	1882	1991	2039	1809	1699	1686	1527	**1805**
S_3 (3 MAS)	1214	1582	1747	1769	888	1626	1501	**1476**	1548	1454	1496	1646	988	1584	1452	**1452**
S_4 (4 MAS)	1183	1520	1256	1367	528	1570	1422	**1264**	1114	1325	1308	1326	805	1622	1261	**1251**
S_5 (5 MAS)	1003	1396	1220	989	500	1006	1211	**1046**	258	1230	1270	1043	669	1513	1170	**1022**
S_6 (6 MAS)	279	620	977	823	262	421	1037	**632**	547	700	817	736	379	538	668	**626**
Mean (P×I×S)	**1221**	**1506**	**1516**	**1451**	**849**	**1360**	**1519**	**1346**	**1247**	**1474**	**1526**	**1437**	**1103**	**1465**	**1365**	**1374**

Factors	**C.D. (5%)**	**SE(m)±**
Interaction P × S	75.79	27.14
Interaction P × I × S	200.52	71.81

P_1: Polyethylene 700 G pouches, P_2: Aluminium laminated pouches, I: Invigoration treatments and MAS: Months after storage

4.1.3.2.4.4 Packing materials × Invigouration treatments × Storage period (P × I × S)

Vigour index - I (Table 8) was found to be significantly affected by the interaction between packing material, invigouration treatments and the storage period (P × I × S). Vigour index - I of both treated and untreated seeds packed in Poly. 700 G as well as Al. pouches, decreased as storage period increased. It ranged from 279 ($P_1I_1S_6$: Poly. 700 G - $CaCl_2$ 50 mM 12 h - 6 MAS) to 2224 ($P_2I_3S_1$: Al. pouches - Kinetin 10 ppm 12 h -1 MAS).

At 1 MAS, VI-I of seeds packed in Al. pouches after treatment with Kinetin 10 ppm 12 h ($P_2I_3S_1$: 2224) was on par with untreated seeds packed in Poly. 700 G ($P_1I_7S_1$: 2196), $P_2I_2S_1$ (Al. pouches - $CaCl_2$ 50 mM 24 h - 1 MAS: 2142), $P_2I_1S_1$ (Al. pouches - $CaCl_2$ 50 mM 12 h - 1 MAS: 2134), $P_2I_7S_1$ (Al. pouches - Untreated - 1 MAS: 2112), $P_2I_5S_1$ (Al. pouches - KH_2PO_4 10^{-1}M 24 h - 1 MAS: 2080), $P_1I_3S_1$ (Poly. 700 G pouches - Kinetin 10 ppm 12 h - 1 MAS: 2066), $P_2I_4S_1$ (Al. pouches - Kinetin 10 ppm 24 h - 1 MAS: 2064), $P_1I_2S_1$ (Poly. 700 G - $CaCl_2$ 50 mM 12 h - 1 MAS: 2066) and $P_2I_3S_2$ (Al. pouches - Kinetin 10 ppm 12 h - 2 MAS: 2039).

VI-I of seeds subjected to I_2 ($CaCl_2$ 50 mM 24 h) and stored in Poly. 700 G pouches (P_1I_2) was found to be on par with the best treatment in the respective months of storage between 1 MAS and 5 MAS [$P_1I_2S_1$ (2044), $P_1I_2S_2$ (1873), $P_1I_2S_3$ (1582), $P_1I_2S_4$ (1520), $P_1I_2S_5$ (1396)]. A similar trend was observed in case of seeds invigourated with *P. fluorescens* 1×10^6 cfu ml^{-1} 12 h (I_6) and stored in Al. pouches during 3 MAS ($P_2I_6S_3$: 1584), 4 MAS ($P_2I_6S_4$: 1622) and 5 MAS ($P_2I_6S_5$: 1513).

Although, packing material alone did not impact the production of vigourous seedlings over storage period, it exerted a marked influence on expression of vigour (VI-I) in combination with the invigouration treatment the seed was subjected to. As observed in the impact of seed invigouration alone, vigourous seedlings were observed in treatments involving invigouration with Kinetin10 ppm for 12 h (I_3) and $CaCl_2$ 50 mM 24 h (I_2). Seeds treated with Kinetin10 ppm for 12 h (I_3) or 24 h (I_4) and packed in aluminium laminated pouches (P_2I_3 and P_2I_4) or polyethylene 700 G pouches (P_1I_3 and P_1I_4) produced high vigourous seedlings. Similarly, seeds subjected to invigouration treatment with $CaCl_2$ 50 mM 24 h and stored either in Poly. 700 G (P_1I_2) or Al. pouches (P_2I_2) also produced vigourous seedlings like the untreated seeds packed in Poly. 700 G pouches (P_1I_7).

Over the course of storage, irrespective of packing material, vigourous seedlings were produced if the seeds were treatments with Kinetin 10 ppm for 12 h (I_3), $CaCl_2$ 50 mM 24 h (I_2) or Kinetin10 ppm for 24 h (I_4).

The results also pointed out that the interactive influence of packing material and storage period on seedling vigour (VI-I) was restricted to early storage period (*i.e.,* at 1 MAS and 2 MAS). The seeds stored in aluminium laminated pouches had registered significantly higher vigour over those packed in polyethylene 700 G pouches, during the first two months after storage and thence after, no such advantage could be discerned.

The interactive impact of packing material, invigouration treatment and the storage period pointed out that vigourous seedlings would result if Kinetin 10 ppm for 12 h (I_3) treated seeds are stored in aluminium laminated pouches (P_2I_3) or Polyethylene 700 G pouches (P_1I_3), or if seeds are packed in Polyethylene 700 G pouches on treatment with $CaCl_2$ 50 mM 24 h (P_1I_2) or if they are left untreated (P_1I_7). In general, storing bio-primed seeds in Al. pouches would be more advantageous for obtaining vigourous seedlings in the long run than storing them in Polyethylene 700 G pouches.

Changes which occur during storage are associated with deterioration such as delayed germination, reduced seedling growth rates, decreased tolerance to adverse conditions and loss of germinability (Abdul - Baki and Anderson, 1973; Vasudevan *et al.,* 2012; Kumar *et al.,* 2017b). Adopting appropriate packaging, ensuring optimum storage environment and priming seeds was found to be beneficial in slowing down the pace of the deteriorative process during storage, maintaining the seed quality and prolonging seed longevity. Priming usually induces early, uniform and produces vigourous sprouting of seed. However, as observed in the present study, earlier workers have reported that the success of seed priming is influenced by the complex interaction of factors including plant species, water potentiality of the priming agent, duration of priming, temperature, seed vigour and dehydration, and storage conditions of the primed seed (Parera and Cantliffe, 1994; Dezfuli *et al.,* 2008).

4.1.3.3 VIGOUR INDEX - II

The results on vigour index - II (VI-II) as influenced by packing materials, invigouration treatments, the storage period and their interaction effects are presented in Table 9, 10 and 11.

4.1.3.3.1 Effect of packing material (P)

Irrespective of the invigouration treatments and the storage period, packing material exerted significant influence on VI-II of stored seeds. Vigour (VI-II) of seeds packed in Aluminium laminated pouches (P_2: 1.56) was significantly superior over Polyethylene 700 G pouches (Poly.700 G, P_1: 1.25) (Table 9).

The results are in concurrence with the findings of earlier workers (Padma and Reddy, 2000; Rao *et al.,* 2006; Lata and Sharma, 2008; Tripathi *et al*., 2014).

4.1.3.3.2 Effect of invigouration treatments (I)

Irrespective of the packing materials and storage period, invigouration treatments exerted significant influence on vigour index - II (Table 9).

Vigour index - II varied between 0.97 (I_5: KH_2PO_4 10^{-1}M 24 h) and 1.73 (I_2: $CaCl_2$ 50 mM 24 h). VI-II in I_2 was found to be on par with that observed in Kinetin 10 ppm 12 h (I_3: 1.53), Kinetin 10 ppm 24 h (I_4: 1.51), Untreated control (I_7: 1.43) and *P. fluorescens* 1×10^6 cfu ml^{-1} 12 h (I_6: 1.38), but differed significantly from all other treatments. Seed invigouration with KH_2PO_4 10^{-1}M 24 h (I_5: 0.97) had resulted in the least vigourous seedling.

The advantage of seed invigouration with $CaCl_2$ (Kathiresan *et al.,* 1984 in sunflower; Bhati and Rathore, 1988 in wheat; Kumari *et al.,* 2017 in maize), kinetin (Afzal *et al.,* 2012 in spring maize; Yarnia *et al.,* 2012 in onion; Nawaz *et al.,* 2013 in tomato; Marutirao, 2016 in green gram ; Zeb *et al.,* 2018 in tomato), biopriming seeds with *P. fluorescens* (Monalisa, 2014 in common bean; Ananthi *et al.,* 2017 in chillies; Jainapur *et al.,* 2018 in chickpea), or storing them untreated (Moradi and Younesi, 2009; Ahmadi *et al*., 2007; Kalsa *et al.,* 2011), in increasing seedling weight and producing vigourous seedlings have been reported earlier.

4.1.3.3.3 Effect of storage period (S)

Irrespective of the packing materials and storage period, invigouration treatments exerted significant influence on vigour index - II.

Vigour index - II (Table 10) decreased over the storage period irrespective of packing materials and invigouration treatments observed. It ranged from 2.98 at 1 MAS (S_1) to 0.49 at 6 MAS (S_6).

The seed moisture, relative humidity and temperature inside the packing material determines the viability and vigour of seed to a great extend (Tripathi *et al*., 2014). Seed being a living entity, deterioration over the period of storage is inevitable and hence reduction in germination potential and the vigour is a natural phenomenon as the storage period increases (Rao *et al*., 2006; Patel *et al*., 2017; Wang *et al*., 2018).

Table 9. Impact of packing material invigoration treatments and their interaction on Vigour index II

Packing materials (P)	Invigoration (I)							Mean P (Packing only)
	I_1 $CaCl_2$ 12 h	I_2 $CaCl_2$ 24 h	I_3 Kinetin 12 h	I_4 Kinetin 24 h	I_5 KH_2PO_4 24 h	I_6 *Pf* 12 h	I_7 Control	
P_1 (Poly. 700G)	1.17	1.35	1.48	1.44	0.76	1.23	1.34	**1.25**
P_2 (Al. bags)	1.44	2.10	1.58	1.57	1.18	1.52	1.53	**1.56**
Mean I (Invigoration only)	**1.31**	**1.73**	**1.53**	**1.51**	**0.97**	**1.38**	**1.43**	

Factors	C.D. (5%)	SE(m)±
Factor P	0.21	0.07
Factor I	0.38	0.14
Interaction P × I	NS	0.19

Table 10. Impact of invigoration treatments, period of storage and their interaction on Vigour index II

Invigoration (I)	Storage period (S)						Mean I (Invigoration only)
	S_1 (1 MAS)	S_2 (2 MAS)	S_3 (3 MAS)	S_4 (4 MAS)	S_5 (5 MAS)	S_6 (6 MAS)	
I_1	3.00	1.84	1.20	0.96	0.53	0.33	**1.31**
I_2	3.76	2.46	1.39	1.18	1.06	0.51	**1.73**
I_3	3.36	1.82	1.33	1.07	0.95	0.65	**1.53**
I_4	2.80	1.89	1.64	1.21	0.86	0.65	**1.51**
I_5	2.59	1.22	0.81	0.59	0.38	0.20	**0.97**
I_6	2.61	1.51	1.42	1.36	0.98	0.39	**1.38**
I_7	2.78	1.52	1.34	1.24	1.01	0.69	**1.43**
Mean S (Storage only)	**2.98**	**1.75**	**1.30**	**1.09**	**0.82**	**0.49**	

Factors	C.D. (5%)	SE(m)±
Factor I	0.38	0.14
Factor S	0.36	0.13
Interaction I × S	NS	0.34

P_1: Polyethylene 700 G pouches, P_2: Aluminium laminated pouches, I: Invigoration treatments and MAS: Months after storage

4.1.3.3.4 Effect of interaction

4.1.3.3.4.1 Packing materials × Invigouration treatments (P × I)

Irrespective of the period of storage, packing material and invigouration treatment (Fig. 3) exercised no significant impact on vigour index - II of stored seeds.

4.1.3.3.4.2 Invigouration treatments × Storage period (I × S)

Irrespective of the packing material, there occurred no significant impact on seedling vigour (VI-II) by the interaction of invigouration treatments and storage period.

4.1.3.3.4.3 Packing materials × Storage period (P × S)

Irrespective of the invigouration treatments, the vigour index - II (Table 11) was significantly influenced by the interaction between Packing materials and Storage period (P × S).

Vigour index - II (Table 10) varied from 0.49 (P_1S_6: Poly. 700 G - 6 MAS) and (P_2S_6: Al. pouches - 6 MAS) to 3.76 (P_1S_1: Poly. 700 G - 1 MAS). Vigour index - II of seeds tended to be higher in both packing materials in initial months of storage. At one month of storage, vigour index - II of seeds packed in Al. pouches, P_2S_1 (3.76) was significantly superior to seeds packed in Poly. 700 G P_1S_2 (2.21). Later, the seeds packed in Poly. 700 G and Al. pouches were on par with each other in the succeeding months of storage.

4.1.3.3.4.4 Packing materials × Invigouration treatments × Storage period (P × I × S)

The interaction between the period of storage, the invigouration treatment and the packing material (P × I × S) exerted no significant impact on vigour index - II (Table 11) of stored seeds.

The packing material, invigouration treatment and storage period individually influenced the seedling vigour of stored seeds. However, the interaction between invigouration and packing material (P × I) and invigouration and storage (I × S) as well as packing material, invigouration treatment and storage period (P × I × S), was ineffective in determining the seedling vigour.

It was observed that storing seeds in aluminium laminated pouches was advantageous over storing in polyethylene 700 G pouches in producing vigourous seedlings for a very short storage period (1 MAS). Thereafter, as the storage period increased no difference in seedling

Fig. 3

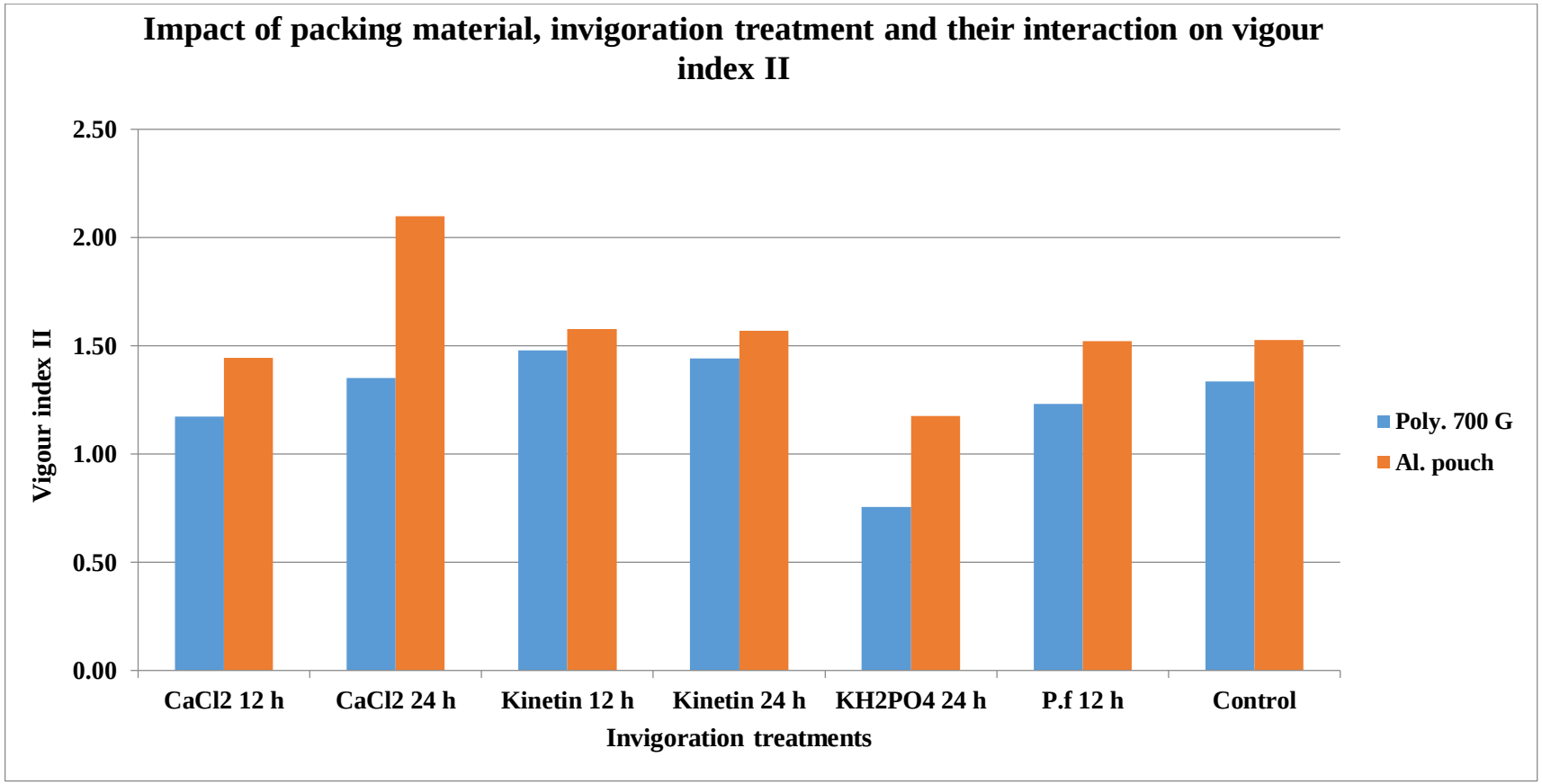

Impact of packing material, invigoration treatment and their interaction on vigour index II
Vigour index II
2.50
2.00
1.50
1.00
0.50
0.00
CaCl2 12 h
CaCl2 24 h
Kinetin 12 h
Kinetin 24 h
KH2PO4 24 h
P.f 12 h
Control
Invigoration treatments
Poly. 700 G
Al. pouch

Table 11. Impact of packing materials, invigoration treatments, storage period and their interaction on Vigour index II

Storage period (S)	Packing material (P) × Invigoration (I)															
	P_1I_1	P_1I_2	P_1I_3	P_1I_4	P_1I_5	P_1I_6	P_1I_7	**Mean (P_1× S_n)**	P_2I_1	P_2I_2	P_2I_3	P_2I_4	P_2I_5	P_2I_6	P_2I_7	**Mean (P_2× S_n)**
S_1 (1 MAS)	2.01	2.40	3.34	2.09	1.71	1.94	1.97	**2.21**	3.98	5.12	3.38	3.51	3.46	3.28	3.59	**3.76**
S_2 (2 MAS)	1.93	1.63	1.56	1.96	0.99	1.56	1.56	**1.60**	1.74	3.28	2.07	1.81	1.44	1.46	1.49	**1.90**
S_3 (3 MAS)	1.05	1.26	1.39	1.89	0.73	1.47	1.37	**1.31**	1.34	1.51	1.27	1.39	0.89	1.38	1.32	**1.30**
S_4 (4 MAS)	1.01	1.22	1.00	1.24	0.48	1.33	1.26	**1.08**	0.91	1.14	1.14	1.17	0.71	1.38	1.22	**1.10**
S_5 (5 MAS)	0.82	1.10	0.90	0.80	0.42	0.80	1.00	**0.83**	0.25	1.03	0.99	0.91	0.35	1.16	1.02	**0.82**
S_6 (6 MAS)	0.22	0.50	0.68	0.67	0.21	0.30	0.85	**0.49**	0.45	0.51	0.61	0.63	0.20	0.47	0.53	**0.49**
Mean (P×I×S)	**1.17**	**1.35**	**1.48**	**1.44**	**0.76**	**1.23**	**1.33**	**1.25**	**1.44**	**2.10**	**1.58**	**1.57**	**1.18**	**1.52**	**1.53**	**1.56**

Factors	**C.D. (5%)**	**SE(m)±**
Interaction P × S	0.50	0.18
Interaction P × I × S	N/A	0.48

P_1: Polyethylene 700 G pouches, P_2: Aluminium laminated pouches, I: Invigoration treatments and MAS: Months after storage

vigour was observed between the two packaging material used for seed storage. This may be the reason for absence of significant impact of interaction between the three factors *viz.*, packing material, invigouration treatment and storage period on seedling vigour (VI-II). The vigour (VI-II) of stored seeds decreased as the storage period increased and it was also observed that the untreated seeds produced seedlings as vigourous as those primed with $CaCl_2$ 50 mM 24 h or Kinetin 10 ppm for 12 h or 24 h and those bio-primed with *P. fluorescens* 1×10^6 cfu ml^{-1} 12 h. Seed invigouration with KH_2PO_4 10^{-1}M 24 h had resulted in the least vigourous seedling.

As observed in the present study, Kalsa *et al.* (2011) also reported that there was no difference in the VI-II between the primed and the unprimed seeds of common vetch (*Vicia sativa*). They suggested that this might be due to the negative impact of priming by reducing the seedling weight and vigour. Similar results were reported by Kaya *et al*. (2006), Ahmadi *et al*. (2007) and Moradi and Younesi (2009). According to them this impairment on priming might be due to the negative effects on both protein synthesis and enzymatic activity. The osmotic effect during imbibition also hampers the respiration processes which occurs during the seed germination.

4.1.3.4 SEED MOISTURE CONTENT (%)

Results pointed out that the individual effects of packing material and invigouration treatment was non-significant although the storage period significantly influenced the moisture. However, their interaction *viz.,* the interaction between packing material and invigouration (P × I), invigouration and storage (I × S), storage and packing material (P × S) and the interaction of all the three factors (P × I × S), did not significantly influence the moisture content of the stored seeds. However, irrespective of packing material, invigouration (Table 12, 13 and 14).

Storing seeds in moisture impervious containers like aluminium laminated pouches and Polyethylene 700 G pouches did not affect the seed moisture content. As observed in the present study, Doijode (2006) found that onion seeds packed at 6.5 per cent moisture in both aluminium laminated pouches and Polyethylene pouches, retained high germination up to seven years. Reducing the seed moisture to as low as possible prior to seed storage in an impermeable, compact and closed containers helps to prevent loss of seed viability and to ensure high per cent of vigourous seedlings (Horky, 1991).

Table 12. Impact of packing material, invigoration treatments and their interaction on seed moisture content (%)

Packing material (P)	Invigoration (I)							Mean P (Packing only)
	I_1 $CaCl_2$ 12 h	I_2 $CaCl_2$ 24 h	I_3 Kinetin 12 h	I_4 Kinetin 24 h	I_5 KH_2PO_4 24 h	I_6 *Pf* 12 h	I_7 Control	
P_1 (Poly. 700G)	7.40	7.40	7.30	7.40	7.30	7.40	7.30	**7.40**
P_2 (Al. bags)	7.50	7.50	7.40	7.40	7.30	7.20	7.40	**7.40**
Mean I (Invigoration only)	**7.40**	**7.40**	**7.30**	**7.40**	**7.30**	**7.30**	**7.30**	

Factors	C.D. (5%)	SE(m)±
Factor P	NS	0.02
Factor I	NS	0.04
Interaction P × I	NS	0.06

Table 13. Impact of invigoration treatments, period of storage and their interaction on seed moisture content (%)

Invigoration (I)	Storage period (P)						Mean I (Invigoration only)
	S_1 (1 MAS)	S_2 (2 MAS)	S_3 (3 MAS)	S_4 (4 MAS)	S_5 (5 MAS)	S_6 (6 MAS)	
I_1	7.30	7.30	7.40	7.40	7.50	7.60	**7.40**
I_2	7.30	7.30	7.40	7.40	7.50	7.60	**7.40**
I_3	7.20	7.30	7.40	7.40	7.40	7.50	**7.30**
I_4	7.30	7.40	7.40	7.40	7.50	7.50	**7.40**
I_5	7.20	7.20	7.30	7.40	7.40	7.50	**7.30**
I_6	7.20	7.20	7.30	7.40	7.40	7.50	**7.30**
I_7	7.20	7.30	7.40	7.40	7.40	7.50	**7.30**
Mean S (Storage only)	**7.20**	**7.30**	**7.30**	**7.40**	**7.40**	**7.50**	

Factors	C.D. (5%)	SE(m)±
Factor I	NS	0.04
Factor S	0.11	0.04
Interaction I × S	NS	0.10

P_1: Polyethylene 700 G pouches, P_2: Aluminium laminated pouches, I: Invigoration treatments and MAS: Months after storage

Table 14. Impact of packing materials, invigoration treatments, storage period and their interaction on seed moisture content (%)

Storage period (S)	Packing material (P) × Invigoration (I)															
	P_1I_1	P_1I_2	P_1I_3	P_1I_4	P_1I_5	P_1I_6	P_1I_7	Mean ($P_1 \times S_n$)	P_2I_1	P_2I_2	P_2I_3	P_2I_4	P_2I_5	P_2I_6	P_2I_7	Mean ($P_2 \times S_n$)
S_1 (1 MAS)	7.30	7.30	7.20	7.40	7.20	7.30	7.20	**7.20**	7.30	7.30	7.20	7.30	7.20	7.10	7.30	**7.20**
S_2 (2 MAS)	7.30	7.30	7.30	7.40	7.20	7.30	7.20	**7.30**	7.40	7.40	7.30	7.40	7.30	7.10	7.40	**7.30**
S_3 (3 MAS)	7.30	7.30	7.30	7.40	7.30	7.40	7.30	**7.30**	7.40	7.40	7.40	7.30	7.30	7.20	7.40	**7.40**
S_4 (4 MAS)	7.40	7.40	7.30	7.50	7.40	7.50	7.30	**7.40**	7.50	7.50	7.50	7.40	7.40	7.30	7.50	**7.40**
S_5 (5 MAS)	7.50	7.40	7.30	7.50	7.40	7.50	7.30	**7.40**	7.60	7.60	7.50	7.40	7.40	7.30	7.50	**7.50**
S_6 (6 MAS)	7.60	7.60	7.40	7.50	7.50	7.60	7.40	**7.50**	7.70	7.70	7.60	7.50	7.50	7.40	7.60	**7.50**
Mean (P×I×S)	**7.40**	**7.40**	**7.30**	**7.40**	**7.30**	**7.40**	**7.30**	**7.40**	**7.50**	**7.50**	**7.40**	**7.40**	**7.30**	**7.20**	**7.40**	**7.40**

Factors	C.D. (5%)	SE(m)±
Interaction P × S	NS	0.055
Interaction P × I × S	NS	0.144

P_1: Polyethylene 700 G pouches, P_2: Aluminium laminated pouches, I: Invigoration treatments and MAS: Months after storage

According to Christensen and Kaufmann (1965), Gupta *et al*. (1973), Rahman *et al*. (1985) and Cook and Veseth (1991), the decrease in seed germination with increasing length of storage period depended to a great extent on the permeability of the seed packing material or storage container, to moisture. Lower the moisture of seeds, lower is the rate of respiration. Similarly, lower the gain in seed moisture during storage, slower is the rate of seed deterioration. This is because the lower rate of respiration and other metabolic activities of the stored seed, slows down the rate at which the seed deteriorates during storage. According to Alam and Rahman (2005) and Ali *et al*. (2014), the thickness of packing material has significant effect on seed moisture during storage. It was reported that higher the thickness of packing material, lower be the moisture absorbed by the seed over the storage period. Thus, seeds packed in moisture impervious packing materials *viz.*, Poly. 700 G and to Al. pouches store longer than those in moisture pervious containers like cloth and jute pouches. Similar results were obtained by Das (1998), FAO (2006) and Nahar (2009) on comparing the longevity of seeds stored in Poly. 700 G and other packing materials.

4.1.3.5 ELECTRICAL CONDUCTIVITY OF SEED LEACHATE (EC) (μSm^{-1})

The results on Electrical conductivity (EC) as influenced by packing materials, invigouration treatments, the storage period and their interaction effects are presented in Table 15, 16 and 17 and Plate 2.

4.1.3.5.1 Effect of packing material (P)

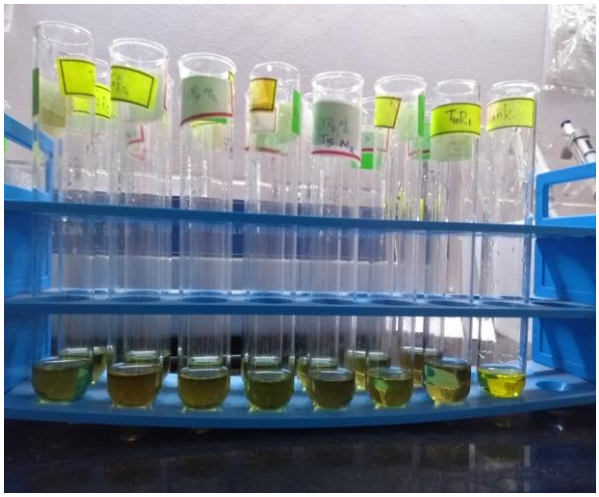
Irrespective of the invigouration treatments and the storage period, packing material exerted significant influence on the electrical conductivity of seed leachate (EC) of stored seeds (Table 15).

EC of seeds stored in the Al. pouches (P_2: 320.07 μSm^{-1}) was the least and differed significantly from that stored in Poly. 700 G pouches (Poly. 700 G, P_1: 326.08 μSm^{-1}).

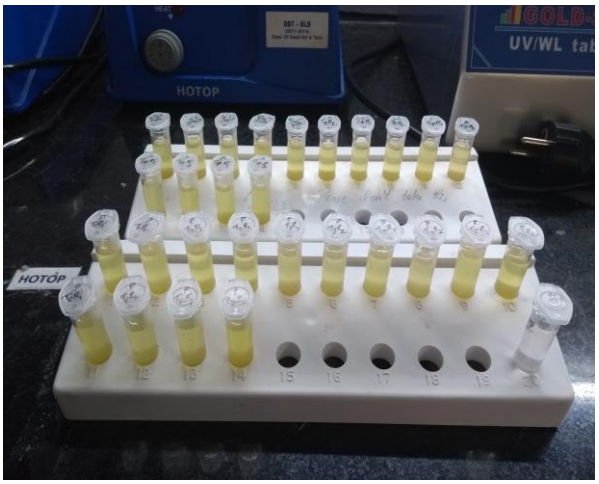
Electrical conductivity of the seed leachate is considered as a measure of membrane integrity. It is considered as a good indicator of the status of seed viability and vigour. Hence, storing seeds in aluminium laminated pouches would be more advantageous than storing them in Polyethylene 700 G pouches. According to Delouche and Baskin (1973), storing the seeds for longer period will cause deterioration as result of lipid peroxidation and further leading to loss of membrane integrity and an increase in seed leachate.

Plate 2: Biochemical analysis of seed leachate

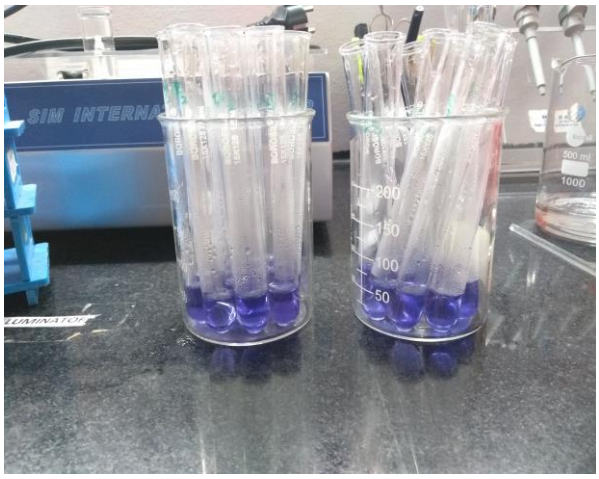
Test for Electrical conductivity of seed leachate



Test for leaching of sugar in seed leachate



Test for lipid peroxidation in seed leachate



Test for leakage of amino acid in seed leachate

4.1.3.5.2 Effect of invigouration treatments (I)

Irrespective of the packing materials and storage period, invigouration treatments exerted significant influence on electrical conductivity of seeds leachate (EC) (Table 15).

The EC varied between 105.54 μSm^{-1} (I_7: Untreated control) and 575.89 μSm^{-1} (I_2: $CaCl_2$ 50 mM 24 h). The electrical conductivity of I_7 (Untreated control) was the least and differed significantly from all other treatments.

Seeds treated with Kinetin 10 ppm 24 h (I_4: 184.50 μSm^{-1}), Kinetin 10 ppm 12 h (I_3: 195.88 μSm^{-1}) and *P. fluorescens* 1×10^6 cfu ml^{-1} 12 h (I_6: 221.31 μSm^{-1}), registered low levels of EC next to untreated seeds but differed significantly from each other.

It was obvious that storing seeds untreated would result in lower EC of seed leachate compared to storing primed seeds. However, among the priming treatments, bio-priming with *P. fluorescens* 1×10^6 cfu ml^{-1} and priming with Kinetin 10 ppm would be more beneficial in reducing EC of seed leachate during storage. As observed in the present study, Nawaz *et al*. (2016) observed improved membrane repair took place when the seeds were treated with kinetin leading to a reduced disintegration of seed membrane and lowering the electrical conductivity. The loss of membrane integrity during storage has been reported to be directly proportional to the increase in electrical conductivity of seed leachate. The lower EC values from the priming of seed treatments are indication of better membrane repair during controlled hydration during priming as reported by Rudrapal and Nakamura (1988) in radish and Basra *et al*. (2005a) in rice. According to Sarika (2013), slow hydration due to the integration of plasma membrane leads to the lower levels of electrical conductivity of seed leachate in primed seeds.

4.1.3.5.3 Effect of storage period (S)

Irrespective of the packing materials and invigouration treatments, storage period exerted significant influence on electrical conductivity (Table 16).

The electrical conductivity of seed leachate increased over the storage period irrespective of packing materials and invigouration treatments. It ranged from 158.59 μSm^{-1} at 1 MAS (S_1) to 467.12 μSm^{-1} at 6 MAS (S_6).

Sharma *et al*. (2011) had opined that the decrease in seed vigour during storage was directly proportional to the increased leakage of Ca, N and K ions, confirming the validity of the leakage test for the evaluation of relationship of membrane integrity and seed vigour as

Table 15. Impact of interaction of packing material and invigoration treatment on EC of seed leachate (µSm^{-1})

Packing materials (P)	Invigoration (I)							Mean P (Packing only)
	I_1 $CaCl_2$ 12 h	I_2 $CaCl_2$ 24 h	I_3 Kinetin 12 h	I_4 Kinetin 24 h	I_5 KH_2PO_4 24 h	I_6 *Pf* 12 h	I_7 Control	
P_1 (Poly. 700G)	435.22	611.67	200.88	188.06	534.00	201.83	117.20	**326.98**
P_2 (Al. bags)	398.17	540.11	190.89	180.94	595.72	240.78	93.89	**320.07**
Mean I (Invigoration only)	**416.70**	**575.89**	**195.88**	**184.50**	**564.86**	**221.31**	**105.54**	

Factors	C.D. (5%)	SE(m) ±
Factor P	1.24	0.45
Factor I	2.32	0.83
Interaction P × I	3.29	1.18

Table 16. Impact of interaction of invigoration treatment and period of storage on EC of seed leachate (µSm^{-1})

Invigoration treatments (I)	Invigoration (I)						Mean I (Invigoration only)
	S_1 (1 MAS)	S_2 (2 MAS)	S_3 (3 MAS)	S_4 (4 MAS)	S_5 (5 MAS)	S_6 (6 MAS)	
I_1	189.00	300.00	380.83	473.67	567.83	588.83	**416.70**
I_2	262.33	430.17	488.33	650.67	770.00	853.83	**575.89**
I_3	118.47	144.00	180.17	215.83	249.50	267.33	**195.88**
I_4	108.17	148.67	171.17	206.00	226.67	246.33	**184.50**
I_5	266.17	395.00	513.50	648.50	733.67	832.33	**564.86**
I_6	107.00	171.83	200.50	246.00	290.17	312.33	**221.31**
I_7	59.00	81.27	85.83	107.33	131.00	168.83	**105.54**
Mean S (Storage only)	**158.59**	**238.71**	**288.62**	**364.00**	**424.12**	**467.12**	

Factors	C.D. (5%)	SE(m) ±
Factor I	2.32	0.83
Factor S	2.15	0.77
Interaction I × S	5.69	2.04

P_1: Polyethylene 700 G pouches, P_2: Aluminium laminated pouches, I: Invigoration treatments and MAS: Months after storage

well as germination. The quantity and intensity of leached material are influenced by seed coat property and storage condition.

4.1.3.5.4 Effect of interaction

4.1.3.5.4.1 Packing materials × Invigouration treatments (P × I)

Significant difference in electrical conductivity was observed (Table 15 and Fig. 4) due to the interaction of packing materials × invigouration treatments (P × I), irrespective of the period of storage.

EC varied between 93.89 μSm^{-1} (P_2I_7: Al. pouches - Untreated) and 611.67 μSm^{-1} (P_1I_2: Poly. 700 G - $CaCl_2$ 50 mM 24 h). The electrical conductivity in P_2I_7 was not only least but also differed significantly from all other treatments.

P_1I_7 (Poly. 700 G - Untreated control: 117.20 μSm^{-1}) followed by P_2I_4 (Al. pouches - Kinetin 10 ppm 24 h: 180.94 μSm^{-1}) and P_1I_4 (Poly. 700 G - Kinetin 10 ppm 24 h: 188.06 μSm^{-1}) registered lower estimates of EC next to P_2I_7.

EC was found to be the highest in seeds packed in Poly. 700 G pouches after treatment with $CaCl_2$ 50 mM 24 h (P_1I_2: 611.67 μSm^{-1}). Next to P_1I_2, seeds treated with KH_2PO_4 10^{-1} M 24 h (I_5) and stored in either of the packing material (P_2I_5: 595.72 μSm^{-1} and P_1I_5: 534.00 μSm^{-1}) registered high EC of seed leachate.

4.1.3.5.4.2 Invigouration treatments × Storage period (I × S)

Interaction between invigouration treatments and storage period (I × S) significantly influenced EC (Table 16), irrespective of the packing material used.

Irrespective of the packing material, EC ranged from 59.00 μSm^{-1} (I_7S_1: Untreated control - 1 MAS) to 853.83 μSm^{-1} (I_2S_6: $CaCl_2$ 50 mM 24 h - 6 MAS). The electrical conductivity in I_7S_1 was the least and differed significantly from all other treatments. The next lower EC estimate was observed in I_6S_1 (*P. fluorescens* 1×10^6 cfu ml^{-1} 12 h: 107.00 μSm^{-1}). I_6S_1 was found to be on par with I_4S_1 (Kinetin 10 ppm 24 h: 108.17 μSm^{-1}).

Seeds treated with I_5 *i.e.*, KH_2PO_4 10^{-1} M 24 h (I_5S_1: 266.17 μSm^{-1}; I_5S_3: 513.50 μSm^{-1}; I_5S_4: 648.50 μSm^{-1}; I_5S_5: 733.67 μSm^{-1}; I_5S_6: 832.33 μSm^{-1}) and I_2 *i.e.*, $CaCl_2$ 50 mM 24 h (I_2S_2: 430.17 μSm^{-1}; I_2S_4: 650.67 μSm^{-1}; I_2S_5: 770.00 μSm^{-1}; I_2S_6: 853.83 μSm^{-1}) had registered high EC of seed leachate during storage.

Fig. 4

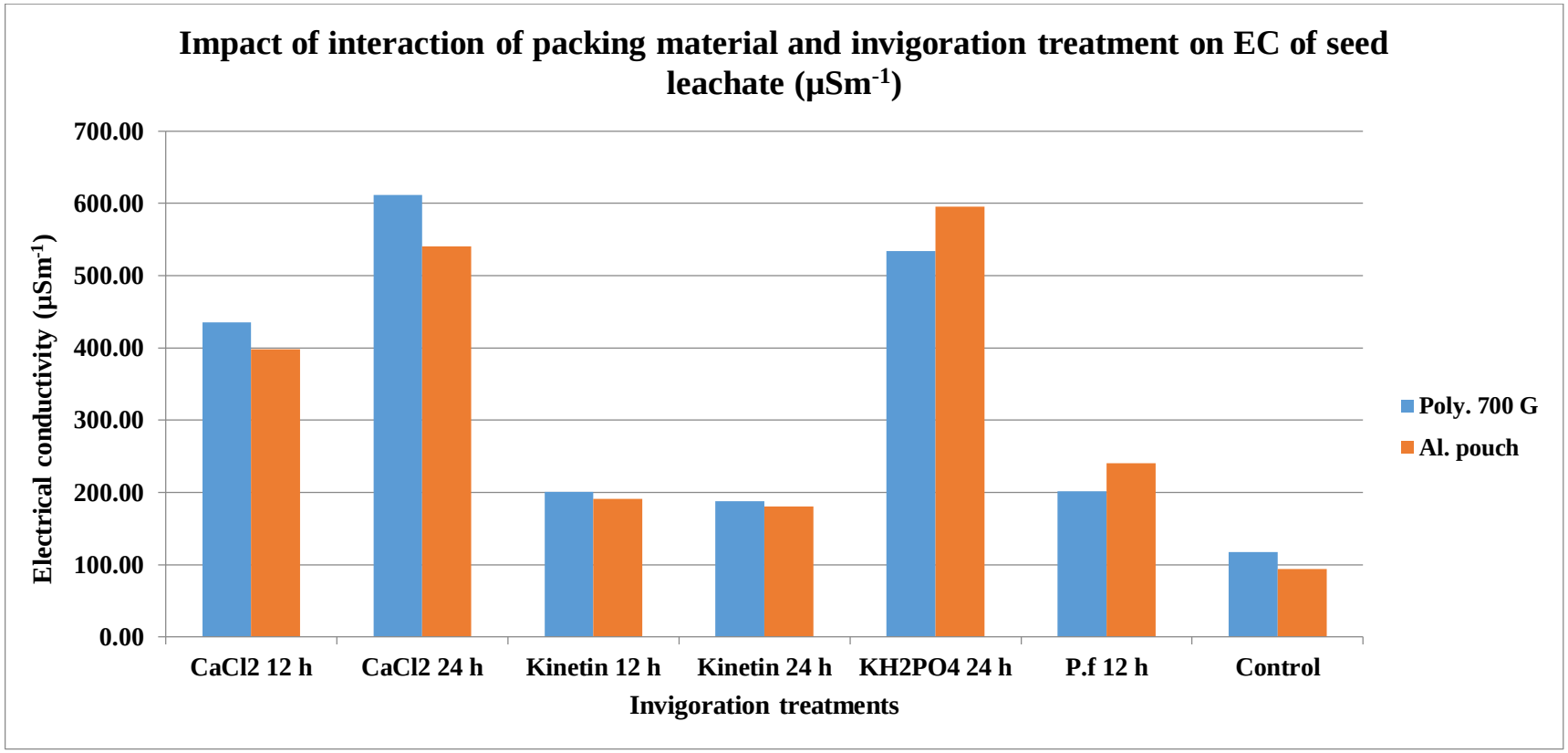

Impact of interaction of packing material and invigoration treatment on EC of seed leachate (µSm-1)
Electrical conductivity (µSm-1)
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
CaCl2 12 h
CaCl2 24 h
Kinetin 12 h
Kinetin 24 h
KH2PO4 24 h
P.f 12 h
Control
Invigoration treatments
Poly. 700 G
Al. pouch

Table 17. Impact of packing materials, invigoration treatments and storage period on electrical conductivity of seed leachate (μSm^{-1})

Storage period (S)	Packing material (P) × Invigoration (I)															
	P_1I_1	P_1I_2	P_1I_3	P_1I_4	P_1I_5	P_1I_6	P_1I_7	Mean ($P_1 \times S_n$)	P_2I_1	P_2I_2	P_2I_3	P_2I_4	P_2I_5	P_2I_6	P_2I_7	Mean ($P_2 \times S_n$)
S_1 (1 MAS)	189.33	327.00	118.60	114.33	271.67	110.33	55.00	169.47	188.67	197.67	118.33	102.00	260.67	103.67	63.00	147.71
S_2 (2 MAS)	317.33	458.67	154.00	156.67	382.33	137.33	70.87	239.60	282.67	401.67	134.00	140.67	407.67	206.33	91.67	237.81
S_3 (3 MAS)	397.33	464.67	191.33	178.00	477.67	163.33	92.33	280.67	364.33	512.00	169.00	164.33	549.33	237.67	79.33	296.57
S_4 (4 MAS)	487.33	678.67	225.00	215.67	595.00	226.33	127.33	365.05	460.00	622.67	206.67	196.33	702.00	265.67	87.33	362.95
S_5 (5 MAS)	607.00	822.33	253.33	224.33	706.33	271.33	156.33	434.43	528.67	717.67	245.67	229.00	761.00	309.00	105.67	413.81
S_6 (6 MAS)	613.00	918.67	263.00	239.33	771.00	302.33	201.33	472.67	564.67	789.00	271.67	253.33	893.67	322.33	136.33	461.57
Mean (P×I×S)	**435.22**	**611.67**	**200.88**	**188.06**	**534.00**	**201.83**	**117.20**	**326.98**	**398.17**	**540.11**	**190.89**	**180.94**	**595.72**	**240.78**	**93.89**	**320.07**

Factors	C.D. (5%)	SE(m) ±
Interaction P × S	3.04	1.09
Interaction P × I × S	8.05	2.88

P_1: Polyethylene 700 G pouches, P_2: Aluminium laminated pouches, I: Invigoration treatments and MAS: Months after storage

4.1.3.5.4.3 Packing materials × Storage period (P × S)

Irrespective of the invigouration treatments, the electrical conductivity of seed leachate (Table 17) was significantly influenced by the interaction between packing materials and storage period (P × S).

EC varied from 147.71 μSm^{-1} (P_2S_1: Al. pouches - 1 MAS) to 472.67 μSm^{-1} (P_1S_6: Poly. 700 G - 6 MAS). EC of seeds stored in both packing materials increased over the storage period.

The electrical conductivity of seeds packed in Al. pouches, P_2S_1 (147.71 μSm^{-1}) was significantly lower than that in Poly. 700 G for most of the storage period *i.e.*, at 1 MAS (P_1S_1:169.47 μSm^{-1}; P_2S_1: 147.71 μSm^{-1}) and 3 MAS (P_1S_3: 280.67 μSm^{-1}; P_2S_3: 296.57 μSm^{-1}), 5 MAS (P_1S_5: 434.43 μSm^{-1}; P_2S_5: 413.81 μSm^{-1}) and 6 MAS (P_1S_6: 472.67 μSm^{-1}; P_2S_6: 461.57 μSm^{-1}). However, it was on par with each other at 2 MAS and 4 MAS.

4.1.3.5.4.4 Packing materials × Invigouration treatments × Storage period (P × I × S)

The electrical conductivity (Table 17) was found to be significantly affected by the interaction between packing material, invigouration treatments and the storage period (P × I × S).

The EC of both treated and untreated seeds packed in Poly. 700 G and Al. pouches increased as storage period increased. It ranged from 55.00 μSm^{-1} ($P_1I_7S_1$: Poly. 700 G - Untreated control - 1 MAS) to 918.67 μSm^{-1} ($P_1I_2S_6$: Poly. 700 G - $CaCl_2$ 50 mM 12 h - 6 MAS). $P_1I_7S_1$ was found to be on par with $P_2I_7S_1$ (Poly. 700 G - Untreated control - 1 MAS: 63.00 μSm^{-1}).

The untreated seeds stored in either of the packing materials registered low EC values compared to the primed seeds as storage period increased. Among priming treatments, seeds primed with Kinetin 10 ppm for 12 h or 24 h and packed in either of the materials recorded lower EC of seed leachate compared to other the priming treatments. The seeds treated with $CaCl_2$ 50 mM for 24 h and those treated with KH_2PO_4 10^{-1} M 24 h and packed either in aluminium laminated pouches or Polyethylene 700 G pouches, registered high EC values with the progression of storage period.

Results thus pointed out that EC of seed leachate increased in both treated and untreated seeds as storage period increased. Parrish *et al*. (1978) suggested that increased leakage associated with aging might be the result of permeable membrane or due to a larger pool of electrolytes in the seed during passage of time. Liu *et al.* (1996) observed a decrease in the

germination and vigour of bean seeds subjected to different days of aging was proportional to the increase in solute leakage from seed cells, suggesting a close relationship between the deterioration of seed membranes and the loss of seed germination and vigour. This decay in the seeds membranes attributes to the loss of seed vigour. Seed storage influence viability of seed vigour and seed vigour depending on duration and conditions of storage (Panobianco *et al*., 2007). Powell *et al*. (1985) reported that increase in leakage of electrolytes from living cells from the early stages of deterioration, increased in later period with a decrease in rate of germination.

It was evident that seeds packed in Al. pouches registered significant lower EC than those packed in Polyethylene 700 G pouches as storage period increased. The result of the present study is in concurrence with that of Hemashree *et al*. (2010). It was reported that at the end of storage, the seeds packed in aluminium pouches were found to be superior in all the seed quality parameters followed by those packed in Polythene pouches. The seeds packed in aluminium pouches had recorded higher germination (68.03%), vigour parameters with lower electrical conductivity compared to seeds stored in Polyethylene 700 G pouches and other containers.

According to Saracco *et al.* (1995), the primed seeds showed an increase in tolerance to deteriorative factors that appear during storage as a consequence of advanced germinative events. However, in the present study, it was evident that the untreated seeds registered lower EC of seed leachate than all primed seeds. Seeds primed with Kinetin 10 ppm had registered lower EC estimate next to unprimed seeds, while, the highest EC was observed in seeds primed with $CaCl_2$ 50 mM for 24 h followed by those primed with KH_2PO_4 10^{-1} M for 24 h. Khan *et al*. (2011) had reported that hormonal priming could significantly lower the EC of seed leachates. They obtained lower values for electrical conductivity in seeds primed with Kinetin 10 ppm for 12 h and 24 h compared to control. The lower EC values indicated that hormonal priming did not damage the seed structure rather it allowed the better membrane repair of the seed. This pointed out that successful membrane and genetic repair and trigger of metabolic activities are promised by seed priming.

The interaction between packing material, invigouration treatment and storage period had exerted a strong influence on EC of seed leachate. Packing untreated seeds or seeds primed with kinetin 10 ppm for 12 h (I_3) or 24 h (I_4) in either aluminium laminated pouches or polyethylene 700 G pouches was found to register low EC of seed leachate during storage. Incidentally, $CaCl_2$ treated seeds in aluminium laminated pouches or Polyethylene 700 G pouches had retained viability for 4 MAS and 3 MAS respectively, while, the KH_2PO_4 primed

seeds in aluminium laminated pouches or Polyethylene 700 G pouches had retained viability for the shortest duration (2 MAS and 1 MAS respectively) compared to all other treatments.

Ferguson *et al.* (1988) and Vieira *et al.* (2001) in their studies on soybean and Pourhadian and Khajehpour (2010) in their study in wheat, observed that there was no definite correlation between reduction in germination and EC of seed leachate. Variation in EC was very slight in comparison to the decrease in seed germinability. Ferguson *et al.* (1988) questioned the use of the EC test to determine germination and seed vigour after storage, especially when seeds are stored at temperatures lower than 10ºC. In the absence of a definite correlation between EC and seed deterioration observed in the study, the present work agrees with the above. Wang *et al*. (2004) in their study in grasses had also observed that EC did not correlate with other germination indices. According to them, EC may not be a good predictor of seed vigour.

4.1.3.6 LEAKAGE OF AMINO ACID (µg leucine eqiv.ml^{-1})

The results on leakage of amino acid (µg leucine eqiv.ml^{-1}) as influenced by packing materials, invigouration treatments, the storage period and their interaction effects are presented in Tables 18, 19 and 20 and Plate 2.

4.1.3.6.1 Effect of packing material (P)

Irrespective of the invigouration treatments and the storage period, packing material exerted significant influence on the leakage of amino acid from the stored seeds.

The leakage of amino acid of seeds stored in the Polyethylene 700 G pouches (Poly. 700 G, P_1: 7.774 µg leucine eqiv.ml^{-1}) was the least and differed significantly from that in seeds packed in aluminium laminated pouches (P_2: 7.896 µg leucine eqiv.ml^{-1}) as represented in Table 18.

According to Manonmani *et al*. (2014) and Sun and Leopold (1995), as a result of seed deterioration the respiratory activities increases leading to an increase in seed leachate as well as amino acid level causing a decreased level of protein content of the seed. The results thus indicated that storing seeds in polyethylene pouches was advantageous over storing them in Al. pouches to reduce the leakage of amino acid and thereby reduce deterioration of seeds over storage.

4.1.3.6.2 Effect of invigouration treatments (I)

Irrespective of the packing materials and storage period, invigouration treatments exerted significant influence on leakage of amino acid (Table 18).

The leakage of amino acid varied between 7.456 µg leucine eqiv.ml^{-1} (I_3: Kinetin 10 ppm 12 h) and 8.207 µg leucine eqiv.ml^{-1} (I_4: Kinetin 10 ppm 12 h). The leakage of amino acid in I_3 was the least and differed significantly from all other treatments.

Next to I_4, $CaCl_2$ 50 mM 24 h (I_2: 7.695 µg leucine eqiv.ml^{-1}) registered lower leakage of amino acid. I_2 was found to be on par with I_5 (KH_2PO_4 10^{-1} M 24 h: 7.700 µg leucine eqiv.ml^{-1}) and I_7 (Untreated control: 7.775 µg leucine eqiv.ml^{-1}). Seed invigouration with I_6 (*P. fluorescens* 1×10^6 cfu ml^{-1} 12 h: 8.203 µg leucine eqiv.ml^{-1}) followed by I_4 (Kinetin 10 ppm 24 h: 8.207 µg leucine eqiv.ml^{-1}) caused high leachate of amino acid during storage.

Subjecting seeds to invigouration with Kinetin 10 ppm 12 h (I_3) was the most advantageous in reducing the leakage of amino acid during storage. Seed invigouration with $CaCl_2$ 50 mM 24 h (I_2) or KH_2PO_4 10^{-1} M 24 h (I_5) or storing them as untreated was the next best option to reduce leakage of amino acid.

Similar results were observed in cytokinin primed seeds on rice (Farooq *et al.*, 2005b) and tomato (Nawaz *et al.*, 2012). Battacharya *et al.* (2015) in soybean found that invigourated seeds showed a reduced level in leakage of amino acid. The studies conducted by Abdul Wahid *et al.* (2008) in sunflower showed that invigouration treatments gave a reduced level of the solute leakage of total soluble protein and soluble sugars and restored the seed metabolism when compared to the unprimed seeds.

4.1.3.6.3 Effect of storage period (S)

Irrespective of the packing materials and invigouration treatments, storage period exerted significant influence on leakage of amino acid (Table 19).

The leakage of amino acid ranged from 1.111 µg leucine eqiv.ml^{-1} at 1 MAS (S_1) to 1.228 µg leucine eqiv.ml^{-1} at 5 MAS (S_5).

It was evident that the leakage of amino acid from seed increased as storage period increased. This might be due to the decrease in cell membrane stability due to seed deterioration. From the studies of Rajjou *et al.* (2012), it was found that regulation of the synthesis and turnover of protein, post-translational modifications and translational activity

Table 18. Impact of packing material, invigoration treatment and interaction on leakage of amino acid of seed leachate (µg leucine eqiv.ml^{-1})

Packing Material (P)	Invigoration (I)							Mean P (Packing only)
	I_1 $CaCl_2$ 12 h	I_2 $CaCl_2$ 24 h	I_3 Kinetin 12 h	I_4 Kinetin 24 h	I_5 KH_2PO_4 24 h	I_6 *Pf* 12 h	I_7 Control	
P_1 (Poly. 700G)	7.699	7.660	7.331	8.330	7.597	8.011	7.789	**7.774**
P_2 (Al. bags)	7.920	7.729	7.581	8.085	7.802	8.395	7.761	**7.896**
Mean I (Invigoration only)	**7.810**	**7.695**	**7.456**	**8.207**	**7.700**	**8.203**	**7.775**	

Factors	C.D. (5%)	SE(m)±
Factor P	0.049	0.017
Factor I	0.092	0.033
Interaction P × I	0.129	0.046

Table 19. Impact of invigoration treatment, period of storage and interaction on leakage of amino acid of seed leachate (µg leucine eqiv.ml^{-1})

Invigoration treatments (I)	Storage period (P)			Mean I (Invigoration only)
	S_1 (1 MAS)	S_3 (3 MAS)	S_5 (5 MAS)	
I_1	7.624	7.824	7.981	**7.810**
I_2	7.536	7.707	7.841	**7.695**
I_3	7.259	7.461	7.649	**7.456**
I_4	7.789	7.936	8.897	**8.207**
I_5	7.552	7.711	7.836	**7.700**
I_6	8.041	8.227	8.341	**8.203**
I_7	7.611	7.792	7.922	**7.775**
Mean S (Storage)	**7.630**	**7.808**	**8.067**	

Factors	C.D. (5%)	SE(m)±
Factor I	0.092	0.033
Factor S	0.060	0.021
Interaction I × S	0.159	0.056

P_1: Polyethylene 700 G pouches, P_2: Aluminium laminated pouches, I: Invigoration treatments and MAS: Months after storage

reduction at the time of germination in dry seeds during storage period resulted in the deterioration and loss of vigour in stored seeds.

4.1.3.6.4 Effect of interaction

4.1.3.6.4.1 Packing materials × Invigouration treatments (P × I)

Significant difference in leakage of amino acid was observed (Table 18 and Fig. 5) due to the interaction of packing materials and invigouration treatments (P × I), irrespective of the period of storage.

The leakage of amino acid varied between 7.331 µg leucine eqiv.ml^{-1} (P_1I_3: Poly. 700 G - Kinetin 10 ppm 12 h) and 8.395 µg leucine eqiv.ml^{-1} (P_2I_6: Al. pouches - *P. fluorescens* 1 × 10^6 cfu ml^{-1} 12 h). The leakage of sugar in P_1I_3 was found to be the least and differed significantly from all other treatments.

The treatments P_2I_3 (Al. pouches - Kinetin 10 ppm 12 h: 7.581 µg leucine eqiv.ml^{-1}), P_1I_5 (Poly. 700 G - KH_2PO_4 10^{-1} M 24 h: 7.597 µg leucine eqiv.ml^{-1}), P_1I_5 (Poly. 700 G - KH_2PO_4 10^{-1} M 24 h: 7.597 µg leucine eqiv.ml^{-1}), P_1I_2 (Poly. 700 G - $CaCl_2$ 50 mM 24 h: 7.66 µg leucine eqiv.ml^{-1}), P_1I_1 (Poly. 700 G - $CaCl_2$ 50 mM 12 h: 7.699 µg leucine eqiv.ml^{-1}) and P_2I_7 (Al. pouches - Untreated control:7.761 µg leucine eqiv.ml^{-1}) were on par with each other and registered lower estimates next to P_1I_3.

Treatments P_2I_6 (Al. pouches - *P. fluorescens* 1 × 10^6 cfu ml^{-1} 12 h: 8.395 µg leucine eqiv.ml^{-1}) followed by P_1I_4 (Poly. 700 G - Kinetin 10 ppm 24 h: 8.330 µg leucine eqiv.ml^{-1}), P_2I_4 (Al. pouches - Kinetin 10 ppm 24 h: 8.085 µg leucine eqiv.ml^{-1}) and P_1I_4 (Poly. 700 G - Kinetin 10 ppm 24 h: 8.011 µg leucine eqiv.ml^{-1}) had registered significant high levels of amino acid leakage during storage.

4.1.3.6.4.2 Invigouration treatments × Storage period (I × S)

Interaction between invigouration treatments and storage period (I × S) significantly influenced leakage of amino acid, irrespective of the packing material used.

The leakage of amino acid (Table 19) ranged from 7.259 µg leucine eqiv.ml^{-1} (I_3S_1: Kinetin 10 ppm 12 h - 1 MAS) to 8.897 µg leucine eqiv.ml^{-1} (I_4S_5: Kinetin 10 ppm 24 h - 5 MAS). The leakage of amino acid in I_3S_1 was the least value and was found to be superior and significantly different from all other treatments. I_3S_1 had also registered the least values for this trait with each succeeding months of storage.

Fig. 5

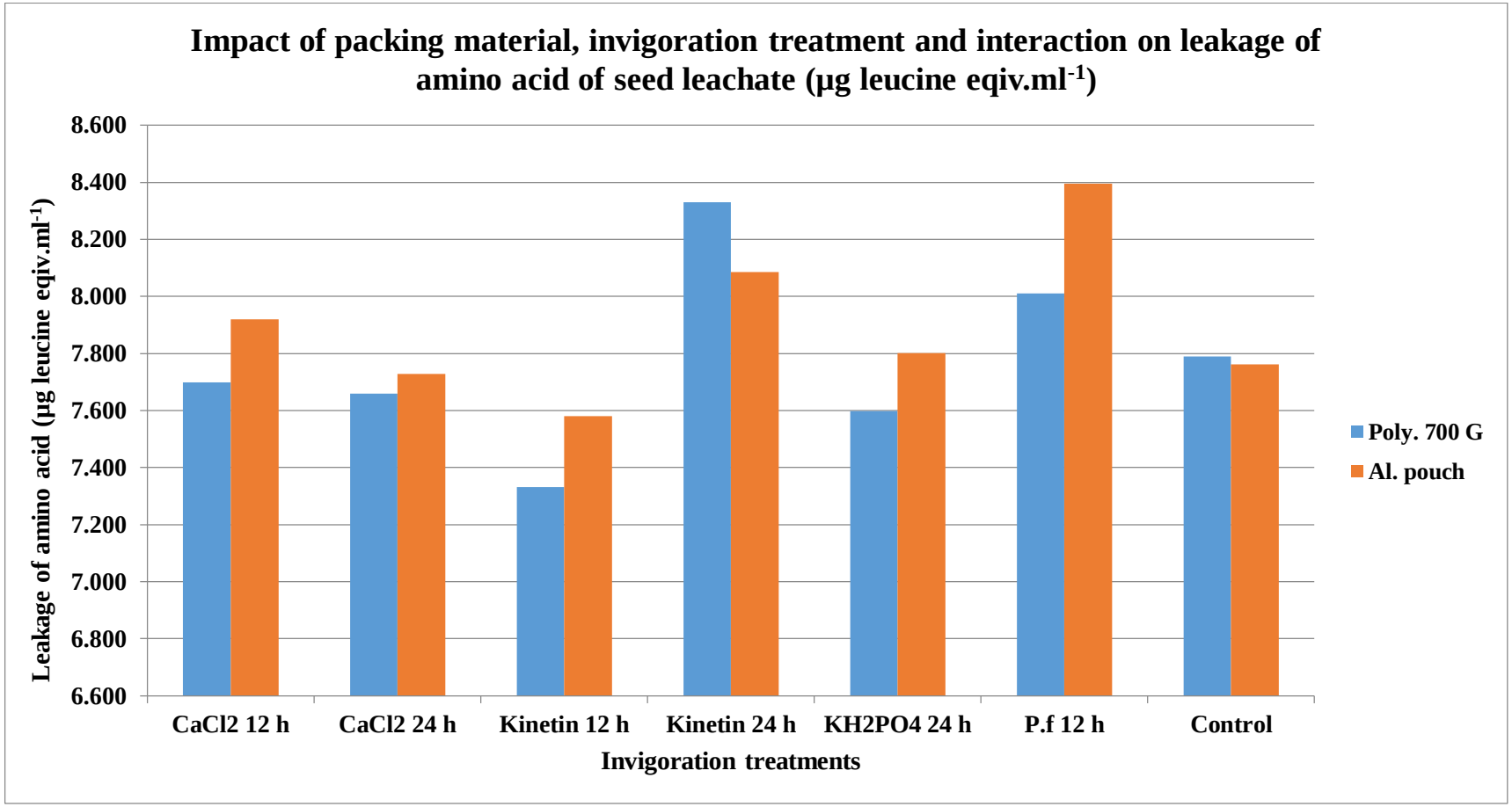

Impact of packing material, invigoration treatment and interaction on leakage of amino acid of seed leachate (µg leucine eqiv.ml-1)
Leakage of amino acid (µg leucine eqiv.ml-1)
8.600
8.400
8.200
8.000
7.800
7.600
7.400
7.200
7.000
6.800
6.600
CaCl2 12 h
CaCl2 24 h
Kinetin 12 h
Kinetin 24 h
KH2PO4 24 h
P.f 12 h
Control
Invigoration treatments
Poly. 700 G
Al. pouch

Table 20. Impact of packing materials, invigoration treatments, storage period and their interaction on leakage of amino acid (µg leucine eqiv.ml^{-1})

Storage period (S)	Packing material (P) × Invigoration (I)															
	P_1I_1	P_1I_2	P_1I_3	P_1I_4	P_1I_5	P_1I_6	P_1I_7	Mean ($P_1 \times S_n$)	P_2I_1	P_2I_2	P_2I_3	P_2I_4	P_2I_5	P_2I_6	P_2I_7	Mean ($P_2 \times S_n$)
S_1 (1 MAS)	7.522	7.502	7.146	7.659	7.479	7.869	7.622	**7.543**	7.726	7.569	7.372	7.919	7.626	8.212	7.599	**7.718**
S_3 (3 MAS)	7.729	7.689	7.336	7.796	7.609	8.019	7.809	**7.712**	7.919	7.726	7.586	8.076	7.812	8.436	7.776	**7.904**
S_5 (5 MAS)	7.846	7.789	7.512	9.536	7.702	8.146	7.936	**8.067**	8.116	7.892	7.786	8.259	7.969	8.536	7.909	**8.067**
Mean (P×I×S)	**7.699**	**7.660**	**7.331**	**8.330**	**7.597**	**8.011**	**7.789**	**7.774**	**7.920**	**7.729**	**7.581**	**8.085**	**7.802**	**8.395**	**7.761**	**7.896**

Factors	C.D. (5%)	SE(m)±
Interaction P × S	0.085	0.03
Interaction P × I × S	0.224	0.08

P_1: Polyethylene 700 G pouches, P_2: Aluminium laminated pouches, I: Invigoration treatments and MAS: Months after storage

I_2S_1 ($CaCl_2$ 50 mM 24 h - 1 MAS: 7.536 µg leucine eqiv.ml^{-1}) and I_5S_1 (KH_2PO_4 10^{-1} M 24 h - 1 MAS: 7.552 µg leucine eqiv.ml^{-1}) and I_7S_1(Untreated control - 1 MAS: 7.611 µg leucine eqiv.ml^{-1}) were on par with each other and registered low amino acid leakage next to I_3S_1.

4.1.3.6.4.3 Packing materials × Storage period (P × S)

Irrespective of the invigouration treatments, the leakage of amino acid (Table 20) was significantly influenced by the interaction between Packing materials and Storage period (P × S).

The leakage of amino acid varied from 7.543 µg leucine eqiv.ml^{-1} (P_1S_1: Poly. 700 G - 1 MAS) to 8.067 µg leucine eqiv.ml^{-1} (P_1S_5: Poly 700 G - 5 MAS and P_2S_5: Al. pouches - 5 MAS). During the initial period of storage (S_1), the leakage of amino acid of seeds packed in Poly. 700 G pouches (P_1S_1: 7.543 µg leucine eqiv.ml^{-1}) was superior to seeds stored in Al. pouches (P_2S_1: 7.718 µg leucine eqiv.ml^{-1}). But as storage period increased (in S_3 and S_5) there was no such variation between the packing materials.

4.1.3.6.4.4 Packing materials × Invigouration treatments × Storage period (P × I × S)

The leakage of amino acid (Table 20) was found to be significantly affected by the interaction between packing material, invigouration treatments and the storage period (P × I × S).

The leakage of amino acid in both treated and untreated seeds packed in Poly. 700 G and Al. pouches increased as the storage period increased. It ranged from 7.146 µg leucine eqiv.ml^{-1} ($P_1I_3S_1$: Poly. 700 G - Kinetin 10 ppm 12 h - 1 MAS) to 9.536 µg leucine eqiv.ml^{-1} ($P_1I_4S_5$: Poly. 700 G - Kinetin 10 ppm 24 h - 5 MAS). At 1 MAS, $P_1I_3S_1$ registered the least value for leakage of amino acid and differed significantly from all other treatments.

Seeds primed with Kinetin 10 ppm 24 h or seeds bioprimed with *P. fluorescens* 1×10^6 cfu ml^{-1} 12 h and stored either in Al. pouches (P_2I_4 and P_2I_6 respectively) or in Poly. 700 G pouches (P_1I_4 and P_1I_6 respectively) had registered significant high estimates of amino acid leakage, over the period of storage.

In general, the leakage of amino acid from seeds increased with increase in storage period in both treated and untreated seeds packed in Poly. 700 G as well as Al. pouches. It was advantageous to store seeds in polyethylene pouches at the start of storage. However, this

advantage was not evident in the later periods. It was clear that the leakage of amino acid could be reduced if invigourated with Kinetin 10 ppm 12 h (I_3). It was also observed that, invigourating seeds with Kinetin 10 ppm 12 h (I_3) and storing them in polyethylene pouches would be most advantageous to check amino acid leakage from seeds during storage, while, seed treatment with either *P. fluorescens* 1×10^6 cfu ml^{-1} 12 h (I_6) or Kinetin 10 ppm 24 h (I_4) and storing them in Al. pouches or polyethylene would be the most detrimental.

Ching and Schoolcraft, (1968) and Parrish and Leopold (1978) were the first to reveal that seed aging can be identified by an increased solute leakage when seeds are imbibed in water, due to membrane deterioration. Number of solutes including amino acids leaks from non-viable seeds, compared to viable seeds (Priestley, 1986). Several workers have confirmed this finding (Helmer *et al*., 1962; Matthews and Bradnock, 1968; Takayanagi and Murakami, 1968; Anderson, 1970; Hendricks and Taylorson, 1974; Verma and Ram, 1987). Abdul-Baki and Anderson (1970) and Khan (1982) confirmed that seed quality was inversely correlated to the seed leachate (for soluble sugars, amino acids, and electrolytes) and can serve as good indicator of membrane damage. Zheng (1991) reported that the quantity of amino acids detected in leachate from the seeds of *Brassica pekinensis* and *Allium tuberosum* registered a negative correlation with seed longevity over storage period. In other words, as the storage period increases, the leakage of different solutes including amino acid increases. The amino acid leachate exudation directly affects the metabolism which includes, respiration and enzymatic activities and also reduce macro molecular synthesis in the seeds.

The studies of Fu *et al*. (1988) on peanut seeds suggested that priming is able to repair DNA, RNA, protein membrane and enzymatic activities. The lower total protein content in seed leachate may be due to the repair attributed by priming over the cellular membrane and increased activities of free radical scavenging enzymes. Rudrapal and Nakamura (1988) attributed improved membrane repair in kinetin treated seeds to be the reason for low seed leachate in primed seeds of eggplant and radish in comparison to unprimed seeds. Farooq *et al.* (2005b) and tomato Nawaz *et al.* (2012) opined that the lesser EC values showed that cytokinin priming allowed better and successful membrane repair and triggered metabolic activities enhancing germination and repair in stored seeds.

4.1.3.7 LIPID PEROXIDATION (OD)

Results pointed out that the individual effects of packing material and invigouration treatment on lipid peroxidation was non-significant although the effect of storage period proved

Table 21. Impact of packing material, invigoration treatment and interaction on lipid peroxidation in seed leachate (OD)

Packing materials (P)	Invigoration (I)							Mean P (Packing only)
	I_1 $CaCl_2$ 12 h	I_2 $CaCl_2$ 24 h	I_3 Kinetin 12 h	I_4 Kinetin 24 h	I_5 KH_2PO_4 24 h	I_6 *Pf* 12 h	I_7 Control	
P_1 (Poly. 700G)	0.099	0.056	0.075	0.059	0.061	0.054	0.052	**0.065**
P_2 (Al. bags)	0.075	0.057	0.053	0.053	0.052	0.054	0.047	**0.056**
Mean I (Invigoration only)	**0.087**	**0.056**	**0.064**	**0.056**	**0.056**	**0.054**	**0.049**	

Factors	C.D. (5%)	SE(m)±
Factor P	NS	0.005
Factor I	NS	0.01
Interaction P × I	NS	0.014

Table 22. Impact of invigoration treatment, period of storage and interaction on lipid peroxidation (OD) in seed leachate

Invigoration treatments (I)	Storage period (P)			Mean I (Invigoration only)
	S_1 (1 MAS)	S_3 (3 MAS)	S_5 (5 MAS)	
I_1	0.050	0.054	0.157	**0.087**
I_2	0.041	0.061	0.067	**0.056**
I_3	0.041	0.059	0.093	**0.064**
I_4	0.049	0.051	0.067	**0.056**
I_5	0.038	0.060	0.071	**0.056**
I_6	0.043	0.048	0.070	**0.054**
I_7	0.039	0.051	0.058	**0.049**
Mean S (Storage only)	**0.043**	**0.055**	**0.083**	

Factors	C.D. (5%)	SE(m)±
Factor I	NS	0.01
Factor S	0.019	0.007
Interaction I × S	NS	0.018

P_1: Polyethylene 700 G pouches, P_2: Aluminium laminated pouches, I: Invigoration treatments and MAS: Months after storage

Table 23. Impact of packing materials, invigoration treatments, storage period and their interaction on lipid peroxidation in seed leachate (OD)

Storage period (S)	Packing material (P) × Invigoration (I)															
	P_1I_1	P_1I_2	P_1I_3	P_1I_4	P_1I_5	P_1I_6	P_1I_7	**Mean ($P_1 \times S_n$)**	P_2I_1	P_2I_2	P_2I_3	P_2I_4	P_2I_5	P_2I_6	P_2I_7	**Mean ($P_2 \times S_n$)**
S_1 (1 MAS)	0.038	0.048	0.044	0.052	0.041	0.047	0.047	**0.045**	0.062	0.035	0.038	0.046	0.035	0.039	0.031	**0.041**
S_3 (3 MAS)	0.042	0.058	0.061	0.052	0.063	0.051	0.050	**0.054**	0.065	0.063	0.057	0.050	0.056	0.046	0.052	**0.056**
S_5 (5 MAS)	0.218	0.061	0.120	0.072	0.079	0.064	0.057	**0.096**	0.097	0.073	0.065	0.061	0.064	0.076	0.058	**0.071**
Mean (P×I×S)	**0.099**	**0.056**	**0.075**	**0.059**	**0.061**	**0.054**	**0.051**	**0.075**	**0.057**	**0.053**	**0.052**	**0.052**	**0.054**	**0.047**	**0.099**	**0.056**

Factors	**C.D. (5%)**	**SE(m)±**
Interaction P × S	NS	0.009
Interaction P × I × S	NS	0.025

P_1: Polyethylene 700 G pouches, P_2: Aluminium laminated pouches, I: Invigoration treatments and MAS: Months after storage

to be significant. However, their interaction *viz.*, the interaction between packing material and invigouration (P × I), invigouration and storage (I × S), storage and packing material (P × S) and the interaction of all the three factors (P × I × S), did not significantly influence the lipid peroxidation in the stored seeds (Table 21, 22 and 23 and Plate 2).

Conversely, the rate of lipid peroxidation in the seeds during storage period or ageing, did not vary between the priming treatments (treated or untreated) it is subjected to nor did it vary with the type of packing material (Poly. 700 G pouches or Al. pouches) it was stored in.

Ageing is known to reduce seed viability in many crop species. This decrease in seed viability is partly attributed to the aging-induced lipid peroxidation, which damages membranes of the seed tissues. The antioxidant system plays a key role in scavenging excess reactive oxygen species (ROS) especially during seed desiccation (Bailly, 2004; Feng *et al.*, 2017). Ageing in soybean seeds inhibited germination and enhanced lipid peroxidation. It was also reported to inhibit the activity of peroxidase, catalase, ascorbate peroxidase, superoxide dismutase and lipoxygenase (Sung, 1995).

4.1.3.8 LEAKAGE OF SUGAR (µg glucose eqiv.ml^{-1})

The results on leakage of sugar (µg glucose eqiv.ml^{-1}) as influenced by packing materials, invigouration treatments, the storage period and their interaction effects are presented in Table 24, 25 and 26 and Plate 2.

4.1.3.8.1 Effect of packing material (P)

Irrespective of the invigouration treatments and the storage period, packing material exerted significant influence on the leakage of sugar from the stored seeds.

Seeds stored in the aluminium laminated pouches (P_2: 1.154 µg glucose eqiv.ml^{-1}) was significantly superior to Polyethylene 700 G pouches (P_1: 1.200 µg glucose eqiv.ml^{-1}) as represented in Table 24.

It was evident from the result that the tight packing provided by the aluminium laminated pouches provided a better environment for the seeds leading to reduced rate of deterioration which in turn reduced the exudation of sugar from the seeds.

Dey and Mukherjee (1986) reported that membrane damage in soybean and sunflower seeds can be assessed from the lower values of seed leachate conductivity and of leakage of

sugar. Simon (1974) has reported that there is a close relationship between membrane permeability and leachate of sugar.

4.1.3.8.2 Effect of invigouration treatments (I)

Irrespective of the packing materials and storage period, invigouration treatments (I) exerted significant influence on leakage of sugar.

The leakage of sugar (Table 24) varied between 1.133 µg glucose eqiv.ml^{-1} (I_1: $CaCl_2$ 50 mM 24 h) and 1.235 µg glucose eqiv.ml^{-1} (I_5: KH_2PO_4 10^{-1} M 24 h). The leakage of sugar in I_1 was the least and differed significantly from all other treatments.

I_2 ($CaCl_2$ 50 mM 24 h: 1.146 µg glucose eqiv.ml^{-1}) followed by I_3 (Kinetin 10 ppm 12 h: 1.171 µg glucose eqiv.ml^{-1}) registered the lower values next to I_1, each being significantly different from each other. Treatment I_5 (KH_2PO_4 10^{-1} M 24 h) registered the highest leakage of sugars followed by I_6 (*P. fluorescens* 1×10^6 cfu ml^{-1} 12 h).

Afzal *et al.* (2009) reported that a significant increase in α-amylase activity was recorded in all haloprimed seeds and maximum response was recorded in seeds primed with $CaCl_2$ 50 mM 24 h. Priming treatments significantly affected total and reducing sugars of the seed leachate. Reducing sugars were increased in seeds which were exposed to halopriming with $CaCl_2$ 50 mM 24 h. The decreased leakage of solute in $CaCl_2$ treatment compared to other priming treatments and control may be because of better membrane repair during hydration as it was already proved by previous works that the Ca^{2+} has a positive influence on seed membranes (Shannon and Francois, 1977). The better performance of osmotic priming has been explained by Ruan (2000a). The results pointed that osmotic priming regulates the entry of water into seed and also provide some nutrients to seed.

Khan *et al.* (2011) recorded that the total soluble sugars vary with that of treatments. They reported that seeds primed with kinetin showed the least value for leachate of sugar compared to other treatments.

4.1.3.8.3 Effect of storage period (S)

Irrespective of the packing materials and invigouration treatments, storage period exerted significant influence on leakage of sugar.

The leakage of sugar (Table 25) increased over the storage period irrespective of packing materials and invigouration treatments. It ranged from 1.111 µg glucose eqiv.ml^{-1} at 1 MAS (S_1) to 1.228 µg glucose eqiv.ml^{-1} at 5 MAS (S_5).

Table 24. Impact of packing material, invigoration treatment and their interaction on Leakage of sugar of seed leachate (µg glucose eqiv.ml^{-1})

Packing materials (P)	Invigoration (I)							Mean P (Packing only)
	I_1 $CaCl_2$ 12 h	I_2 $CaCl_2$ 24 h	I_3 Kinetin 12 h	I_4 Kinetin 24 h	I_5 KH_2PO_4 24 h	I_6 *Pf* 12 h	I_7 Control	
P_1 (Poly. 700G)	1.155	1.157	1.185	1.195	1.268	1.225	1.214	**1.200**
P_2 (Al. bags)	1.112	1.135	1.157	1.155	1.202	1.182	1.135	**1.154**
Mean I (Invigoration only)	**1.133**	**1.146**	**1.171**	**1.175**	**1.235**	**1.203**	**1.174**	

Factors	C.D. (5%)	SE(m)±
Factor P	0.001	0.001
Factor I	0.003	0.001
Interaction P × I	0.004	0.001

Table 25. Impact of invigoration treatment, period of storage and their interaction on Leakage of sugar of seed leachate (µg glucose eqiv.ml^{-1})

Invigoration treatments (I)	Storage period (P)			Mean I (Invigoration only)
	S_1 (1 MAS)	S_3 (3 MAS)	S_5 (5 MAS)	
I_1	1.055	1.153	1.192	**1.133**
I_2	1.096	1.160	1.184	**1.146**
I_3	1.104	1.199	1.210	**1.171**
I_4	1.144	1.160	1.222	**1.175**
I_5	1.167	1.243	1.296	**1.235**
I_6	1.139	1.219	1.253	**1.203**
I_7	1.071	1.214	1.238	**1.174**
Mean S (Storage)	**1.111**	**1.193**	**1.228**	

Factors	C.D. (5%)	SE(m)±
Factor I	0.003	0.001
Factor S	0.002	0.001
Interaction I × S	0.005	0.002

P_1: Polyethylene 700 G pouches, P_2: Aluminium laminated pouches, I: Invigoration treatments and MAS: Months after storage

It was evident that the sugar in seed leachate increased with the increase in duration of storage. This may be attributed to the deterioration of seeds as the storage period prolong. During the storage period, temperature and moisture content increases leading to an increased rate of lipid peroxidation and other biochemical changes. This in turn results in high seed leachate and the loss of seed quality parameters of the stored seed (Rajasree and Jirali, 2017).

4.1.3.8.4 Effect of interaction

4.1.3.8.4.1 Packing materials × Invigouration treatments (P × I)

Significant difference in leakage of sugar was observed (Table 24 and Fig. 6) due to the interaction of packing materials × invigouration treatments (P × I), irrespective of the period of storage.

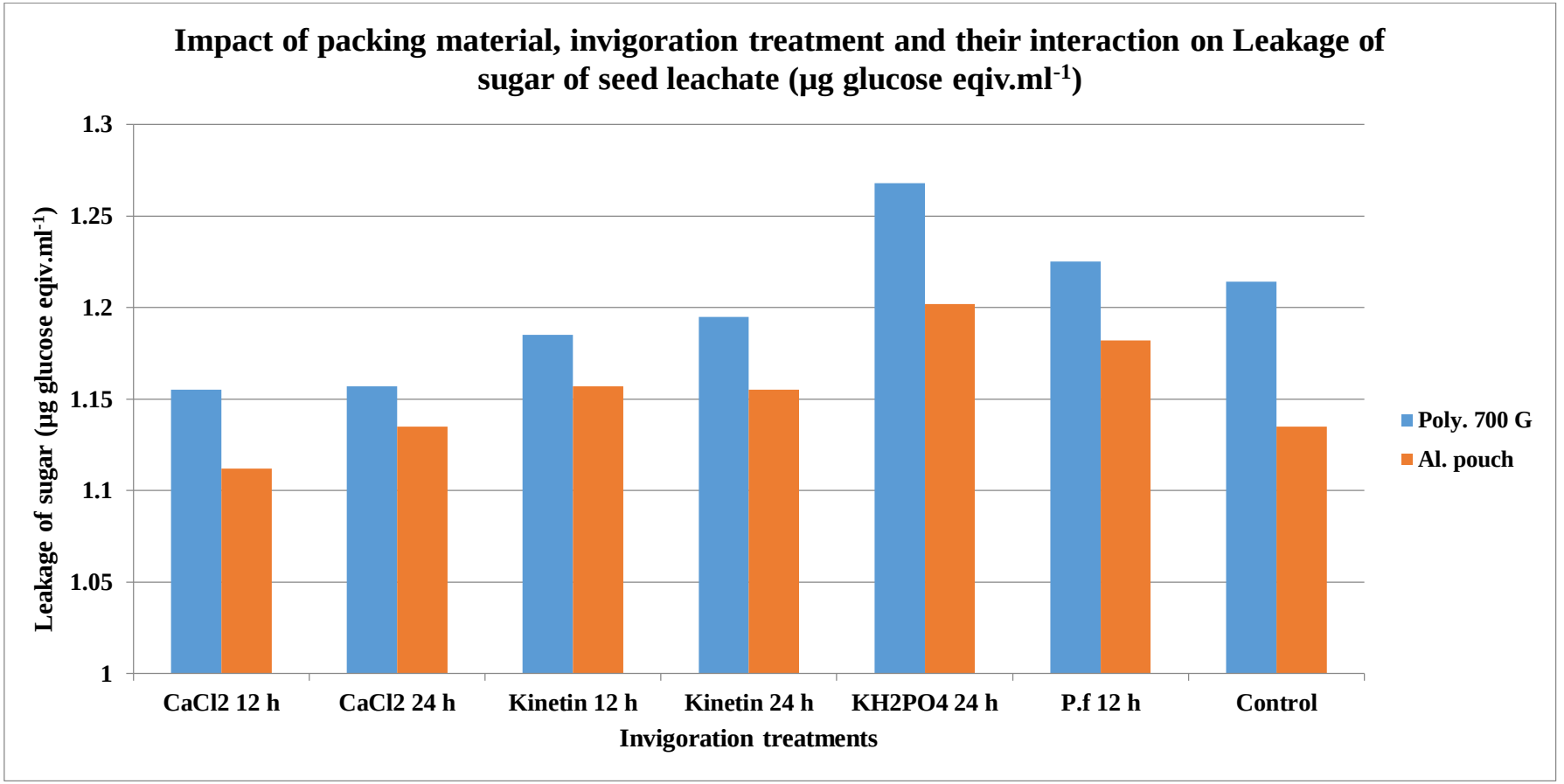
Irrespective of the period of storage, the leakage of sugar (Table 24) varied between 1.112 µg glucose eqiv.ml^{-1} in P_2I_1 (Al. pouches - $CaCl_2$ 50 mM 12 h) and 1.268 µg glucose eqiv.ml^{-1} in P_1I_5 (Poly. 700 G - KH_2PO_4 10^{-1} M 24 h). P_2I_1 was found to differ significantly from all other treatments and the leakage of sugar was found to be the least.

Treatments P_2I_2 (Al. pouches - $CaCl_2$ 50 mM 12 h: 1.135 µg glucose eqiv.ml^{-1}) and P_2I_7 (Al. pouches - Untreated control: 1.135 µg glucose eqiv.ml^{-1}) were on par with each other and also registered lower estimates for leakage of sugar next to P_2I_1.

P_1I_5 (Poly. 700 G - KH_2PO_4 10^{-1} M 24 h: 1.202 µg glucose eqiv.ml^{-1}), P_1I_6 (Poly. 700 G - *P. fluorescens* 1×10^6 cfu ml^{-1} 12 h: 1.225 µg glucose eqiv.ml^{-1}), P_1I_7 (Poly. 700 G - Untreated control: 1.214 µg glucose eqiv.ml^{-1}) and P_2I_5 (Al. pouches - KH_2PO_4 10^{-1} M 24 h: 1.202 µg glucose eqiv.ml^{-1}), registered high values for this trait and were significantly different from each other as well as from other treatments.

Results pointed out that storing seeds in aluminium laminated pouches after invigouration with $CaCl_2$ 50 mM 12 h (I_1) was most advantageous in curbing leachate of sugars, followed by storing seeds either as untreated (I_7) or after invigouration with Kinetin (I_3) in aluminium laminated pouches. It would be detrimental to treat seeds with KH_2PO_4 (I_5) and store them in either containers. A similar negative impact was also observed on storing untreated seeds in Al. pouches.

Fig. 6


Impact of packing material, invigoration treatment and their interaction on Leakage of sugar of seed leachate (µg glucose eqiv.ml-1)
Leakage of sugar (µg glucose eqiv.ml-1)
1.3
1.25
1.2
1.15
1.1
1.05
1
CaCl2 12 h
CaCl2 24 h
Kinetin 12 h
Kinetin 24 h
KH2PO4 24 h
P.f 12 h
Control
Invigoration treatments
Poly. 700 G
Al. pouch

4.1.3.8.4.2 Invigouration treatments × Storage period (I × S)

Interaction between invigouration treatments and storage period (I × S) significantly influenced leakage of sugar, irrespective of the packing material used.

The leachate of sugar increased as storage period advanced in both invigourated and untreated seeds. Irrespective of the packing material, leakage of sugar (Table 25) ranged from 1.055 µg glucose eqiv.ml^{-1} in I_1S_1 ($CaCl_2$ 50 mM 12 h - 1 MAS) to 1.296 µg glucose eqiv.ml^{-1} in I_5S_5 (KH_2PO_4 10^{-1} M 24 h - 5 MAS). The treatment I_1S_1 registered the least leachate of sugar and differed significantly from all other

Seeds treated with $CaCl_2$ 50 mM 12 h (I_2S_1: 1.096 µg glucose eqiv.ml^{-1}, I_2S_3: 1.184 µg glucose eqiv.ml^{-1} and I_2S_5:1.184 µg glucose eqiv.ml^{-1}), Kinetin 10 ppm 12 h (I_3S_1: 1.104 µg glucose eqiv.ml^{-1}, I_3S_3: 1.199 µg glucose eqiv.ml^{-1} and I_3S_5: 1.210 µg glucose eqiv.ml^{-1}) along with untreated seeds (I_7S_1: 1.071 µg glucose eqiv.ml^{-1}, I_7S_3: 1.214 µg glucose eqiv.ml^{-1}, I_7S_5: 1.238 µg glucose eqiv.ml^{-1}) registered low estimates with progression in storage period *i.e.*, at 3 MAS and 5 MAS.

4.1.3.8.4.3 Packing materials × Storage period (P × S)

Irrespective of the invigouration treatments, the leakage of sugar (Table 26) was significantly influenced by the interaction between Packing materials and Storage period (P × S).

Leakage of sugar (Table 25) increased with period of storage and varied from 1.095 µg glucose eqiv.ml^{-1} (P_1S_1: Poly. 700 G - 1 MAS) to 1.259 µg glucose eqiv.ml^{-1} (P_1S_5: Poly. 700 G - 5 MAS).

The sugar in seed leachate of seeds packed in Al. pouches tended to be lower and significantly superior to those packed in poly 700 G pouches with progression in storage period.

4.1.3.8.4.4 Packing materials × Invigouration treatments × Storage period (P × I × S)

The leakage of amino acid (Table 26) was found to be significantly affected by the interaction between packing material, invigouration treatments and the storage period (P × I × S).

The leakage of sugar of both treated and untreated seeds packed in Poly 700 G and Al. pouches increased as the storage period increased. It ranged from 1.026 µg glucose eqiv.ml^{-1}

Table 26. Impact of packing materials, invigoration treatments, storage period and their interaction on Leakage of sugar of seed leachate (µg glucose eqiv.ml^{-1})

Storage period (S)	Packing material (P) × Invigoration (I)															
	P_1I_1	P_1I_2	P_1I_3	P_1I_4	P_1I_5	P_1I_6	P_1I_7	Mean ($P_1 \times S_n$)	P_2I_1	P_2I_2	P_2I_3	P_2I_4	P_2I_5	P_2I_6	P_2I_7	Mean ($P_2 \times S_n$)
S_1 (1 MAS)	1.026	1.075	1.099	1.154	1.217	1.201	1.112	**1.126**	1.083	1.116	1.109	1.134	1.117	1.076	1.029	**1.095**
S_3 (3 MAS)	1.215	1.191	1.217	1.169	1.242	1.223	1.245	**1.215**	1.092	1.128	1.18	1.15	1.244	1.214	1.183	**1.170**
S_5 (5 MAS)	1.223	1.206	1.238	1.263	1.345	1.25	1.285	**1.259**	1.161	1.161	1.181	1.181	1.246	1.256	1.192	**1.197**
Mean (P×I×S)	**1.155**	**1.157**	**1.185**	**1.195**	**1.268**	**1.225**	**1.214**	**1.200**	**1.112**	**1.135**	**1.157**	**1.155**	**1.202**	**1.182**	**1.135**	**1.154**

Factors	**C.D. (5%)**	**SE(m)±**
Interaction P × I	0.003	0.001
Interaction P × I × S	0.007	0.002

P_1: Polyethylene 700 G pouches, P_2: Aluminium laminated pouches, I: Invigoration treatments and MAS: Months after storage

($P_1I_1S_1$: Poly. 700 G - $CaCl_2$ 50 mM 12 h - 1 MAS) to 1.345 µg glucose eqiv.ml^{-1} ($P_1I_5S_5$: Poly. 700 G - KH_2PO_4 10^{-1} M 24 h - 5 MAS). The leakage of sugar of $P_1I_1S_1$ was found to be on par with $P_2I_7S_1$ (Al. pouches - Untreated control - 1 MAS: 1.029 µg glucose eqiv.ml^{-1}). P_2I_7 had also registered low estimates for this trait at S5 ($P_2I_7S_1$: 1.192 µg glucose eqiv.ml^{-1})

Seeds primed with $CaCl_2$ 50 mM 12 h (I_1) and stored in Al. pouches consistently registered low values of sugar in seed leachate ($P_2I_1S_1$: 1.083 µg glucose eqiv.ml^{-1}, $P_2I_1S_3$:1.092 µg glucose eqiv.ml^{-1}, $P_2I_1S_5$: 1.161 µg glucose eqiv.ml^{-1}) with progression in storage. However, in case of seeds treated with KH_2PO_4 10^{-1} M 24 h (I_5) and stored in Poly 700 G pouches ($P_1I_5S_1$: 1.217 µg glucose eqiv.ml^{-1}, $P_1I_5S_3$: 1.242 µg glucose eqiv.ml^{-1}, $P_1I_5S_3$: 1.345 µg glucose eqiv.ml^{-1}), the sugar in seed leachate was consistently the highest at the end of each storage month.

Hence, it was evident that storing seeds in Al. pouches after priming with $CaCl_2$ 50 mM for 12 h (I_1) or 24 h (I_2) is most advantageous in reducing leachate of sugar during storage, while it was highly detrimental to invigourate seeds with KH_2PO_4 10^{-1} M 24 h (I_5) or bioprime seeds or store untreated seeds in Poly. 700 G pouches.

As in the present study, Basra *et al*. (1989), Thanki *et al*. (1993), Thasni (2003), Nagarajan *et al*. (2005) and Pandita *et al*. (2007), found that not all priming treatments exhibited positive effect. A significant increase in DNA content and enzymatic activities and reduction in seed leachates content was observed only in a few priming treatments. The beneficial effect of priming treatments in delaying ageing process and viability maintenance are evident from the studies on pepper seeds (Georghiou *et al*., 1987; Thanos *et al*., 1989) and onion seeds (Dearman *et al*., 1986) using various osmo-conditioning solutions. However, contrary results were also obtained in tomato seeds (Argerich *et al.,* 1989) and leek seeds (Clarke and James, 1991), in which the priming treatment brought increased deterioration during storage, especially under adverse storage conditions. Bhattacharya *et al.,* (2015), had also observed that the germination in primed seeds of sesame was low compared to untreated control even though the former had registered low leakage of electrolytes, sugars and amino acid than in unprimed control.

To summarize, the results of storage studies revealed that irrespective of the packaging material, germination and vigour indices I and II in both treated and untreated seeds, decreased progressively over the storage period. However, there was an increase in electrical conductivity of seed leachate, leachate of sugar and amino acid towards the end of storage period.

Storing seeds either in Poly 700 G pouches (P_1) or Al. pouches (P_2) did not influence seed viability and longevity, moisture content or rate of lipid peroxidation of packed seeds. However, storing seeds in aluminium laminated pouches was advantageous in improving vigour of seedlings (VI-II), lowering the EC of seed leachate and leakage of sugars from seeds during storage.

The various seed priming treatments differed in their effect on germination, vigour and other quality parameters studied. Considering the influence of invigouration treatment alone, for most evaluated germination parameters, priming seeds with kinetin 10 ppm either for 12 h (I_3) or 24 h (I_4) or storing them untreated (I_7) was found to be the best. Generally, the performance of KH_2PO_4 10^{-1} M 24 h primed seeds was poor for most of the parameters, possibly due to low osmotic potential or long priming period.

Existence of a strong influence of the interaction between packing material, invigouration treatment and storage period on seed quality and longevity of packed seeds was discerned. Under ambient storage environment, it would be best to pack seeds in aluminium pouches after invigouration with *P. fluorescens* 1×10^6 cfu ml^{-1} for 12 h (P_2I_6), as this was found to prolong seed longevity the farthest *i.e.*, until five months after storage (5 MAS). However, the bioprimed seeds if stored in polyethylene 700 G pouches (P_1I_6) had retained viability above the minimum standards of seed certification (MSCS) for 4 MAS only. Similar to P_1I_6, storing untreated seeds in polyethylene (P_1I_7) or seeds primed with $CaCl_2$ 50 mM for 24 h in polyethylene 700 G pouches (P_1I_2) retained viability above MSCS for 4 MAS. However, storing them in Al. pouches (P_2I_7 and P_2I_2) was less effective.

In addition, the interaction between packing material, invigouration treatment and storage period had exerted a strong influence on EC of seed leachate, leakage of amino acid and sugars from the stored seeds. Packing untreated seeds or seeds primed with kinetin 10 ppm for 12 h (I_3) or 24 h (I_4) in either aluminium laminated pouches or Polyethylene 700 G pouches was found to register low EC of seed leachate and low leakage of amino acid during storage. Incidentally, kinetin treated seeds had retained viability for 3 months only, whereas, $CaCl_2$ 50 mM 24 h treated seeds in aluminium laminated pouches or Polyethylene 700 G pouches had retained viability for 4 MAS and 3 MAS respectively, in spite of these registering the highest estimate for EC of seed leachate.

Hence, no definite correlation between germination and vigour, and deteriorative events like EC of seeds leachate, leakage of amino acid and sugars could be delineated in the present

study indicating that an increase in EC of seed leachate, leakage of sugars and amino acids cannot be used as indicators of seed deterioration. This is contradictory to the findings of Saracco *et al.* (1995). According to them the primed seeds showed an increase in tolerance to deteriorative factors that appear during storage as a consequence of advanced germinative events. Ferguson (1988) and Vieira *et al.* (2001) however, in their studies in soybean observed that there was no definite correlation between reduction in germination and EC of seed leachate. They found that variation in EC was very slight in comparison to the decrease in seed germinability. Ferguson (1988) questioned the use of the EC test to determine germination and seed vigour after storage especially when seeds are stored at temperatures lower than 10ºC. In the absence of a definite correlation between EC, leakage of sugars and amino acids and seed deterioration in terms of germination and vigour observed in the study, the present work agrees with this thought.

4.2 Experiment II: Field performance of primed seeds

4.2.1 Analysis of variance

Results indicated existence of wide variability in the field performance of primed seeds owing to the influence of time of invigouration, invigouration treatments and their interaction.

4.2.2.1 DAYS TO FIRST FLOWERING (FEMALE FLOWER)

The results on days to first flowering (female flower) as influenced by time of invigouration, invigouration treatments and their interaction (Table 27, Fig. 7 and Plate 3).

4.2.2.1.1 Effect of time of invigouration (T)

Irrespective of the invigouration treatments, the number of days required for the emergence of first female flower in pre-storage treatment (T_2: 97.95 days) and pre-sowing treatment (T_1: 97.43 days) did not vary significantly from each other.

4.2.2.1.2 Effect of invigouration treatments (I)

Irrespective of the time of invigouration, invigouration treatments exerted significant influence on female flowering of ash gourd.

Plate 3: Growth stages of ash gourd

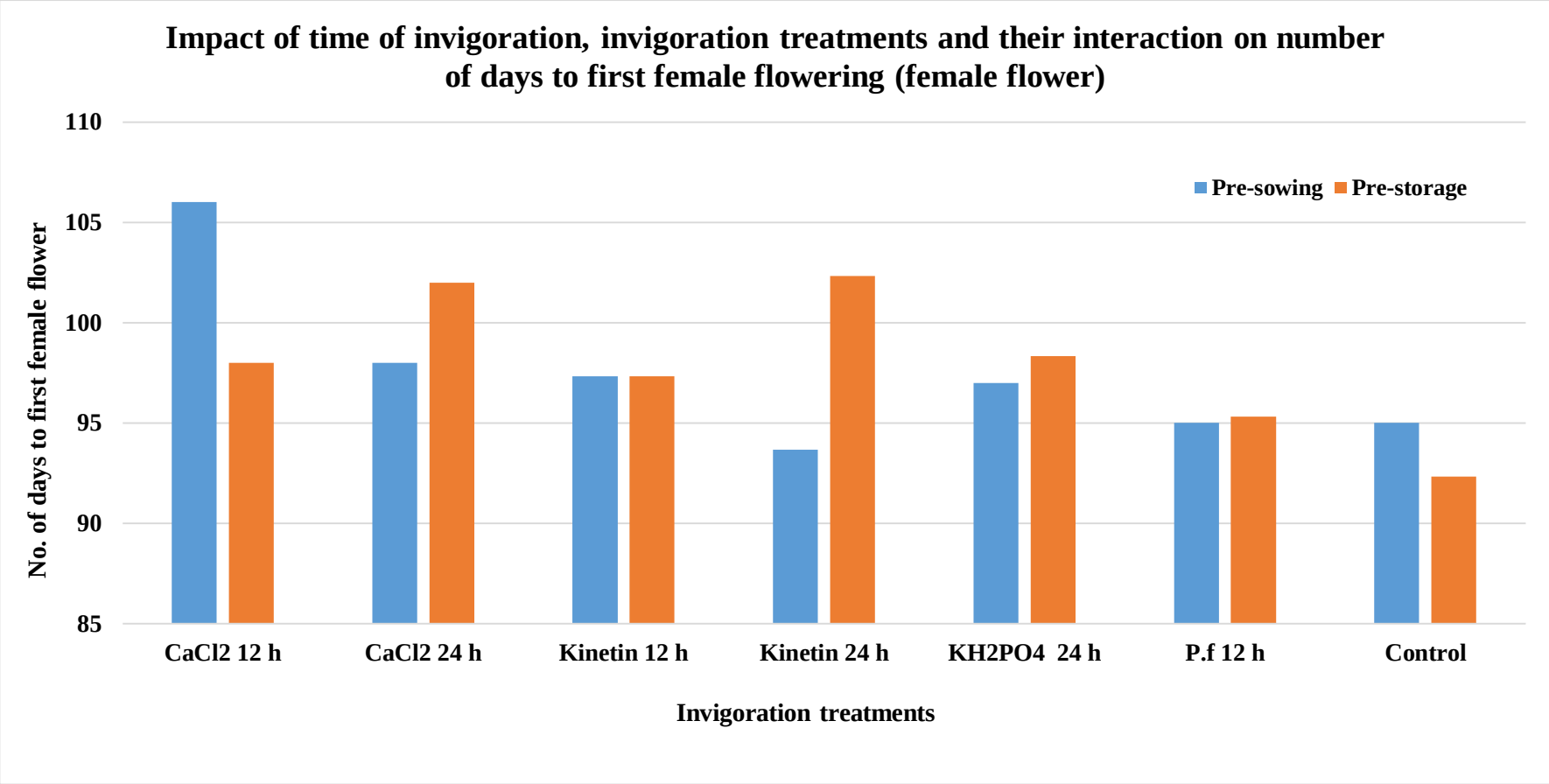
Table 27. Impact of time of invigoration, invigoration treatments and their interaction on days to first flowering (female flower).

Time of invigoration (T)	Invigoration (I)							Mean T (Time of invigoration only)
	I_1 $CaCl_2$ 12 h	**I_2 $CaCl_2$ 24 h**	**I_3 Kinetin 12 h**	**I_4 Kinetin 24 h**	**I_5 KH_2PO_4 24 h**	**I_6 *Pf* 12 h**	**I_7 Control**	
T_1 (Pre-sowing)	106.00	98.00	97.33	93.67	97.00	95.00	95.00	**97.43**
T_2 (Pre-storage)	98.00	102.00	97.33	102.33	98.33	95.33	92.33	**97.95**
Mean I (Invigoration only)	**102.00**	**100.00**	**97.33**	**98.00**	**97.67**	**95.17**	**93.67**	

Factors	**C.D. (5%)**	**SE(m)±**
Factor T	NS	0.43
Factor I	2.34	0.80
Interaction T × I	3.31	1.14

T_1: Pre-sowing, T_2: Pre-storage and I: Invigoration treatments

Fig. 7


Impact of time of invigoration, invigoration treatments and their interaction on number of days to first female flowering (female flower)
110
105
100
95
90
85
No. of days to first female flower
Pre-sowing
Pre-storage
CaCl2 12 h
CaCl2 24 h
Kinetin 12 h
Kinetin 24 h
KH2PO4 24 h
P.f 12 h
Control
Invigoration treatments

The number of days required for the emergence of first female flower varied from 93.67 (I_7: Untreated control) to 102 days (I_1: $CaCl_2$ 50 mM 12 h). I_7 (Untreated control) recorded the least value and was found to be on par with I_6 (*P. fluorescens* 1×10^6 cfu ml^{-1} 12 h: 95.17 days).

I_3 (Kinetin 10 ppm 24 h: 102.33 days) was found to be on par with I_6, while, flowering was delayed the farthest in I_1 ($CaCl_2$ 50 mM 12 h: 102.00 days) and I_2 ($CaCl_2$ 50 mM 24 h: 100.00 days).

4.2.2.1.3 Effect of interaction

Time of invigouration × Invigouration treatments (T × I)

Significant difference in female flowering was observed due to the interaction of time of invigouration × invigouration treatments (T × I).

The number of days required for the emergence of first female flower of pre-sowing treatment as well as pre-storage treatments ranged from 92.33 (T_2I_7: Pre-storage treatment - Untreated control) to 106.00 days (T_1I_1: Pre-sowing treatment - $CaCl_2$ 50 mM 12 h). The treatment T_2I_7 (Pre-storage treatment - Untreated control) recorded the least number of days for the emergence of first female flower and was found to be significantly earlier to all other treatments.

T_1I_4 (Pre-sowing treatment - Kinetin 10 ppm 24 h: 93.67 days) induced early emergence of female flowers next to T_2I_7 and was also on par with it. T_1I_4 however, was found to be on par with T_1I_6 (Pre-sowing treatment - *P. fluorescens* 1×10^6 cfu ml^{-1} 12 h: 95.00 days) and T_1I_7 (Pre-sowing treatment - Untreated control: 95.00 days).

T_1I_1 (Pre-sowing treatment - $CaCl_2$ 50 mM 12 h: 106.00 days) delayed flowering the most followed by T_2I_4 (Pre-storage treatment - Kinetin 10 ppm 24 h: 102.33 days) and T_2I_2 (Pre-storage treatment - $CaCl_2$ 50 mM 24 h: 102.00 days).

Results thus pointed out that sowing unprimed seeds would induce early flowering (female flower) or conversely priming delays production of female flowers in ash gourd. The delay was farthest when seeds were primed with $CaCl_2$ 50 mM for 12 h. However, earlier studies had indicated that priming was effective in inducing earlier flowering (Harris *et al*., 1999; Narendrabhai, 2018; Venkateshbabu, 2018). Chavan *et al.* (2014) and Rehman *et al.* (2014b) had reported that seeds osmo-primed with $CaCl_2$ exhibited a significant effect on flowering. It induced early flowering compared to other priming treatments due to their effect

in the fast emergence of the seeds at the beginning. From the studies of Bhargava *et al.* (2015) and Negi (2009), it was evident that, the earliness of flowering due to $CaCl_2$ priming was due to the easy uptake of nutrients and simultaneously transport of growth promoting substances to the axillary buds resulting in breakage of apical dominance. Ultimately, priming had resulted in better sink for faster mobilization of photosynthates and early transformation of plant parts from vegetative to reproductive phase. Similar results were also obtained by Barlow and Haigh (1986) on priming of tomato seeds.

4.2.2.2 FRUIT LENGTH (cm)

The results on fruit length (cm) in ash gourd as influenced by time of invigouration, invigouration treatments and their interaction effects are presented in Table 28 and Fig. 8.

4.2.2.2.1 Effect of time of invigouration (T)

Irrespective of the invigouration treatments, fruit length of ash gourds harvested from the pre-sowing treatment (T_1: 23.82 cm) was significantly superior over pre-storage treatment (T_2: 23.07 cm).

4.2.2.2.2 Effect of invigouration treatments (I)

Irrespective of the time of invigouration, invigouration treatments exerted significant influence on fruit length.

Fruit length of ash gourds varied between 15.93 cm (I_1: $CaCl_2$ 50 mM 12 h) and 29.21 cm (I_4: Kinetin 10 ppm 24 h). The length of fruit in I_4 (Kinetin 10 ppm 24 h) was the highest and found to be significantly superior to all other treatments.

Fruit length in I_2 ($CaCl_2$ 50 mM 24 h: 25.85 cm) was next highest in length to I_4 and significantly superior to I_5 (KH_2PO_4 10^{-1} M 24 h: 24.78 cm).

I_1 ($CaCl_2$ 50 mM 12 h: 15.93 cm) produced short fruits followed by treatments I_3 (Kinetin 10 ppm 12 h: 22.55 cm), I_7 (Untreated control: 22.72 cm) and I_6 (*Pseudomonas fluorescens* 1 $\times 10^6$ cfu. ml^{-1} 12 h: 23.06 cm) were found to be on par with each other.

4.2.2.2.3 Effect of interaction

Time of invigouration × Invigouration treatments (T × I)

No significant difference in fruit length was observed due to the interaction of time of seed treatment and invigouration treatments (T × I).

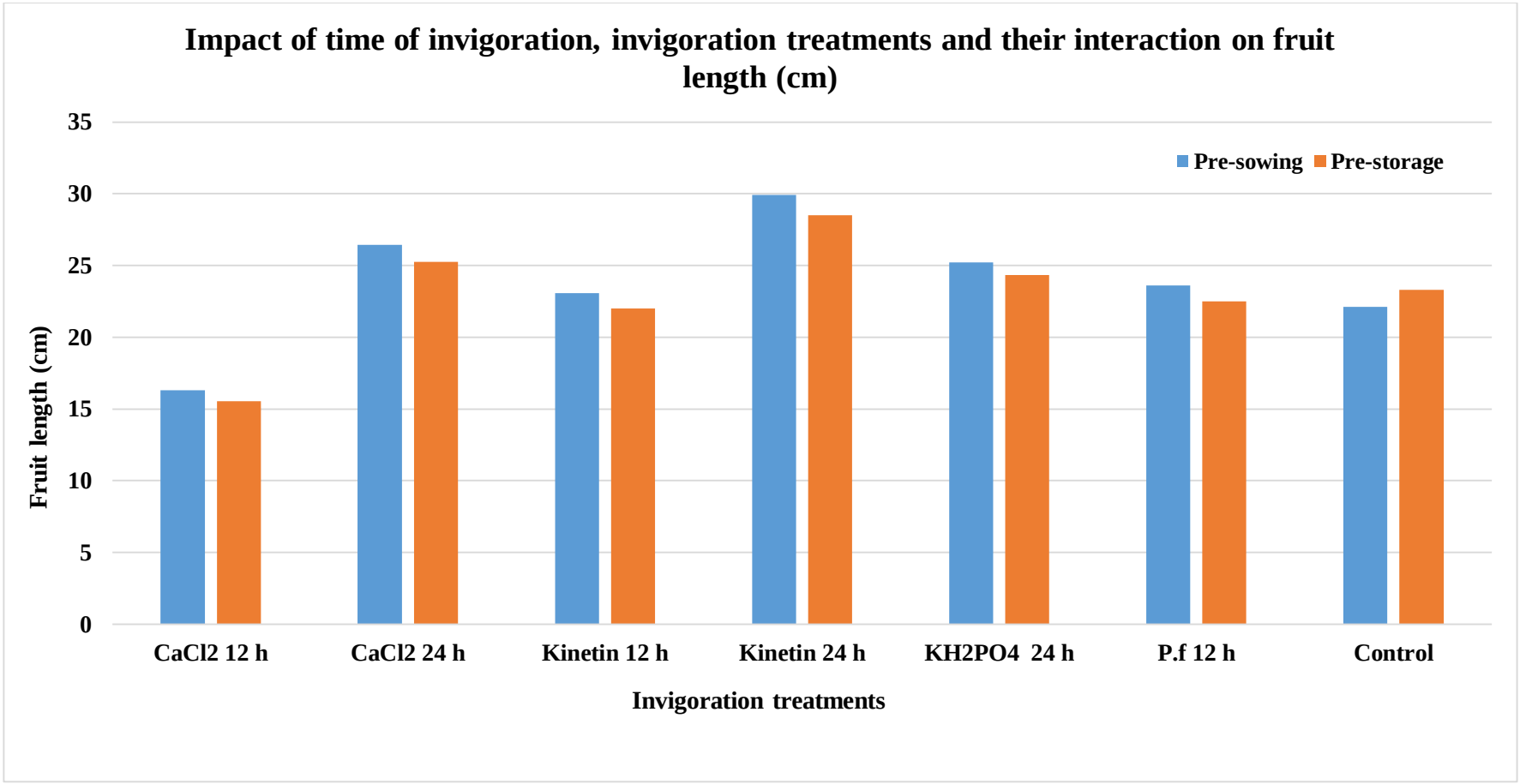
Table 28. Impact of time of invigoration, invigoration treatments and their interaction on fruit length (cm).

Time of invigoration (T)	Invigoration (I)							Mean T (Time of invigoration only)
	I_1 $CaCl_2$ 12 h	**I_2 $CaCl_2$ 24 h**	**I_3 Kinetin 12 h**	**I_4 Kinetin 24 h**	**I_5 KH_2PO_4 24 h**	**I_6 *Pf* 12 h**	**I_7 Control**	
T_1 (Pre-sowing)	16.30	26.46	23.08	29.91	25.23	23.62	22.12	**23.82**
T_2 (Pre-storage)	15.55	25.25	22.02	28.50	24.33	22.50	23.32	**23.07**
Mean I (Invigoration only)	**15.93**	**25.85**	**22.55**	**29.21**	**24.78**	**23.06**	**22.72**	

Factors	**C.D. (5%)**	**SE(m)±**
Factor T	0.45	0.15
Factor I	0.84	0.29
Interaction T × I	NS	0.41

T_1: Pre-sowing, T_2: Pre-storage and I: Invigoration treatments

Fig. 8


Impact of time of invigoration, invigoration treatments and their interaction on fruit length (cm)
Pre-sowing
Pre-storage
Fruit length (cm)
35
30
25
20
15
10
5
0
CaCl2 12 h
CaCl2 24 h
Kinetin 12 h
Kinetin 24 h
KH2PO4 24 h
P.f 12 h
Control
Invigoration treatments

Although there was no evidence of interaction between the time of invigouration and the treatment, considering the individual effects of these factors, results indicated that pre-sowing priming of seeds is more effective in increasing length of fruit in ash gourd than pre-storage priming. It was also evident that seed treatment with kinetin 10 ppm 24 h was most beneficial for obtaining long fruits.

The favourable impact of priming has been associated with various, cellular, molecular and biochemical events including synthesis of DNA and proteins (Bewley and Black, 1994). Priming was also found to help in increasing enzyme activity and neutralize the effects of seed ageing (Nawaz *et al.*, 2012). Endogenous levels of cytokinins have been linked with fruit growth (Gillaspy *et al*., 1993; Srivastava and Handa, 2005) and therefore, may play an important role in fruit development. As in the present study, Bairwa *et al.* (2016) had also found that kinetin treated seeds displayed the highest fruit length values than that of a normal ash gourd cultivar.

Leskovar and Sims (1987) had observed that the length of the fruits obtained from primed seed was higher than the control. However, unlike the results of the present study, priming with $CaCl_2$ had enhanced the mobilisation of substrate for amino acid and protein synthesis (Farooq *et al*., 2010). They also reported that all the seed priming techniques significantly improved the α-amylase activity, soluble sugars and dehydrogenase activity of the seeds compared with untreated control. Activity of dehydrogenase enzyme, an index of tissue respiration and metabolism, and that of α -amylase as starch hydrolyzing enzyme were stimulated by the priming. Takhti and Shekafandeh (2012) had also observed similar results of priming on fruit growth.

4.2.2.3 FRUIT DIAMETER (cm)

The results on fruit diameter (cm) as influenced by time of invigouration, invigouration treatments and their interaction effects are presented in Table 29 and Fig. 9.

4.2.2.3.1 Effect of time of invigouration (T)

Irrespective of the invigouration treatments, fruit diameter of ash gourds harvested from the Pre-sowing treatment (T_1: 10.81 cm) was found to be significantly superior over Pre-storage treatment (T_2: 10.55 cm).

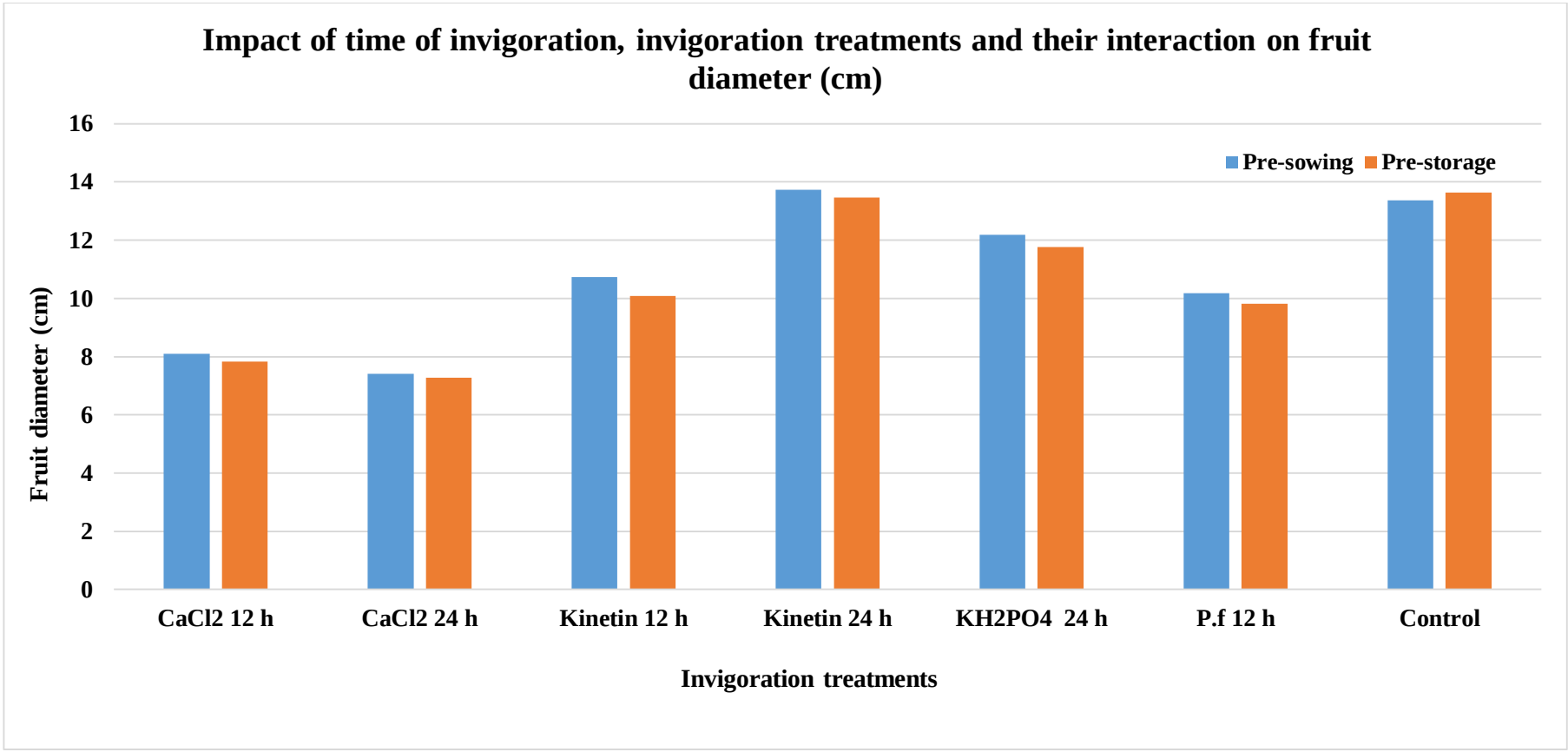
Table 29. Impact of time of invigoration, invigoration treatments and their interaction on fruit diameter (cm).

Time of invigoration (T)	Invigoration (I)							Mean T (Time of invigoration only)
	I_1 $CaCl_2$ 12 h	I_2 $CaCl_2$ 24 h	I_3 Kinetin 12 h	I_4 Kinetin 24 h	I_5 KH_2PO_4 24 h	I_6 *Pf* 12 h	I_7 Control	
T_1 (Pre-sowing)	8.09	7.40	10.74	13.74	12.18	10.18	13.36	**10.81**
T_2 (Pre-storage)	7.83	7.27	10.09	13.47	11.77	9.81	13.63	**10.55**
Mean I (Invigoration only)	**7.96**	**7.33**	**10.42**	**13.61**	**11.98**	**9.99**	**13.50**	

Factors	**C.D. (5%)**	**SE(m) ±**
Factor T	0.09	0.03
Factor I	0.17	0.06
Interaction T × I	0.24	0.08

T_1: Pre-sowing, T_2: Pre-storage and I: Invigoration treatments

Fig. 9


Impact of time of invigoration, invigoration treatments and their interaction on fruit diameter (cm)
Pre-sowing
Pre-storage
Fruit diameter (cm)
16
14
12
10
8
6
4
2
0
CaCl2 12 h
CaCl2 24 h
Kinetin 12 h
Kinetin 24 h
KH2PO4 24 h
P.f 12 h
Control
Invigoration treatments

4.2.2.3.2 Effect of invigouration treatments (I)

Irrespective of the time of invigouration, the treatment effect on fruit diameter of ash gourds varied between 7.33 cm (I_2: $CaCl_2$ 50 mM 24 h) and 13.61 cm (I_4: Kinetin 10 ppm 24 h). The diameter of fruits in I_4 (Kinetin 10 ppm 24 h) was found to be superior and significantly different from all other treatments. The treatment I_4 was followed by untreated control (I_7: 13.50 cm).

4.2.2.3.3 Effect of interaction

Time of invigouration × Invigouration treatments (T × I)

Significant difference in fruit diameter was observed due to the interaction of time of invigouration and invigouration treatments (T × I).

The fruit diameter of the ash gourds from Pre-sowing treatment and Pre-storage treatments ranged from 7.27 cm (T_2I_2: Pre-storage treatment - $CaCl_2$ 50 mM 24 h) to 13.74 cm (T_1I_4: Pre-sowing treatment - Kinetin 10 ppm 24 h) respectively. The treatment T_1I_4 (Pre-sowing treatment - Kinetin 10 ppm 24 h) recorded the highest value and was on par with T_2I_7 (Pre-storage treatment - Untreated control: 13.63 cm), but was superior to all other treatments.

T_2I_4 (Pre-storage treatment - Kinetin 10 ppm 24 h: 13.47 cm) and T_1I_7 (Pre-storage treatment - Untreated control: 13.36 cm) were next best to T_1I_4.

Hence, it was evident that priming with Kinetin 10 ppm 24 h either prior to sowing or before storage, was beneficial in increasing fruit diameter. Priming with kinetin in tomato had increased the fruit diameter compared to control (Deepak *et al*., 2018). Role of kinetin is connected with the growth and development of plants *i.e.*, cell division. According to Duszka *et al*. (2009), kinetin influences chloroplast differentiation and chlorophyll (Chl) biosynthesis by stimulation of 5- aminolevulinc acid synthesis for the assimilation of energy. This may lead to increased photosynthetic assimilation and better source-sink transport of synthesised food resulting in increased fruit size. Kinetin treatment in Loquat had resulted not only in production of higher amounts of chlorophyll but also helped maintain or improve loquat fruit quality characters and increase fruit yield (Sayed, 1999). Furthermore, Gillaspy *et al*. (1993) and Srivastava and Handa (2005) had also observed that the endogenously applied kinetin exhibited a strong linked with fruit growth characters.

4.2.2.4 FRUIT WEIGHT (kg)

The results on fruit weight (kg) as influenced by time of invigouration, invigouration treatments and their interaction effects are presented in Table 30 and Fig. 10.

4.2.2.4.1 Effect of time of invigouration (T)

Irrespective of the invigouration treatments, fruit weight of ash gourd harvested from pre-sowing treatment (T_1: 0.87 kg) was significantly superior over pre-storage treatment (T_2: 0.74 kg).

4.2.2.4.2 Effect of invigouration treatments (I)

Irrespective of the time of invigouration, invigouration treatments exerted significant influence on fruit weight.

Fruit weight of ash gourd varied between 0.17 kg (I_4: Kinetin 10 ppm 24 h) and 2.12 kg (I_7: Untreated control). I_7 was found to be significantly different from all other treatments.

I_5 (KH_2PO_4 10^{-1} M 24 h) with a fruit weight of 1.43 kg followed by I_3 (Kinetin 10 ppm 12 h: 0.66 kg) were found next best to I_7. I_1 ($CaCl_2$ 50 mM 12 h: 0.22 kg) produced fruits of low weight next to I_4 which had produced fruits of the least weight.

4.2.2.4.3 Effect of interaction

Time of invigouration × Invigouration treatments (T × I)

Significant difference in fruit weight was observed due to the interaction of time of invigouration and invigouration treatments (T × I).

The fruit weight ranged from 0.14 kg (T_2I_4: Pre-storage treatment - Kinetin 10 ppm 24 h) to 2.22 kg (T_2I_7: Pre-storage treatment - Untreated control). T_2I_7 was found superior to all other treatments.

T_1I_7 (Pre-sowing treatment - Untreated control: 2.01 kg) and T_1I_5 (Pre-sowing treatment - KH_2PO_4 10^{-1} M 24 h: 1.65 kg) were found next best to T_2I_7 and but were significantly different from each other. T_2I_5 (Pre-storage treatment - KH_2PO_4 10^{-1} M 24 h: 1.21 kg) was next best to T_1I_5 and T_1I_7.

T_2I_1 (Pre-storage treatment - $CaCl_2$ 50 mM 12 h: 0.18 kg) and T_1I_4 (Pre-sowing treatment - Kinetin 10 ppm 24 h: 0.19 kg) were on par with T_2I_4 which registered the lowest fruit weight.

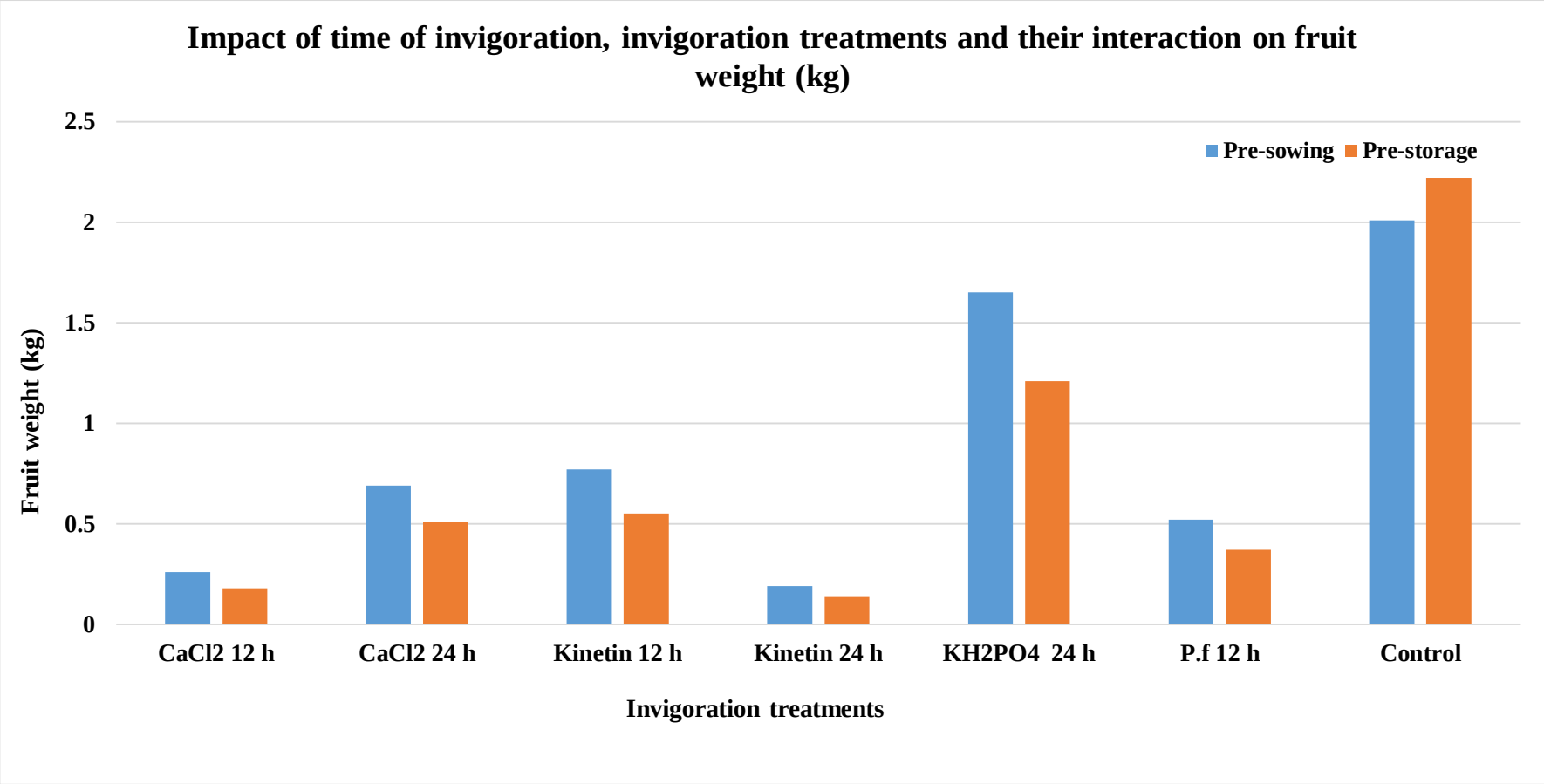
Table 30. Impact of time of invigoration, invigoration treatments and their interaction on fruit weight (kg).

Time of invigoration (T)	Invigoration (I)							Mean T (Time of invigoration only)
	I_1 $CaCl_2$ 12 h	**I_2 $CaCl_2$ 24 h**	**I_3 Kinetin 12 h**	**I_4 Kinetin 24 h**	**I_5 KH_2PO_4 24 h**	**I_6 *Pf* 12 h**	**I_7 Control**	
T_1 (Pre-sowing)	0.26	0.69	0.77	0.19	1.65	0.52	2.01	**0.87**
T_2 (Pre-storage)	0.18	0.51	0.55	0.14	1.21	0.37	2.22	**0.74**
Mean I (Invigoration only)	**0.22**	**0.60**	**0.66**	**0.17**	**1.43**	**0.45**	**2.12**	

Factors	**C.D. (5%)**	**SE(m)±**
Factor T	0.01	0.00
Factor I	0.01	0.01
Interaction T × I	0.02	0.01

T_1: Pre-sowing, T_2: Pre-storage and I: Invigoration treatments

Fig. 10


Impact of time of invigoration, invigoration treatments and their interaction on fruit weight (kg)
Pre-sowing
Pre-storage
Fruit weight (kg)
2.5
2
1.5
1
0.5
0
CaCl2 12 h
CaCl2 24 h
Kinetin 12 h
Kinetin 24 h
KH2PO4 24 h
P.f 12 h
Control
Invigoration treatments

Results thus pointed out that sowing untreated seeds would be more advantageous in realising higher fruit weight in ash gourd. Jamshidian and Talat (2017) observed that yield and yield components in crop raised from the untreated seeds of coriander was better than those from the osmo-primed and hormone-primed seeds. The performance degradation of priming treatments was attributed to the concentration as well as the physiological characters of the treated seeds. Murungu *et al*. (2004) opined that effect of seed priming depends on the crop species. They observed that priming adversely affected the field emergence, growth and seed yield in cotton compared to maize. Contrary to the above results, Nafziger *et al*. (1991) and Wade and Meinke (1994) stated that priming would be expected to show a positive response indirectly through its effect on the crop stand because stands with even emergence gave higher yields than stands with uneven time of emergence.

4.2.2.5 FRUIT YIELD PER VINE (kg)

The results on fruit yield per vine (kg) as influenced by time of invigouration, invigouration treatments and their interaction effects are presented in Table 31, Fig. 11 and Plate 3.

4.2.2.5.1 Effect of time of invigouration (T)

Irrespective of the invigouration treatments, fruit yield per vine in ash gourd harvested from pre-sowing treatment (T_1: 3.78 kg) was significantly superior over pre-storage treatment (T_2: 2.30 kg).

4.2.2.5.2 Effect of invigouration treatments (I)

Irrespective of the time of invigouration, invigouration treatments exerted significant influence on fruit yield in ash gourd.

Fruit yield per vine of ash gourd varied between 0.66 kg (I_4: Kinetin 10 ppm 24 h) and 7.05 kg (I_7: Untreated control). I_7 registered high yield and differed significantly from all other treatments.

I_5 (KH_2PO_4 10^{-1} M 24 h) with fruit yield of 5.71 kg per vine was found next best to I_7. However, it was found to be superior to I_3 (Kinetin 10 ppm 12 h: 2.61 kg). I_1 ($CaCl_2$ 50 mM 12 h: 0.87 kg) was found to on par with I_4 that had registered the least fruit yield per vine.

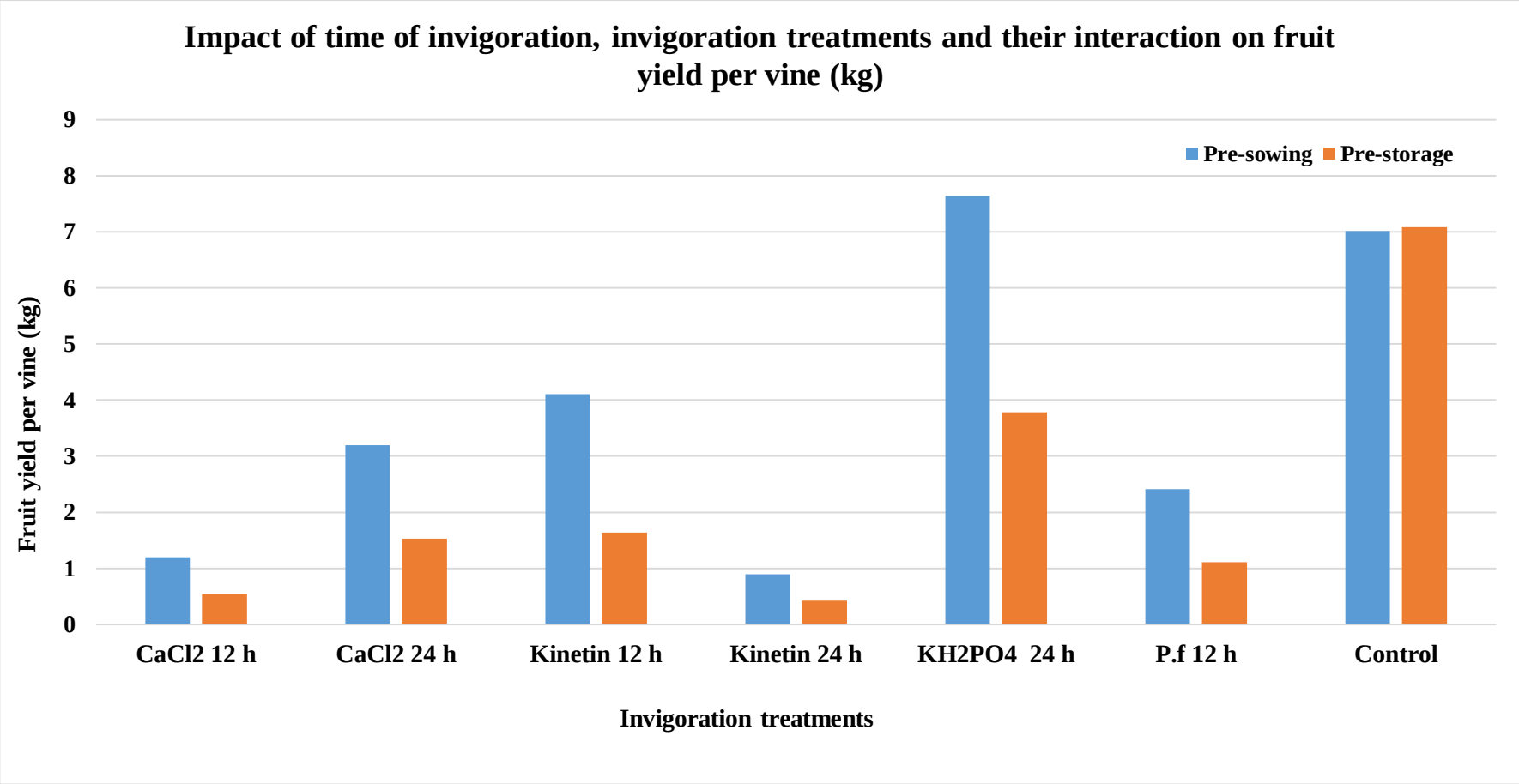
Table 31. Impact of time of invigoration, invigoration treatments and their interaction on fruit yield per vine (kg).

Time of invigoration (T)	Invigoration (I)							Mean T (Time of invigoration)
	I_1 $CaCl_2$ 12 h	I_2 $CaCl_2$ 24 h	I_3 Kinetin 12 h	I_4 Kinetin 24 h	I_5 KH_2PO_4 24 h	I_6 *Pf* 12 h	I_7 Control	
T_1 (Pre-sowing)	1.20	3.20	4.11	0.89	7.64	2.41	7.01	**3.78**
T_2 (Pre-storage)	0.54	1.53	1.64	0.42	3.78	1.11	7.08	**2.30**
Mean I (Invigoration only)	**0.87**	**2.36**	**2.88**	**0.66**	**5.71**	**1.76**	**7.05**	

Factors	C.D. (5%)	SE(m)±
Factor T	0.10	0.04
Factor I	0.19	0.07
Interaction T × I	0.27	0.09

T_1: Pre-sowing, T_2: Pre-storage and I: Invigoration treatments

Fig. 11


Impact of time of invigoration, invigoration treatments and their interaction on fruit yield per vine (kg)
Pre-sowing
Pre-storage
Fruit yield per vine (kg)
0
1
2
3
4
5
6
7
8
9
CaCl2 12 h
CaCl2 24 h
Kinetin 12 h
Kinetin 24 h
KH2PO4 24 h
P.f 12 h
Control
Invigoration treatments

4.2.2.5.3 Effect of interaction

Time of invigouration × Invigouration treatments (T × I)

Significant difference in fruit yield per vine was observed due to the interaction of time of invigouration and invigouration treatments (T × I).

The fruit yield per vine ranged from 0.42 kg (T_2I_4: Pre-storage treatment - Kinetin 10 ppm 24 h) to 7.64 kg (T_1I_5: Pre-sowing treatment - KH_2PO_4 10^{-1} M 24 h). T_1I_5 differed significantly from all other treatments.

T_2I_7 (Pre-storage treatment - Untreated control: 7.08 kg) and T_1I_7 (Pre-sowing treatment - Untreated control: 7.01 kg) were found to be next best to T_1I_5 and superior to all other treatments. T_2I_1 (Pre-storage treatment - $CaCl_2$ 50 mM 12 h: 0.54 kg) was found to be on par with T_2I_4 that had registered the least fruit yield per vine.

Although, considering the effect of invigouration alone, it was concluded that sowing untreated seeds would be more advantageous than sowing primed seeds to obtain high fruit yield in ash gourd, a strong interaction between the time of invigouration and the treatment administered was evident. Results indicated that pre-sowing priming of aged seeds with KH_2PO_4 10^{-1} M for 24 h would be more advantageous to acquire higher fruit yield per vine (7.64 kg), although the weight of single fruits in this treatment (1.65 kg) was less than that observed in untreated seeds (2.01 kg to 2.22 kg). This was attributed to the higher number of fruits per vine (4.63 Nos.) produced in KH_2PO_4 primed seeds compared to untreated seeds (3.19 Nos. to 3.49 Nos.) (Appendix).

Results pointed towards the effectiveness of pre-sowing priming of ash gourd seeds with KH_2PO_4 10^{-1} M 24 h over untreated as well as other priming treatments in increasing the fruit yield per vine. Miraj *et al*. (2013) reported that osmo-priming with KH_2PO_4 resulted in an increased cob, grain and straw yield in maize compared to the unprimed plants. Seeds primed with KH_2PO_4 not only exhibited higher vigour than unprimed seeds in terms of increased fresh and dry shoot weights, fresh shoot height and shoot P content but also the nutrient uptake of seedling was increased four times due to priming with KH_2PO_4. Similar results were also obtained by Rashid *et al*. (2004) in mung bean, Hagpanah *et al*. (2009) in sorghum and Ali *et al*. (2008) in wheat. They attributed the advantage of KH_2PO_4 priming to the improved metabolic repair process and its impetus on building up of germination metabolites or adjusting osmotic variation during priming.

4.2.2.6 SEEDS PER FRUIT

The results on seeds per fruit as influenced by time of invigouration, invigouration treatments and their interaction effects are presented in Table 32 and Fig. 12.

4.2.2.6.1 Effect of time of invigouration (T)

Irrespective of the invigouration treatments, the number of seeds per fruit in ash gourd in pre-storage treatment (T_2: 275.09) was significantly superior to pre-sowing treatment (T_1: 226.76).

4.2.2.6.2 Effect of invigouration treatments (I)

Irrespective of the time of invigouration, invigouration treatments exerted significant influence on number of seeds per fruit.

The number of seeds per fruit varied from 135.50 (I_2: $CaCl_2$ 50 mM 24 h) to 651.50 (I_5: KH_2PO_4 10^{-1} M 24 h). I_5 was found to be superior to all other treatments.

I_7 (Untreated control: 270.67) was found next best to I_5 but superior to I_3 (Kinetin 10 ppm 12 h: 208.67). The number of seeds produced in I_1 ($CaCl_2$ 50 mM 12 h: 142.17) was low. However, I_1 differed significantly from I_2, which had registered the least value for this trait.

4.2.2.6.3 Effect of interaction

Time of invigouration × Invigouration treatments (T × I)

Significant difference in seeds per fruit was observed due to the interaction of time of invigouration and invigouration treatments (T × I).

The number of seeds per fruit ranged from 115.00 (T_1I_3: pre-sowing treatment - Kinetin 10 ppm 12 h) to 693.00 (T_2I_5: pre-storage treatment - KH_2PO_4 10^{-1} M 24 h). T_1I_5 (Pre-sowing treatment - KH_2PO_4 10^{-1} M 24 h: 610.00) was found to be next best to T_2I_5. However, it differed significantly from T_2I_3 (Pre-storage treatment - Kinetin 10 ppm 12 h: 302.33).

T_1I_2 (Pre-sowing treatment - $CaCl_2$ 50 mM 24 h: 127.00), T_1I_1 (Pre-sowing treatment - $CaCl_2$ 50 mM 12 h: 133.00), T_2I_2 (Pre-storage treatment - $CaCl_2$ 50 mM 24 h: 144.00) and T_2I_1 (Pre-storage treatment - $CaCl_2$ 50 mM 12 h: 151.33) were on par with each other and with T_1I_3 that had registered the least value for this trait.

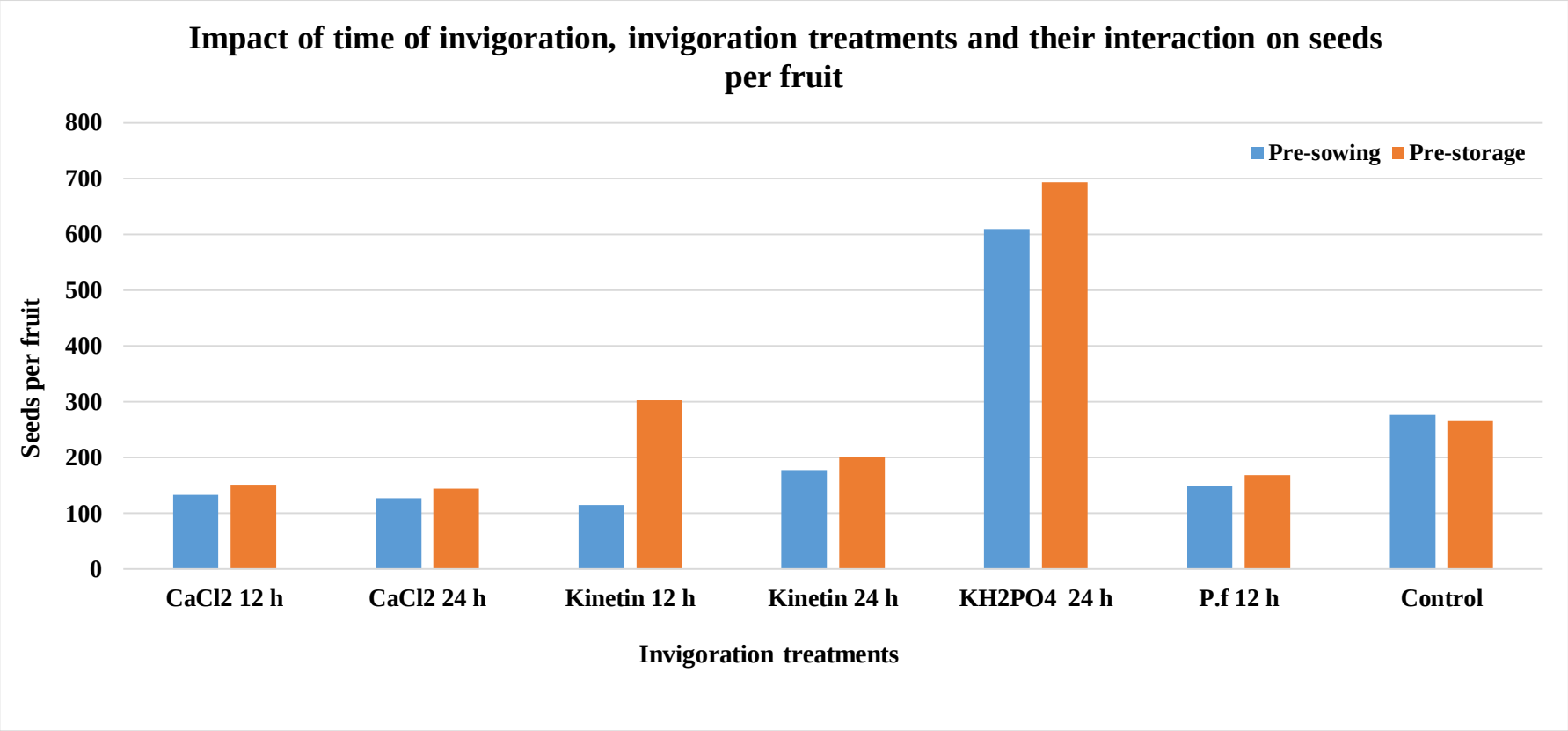
Table 32. Impact of time of invigoration, invigoration treatments and their interaction on number of seeds per fruit.

Time of invigoration (T)	Invigoration (I)							Mean T (Time of invigoration)
	I_1 $CaCl_2$ 12 h	I_2 $CaCl_2$ 24 h	I_3 Kinetin 12 h	I_4 Kinetin 24 h	I_5 KH_2PO_4 24 h	I_6 *Pf* 12 h	I_7 Control	
T_1 (Pre-sowing)	133.00	127.00	115.00	178.00	610.00	148.00	276.33	**226.76**
T_2 (Pre-storage)	151.33	144.00	302.33	202.00	693.00	168.00	265.00	**275.09**
Mean I (Invigoration only)	**142.17**	**135.50**	**208.67**	**190.00**	**651.50**	**158.00**	**270.67**	

Factors	C.D. (5%)	SE(m)±
Factor T	26.00	8.90
Factor I	48.64	16.64
Interaction T × I	68.79	23.53

T_1: Pre-sowing, T_2: Pre-storage and I: Invigoration treatments

Fig. 12


Impact of time of invigoration, invigoration treatments and their interaction on seeds per fruit
800
700
600
500
400
300
200
100
0
Seeds per fruit
Pre-sowing
Pre-storage
CaCl2 12 h
CaCl2 24 h
Kinetin 12 h
Kinetin 24 h
KH2PO4 24 h
P.f 12 h
Control
Invigoration treatments

The above results clearly point to the beneficial effect of administering seed priming with KH_2PO_4 10^{-1} M 24 h either as pre-sowing or pre-storage treatment on increasing the number of seeds per fruit in ash gourd.

The positive impact of osmopriming with KH_2PO_4 on yield parameters of *Cucurbita pepo* L. was observed (Mauromicale and Cavallaro, 1997). They reported that there was a beneficial increment on seeds per fruit on priming the seeds. Similar results were also observed musk melon by Nerson and Govers (1986). They reported that priming the seed with KH_2PO_4 not only enhanced the crop growth but also enhanced seed yield parameters like seed weight and seeds per fruit. Clark and James (1991) found that pre-treatment of canola seeds resulted in significant increase in the number of seeds owing to the availability of photosynthates for plant as well as high concentrations of sucrose. Similar results were obtained by Kaur *et al.* (2005) in chickpea seeds. They reported that osmo-priming of seeds resulted in an increase number of seeds per pod and seed weight. They attributed this increase to the change in hormonal balance after priming.

4.2.2.7 FRESH WEIGHT (g)

The results on fresh weight (g) as influenced by time of invigouration, invigouration treatments and their interaction effects are presented in Table 33 and Fig. 13.

4.2.2.7.1 Effect of time of invigouration (T)

Irrespective of the invigouration treatments, fresh weight of seeds extracted from the ash gourds in pre-sowing treatment (T_1: 32.67 g) was significantly superior over pre-storage treatment (T_2: 28.01 g).

4.2.2.7.2 Effect of invigouration treatments (I)

Irrespective of the time of invigouration, invigouration treatments exerted significant influence on fresh weight of seeds.

The fresh weight of seeds varied between 6.23 g (I_1: $CaCl_2$ 50 mM 12 h) and 56.40 g (I_5: KH_2PO_4 10^{-1} M 24 h). I_5 was found to be superior to all other treatments.

I_4 (Kinetin 10 ppm 24 h: 54.87 g) was next best to I_5 and significantly superior to I_7 (Untreated control: 34.00 g). I_6 (*P. fluorescens* 1×10^6 cfu ml^{-1} 12 h: 7.52 g) was on par with I_1 which registered the least value for this trait.

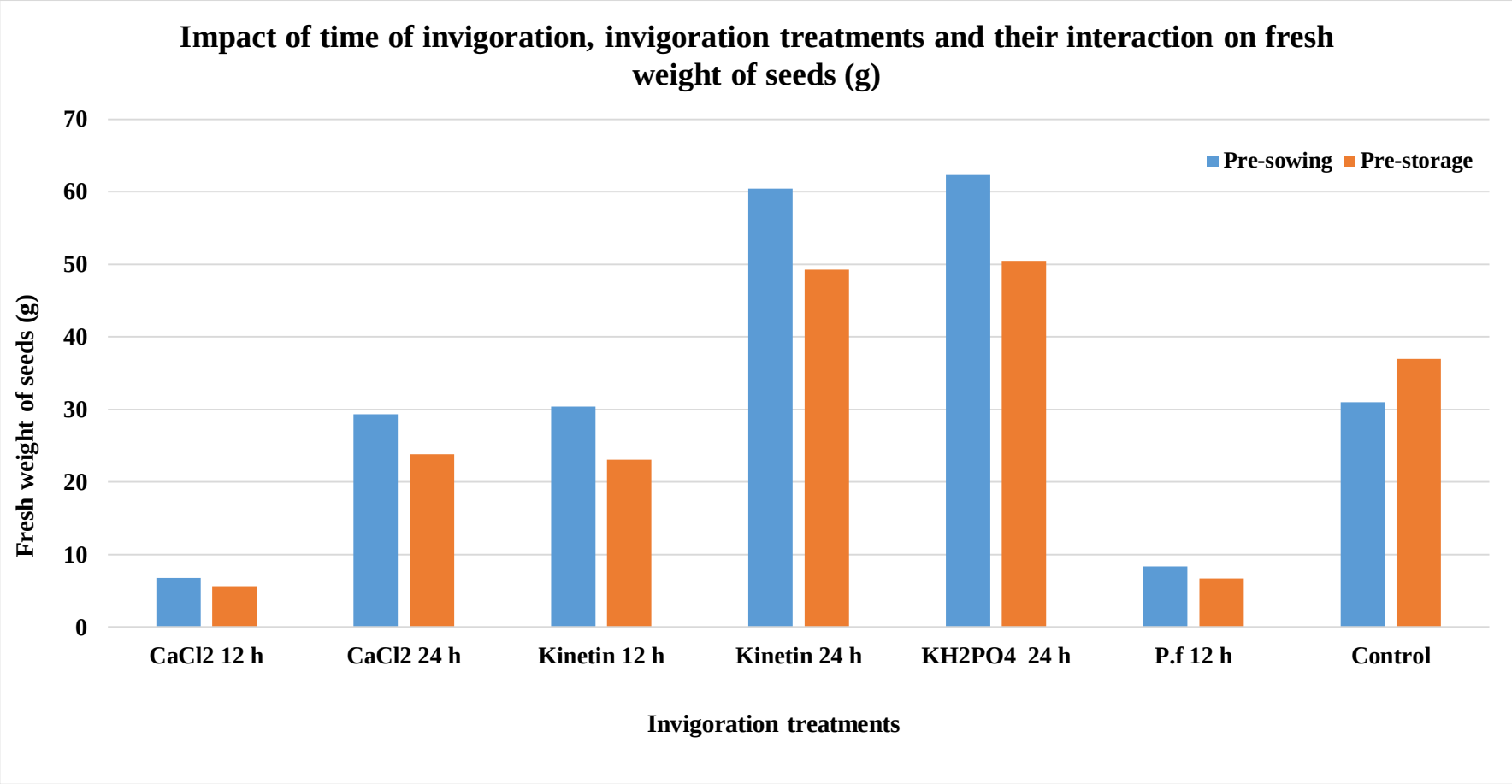
Table 33. Impact of time of invigoration, invigoration treatments and their interaction on fresh weight of seeds per fruit (g).

Time of invigoration (T)	Invigoration (I)							Mean T (Time of invigoration only)
	I_1 $CaCl_2$ 12 h	**I_2 $CaCl_2$ 24 h**	**I_3 Kinetin 12 h**	**I_4 Kinetin 24 h**	**I_5 KH_2PO_4 24 h**	**I_6 *Pf* 12 h**	**I_7 Control**	
T_1 (Pre-sowing)	6.80	29.37	30.43	60.43	62.30	8.33	31.00	**32.67**
T_2 (Pre-storage)	5.67	23.80	23.07	49.30	50.50	6.70	37.00	**28.01**
Mean I (Invigoration only)	**6.23**	**26.58**	**26.75**	**54.87**	**56.40**	**7.52**	**34.00**	

Factors	**C.D. (5%)**	**SE(m)±**
Factor T	0.39	0.13
Factor I	0.73	0.25
Interaction T × I	1.03	0.35

T_1: Pre-sowing, T_2: Pre-storage and I: Invigoration treatments

Fig. 13


Impact of time of invigoration, invigoration treatments and their interaction on fresh weight of seeds (g)
Fresh weight of seeds (g)
70
60
50
40
30
20
10
0
Pre-sowing
Pre-storage
CaCl2 12 h
CaCl2 24 h
Kinetin 12 h
Kinetin 24 h
KH2PO4 24 h
P.f 12 h
Control
Invigoration treatments

4.2.2.7.3 Effect of interaction

Time of invigouration × Invigouration treatments (T × I)

Significant difference in fresh weight of seeds was observed due to the interaction of time of invigouration and invigouration treatments (T × I).

The fresh weight of the seeds extracted ranged from 5.67 g (T_2I_1: pre-storage treatment - $CaCl_2$ 50 mM 12 h) to 62.30 g (T_1I_5: pre-sowing treatment - KH_2PO_4 10^{-1} M 24 h). T_1I_5 was found to be superior to all other treatments.

T_1I_4 (pre-sowing treatment – Kinetin 10 ppm 24 h: 60.43 g) followed by T_2I_5 (pre-storage treatment - KH_2PO_4 10^{-1} M 24 h: 50.50 g) were next best to T_1I_5.

T_2I_6 (pre-storage treatment - *P. fluorescens* 1×10^6 cfu ml^{-1} 12 h: 6.70 g) was found to be on par with T_2I_1 that had registered the least value for this trait. T_1I_1 (pre-sowing treatment - $CaCl_2$ 50 mM 12 h: 6.80 g), was on par with T_2I_6.

The above results indicated that pre-sowing priming in ash gourd with KH_2PO_4 10^{-1} M 24 h increased the seed yield per fruit on fresh weight basis followed by pre-sowing priming with Kinetin 10 ppm 24 h.

Pre-sowing treatment with inorganic salts not only promoted seed germination in most crops, but also stimulates faster growth, metabolic processes and hence, ultimate crop yield (Sallam, 1999). Earlier workers have reported the advantage of osmo-priming with KH_2PO_4 in increasing the seed weight in maize (Miraj *et al.*, 2013 and Soleimanzadeh, 2013). Priming seeds had resulted in advanced metabolic processes and higher germination per cent and germination rate, compared to unprimed seeds. This suggested that there was no detrimental effect of KH_2PO_4, due to ion accumulation in the embryo (Demir and Venter, 1999).

4.2.2.8 DRY WEIGHT (g)

The results on dry weight (g) as influenced by time of invigouration, invigouration treatments and their interaction effects are presented in Table 34 and Fig. 14.

4.2.2.8.1 Effect of time of invigouration (T)

Irrespective of the invigouration treatments, dry weight of seeds extracted from the ash gourds of pre-sowing treatment (T_1: 5.06 g) and pre-storage treatment (T_2: 5.20 g) did not vary significantly from each other.

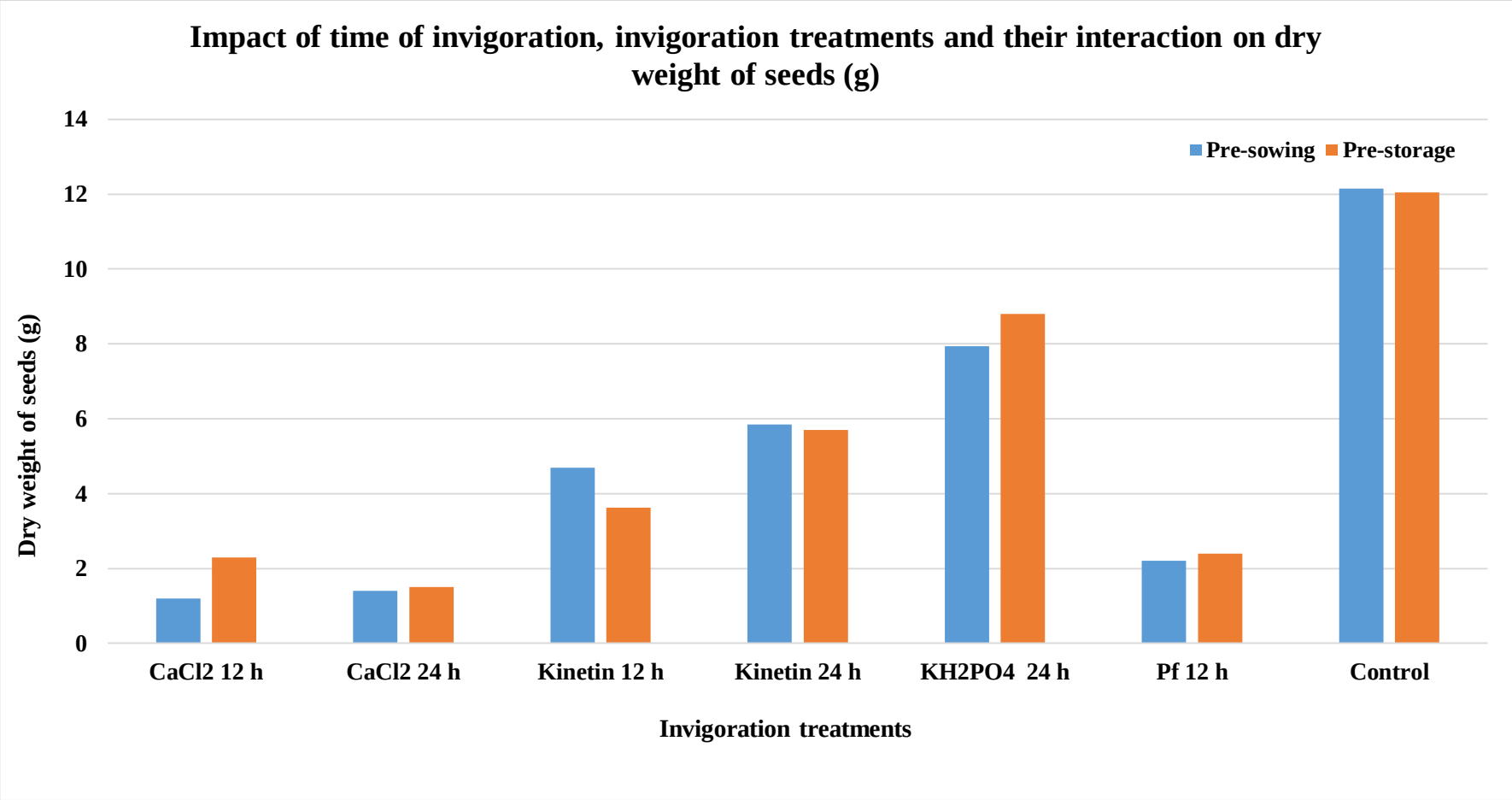
Table 34. Impact of time of invigoration, invigoration treatments and their interaction on dry weight of seeds per fruit (g).

Time of invigoration (T)	Invigoration (I)							Mean T (Time of invigoration)
	I_1 $CaCl_2$ 12 h	I_2 $CaCl_2$ 24 h	I_3 Kinetin 12 h	I_4 Kinetin 24 h	I_5 KH_2PO_4 24 h	I_6 *Pf* 12 h	I_7 Control	
T_1 (Pre-sowing)	1.19	1.40	4.69	5.85	7.94	2.20	12.15	**5.06**
T_2 (Pre-storage)	2.30	1.50	3.63	5.70	8.80	2.40	12.05	**5.20**
Mean I (Invigoration only)	**1.75**	**1.44**	**4.16**	**5.78**	**8.37**	**2.30**	**12.10**	

Factors	C.D. (5%)	SE(m)±
Factor T	NS	0.09
Factor I	0.51	0.17
Interaction T × I	0.72	0.25

T_1: Pre-sowing, T_2: Pre-storage, and I: Invigoration treatments

Fig. 14


Impact of time of invigoration, invigoration treatments and their interaction on dry weight of seeds (g)
Pre-sowing
Pre-storage
Dry weight of seeds (g)
0
2
4
6
8
10
12
14
CaCl2 12 h
CaCl2 24 h
Kinetin 12 h
Kinetin 24 h
KH2PO4 24 h
Pf 12 h
Control
Invigoration treatments

4.2.2.8.2 Effect of invigouration treatments (I)

Irrespective of the time of invigouration, invigouration treatments exerted significant influence on dry weight of seeds.

The dry weight of seeds varied between 1.44 g (I_2: $CaCl_2$ 50 mM 24 h) and 12.10 g (I_7: Untreated control). I_7 was superior to all other treatments.

I_5 (KH_2PO_4 10^{-1} M 24 h: 8.37 g) followed by I_4 (Kinetin 10 ppm 24 h: 5.78 g) were found next best to I_7. I_1 ($CaCl_2$ 50 mM 12 h: 1.75 g) and I_6 (*P. fluorescens* 1×10^6 cfu ml^{-1} 12 h: 2.30 g) registered low dry weight of seeds per fruit. They differed significantly from I_2 that had registered the least value for this trait.

4.2.2.8.3 Effect of interaction

Time of invigouration × Invigouration treatments (T × I)

Significant difference in dry seed weight of seeds was observed due to the interaction of time of invigouration and invigouration treatments (T × I).

The dry weight of the seeds extracted ranged from 1.19 g (T_1I_1: pre-sowing treatment - $CaCl_2$ 50 mM 12 h) to 12.15 g (T_1I_7: pre-sowing treatment - Untreated control). T_1I_7 was found to be superior to all other treatments.

T_2I_7 (pre-storage treatment - Untreated control: 12.05 g) followed by T_2I_5 (pre-storage treatment - KH_2PO_4 10^{-1} M 24 h: 8.80 g) were next best to T_1I_7. The dry weight of seed in T_1I_2 (pre-sowing treatment - $CaCl_2$ 50 mM 24 h: 1.40 g) and T_2I_2 (pre-storage treatment - $CaCl_2$ 50 mM 24 h: 1.50 g) was next to T_1I_1 that had recorded the least value for this trait.

From the above, it was evident that sowing untreated seeds was more advantageous in realising higher seed yield per fruit on dry weight basis although, priming with KH_2PO_4 10^{-1} M 24 h has resulted in higher seed weight per fruit on wet weight basis as well as higher number of seeds per fruit. This may be either due to increased moisture content of seeds or small sized seeds obtained in crop raised from KH_2PO_4 10^{-1} M 24 h primed seeds.

However, the result is contradictory to the findings of Nerson and Grover (1986) and Kaur *et al.* (2005) in chickpea, Miraj *et al.* (2013) and Soleimanzadeh *et al.* (2013) in maize. On priming with KH_2PO_4 Miraj *et al.* (2013) had realised 157 per cent increase in grain over control. Meena *et al*. (2018) found that seed hardening in lentil with KH_2PO_4 led to significant

increase in germination per cent, seedling length, root length, shoot length, seedling dry weight seedling fresh weight, number of nodules per plant, nodules fresh weight, nodules dry weight, number of pod per plant, number of seed per plant, 100 seed weight and seed yield, over untreated seeds.

4.2.2.9 100 SEED WEIGHT (g)

The results on 100 seed weight (g) in ash gourd as influenced by time of invigouration, invigouration treatments and their interaction effects are presented in Table 35 and Fig. 15.

4.2.2.9.1 Effect of time of invigouration (T)

Irrespective of the invigouration treatments, 100 seed weight in pre-sowing treatment (T_1: 2.11 g) and pre-storage treatment (T_2: 2.05 g) did not vary significantly from each other.

4.2.2.9.2 Effect of invigouration treatments (I)

Irrespective of the time of invigouration, invigouration treatments exerted significant influence on 100 seed weight.

The 100 seed weight varied between 0.92 g (I_1: $CaCl_2$ 50 mM 12 h) and 4.67 g (I_7: Untreated control). I_7 was superior to all other treatments.

I_2 ($CaCl_2$ 50 mM 24 h: 4.02 g) followed by I_6 (*P. fluorescens* 1×10^6 cfu ml^{-1} 12 h: 1.35 g) were next best to I_7. I_5 (KH_2PO_4 10^{-1} M 24 h) and I_3 (Kinetin 10 ppm 12 h) with a 100 seed weight of 1.25 g each were found to be next best to the above treatments. I_4 (Kinetin 10 ppm 24 h: 1.12 g) was found to be on par with I_1 that had registered the least 100 seed weight (0.92 g).

4.2.2.9.3 Effect of interaction

Time of invigouration × Invigouration treatments (T × I)

Significant difference in 100 seed weight was observed due to the interaction of time of invigouration and invigouration treatments (T × I).

The 100 seed weight ranged from 0.90 g (T_2I_1: pre-storage treatment - $CaCl_2$ 50 mM 12 h) to 4.80 g (T_2I_7: pre-storage treatment - Untreated control). T_2I_7 was found to be superior to all other treatments.

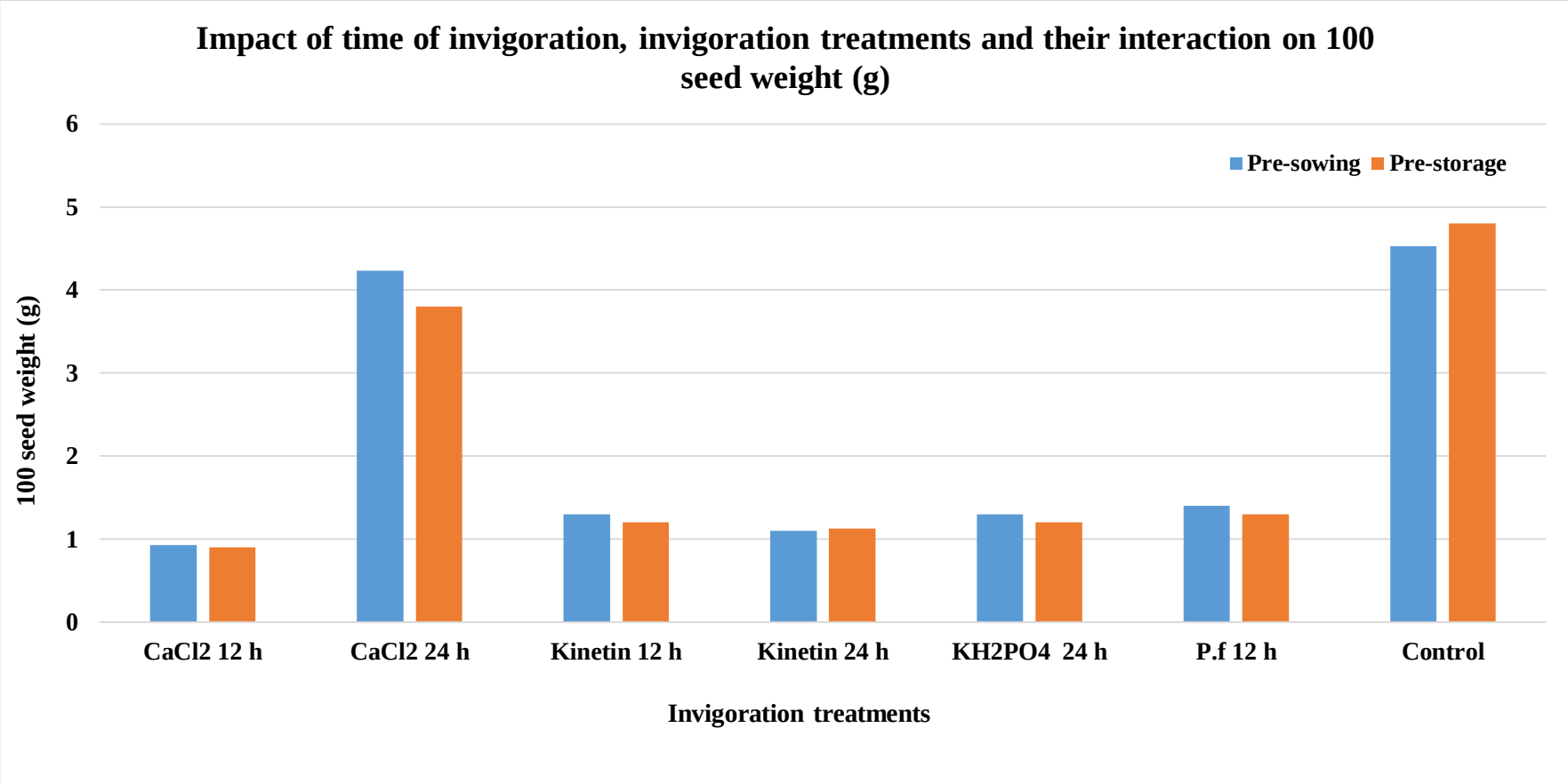
Table 35. Impact of time of invigoration, invigoration treatments and their interaction on 100 seed weight (g).

Time of invigoration (T)	Invigoration (I)							Mean T (Time of invigoration only)
	I_1 $CaCl_2$ 12 h	**I_2 $CaCl_2$ 24 h**	**I_3 Kinetin 12 h**	**I_4 Kinetin 24 h**	**I_5 KH_2PO_4 24 h**	**I_6 *Pf* 12 h**	**I_7 Control**	
T_1 (Pre-sowing)	0.93	4.23	1.30	1.10	1.30	1.40	4.53	**2.11**
T_2 (Pre-storage)	0.90	3.80	1.20	1.13	1.20	1.30	4.80	**2.05**
Mean I (Invigoration only)	**0.92**	**4.02**	**1.25**	**1.12**	**1.25**	**1.35**	**4.67**	

Factors	**C.D. (5%)**	**SE(m)±**
Factor P	NS	0.03
Factor T	0.18	0.06
Interaction P × T	0.25	0.09

T_1: Pre-sowing, T_2: Pre-storage and I: Invigoration treatments

Fig. 15


Impact of time of invigoration, invigoration treatments and their interaction on 100 seed weight (g)
Pre-sowing
Pre-storage
100 seed weight (g)
0
1
2
3
4
5
6
CaCl2 12 h
CaCl2 24 h
Kinetin 12 h
Kinetin 24 h
KH2PO4 24 h
P.f 12 h
Control
Invigoration treatments

T_1I_7 (pre-sowing treatment - Untreated control: 4.53 g) followed by T_1I_2 (pre-sowing treatment - $CaCl_2$ 50 mM 24 h: 4.23 g) were next best to T_2I_7 and also varied significantly from each other.

T_1I_1 (pre-sowing treatment - $CaCl_2$ 50 mM 12 h: 0.93 g), T_1I_4 (pre-sowing treatment - Kinetin 10 ppm 24 h: 1.10 g) and T_2I_4 (pre-storage treatment - Kinetin 10 ppm 24 h: 1.13 g) were on par with each other as well as with T_2I_1 which had registered the least value for this trait.

It was evident that all the priming treatments except I_7 had a negative effect on 100 seed weight which was similar to the results obtained by Mohammadi (2009) in sesame, Hardegree and Emmerich (1992) in grasses. Grey *et al.* (1990) stated that the negative effect of priming may be due to the osmotic adjustment that occurs during imbibition and that the rate of imbibition primed seeds play a crucial factor in the success of a priming.

Dodd and Donovan (1999) based on their studies on desert shrubs stated that the negative response to priming might be due to the variation of water potential gradient between the embryo and the substrate due to priming. Contrary to the above results, Korkmaz and Pill (2003) and Ghana and Schillinger (2003) stated that priming produced a positive response in seed yield.

To summarise, the results of the field experiment indicated that administering the seed treatment prior to sowing *i.e.,* as a pre-sowing treatment was more beneficial for plant growth and yield, rather than applying the same as a pre-storage treatment.

The fruit weight, seed yield per fruit on dry weight basis as well as 100 seed weight, was the highest when the crop was raised from unprimed seeds. In addition, the production of female flowers was observed to be the earliest in the crop raised from untreated seeds.

The fruit yield per vine (kg) from unprimed seeds was found next best to priming with KH_2PO_4 10^{-1} M 24 h. However, pre-sowing priming of seeds with KH_2PO_4 10^{-1} M 24 h (I_5) had registered the highest fruit yield per vine. Although the weight of single fruits in this treatment (1.65 kg) was less than that observed in untreated seeds (2.01 kg to 2.22 kg), higher number of fruits per vine (4.63 Nos.) was produced in KH_2PO_4 primed seeds compared to that in untreated seeds (3.19 Nos. to 3.49 Nos.)

The seed yield per fruit in KH_2PO_4 10^{-1} M 24 h primed seeds was the highest, next to using unprimed seeds.

Hence, considering the impact of priming treatment, time of seed priming and their interaction, it was evident that sowing untreated seeds was most advantageous to raise a seed crop from aged seeds. However, pre-sowing seed priming of aged seeds with KH_2PO_4 10^{-1} M 24 h would help realise better fruit yield per vine.

Summary

5. SUMMARY

A study was conducted to elucidate the effect of seed invigouration on field performance in ash gourd and to assess the impact of packaging material on seed quality and longevity. Ash gourd variety KAU Local was used and the study was conducted at College of Horticulture, Vellanikkara, Thrissur, during 2016-2018. The results obtained are summarized below.

I. **Experiment I: Seed storage studies**

I(a). Seed quality before storage

1. It was evident that before the initiation of storage, the quality parameters of the primed and unprimed seeds varied among themselves.
2. Seeds invigourated with Kinetin 10 ppm 12 h had registered the highest germination (88 %) and vigour index – I (1979.00).

I(b) Seed quality during storage

1. Results indicated existence of wide variability in the impact of packing materials, invigouration treatments, storage period and their interaction on most of the seed indices during the period of seed storage.

A. Seed quality and longevity of ash gourd variety KAU Local as influenced by packing material

1. Irrespective of the invigouration treatments and storage period, storing seeds in either polyethylene 700 gauge bags or in aluminium laminated pouches did not influence germination, vigour (VI-I), moisture content and lipid peroxidation in the packed seeds.
2. Seeds stored in aluminium laminated pouches registered significantly high vigour index-II and the low leakage of sugars in seed leachate, while, those stored in polyethylene pouches 700 G exhibited significantly low leakage of amino acid in seed leachate.

B. Seed quality and longevity of ash gourd variety KAU Local as influenced by invigouration treatment

1. Irrespective of the packing materials and storage period, invigouration treatments exerted significant influence on germination and other seed indices except, seed moisture content and lipid peroxidation.

2. In general, seeds invigourated with Kinetin 10 ppm for 12 h or 24 h or biopriming seeds (*P. fluorescens* 1×10^6 cfu ml^{-1} 12 h) or storing untreated registered high germination and vigour although the other seed indices in these treatments were not consistently superior.

3. Untreated seeds had registered the least EC of seed leachate, while, recording the highest leakage of amino acid in seed leachate

4. The seeds treated with Kinetin 10 ppm for 12 h recorded high estimates for EC of seed leachate and leakage of sugars in seed leachate, but the least leakage of amino acid in seed leachate and.

5. Bioprimed seeds had registered the high estimates for EC of seed leachate, leakage of amino acid and sugars in seed leachate

C. Seed quality and longevity of ash gourd variety KAU Local as influenced by storage period

1. Irrespective of the packing materials and invigouration treatments, storage period exerted significant influence on all the seed indices studied.

2. Germination, vigour indices I and II, in both treated and untreated seeds decreased progressively over the storage period.

3. There was an increase in seed moisture content, lipid peroxidation, electrical conductivity of seed leachate, leachate of sugar and amino acid towards the end of storage period.

D. Seed quality and longevity of ash gourd variety KAU Local as influenced by interaction between packing material, invigouration treatment and storage period

1. Results indicated existence of wide variability in the impact of interaction between packing material, invigouration treatments and storage period on all the seed indices during seed storage.

2. Based on the influence of interaction between packing material and invigouration treatment on germination and other seed indices, it can be concluded that, seed invigouration followed by packing seeds in moisture and vapour impervious container is advantageous.

3. Under ambient condition, it would be advantageous to bioprime the seeds with *P. fluorescens* 1×10^{6} cfu ml^{-1} 12 h and storing them in aluminium laminated pouches. Viability of seeds on biopriming was retained above MSCS (> 60 %) for 5 MAS.

4. Biopriming seeds and packing them in aluminium laminated pouches was also found to be the best to obtain vigourous seedlings in the long run than storing them in polyethylene 700G bags.

5. Packing the seeds in polyethylene 700 gauge pouches after invigouration with *P. fluorescens* 1×10^{-6} cfu ml^{-1} for 12 h or with $CaCl_2$ 50 mM for 24 h or storing them untreated helped retain germination above MSCS for 4 MAS.

6. Seeds invigourated with Kinetin 10 ppm either for 12 h or for 24 h followed by storing them in either Polyethylene 700 gauge bags or Aluminium laminated pouches had retained viability above MSCS up to 3 MAS only.

7. The interaction between packing materials, invigouration treatments and storage period have strong impact on electrical conductivity of seed leachate, leakage of amino acid and sugars from the stored seeds. Packing untreated seeds or seeds primed with kinetin 10 ppm for 12 h or 24 h in either polyethylene 700G pouches or aluminium laminated

pouches would be most advantageous to acquire low EC of seed leachate and low leakage of amino acid during storage.

8. Considering the impact of packing material, invigouration treatments, storage period and their interaction, it was evident that under ambient storage environment, it would be best to pack seeds in aluminium laminated pouches after invigouration with *P. fluorescens* 1×10^6 cfu ml^{-1} for 12 h, as this was found to prolong seed longevity the farthest *i.e.*, until five months after storage (5 MAS).

E. Field performance of ash gourd variety KAU Local as influenced by time of invigouration, invigouration treatments and their interaction

1. Results indicated that the pre-sowing treatment was more beneficial for plant growth (days to appearance of first female flower), fruit (fruit length, diameter, weight, number of fruits per vine) and seed (seed yield per fruit on wet weight and dry weight basis, 100-seed weight) yield parameters, rather than administering the same invigouration treatments as a pre-storage treatment.

2. The crop raised from untreated seeds produced female flowers earlier than primed seeds.

3. The fruit (weight and yield per vine on weight basis) and seed yield per fruit on dry weight basis as well as 100 seed weight, was the highest when the crop was raised from untreated seeds.

4. Seed invigouration with KH_2PO_4 10^{-1} M for 24 h had produced the highest number of seeds per fruit and ranked the next best to untreated seeds with respect to fruit diameter, fruit weight and seed yield per fruit on dry weight basis.

5. Priming seeds with Kinetin 10 ppm for 24 h had resulted in production of fruits with the highest length and diameter. However, the weight of single fruit and fruit yield per vine was the least in this treatment.

6. Results of the interaction between time of seed invigouration and the invigouration treatment indicated that pre-sowing priming of aged seeds with KH_2PO_4 10^{-1} M for

24 h would be more advantageous to acquire higher fruit yield per vine (7.64 kg), although the weight of single fruits in this treatment (1.65 kg) was less than that observed in untreated seeds (2.01 kg to 2.22 kg). This was attributed to the higher number of fruits per vine (4.63 Nos.) produced in KH_2PO_4 primed seeds compared to untreated seeds (3.19 Nos. to 3.49 Nos.)

7. The seed yield per fruit on dry weight basis was the highest (12.05 g to 12.15 g) in untreated seeds although the seed yield per fruit on fresh weight was high in pre-sowing priming of seeds with KH_2PO_4 10^{-1} M for 24 h.

8. Considering the impact of time of seed priming, invigouration treatments and their interaction, it was evident that using untreated seeds was the most advantageous to raise a seed crop from aged seeds. However, pre-sowing seed priming of aged seeds with KH_2PO_4 10^{-1} M 24h would help realise better fruit yield per vine.

9. The increase in cost of seed invigouration with calcium chloride amounts to Rs. 95.00 per kilogram of seed. Hence a 7.60 per cent escalation in the cost of production of seeds invigourated with calcium chloride occurs over untreated control. However, by incurring this in expenditure, the fruit yield per vine can be increased compared to untreated control.

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Appendices

APPENDIX-I

Table 36. Total number of fruits harvested per vine

Packing material (P)	Number of fruits							Mean P (Packing only)
	I_1 $CaCl_2$ 12 h	I_2 $CaCl_2$ 24 h	I_3 Kinetin 12 h	I_4 Kinetin 24 h	I_5 KH_2PO_4 24 h	I_6 *Pf* 12 h	I_7 Control	
P_1 (Poly. 700G)	4.62	4.64	5.34	4.68	4.63	4.63	3.49	**4.58**
P_2 (Al. bags)	3.00	3.00	2.98	3.00	3.12	3.00	3.19	**3.04**
Mean I (Invigoration only)	**3.81**	**3.82**	**4.16**	**3.84**	**3.88**	**3.82**	**3.34**	

SEED INVIGORATION FOR IMPROVED FIELD PERFORMANCE AND STORABILITY IN ASH GOURD (*Benincasa hispida* (Thunb.) Cogn.)

By

BENNETT THOMAS. K

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ABSTRACT OF THE THESIS

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ABSTRACT

Ageing of seeds is an inevitable natural phenomenon, subsequently resulting in loss of vigour and viability. Adopting appropriate packaging, ensuring optimum storage environment and priming ash gourd seeds was found to be beneficial in slowing down the pace of the deteriorative process during storage, maintaining the seed quality and prolonging seed longevity. Despite the improvements in seed performance following priming treatments, there have been contrasting reports on its impact on seed storage potential and crop performance. Considering the above, a study to elucidate the effect of seed priming on field performance in ash gourd variety KAU Local and to assess the impact of packing material on seed quality and longevity, was conducted at College of Horticulture, Vellanikkara, Thrissur, during 2016 - 2019.

The effect of seed invigouration and packing material on seed viability and seed quality parameters under ambient storage environment was assessed following a completely randomized design with three replications and seven priming treatments (I_1 to I_7). Freshly extracted seeds were separately primed using $CaCl_2$ (50 mM) for 12 h (I_1), $CaCl_2$ (50 mM) for 24 h (I_2), kinetin (10 ppm) for 12 h (I_3), kinetin (10 ppm) for 24 h (I_4), KH_2PO_4 (10^{-1} M) for 24 h (I_5) and *Psuedomonas fluorescens* (1×10^6 cfu.ml^{-1}) for 12 h (I_6). Untreated seeds (I_7) served as control. Both treated and untreated seeds were dried to $\leq$ 8 per cent moisture content and packed in polyethylene 700 gauge pouches (P_1: Poly 700G pouches) and in aluminium laminated pouches (P_2: Al. pouches). The seed quality parameters were recorded after treatment from the start of storage and at monthly intervals for a period of six months of storage (MAS). At bimonthly intervals, quantification of lipid peroxidation, sugar and amino acids leached out from the seeds were also done.

In addition, the performance of crop raised from seeds, stored for five months and subjected to priming just prior to sowing (T_1: Pre-sowing treatment) and prior to storage (T_2: Pre-storage treatment), was also assessed following a randomized block design with three replications and seven priming (I_1 to I_7)

treatments as detailed in the laboratory studies. The fruit as well as seed yield parameters were recorded from the crop raised.

Results of storage studies revealed that irrespective of the packaging material, germination and vigour indices I and II in both treated and untreated seeds decreased progressively over the storage period. However, there was an increase in and lipid peroxidation, electrical conductivity of seed leachate, leachate of sugar and amino acid towards the end of storage period. Irrespective of the invigouration treatment and the storage period, storing seeds either in Poly 700 G pouches (P_1) or Al. pouches (P_2) did not influence seed viability, vigour (VI-I), moisture content and lipid peroxidation in packed seeds.

Considering the influence of invigouration treatment alone, priming seeds with kinetin 10 ppm either for 12 h or 24 h or biopriming seeds (*P. fluorescens* 1×10^6 cfu ml^{-1} 12 h) or storing them untreated, was found to be the best.

Existence of a strong influence of the interaction between packing material, invigouration treatment and storage period on seed quality and longevity of packed seeds was discerned. Under ambient storage environment (72% RH and 32 ^{0}C), it would be best to pack seeds in aluminium foil pouches after invigouration with *P. flourescens* 1×10^6 cfu ml^{-1} for 12 h (P_2I_6), as this was found to prolong seed longevity the farthest *i.e.,* until five months after storage (5 MAS). However, the bioprimed seeds if stored in polyethylene 700G pouches (P_1I_6) had retained viability above the minimum standards of seed certification (MSCS) for 4 MAS only. Similar to P_1I_6, storing untreated seeds in polyethylene (P_1I_7) or seeds primed with $CaCl_2$ 50 mM for 24 h in polyethylene 700G pouches (P_1I_2) retained viability above MSCS for 4 MAS. However, storing them in Al. pouches (P_2I_7 and P_2I_2) was less effective.

From the results of the field experiment it was evident that administering the seed treatment prior to sowing *i.e.,* as a pre-sowing treatment was more beneficial for plant growth and yield, rather than applying the same as a pre-storage treatment. Seed yield per fruit on dry weight basis was the highest (I_7: 12.10 g) when the crop was raised from unprimed seeds [(both pre-sowing (T_1I_7: 12.15 g) and pre-storage

(T_2I_7: 12.05g)], while the fruit yield per vine from unprimed seeds ranged between 7.01 kg (T_1I_7) and 7.08 kg (T_2I_7). In addition, the production of female flowers was observed to be the earliest (I_7: 93.67 days; T_1I_7: 95.00 days and T_2I_7: 92.33 days) in the crop raised from untreated seeds. The single fruit weight (I_7: 2.12 kg; T_1I_7: 2.01 kg and T_2I_7: 2.22 kg) and 100-seed weight (I_7: 4.67 g T_1I_7: 4.53 g and T_2I_7: 4.80 g), were also high on using untreated seeds.

Pre-sowing priming seeds with KH_2PO_4 10^{-1} M 24 h (I_5) had registered the highest fruit yield per vine (T_1I_5: 7.64 kg), although the weight of single fruits in this treatment (1.65 kg) was less than that observed in untreated seeds (2.01 kg to 2.22 kg). This was attributed to the higher number of fruits per vine (4.63 Nos.) produced in KH_2PO_4 primed seeds compared to untreated seeds (3.19 Nos. to 3.49 Nos.)

The seed yield per fruit in KH_2PO_4 10^{-1} M 24 h primed seeds (I_5: 8.37 g; T_1I_5: 7.93 g and T_2I_5: 8.80 g) was the highest, next to using unprimed seeds.

Hence, considering the impact of priming treatment, time of seed priming and their interaction, it was evident that using untreated seeds was most advantageous to raise a seed crop from aged seeds. However, pre-sowing seed priming of aged seeds with KH_2PO_4 10^{-1} M 24 h would help realise better fruit yield per vine.