

Impact of biopriming on seed quality and longevity in Rice (*Oryza sativa* L.)

By

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(2019-11-028)



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THESIS

Submitted in partial fulfilment of the requirements for the degree of

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DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

COLLEGE OF AGRICULTURE

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INDIA

2021

DECLARATION

I hereby declare that this thesis entitled "**Impact of biopriming on seed quality and longevity in rice (*Oryza sativa* L.)**" 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 to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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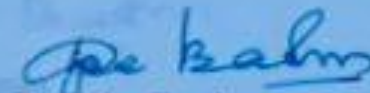
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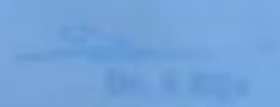
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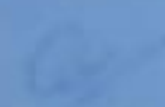
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LIST OF ABBREVIATIONS

ISTA	: International Seed Testing Association
mL	: milli litre
H	: hours
EC	: electrical conductivity
mg	: milli gram
Kg	: kilo gram
$\mu\text{S cm}^{-1}$: micro-Siemens per centimetre
ppm	: Parts per million
mm	: milli metre
nm	: nano metre
M	: metre
μm	: micro metre
$^{\circ}\text{C}$: degree Celsius
Pa	: Pascal
L	: Litre
Ha	: Hectare
Cc	: cubic centimeters
ANOVA	: Analysis of Variance

ZnO	: zinc oxide
nZnO	: nano-zinc oxide
TiO ₂	: titanium dioxide
nTiO ₂	: nano-titanium dioxide
SE _m	: standard error of error mean sum of squares
CD	: critical difference
var.	: variety
cv.	: cultivar
NP	: nano particle
IMSCS	: Indian Minimum Seed Certification Standard
mg/kg	: milligram per kilogram
°N	: degrees North
°E	: degrees East
G	: gauge
DAT	: Days after transplanting
MAS	: months after storage
%	: per cent
<i>et al.</i>	: <i>et alia</i> (Latin: „and others“)
V.I.	: vigour index
PDA	: Potato Dextrose Agar
VFPCCK	: Vegetable and Fruit Promotion Council Kerala

Introduction

I. INTRODUCTION

Rice (*Oryza sativa* L.) is life for more than half of the world's population. It is a wealthy source of carbohydrates, proteins, vitamins, minerals, and dietary fibre. India is the second largest rice producer after China (Saikrishna *et al.*, 2018). Asian countries consume about 90 per cent of the rice grown and produced in the world and it feeds more than 65% of the population (Ministry of Agriculture, Cooperation and Agricultural Welfare 33, 2017).

The area of rice production and productivity in Kerala in 2020-21 estimated as 56,465 hectares and 149,260 tons (Kerala State Bureau of Economics and Statistics, 2021)

Rice is grown in humid, tropical and sub-tropical climates where regional cultivars are extremely favoured for their taste and agricultural suitability. But these ideal growing conditions are in contrary with rice storage conditions where warm, humid climates can lead to rapid seed deterioration (Emam, 2007), Kerala's tropical climate poses a particular challenge for seed storage as the seed is stored in bags with little control over humidity, temperature or air circulation.

Seed quality deterioration occurs mainly during storage and affects the vigour and viability of seeds. However, the degree of spoilage depends on differences in the inherent genetic makeup, production environment, phytonutrients, storage conditions and other factors. The preservation capacity of the seed lot is predetermined by the original quality and the degree of deterioration at the time of storage.

The era of synthetic chemicals produced a number of pesticides and fungicides to successfully control infections caused by insects, moulds and other microorganisms on seeds during storage. However, its residual toxicity on non-target animals, including humans, is high. Therefore, a safe approach which is environmentally friendly, economical and readily available is to the farmers is to treat seeds with organics (Gajendra, 2015).

Seed priming is mainly a seed treatment before sowing. However, the primed seeds can be dried to their original moisture content and stored for different periods of time depending on the species. Primed seeds usually germinate faster and more evenly when soaked in

water, especially under adverse environmental conditions. The method of drying seeds after biological priming, known as dehydration or re-drying is used to remove moisture in the seeds to a level suitable for storage and also to retain the beneficial effects of the seed, benefits of processing, without the loss of quality caused by rapid grain recession (Arvindkumar *et al.*, 2015).

During storage, seed activity reduced. Ageing (or loss of vigour) may manifest as delayed seed germination and seedling emergence, slower growth and increased sensitivity to environmental stressors (Abdul-Baki and Anderson, 1972).

Seed senescence is a serious problem, but little is known about its mechanism (s) or event (s) leading to loss of viability and vitality in different species. It may be quantified by a stress test known as the Accelerated Ageing (AA) test developed by Delouche (1965) at Mississippi State University. It is assumed that the degradation process under AA conditions is similar to the natural ageing process. The results of standard germination tests obtained from a set of varieties or seed lots stored at ambient conditions are compared with the results of the accelerated ageing test to predict the relative storage capacity of the seeds. The information generated will be used to decide whether a particular variety or lot of seeds can be saved or discarded.

However, our understanding of the role of organic compounds in seed quality maintenance and relative conservation is limited and incomplete. Keeping the above points in mind, this study entitled “Impact of bioprimering on seed quality and longevity in rice” (*Oryza sativa* L.) was conducted with the following objectives:

- 1) To study the effect of different seed priming treatments on seed quality attributes.
- 2) To study the effect of seed priming treatments on seed longevity when stored at ambient temperature.
- 3) To assess the quality attributes and storage capacity of rice grains through accelerated aging tests.
- 4) To study the effects of seed priming on seed health

Review of Literature

II. REVIEW OF LITERATURE

Organic farming is not just an agrochemical non-chemism, but a systematic farming based on an intrinsic relationship with nature (Lampkin, 1990). The seed being a hygroscopic entity, during its storage undergoes numerous cytological, physical, physiological and biochemical changes which lead to a reduced vitality and to terminal death (Jyothi and Malik, 2013).

Seed deterioration is unequivocally associated with impaired protein and nucleic acid synthesis, loss of membrane integrity and change in enzyme activity during storage (Mc Donald, 1999). The effects of seed ageing may be delayed by slowly percolating the seed primer through the seed and rehydrating the seeds to their original moisture content (Tilden and West, 1985).

Literature pertaining to the effect of seed priming on seed quality and longevity have been reviewed in this chapter.

2.1 Seed priming

2.1.1 Biopriming

2.1.2 Orgo priming

2.1.3 Hydro priming

2.2 Efficiency of seed priming on seed quality during storage

2.2.1 Efficiency of biopriming on seed quality

2.2.2 Efficiency of organic priming on seed quality

2.2.3 Efficiency of hydro priming on seed quality

2.3 Seed infection across storage period

2.4 Storability evaluation through artificial ageing

2.1 Seed priming

Seed priming is a treatment performed prior to seeding that involves hydration of seeds that initiate metabolic events that occur prior to germination to prevent the emergence of seed radicles (Nascimento *et al.*, 2004 and Rehman *et al.*, 2011). Initiation involves treating seeds with different organic or inorganic chemicals and at high or low temperature (Kamiti *et al.*, 2016). It requires the seeds to be soaked in different solutions for a specified period of time under specified controlled conditions, and then dried back to their original moisture content to prevent the radicle emergence before sowing. This stimulates various metabolic processes, thereby improving the germination ability and the emergence rate of various seed types, especially vegetable seeds, grasses and ornamental species (Tavili *et al.*, 2011), while also reversing this effect (Golezani *et al.*, 2012). Seed perfusion is considered to be an easy, very effective, inexpensive and low-risk technique. Primed seeds also have many advantages, such as uniformity, earlier and faster emergence (Musa *et al.*, 1999), germination over a wide temperature range, uniform crop establishment, efficient use of water, increased length of root development, allowing germination of dormant seeds by increasing metabolism. Initiation of events that help in development of organs for reproduction (Soleimanzadeh, 2013), early flowering and maturation (Singh *et al.*, 2015), better compete with weeds, resist abiotic stresses (Aghbolaghi and Sedghi, 2014) and diseases caused by soil-borne destructives (*R. solani*, *Fusarium* spp., *Sclerotium rolfsii*, etc.) (Rafi *et al.*, 2015).

Seed priming can induce salt tolerance in pepper seedlings, while the average germination time without primer treatment is reduced. Seeds soaked in potassium nitrate need the minimum time to germinate, i.e., 5.63 days, while the time for unprimed seeds is 14 days. The final germination rate of the hydrogen-primed and halo-inhibited seeds was 96 per cent, while those that were not soaked showed a germination rate of 70 per cent (Muhammad *et al.*, 2007).

The improved yield of priming seed plots could be attributed to the early appearance and improvement of the priming treatments, which ultimately resulted in reporting higher yields in PEG 8000 lined soybean for 6 h improved emergence rate and grain yield (Arif *et al.*, 2008).

Seed priming improved seedling emergence, seedling growth, and seedling appearance synchronized with pre-metabolism. It also improved bud and root length, dry weight of seedlings, root score, α -amylase activity, soluble sugar and dehydrogenase activity. If the seeds need to be stored for a long time before sowing, it is best to re-dry the primed seeds that can better establish a uniform support for direct seeded aerobic cultivation (Farooq *et al.*, 2009).

Ageing is a common phenomenon during storage. Study by Siadat *et al.* (2012) reported that seed priming with 100 ppm Gibberellin solution for 12h in mature corn kernels improved the germination characteristics and growth performance of old and low-quality corn kernels.

The shelf life of primed seeds is often affected by the surrounding storage conditions, this was reported in the work of Hussain *et al.* (2015) in which the seeds of rice and tomato were stored at a temperature of 25°C were more effective than unprimed seeds. The germination rate was higher for up to 12 months.

Rehman *et al.* (2015) reported that direct seeded rice when integrated with seed priming showed increased crop performance in terms of crop stand, tillering, yield and increased grain quality.

The results of the study conducted by Hussain *et al.* (2016) clarified that seed priming not only promotes processes related to germination but also involves other specific mechanisms that improve seedling vigour and enable rice to cope with

stress. The increased antioxidant activity regulated by seed priming may protect ROS-induced degradation of enzymes by cooling and maintaining membrane integrity. In addition to its protective effect against oxidative stress, seed priming is also involved in regulating respiration rate and starch metabolism in rice, which may also be an important manifestation of increased vigour and stress tolerance in plants.

As per the results reported by Herbert *et al.* (2021) halogen priming of chilli seeds has improved the quality of seeds in terms of germination, vigour in addition to yield under field conditions and maintained the same up to 6 months of storage.

Nano priming with ZnO at 1300 mgkg⁻¹ from seeds and Nano TiO₂ at 900 mgkg⁻¹ showed superior results during the experiment. The results indicated that the seeds containing nanoparticles of zinc oxide and titanium dioxide in chilli is effective in improving field performance and chilli yield (Mathew *et al.*, 2021).

2.1.1 Biopriming

Biological priming is a biological seed treatment process that refers to the combination of seed hydration (physiological aspect of disease control) and inoculation (biological aspect of disease control) in seeds with organisms beneficial to seed protection (Sivasubramaniam *et al.*, 2011). Seeds treated with microbes may be of greatest benefit when used in conditions where pathogens are known to cause specific problems. The combination of bacteria and fungi can be grown simultaneously on carrot and onion seeds under laboratory conditions for best results (Bennett *et al.*, 2009).

Trichoderma conidia germinate on the surface of the seed and form a layer around the biologically incubated seed. These seeds are more tolerant of various adverse soil conditions. Biological priming can also reduce the amount of biological control agent applied to the seed. Bio-seed primers used successfully on tomato, brinjal, soybean and mung beans showed higher germination rates and better plant growth compared to the control plants that did not use biopriming (Kumar *et al.*, 2014). Biological priming leads to biochemical changes, increased production of proteins, hormones, phenols and flavonoid compounds which contribute to better plant growth and better developmental performance. The

percentage of soluble protein in seeds and seedlings that received biopriming was higher when compared to other types of priming (Sukanya *et al.*, 2018).

When biocontrol agents such as *Trichoderma harzianum*, *Trichoderma viride* and *Pseudomonas fluorescens* are used as primers, it is highly effective for controlling of seed borne diseases, apart from improving germination rate, uniformity of appearance and reducing emergence time like other priming methods, it also controls plant diseases (Goswami, 2019). The synergistic effect of the microbial complex may provide plants with better growth and better tolerance to abiotic stress in plants. The microbial complex may also provide the ability to enhance resistance responses in plants against a variety of soil and seed pathogens. It can be suggested that the combined application of hydrolytic priming and seed coating with biological control agents would be a reasonable alternative to chemical seed treatment (Singh, 2016).

2.1.1.1 *Trichoderma*

Sl.No	Crop	Experimental details	Reference
1	Wheat	Seed priming with <i>Trichoderma</i> isolates promoted seed germination percentage (87per cent) which is far higher than control (72.7per cent), vigour index, and root shoot growth. Based on the results, it is concluded that <i>Trichoderma</i> isolates can be used as alternative plant growth promoters for synthetic molecules and chemical fertilizers	Anjum <i>et al.</i> (2020)
2	Okra	Studies have shown that bio-primed seeds containing <i>T. viride</i> are the best treatment methods to obtain better seed quality. Carbohydrate content increased after inoculation with <i>T. viride</i> .	Rai <i>et al.</i> (2019)
3	Chick pea	Bio-seed priming with <i>T. viride</i> or <i>T. harzianum</i> provide better disease control (root rot and wilt) and improved crop yields. Use of talc formulation of <i>Trichoderma</i> spp. and <i>P. fluorescens</i> germination was increased by individual liquid fermentation (2×10^8 cfu / g) at 50 g of product / 250 ml of water / kg of seed for 10h	Pandey <i>et al.</i> (2017)

4	Rice	In a group of rice lines with the minimum and maximum root uprooting force, the differential response of <i>Trichoderma</i> seed treatment to root parameters was observed. <i>T. viride</i> isolate showed greater increase in root length, secondary branches of the root	Sureshrao <i>et al.</i> (2016)
6	Chilli	Seeds bio-primed with 60% (w/v) <i>T. viride</i> with a soaking of 3 hours germinated faster with 93% germination and have also been shown to be superior to control pathogen-transmitted diseases during germination, as well as improving seed germination and seedling vigour by incorporating a variety of biological and physiological changes during germination	Ananthi <i>et al.</i> (2014)
7	Rice	Rice plants inoculated with <i>Trichoderma</i> showed significantly higher plant height, photosynthesis rate, chlorophyll a and b content, stomatal conductivity, number of panicles. The grain yield of rice plants inoculated with <i>Trichoderma</i> fungus was 30% higher than that of the SRI control plants.	Doni <i>et al.</i> (2007)

2.1.1.2 *Pseudomonas*

Sl.No	Crop	Experimental details	Reference
1	Vegetable crops	Seeds bio-primed with <i>Pseudomonas fluorescens</i> for 12 h exhibited minimum pre- and post-emergence mortality, maximum germination (84.89%), maximum root and shoot length (15.49 cm and 12.14 cm), root weight and minimum days for 50% germination (3.72 d) and seedling vitality index (2373)	Jaiman <i>et al.</i> (2020a)

2	Okra	Plants grown from PF-treated seeds developed better tolerance to water stress by maintaining RWC, enhancing the activity of enzymatic, non-enzymatic antioxidants, and increasing accumulation of metabolites, suggesting that biochemical and physiological changes in okra enhance growth under water stress	Pravisya <i>et al.</i> (2019)
3	Kodo millet barnyard millet	<i>Pseudomonas fluorescens</i> 20% for 6 hours was considered as the best treatment to improve the physiology of seeds and quality parameters, namely germination rate, shoot and root length, dry matter production, vitality index and metabolic efficiency of kodo millet and barnyard millet seeds.	Sridevi and Manomani (2019)
4	Chilli	<i>Pseudomonas fluorescens</i> 60% biological pepper for 12 hours can withstand up to 0.50% NaCl salt stress with increased germination (95%), vigour index (2242) and increased seed length (36.43 cm)	Ananthi <i>et al.</i> (2014)
5	Barley	Seeds treated with <i>Pseudomonas</i> 20% showed an increase in root mass (17.56 cm), root dry weight (0.256 g), plant height when compared to other treated seeds	Jalal <i>et al.</i> (2014)

2.1.2 Orgo priming

Orgo priming refers to the immersion of seeds in various organic solutions alone or in combination, namely coconut water, leaf extract, cow urine, panchagavya, vermiwash etc. (Khan, 1992). When absorbed by seeds, priming helps to promote germination (Harish *et al.*, 2014). Coconut water is an essential growth supplement for plant tissue culture/micro propagation (Neumann *et al.*, 2009). The cytokines identified in coconut water are N₆-isopentenyl adenine, dihydrozeatin, trans-zeatin, kinetin,

orthotopolin, dihydrozeatin O-glucoside, trans-zeatin O-glucoside, trans-zeatin nucleoside, kinetin nucleoside and trans-zeatin nucleoside-5-monophosphate (Yong *et al.*, 2009). In seed preparation, the role of cytokinin is to mobilize the food reserve in the seed coat, causing the seed coat to absorb water. The potential of natural products such as coconut water and coconut milk as alternative primers need to be explored.

Sl.No	Crop	Experimental details	Reference
1	Aromatic crops	Treatment of seeds with 25% coconut water for 6 hours improved the emergence rate and seed quality of seedlings in the nursery. The parameters of germination, vigour and dry weight of seedlings has been increased than untreated seeds	Nagaraju <i>et al.</i> (2011)
2	Brinjal	Coconut water (50%) induced maximum germination rate (88%), shoot length (6.3 cm), seedling dry matter production (38.1 mg) and vigour index (1100). Growth hormones in coconut water may have increased the average dry weight of plants, which may be due to increased cell division in the apical meristem of seedling roots	Bhavyasree and Vinothini (2019)
3	Black gram	50ml of coconut water solution are taken in the measuring bottle and add distilled water to 1000ml. Seeds soaked in the above solution increased the germination per cent (80%), vigour indices (2942, 2773), speed of germination (23.39).	Pradhan <i>et al.</i> (2017)

4	Spanish Red Cedar	The cytokines present in CW induce the formation of adventitious shoots on rhizomes, roots and leaves, providing or activating the biosynthesis of these molecules, thus affecting the quantity and quality of protoplasmic clusters	Pena-Ramirez <i>et al.</i> (2010)
5	Rice	Seeds soaked in organic solution of coconut water (100%) for 16 h with the solution ratio of 1:0 improved seed germination and vigour in rice seeds	Sundaralingam, (2005)

Coconut water is famous for its enzymes and growth promoting substances, especially its cytokinin content. The combination of these beneficial factors can help improve germination. Mamaril and Lopez (1997) reported the positive effect of coconut water on increasing biomass and fruit yield of sweet peppers.

2.1.3. Hydro priming

Hydro priming is a simple, inexpensive, eco-friendly seed starter technology that increases the initial moisture content before and after sowing and seeding (Singh *et al.*, 2015). Humidification is increased by continuous oxygen supply and is a metabolic process to further develop food reserves (carbohydrates, proteins and fats) by creating accumulation of enzymes hydrolyzed (amylase, cellulose and xylose) to single substance (ATP) (Zulueta-Rodriguez *et al.*, 2015).

Hydro priming helps in uniform germination, early plant establishment and crop growth in chickpeas (Kaur *et al.*, 2002), faba beans (Damalas *et al.*, 2019), basil (Nahid *et al.*, 2018) and also in stressful conditions like drought (Adinde *et al.*, 2020 and Rambod *et al.*, 2016). Despite the advantages, the uncontrolled water uptake of the seed is the main disadvantage of this method because the adsorption performs the pairing of seed tissue in water. Setting the exact amount, temperature and duration of the desired humidity level to prevent radical projection is strenuous. Hydro priming is a controlled hydration process in which the seeds are maintained at a water

potential that allows absorption but prevents the protrusion of radicals. According to reports, the 16-hour hydrothermal effect leads to the maximum emergence of seedlings and greater oxidation of the seed cover (Sinha *et al.*, 2021).

Sl.No	Crop	Experimental details	Reference
1	Wheat	Wheat kernels were soaked in ordinary tap water at a ratio of 1:2 (grain: water ratio) for 12 hours, then dried in the shade. Improve the seed germination and viability parameters of wheat seed seedlings	Sharma <i>et al.</i> (2020)
2	Sunflower	Hydro-priming preserved sunflower seeds from damage due to abiotic stresses, salinity and drought	Mustafa <i>et al.</i> (2017)
3	Maize	Immersion of seeds in water for 24 hours reduced the risk of poor growth. Results showed that among the seed stimulation techniques, the hydro prime is useful in improving the seeding rate of corn. This could improve seedling establishment and field yield of this important grain	Khan <i>et al.</i> (2017)
4	Rice	16hour treatment was the most effective for crop to increase seed quality parameters for storage of seeds for longer periods	Mondo <i>et al.</i> (2016)
5	Capsicum	Seeds soaked in water for 6 hours improved physiological quality and seedling vigour	Adebisi <i>et al.</i> (2013)
6	Sorghum	Hydro priming with distilled water for 10h, improved germination and growth of sorghum plants as well as the number of leaves	Shehzad <i>et al.</i> (2012)
7	Rice	Priming with water in the grains of rice for 40 or 48 hours was the most effective of grain moisture at 9.5 (dry weight) percent, as determined by the high oven method. For the hydrolytic primers, 250g granules were soaked in aerated tap water at $25 \pm 2^{\circ}\text{C}$ then dried at the initial humidity in the shade. The dried seeds were packed in polythene bags at room	Prasad (2012)

		temperature $25 \pm 2^{\circ}\text{C}$ for later use	
8	Onion Carrot Beet root Tomato	Carrot seeds, soaked in water (36 hours) in double the weight of seeds these priming treatments can be considered a powerful tool for seed invigourating as the treated seeds can be dried to their original moisture content and stored or transported without losing the benefit of treatment measures Tomato seeds, soaked in water for 16-18 hours recorded the highest percentage of germination. Root beet, hydro priming (12 h in double seed mass) is optimal Onion seed protrusion increased steadily, 50% germination date, maximum germination date, speed germination, germination rate when primed up to 24h of soaking	Selvarani and Umarani (2011)
9	Rice	The solution ratio is 1:5 the seed were kept under water for 12 hours, then it is dried again and sealed in a polyethylene bag and stored in the refrigerator for future use	Rehman <i>et al.</i> (2011)
10	Coriander	Hydro priming improves the germination and vigour of mature and immature seeds, and its effect depends on the vigour of the seeds. Seeds aged 0, 24, and 48 hours with or without priming showed high germination rates. Increasing the ageing time to 72 hours will cause the germination rate to decrease, and the germination rate of germinated seeds is higher than that of ungerminated seeds	Rithichai <i>et al.</i> (2009)

2.2 Efficiency of seed priming on seed quality during storage

Seed priming is a controlled hydration process that involves exposing the seeds to a low water potential that restricts germination, but allows physiological and biochemical changes prior to germination (Rinku *et al.*, 2017). The harvest time of the quality of the seed of any crop depends on its time of maturity and physiological maturity. Collecting seeds at the optimum stage of maturity helps to obtain better quality seeds. The harvest stage affects the quality of the seed related to germination, vigour, vigour and storage capacity (Khatun *et al.*, 2009).

Roberts (1973) classified seeds according to their comparative physiological response to survival during storage. He introduced two types of seeds, namely orthodox seeds and stubborn seeds. Rice exhibits orthodox seed storage behaviour, which means that seeds can be dried and stored under low temperature and low moisture (Hay *et al.*, 2013).

2.2.1 Efficiency of Biopriming on seed quality

Trichoderma

Germination

Sl.No	Crop	Experimental details	Reference
1	Rice	Seeds primed with <i>Trichoderma</i> had significantly higher germination rates (93.3%), germination energy and minimum average germination time (5.7d). <i>Trichoderma</i> powder is poured into 100 g of rice grain and a small amount of water is added to create a suitable coating. Seeds are immersed in a solution corresponding to a specific priming concentration and time. Seed priming with <i>Trichoderma</i> increases seed yield and water yield at all soil potential levels	Das <i>et al.</i> (2021)

2	French bean	Seeds treated with <i>T. viride</i> (10%) showed high-quality germination parameters (76.5%) Seedling length (61.06) Vigour index (2685.15)	Dhal <i>et al.</i> (2020)
3	Rice	Biological priming for rice seeds with isolates of <i>Trichoderma</i> showed that the seedling vitality index increased significantly compared to the control in addition to <i>T. viride</i> (86.33%) and the least percentage of seed germination were observed in the control	Devi <i>et al.</i> (2019)
4	Okra	Treatment of okra seeds with <i>Trichoderma</i> along with polykote resulted in highest germination (72 per cent) than all other treatments even after 12 months of storage at ambient storage conditions.	Rosna, (2019)
5	Chilli	The probiotic <i>T. viride</i> had a significant effect on inducing higher germination at first count (75.6%) than control in all situations, in addition to increasing root length (8.74 cm)	Rai <i>et al.</i> (2018)
6	Red gram	The growth rate of <i>T. viride</i> in the talc medium also indicated that seedling germination and length as well as seedling dry weight are important attributes that determine the seed quality of any seed lot. In addition to these seed quality parameters, the seed vigour index also plays a very important role in predicting the fate of any seed lot under conditions of biotic and abiotic stress	Shahid <i>et al.</i> (2014)
7	Soya bean	<i>Trichoderma</i> spp., early germination as well as high germination rates have been reported. Higher GI results (6.84) indicate higher seed quality and better performance under a period of 4 months	Mukhtar <i>et al.</i> (2012)

8	Chickpea	Seed priming significantly improved the germination and vigour of chickpea seedling compared to seed without primer. Seeds lined with bio control agents resulted in high seedling emergence at 96% when treated with <i>T. viride</i> . Treatment with <i>T. viride</i> increased the maximum plant height (56.25 cm and 53.75 cm, respectively)	Reddy <i>et al.</i> (2011)
9	Faba bean	Field pea seeds were soaked in each prepared primer solution for 16 h. Then, the bio-primed particles were air-dried. The results confirm the current results that the antagonistic microorganisms evaluated were able to survive and maintain their antagonistic viability throughout the 6 MAS	Mougy <i>et al.</i> (2008)

Vigour

Sl.No	Crop	Experimental details	Reference
1	Baby corn	The seeds which were bio fertilized with the TV + GA combination with 75% RDF was the most effective in improving the growth of young corn roots.	Madane <i>et al.</i> (2019)
2	Seeds irrespective of crops	Biological seed treatment and seed treatment with <i>Trichoderma</i> spp. activates the release and / or production of enzymes and phytohormones and indirectly by modifying the soil micro biome and the availability of soil nutrients involved in seed germination. It also improves the germination rate and seedling vigour.	Singh <i>et al.</i> (2018)
3	Common bean	The seeds were soaked in a suspension twice the volume of the biological control agent for 4 h, then dried to the original moisture content reported higher values for germination index (9.2), root and shoot length, dry	Monalisa <i>et al.</i> (2017)

		matter yield and vigour index than control.	
4	Chick pea	Seed biology and soil bio medication showed the lowest wilt and root rot rates (8.59%) and the highest seed germination rates (96.69%), vigour index (2734) and grain yield (1535 kg / ha) at 10 h of treatment with a talc-based suspension (2x10 ⁸ cfu / g) of <i>T. viride</i> and prevents loss of content in oil for up to 6 MAS.	Pandey <i>et al.</i> (2017)
5	Bean	Seeds inoculated with <i>Trichoderma</i> spp., improved seedling yield compared with other treatments, demonstrating the continued benefit of biological treatment for seedlings already grown in soil. Treatments did not significantly affect root growth, but suspensions and physiological conditioning techniques increased root dry mass.	Junges <i>et al.</i> (2016)
6	Mung bean	The seed treatment of <i>T. viride</i> recorded the maximum volume of viability index (81.17), in addition to the good formation and adhesion of the fungi which increased the quality of the seeds.	Supriya <i>et al.</i> (2014)
7	Maize	Study reported effect of seed treatment with liquid biofertilizer on germination and seedling vigour and seeds bio fertilized with <i>T. viride</i> 60% for 12h gave highest germination rate up to 4 months of storage of hybrid	Karthika and Vanagamudi (2013)

Moisture

Sl. No	Crop	Experimental details	Reference
1	Rice	Seeds were bio incubated with <i>Trichoderma</i> (@5 g/kg seeds) and stored in IRRI super bags for 6 to 12 months of storage at 8% humidity	Dar <i>et al.</i> (2019)
2	Soya bean	The drying process consists of heated air passing through the grain and dehumidifying the outermost layers of the grain, reducing the grain's moisture content. During storage period of 6 to 12 months, soybeans should be dried at 13% humidity and for longer storage period soybeans should be dried at humidity between 10 and 11%	Mutai, (2018)
3	Okra	Seeds treated with <i>Trichoderma</i> can be stored for up to 5 months with soaking time of 16 hours before sowing. Seed treatment improved germination and seedling vigour. Wrapping okra seeds in polyethylene bags (gauge 700) increased shelf life up to 7 months.	Singh <i>et al.</i> (2014)
4	Rice	Seeds treated with <i>T. viride</i> @ 67g per kg were collected in a plastic container soaked in twice the amount of water needed for chemicals and water for about 12 hours at 25°C completely dried to 8 percent moisture	Priya <i>et al.</i> (2018)

Pseudomonas

Germination

Sl. No	Crop	Experimental details	Reference
1	Sorghum	The higher germination percentage (91.44%) recorded in the present study may be due to the fact that seeds primed with 20% <i>P. fluorescens</i> showed increased seed	Prakash <i>et al.</i> (2021)

		metabolic efficiency compared to unprimed seeds. Higher metabolic efficiency leads to the mobilization of food reserves for embryos to initiate early germination	
2	Rice	19 isolates of <i>P. fluorescens</i> as biological bait from rice seeds showed a stimulating effect on plant growth. Inoculation of pseudomonas also improved the fresh and dry weight of shoots and roots by 95.68%, ultimately increasing plant yield.	Tomer <i>et al.</i> (2020)
3	Proso millet	<i>P. fluorescens</i> will increase the production of growth hormones such as gibberellins, which further activate the enzyme amylase. These enzymes play an important role in promoting early germination (90.13%) by increasing starch uptake. <i>Pseudomonas fluorescens</i> 20% had a higher germination rate than unmarked seeds with low seed leachates ($26.3\mu\text{Scm}^{-1}$) indicating increase of storage period	Sridevi and Manomani (2019)
4	Chilli	Seed bioprimering with 60% (w/v) <i>P. fluorescens</i> preparation for 3 h or 12 h, respectively, can be used to improve seed germination (95%) seedlings had higher biomass accumulation ($4.7\text{ mg seedlings}^{-1}$) and vigour of chili seedlings	Ananthi <i>et al.</i> (2014)
5	Vegetables	<i>P. fluorescens</i> persists on seeds for at least 180 days. In addition, population growth and viability of bait resistant seeds during storage have been reported for viability of <i>P. fluorescens</i> without significant decrease during storage for up to 10 months with final germination (84.89%)	Abdel-Khader <i>et al.</i> (2012)

Vigour

Sl.No	Crop	Experimental details	Reference
1	Barnyard Millet	The dehydrogenase and β -amylase enzyme activities were higher in the bait seeds of <i>P. fluorescens</i> . The improvement in seedling growth enzyme activity and microbial counts seen in this study may be due to the suppression of harmful microorganisms, pathogens, production of plant growth regulators such as Gibberellic acid (GA), cytokinin, Indole Acetic Acid (IAA) availability of minerals and other ions as well as the ability to absorb more water also improves the mobilization of nutrients from the seed.	Iswariya <i>et al.</i> (2019)
2	Rice	Rice seeds soaked in an equal volume of liquid microbial service, i.e., for 18 h, showed higher germination and vigour. The viability and viability of biological seeds were not significantly affected during 3 months of storage	Raja <i>et al.</i> (2017)
3	Chilli	This study included that the seeds primed with pseudomonas (10g/kg) showed increased vigour, seedling length and seedling dry weight with a storability potential up to 9 months when stored in 700-gauge polythene bags	Parisa, (2013)
4	Sunflower	This study showed that bio culture with <i>P. fluorescens</i> improved the vigour of sunflower seeds and uniformly grown seedlings. It can be concluded that microorganisms have the potential to proliferate, proliferate and produce PGR during priming	Moeinzadeh <i>et al.</i> (2010)

Moisture

Sl. No	Crop	Experimental details	Reference
1	Pepper	The use of <i>Pseudomonas</i> and <i>Bacillus</i> strains for biopriming in this study can stimulate rapid and uniform germination and faster seedling growth. 7-7.5% moisture and dry seeds are kept at 4°C in an aluminum foil bag until use	Yildirim <i>et al.</i> (2021)
2	Chilli	The study reported that pepper seeds bio primed with <i>P. fluorescens</i> for 60 hours can tolerate low (20% WHC) and high (80% WHC) moisture regimes	Ananthi <i>et al.</i> (2019)
3	Faba bean	The present results demonstrate that bean senescence is associated with changes in the activity of antioxidant enzymes and electrolyte leakage. At the end of the storage period, temperatures of 25 and 35°C, and humidity of 18 and 22%, exhibited the lowest levels of antioxidant enzymes and the highest electrolyte leakage, concomitant with a decrease in the germ rebound rate at this temperature and humidity	Kooshki <i>et al.</i> (2018)
4	Rice	<i>P. fluorescens</i> @ 20% with corresponding immersion time of 18 h at 25°C. Seeds, after priming and drying to initial moisture content, were packed in cloth bags and stored at room temperature for a period of 6 months with germination recorded at least by the sixth month of storage.	Nithya and Geetha (2017)

2.2.2 Efficiency of Organic priming on seed quality

Coconut water

Germination

Sl.No	Crop	Experimental details	Reference
1	Cucumber	Percent germination, root length and shoot length increased with 100 percent CW for 24 h, resulting in better seedling formation. Early emergence in treated seeds may be due to the production of faster germinating metabolites and better genetic repair.	Shakuntala <i>et al.</i> (2020)
2	Ground nut	50% coconut water soaked for 6h induced the maximum germination rate (91%), shoot length, seedling dry matter yield and vigour index. Therefore, 50 percent coconut water can be used effectively to determine the biological value.	Vinothini and Bhavyasree (2019)
3	Chilli	Primer with coconut water (3%) increased germination (%) and seed vigour in chili peppers. Coconut water (3%) had a high mean value for seedling properties such as seed germination rate (85.5%), germination energy (46%), germination rate (16), root length (5.125 cm), shoot length (5.625 cm), seedling length (10.8), seedling fresh weight (2.0 g) and seedling dry weight (0.023 g), Seed Viability Index I (929.2) and Seed Vitality Index II (1998), compared to other treatments.	Reddy <i>et al.</i> (2018)
4	Rice	Effect of seed priming for 12 hours with coconut water has improved the germination rate of rice. The ideal rice plant height for transplanting is 18-20 cm achieved on the 13th day after sowing on seeds soaked in tap water for 12 hours. Primer for 12 hours has improved seed germination and early sprouting of rice	Gapasin-Catada <i>et al.</i> (2016)

Vigour

Sl.No	Crop	Experimental details	Reference
1	Bitter kola	Seedling appearance, shoots dry weight (0.97g/plant) and root dry weight (0.40g/plant) were improved by priming. Dried bitter kola seeds were soaked in coconut water for early seedling growth and development.	Nwonuala and Christo (2021)
2	Carrot	Fresh seed lots with high vigour, higher enzyme activity, and even better priming performance could be attributed to a change in ribonucleic acid by increased enzyme activity in seeds primed with coconut water @ 12.5% which showed storage up to 3-4 months	Sowmeya <i>et al.</i> (2018)
3	Pepper	The pepper was soaked in coconut water for 12 hours. Plants fertilized with GA and CW had the highest germination rate, number of leaves and plant height while the control (without fertilizer) had the lowest. Gibberellic acid and coconut water have high seed vigour enhancing abilities resulting in high germination and growth rates of pepper plants.	Chuwang <i>et al.</i> (2018)

Moisture

Sl.No	Crop	Experimental details	Reference
1	Rice	Coconut water (75%) for 12 hours at 11-12% humidity its effect on genetically diverse genotypes to prolong seed life. Therefore, study carried out showed it as the best seed priming method to increase the germination and vigour of rice seeds.	Somasundaram and Bhaskaran (2017)

2	Rice	Priming is made by soaking rice grain coconut water for 12 and 24 hours. Subsequently, the ratio of 1 g / 100 ml (%) to the weight of the rice grain and of the primers used was. The seeds were removed from the primers, put on head of a clean cloth, and air dry in about 5 hours. Then the seed is sown.	Catada <i>et al.</i> (2016)
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2.2.3 Efficiency of Hydro priming on seed quality

Germination

Sl.No	Crop	Experimental details	Reference
1	Faba bean	Seed hydro priming for 16h accelerated faba bean germination (97.3%), mean germination time (5.96 d) and seedling emergence in the field,	Damalas <i>et al.</i> (2019)
2	Bitter gourd	Seed soaking in hot water (45°C) for 5 min effectively enhanced germination (87%), seedling vigour (63.2), other growth parameters such as primary branch length, leaf area, etc. exhibited better performance than the untreated control seeds.	Tania <i>et al.</i> (2019)
3	Onion	Seeds of onion cv. CO5 with 8% moisture content were soaked in equal volume of water for 6h. Priming induces metabolic activities of germination and the resulting sugars can be used for protein synthesis during germination which improves germination rate (88%) and uniform growth of the plant	Saranya <i>et al.</i> (2017)

4	Rice	Hydro priming as a successful technique for increasing and hastening of seed germination (100), germination index (9.36) and better crop stand under rainfed conditions.	Subedi <i>et al.</i> (2015)
5	Rice	Priming rice grains for 48h in water improved germination (72.54%) and seedling growth (56.44 cm) under drought and salinity stress conditions.	Khafagy <i>et al.</i> (2014)
6	<i>Salvia officinalis</i> L	Seed hydropriming is a very effective technique in improving seed germination and the growth of seedlings of <i>S. officinalis</i> L. Hydropriming for 12 h at temperature 30°C was the most effective treatment, while hydropriming for 48h at this temperature resulted in lowest germination. Improved seed germination due to hydropriming may be explained by an increased water uptake and rate of cell division.	Dastanpoo <i>r et al.</i> (2013)
7	Rice	Hydro primed seeds (10h soak period) have shown a viability period of 7.8 months which prolonged storage life of 4.7 months when compared to unprimed seeds stored under ambient conditions.	Guzman and Aquino (2007)

Vigour

Sl. No	Crop	Experimental details	Reference
1	Rice	Hydro primed seeds showed more shoot and root length along with vigour of seedlings compared to other treatments for 24 hours soak in ratio of 1:2 (kg of seeds/volume of solution)	Afreen <i>et al.</i> (2021)

2	Rice	Hydrogen priming increased α -amylase activity and total sugar by soaking and drying the seeds. In primed seeds, significant correlations were observed between α -amylase activity and mean emergence time, α -amylase activity and seedling dry weight of seedlings.	Nakao <i>et al.</i> (2020)
3	Chick pea	Primed seeds with high rate of germination also resulted in high emergence index while a negative relationship was demonstrated by root length and electrical conductivity. A significant correlation between seedling emergence index and other vigour tests for a period of 6 months	Sori, (2014)
4	Maize	Hydropriming for short durations (3 to 9 h) may prevent reductions in the vigour of primed seeds as they age in initial storage period. The amelioration of the ageing process is likely due to reduction in oxidative stress by antioxidant enzymes (SOD and APX).	Wattanakupakin <i>et al.</i> (2012)
5	Basil	The seedling vigour, germination percentage and seedling dry weight increased with increasing in treatment duration. Also, highest seedling vigour, germination percentage and seedling dry weight were achieved by hydropriming came up to 12 hours	Farahani and Maroufi (2011)
6	Common vetch	The performance of two-year aged seeds and freshly harvested seeds were on par as compared to freshly harvested seeds. This proves the effect of 24 h duration of hydropriming stored under room temperature initial moisture 10-11%	Kalsa <i>et al.</i> (2011)

7	Maize	Hydro-priming for 18h was the best treatment for improving germination index (26.43%), T ₅₀ (2 d), seedling vigour and field emergence of maize. These effects can improve seedling establishment and field performance of this important cereal with a storage up to 6 months	Mahmoodi <i>et al.</i> (2011)
8	Maize	Priming with water for 36 h was better than other priming media tested for high vigour (96.88) and rapid seed germination (82%)	Dezfuli <i>et al.</i> (2008)

Moisture

Sl. No	Crop	Experimental details	Reference
1	Rice	Hydro-priming for 72 h to enhance the seed quality. Further, seeds of these two varieties could be stored in polythene bags at 8°C (normal refrigerator temperature) as it can maintain seed viability at 85 % up to 6 months	Galappaththi <i>et al.</i> (2020)
2	Rapeseed	Seeds held at 15 and 25°C were stored for up to eight months and seeds held at 35 and 45°C for up to 4 months at 9% moisture after the treatment	Malek <i>et al.</i> (2019)
3	Rice	The interrelationship between seed moisture content and storage temperature plays more important role in influencing seed viability during storage. if primed seeds were stored at vacuum or low RH or low temperature conditions, there will be no negative	Wang <i>et al.</i> (2018)

		effect on seed viability within 60 days of storage	
4	Ground nut	Seeds were stored in HDPE bags under ambient conditions for a period of 5 months. Hydropriming has been reported to be effective in improving seed quality at 7% moisture	Das and Mohanty (2018)
5	Okra	Priming for 12 h, re-dried to 8% moisture and stored in aluminum pouches for medium term storage	Sharma <i>et al.</i> (2014)
6	Maize	The medium vigour (Six-month-old) seed lots of five maize hybrids having germination up to minimum seed certification standard (MSCS) were dried to 9% moisture are used for the study during 2010-11. The seeds were subjected to priming at 25°C with distilled water soaking in same quantity of water/aqueous solution of chemical (1:1 seed and water/solution) for 18 hours at ambient temperature followed by drying back to original moisture content under shaded condition	Kumar <i>et al.</i> (2013)
7	Wheat	Hydro primed seeds are re-dried closer to their original weight up to 9.12% moisture under shade with forced air at 27 ± 3,	Jafar <i>et al.</i> (2012)

		sealed in polythene bags and stored in a refrigerator until use	
8	Rice	Hydro primed seeds dried back closer to original moisture level under forced air at $27 \pm 3^{\circ}\text{C}$, sealed in polythene bags and stored in a refrigerator at 5°C until use	Farooq <i>et al.</i> (2008)
9	Rice	The initial seed moisture contents were 8.04% and 8.43% (dry weight basis) in coarse and fine rice, respectively. Hydro priming was achieved using a weighed quantity of seeds (250 g) that was soaked in aerated tap water at $27 \pm 2^{\circ}\text{C}$ for 12, 24, 36, 48 and 60h followed by drying to initial moisture under shade with forced air (with electric ceiling fan) Seeds were then sealed in polythene bags and stored at 5°C for further use	Farooq <i>et al.</i> (2006a)
10	Rice	Seed moisture contents of coarse and fine rice were 7.89% and 8.06%, respectively re-dried to original weight with forced air under shade at $27 \pm 3^{\circ}\text{C}$. These seeds were then sealed in polythene bags and stored in refrigerator at 5°C before further use.	Basra <i>et al.</i> (2006)
11	Rice	Primed seeds were given re-dried closer to original moisture (8%) under forced air at $27^{\circ}\text{C} \pm 3$. These seeds were put in polythene bags	Farooq <i>et al.</i> (2006b)

		and stored in a refrigerator at $5 \pm 1^\circ\text{C}$ until used.	
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2.3 Seed infection across storage period

Sl. No	Crop	Experimental details	Reference
1	Rice	Due to the coating of the seeds with the antagonist spore mass (<i>Trichoderma</i> spp.), the germination rate and healthy seedlings are improved. In addition, leaf infection, crown infection, root infection and seedling leaf blight were significantly reduced compared to the untreated control. Bio control agents can compete with and control the growth of pathogens and the production of plant growth promoting hormones	Deb and Khair (2020)
2	Tomato	Seed priming and seed fertilization with antagonistic microorganisms in controlling root rot rates compared with other treatments, i.e., seeds treated with <i>Pseudomonas</i> showed only the least percentage of infected seeds (14%) with increased germination and vigour	Rajaput <i>et al.</i> (2019)
3	Green gram	10 g of biological agent (10^8 cfu/g) was suspended in two liters of sterile distilled water. 1 kg of seeds collected in suspension in the above solution and left to soak for 8 h is recommended for the meaningful management of the <i>Alternaria</i> leaf spot disease	Deshmukh and Sabalpra (2019)
4	Sweet potato	<i>Trichoderma</i> species are known to produce enzymes such as chitinase, pectolytic, and amylase which help them break down the chitin component present in the cell wall of invading fungi and facilitate their entry. Although the <i>Trichoderma</i> species	Dania, (2019)

		evaluated in this study significantly reduced ($p < 0.05$) tuber rot rates in in vivo tests, however, they were not able to completely suppress diseased germicidal activity	
5	Sorghum	<i>T. viride</i> has been shown to be effective in reducing fungal diseases in sorghum. Esp. inhibits linear growth and microsclerotia in addition to being able to test growth of <i>Macrophomina phaseolina</i> in vitro	Vaja <i>et al.</i> (2018)
6	Maize	Seed priming with coconut water (3%) not only reduced disease infection but also increased plant growth characteristics. It has been suggested that CW can be effectively used as a corn seed treatment to promote plant growth and reduce infection by plant diseases, including nematodes	Kumhar (2018)
7	Cabbage	Disease rates in cabbages resistant to Pusa mukta were reduced by 4 to 3% when seeds were treated with <i>P. fluorescens</i> , while in cabbage varieties susceptible to disease rates were reduced from 66 to 40% when seed treatment with <i>P. fluorescens</i>	Umesha <i>et al.</i> (2017)
8	Seeds irrespective of crop	<i>Trichoderma</i> treatment can also be used to correct various fungal diseases originating in the soil. The many plant pathogens for which this biological control agent is effective include <i>Botrytis cinerea</i> , <i>Pythium</i> spp., <i>Sclerotium rolfsii</i> , <i>Rhizoctonia solani</i> , <i>Sclerotinia homoeocarpa</i> and <i>Fusarium</i> spp.	Arora <i>et al.</i> (2017)
9	Sorghum	Coconut water as a seed primer covers the micro cracks and aberrations that cover the seed coat, preventing the entry of fungi. It can also act as a physical barrier, reducing the leaching of inhibitors from the seed coat and limiting the movement of oxygen and thus reducing embryonic respiration, thus reducing the impact of ageing for	Arvind Kumar <i>et al.</i> (2015)

		coconut water (88.40%) seed germination rate, seedling dry weight, germination rate, seed vigour length, seedling length, root length, test bud length	
10	Chick pea	Seeds treated with only probiotics @ 5% (<i>Trichoderma</i> @ 5g/kg) is considered as best seed treatment to improve seed quality, especially by reducing the incidence of wilt disease. Therefore, instead of treating seeds successively with different concentrations of probiotics, fungicides and pesticides	Kumar <i>et al.</i> (2014b)
11	Rice	Research has shown that <i>P. fluorescens</i> can reduce bacterial blight caused by seed treatment. Disease rates were significantly reduced when seeds were treated with <i>P. fluorescens</i> and then inoculated with the pathogen	Shivalingai ah and Umesha (2013)
12	Onion	Primed and non-primed onion seeds were stored in air-tight plastic containers for 6 and 12 months at 4 and 20°C. Deterioration of germination after 12 months storage was connected with a significant increase in the percentages of diseased and deformed abnormal seedlings where <i>Penicillium</i> spp., <i>Botrytis</i> spp. was observed on infected seeds	Dorna <i>et al.</i> (2013)
13	Soya bean	The hydropriming is beneficial to improve the quality of soybean seeds with low incidence of storage fungi (<i>Aspergillus</i> spp., <i>Pencillium</i> spp.) with increments on speed of germination (first count) and seed germination after accelerated ageing test. The high incidence of microorganisms can reduce the hydropriming benefits	Costa <i>et al.</i> (2013)
14	Okra	Seeds treated with <i>Trichoderma viride</i> along with polykote resulted in less seed infection when compared to untreated seeds and have observed	Reshma, (2018)

		<i>Aspergillus flavus</i> , <i>Aspergillus niger</i> and <i>Rhizopus</i> spp. in infected seeds.	
15	Chilli	Seeds when treated with botanicals has stored till 14 months with less seed infection of 13.33 per cent when compared with untreated seeds. Storage microbes such as <i>Aspergillus</i> sps., <i>Pencillium</i> sps., <i>Alternaria</i> sps. were observed in infected seeds.	Sandhya, (2016)

2.4 Storability evaluation through artificial ageing

Biochemical and physiological deterioration during seed ageing occurs mainly under accelerated ageing conditions using high temperatures and high grain moisture content (Mc Donald, 1999). Several studies indicate that lipid peroxidation and degradation of phospholipid membranes are the main causes of grain senescence under conditions of accelerated ageing (Hsu and Sung, 1997). Rapid ageing of seeds within days of exposure to high temperature and high relative humidity is a good predictor of grain storage capacity (Sung and Jeng, 1994). Seeds that deteriorate rapidly due to rapid ageing will perform less well in long-term storage (Delouche and Baskin, 1973). The processes used in seed storage induce seed senescence and induce radical-mediated lipid peroxidation, inactivation or proteolysis of enzymes, disruption of cell membrane and damaged genetic material such as nucleic acids (McDonald, 1999; Murthy *et al.*, 2003) leads to poor germination.

To speed up the ageing process, the seeds were exposed to 45°C and 100% Relative Humidity (RH) for 24, 48, 72, 96 and 120 hours. The gradual increase in lipid peroxidation products corresponds to the ageing time, a sign of lipid degradation. This degradation is particularly relevant for the storage and germination of seeds because it is assumed that these lipid fractions are degraded from the cell membrane of seeds. Cell membranes containing a high percentage of lipids are susceptible to oxidative damage and free radicals are generated in the cell membrane during the rapid ageing process and are very harmful to the cell membrane. Loss of seed viability with accelerated ageing in the eight rice varieties tested was associated

with increased grain moisture content (grain electrical conductivity), electrolyte leakage and lipid peroxidation (Bijanazadeh *et al.*, 2015).

Sesame seeds are quickly incubated by incubating the seeds at $45 \pm 1^\circ\text{C}$ and 100% relative humidity. Seed samples were taken periodically after 1-8 days, dried and stored in a desiccator with calcium chloride for 5 days to balance the moisture content of the seeds. Aged seeds with varying degrees of vigour and viability were used. The seeds were germinated in a filter paper lined incubator for 96 h in a BOD incubator under light. Only seeds that produced normal seedlings were considered germinated; germination rate, seedling length, fresh and dry weight were recorded (Saxena *et al.*, 1985).

The results suggest the need to be careful when increasing the MC of prepared lettuce seeds to accelerate their ageing process. Commercially prepared lettuce seed quantities are often bagged to prevent moisture migration between the seed and the medium. Increasing the MC of primed lettuce seeds on top of seeds in which they have been packed may not provide an accurate prediction of their potential shelf life, especially when comparing seed products that have been are primed differently (Hill *et al.*, 2008).

The rapid ageing of the control seeds of sorghum by permeation and hydrolysis was obtained by incubating the seeds in a sealed plastic chamber at 40°C and 100% relative humidity for 6 days. After ageing, the seeds were air-dried at 25°C until the original weight was restored. The negative effects of accelerated ageing on the parameters of the primed seeds increased with longer priming time and the lowest occurrence rate was observed with water priming for 36 h (Moradi and Younasi, 2009).

By equilibrating the seeds from $40\pm 1^\circ\text{C}$ to about 100% RH for 72 hours, the seeds were applied to controlled degradation (ageing) and dried at room temperature to reach their original water content. These weaknesses have sparked interest in

vitality testing to provide information on seed quality and longevity. Okra varieties evaluated seed vitality based on seedling length and dry weight of the seedlings. Although seed vitality decreased even after accelerated ageing, Varsha, Uphar recorded maximum vitality, indicating that this variety well resists stress conditions in both of the three varieties (Raj *et al.*, 2013).

The seeds of Bitter gourd were checked for deterioration (rapid ageing) by equilibrating the seeds at about 90% relative humidity at $40 \pm 2^{\circ}\text{C}$ for 72 h in a desiccator. The seeds were then dried again at room temperature to reach a moisture content of 80%. Seed priming improved germination and other seed quality parameters of viable seeds (Kanwar *et al.*, 2014).

The corn seeds were artificially incubated at two different temperatures (40 and 42°C), four relative humidity levels (85, 90, 95 and 100%) and four periods (3, 6, 9 and 12 days) and the observations were recorded after ageing. In the case of natural ageing, observations were recorded monthly for corn kernels stored in cloth bags at ambient conditions for up to one year. Decreased germination of older legume seeds was correlated with decreased activity in enzyme antioxidant studies. These results support the hypothesis that a decrease in antioxidant enzymes is associated with increased lipid peroxidation and accelerated ageing. Next, a positive relationship has been proposed between the antioxidant capacity of enzymes and the vigour of seeds (Radha *et al.*, 2014).

The accelerated ageing process was carried out with thirty grams of cucumber seeds were placed on wire mesh trays, then placed in distilled water in a closed incubation chamber to reach 45°C and 100% relative humidity for 3, 6, 9, 12, 15, 18 and 21 days. Then all the seeds were dried at 35°C for 24 h using an improved air dryer until the moisture content of the kernels decreased to the initial moisture content of 8.1%. While total peroxide decreased the most to 36.9% during 6 days of accelerated ageing (Kairnat *et al.*, 2015).

Accelerated ageing was developed as a test method for estimating rice seed longevity under various storage conditions. The increased MDA (malondialdehyde) content of rice seeds after accelerated seed ageing treatment resulted in increased cell division and seedling elongation of rice seedlings (Kanto *et al.*, 2015).

Fresh black gram seeds with 98% germination rate were rapidly incubated for 4 days. It is done by packing the seeds in a paper bag with a uniform pinhole size and placing in an incubator containing 100ml of double distilled water to maintain a relative humidity of $98\pm 2\%$ and the whole being kept in an incubator maintained at $40\pm 1^\circ\text{C}$ for four days to reduce germination by about 80%. Seed priming in the case of mature seeds to reduce the harmful effects of ageing and subsequently improve its physiological performance during germination (Ramanujan *et al.*, 2017)

The rice seeds were exposed to stress for 72h at $43\pm 2^\circ\text{C}$ and 100% humidity. The results reported that the larger grain size achieved the highest germination rate in ageing rate while the smaller grain size recorded a lower average of this trait. Cytokinin is a seed activator and improves the yield and germination of large seeds (Saudi, 2017).

The accelerated ageing test using saturated NaCl solution at 45°C for 48 hours is effective in assessing the quality of sesame seeds. There are variations in the pattern of expression of the enzyme's superoxide dismutase, catalase, esterase, malate dehydrogenase, alcohol dehydrogenase, in the process of accelerated ageing of sesame seeds. The esterase profile of sesame seeds conventionally incubated for 48 h can differentiate cultivars (Nery *et al.*, 2018).

Seeds of hybrid maize (*Zea mays* L.) were purchased for accelerated ageing; the seeds were dried at a high temperature of $45 \pm 1^\circ\text{C}$ and about 98% humidity in an airtight desiccator. The aged seeds were pre-treated with distilled water for 12 and 24 h at $20 \pm 2^\circ\text{C}$ in the dark. After drying the seeds for 10 h at room temperature, the seeds were used for germination characteristics assessment and biochemical analysis.

The results suggest that the antioxidant defense system can have a major impact on seed vigour. Therefore, seed soaking for 12h and 24h in water can be considered as a good hydrolytic treatment to improve germination and growth of mature corn kernels under unfavorable storage conditions (Ghahfarokhi *et al.*, 2019).

Accelerated ageing sharply reduces seed vitality and viability by exposing the seed to two seed-deteriorating variables: high temperature and high relative humidity. Highly vibrant seeds deteriorate at a slower rate than their vibrant seeds. Onion seeds were wrapped in a muslin cloth bag and placed on a wire mesh in a closed dryer filled with demineralized water. Be careful not to let the seeds come in contact with water. The desiccator was placed in an incubator maintained at 45°C and 100% RH for 72 hours. Primed seeds have been reported to shift the effects of accelerated ageing and improve the shelf life and performance of onion seeds under adverse storage conditions (Yalamallae *et al.*, 2019).

Black bean seeds (cultivar: Arka garima) were collected at IIHR and artificially aged for 4 days at 45°C and 75% relative humidity (RH) according to ISTA procedures. Results showed that priming restores the vitality of the lost seeds of the aged seeds due to the reactivation of the aged seed proteins, and that expression of specific proteins in the priming treatment is related to inhalation-induced proteins in the priming treatment, unlike the lack of the aged seeds required for germination which interprets the longevity of seeds (Arun *et al.*, 2021)

Material and Methods

III. MATERIAL AND METHODS

This study entitled “Impact of bioprimering on seed quality and longevity in Rice” (*Oryza sativa* L.) was undertaken at the Department of Seed Science and Technology, College of Agriculture, Vellanikkara from December 2020 to August 2021 as detailed below.

3.1 Location

The area is located at latitude 10^o 54 N and longitude 76^o 28 E at an altitude of 40 m above sea level, the area has a typical hot and humid climate and receives an average rainfall of 2663 mm per year. The site experiences humid tropical climate. The monthly mean meteorological data recorded at Meteorological Observatory, College of Agriculture, Vellanikkara during the course of study (December 2020 to September 2021) is presented in Table I.

3.2 Climatic condition

Average weather data from December 2020 to September 2021 revealed the maximum temperature average of 32.52°C and average minimum temperature of 22.88°C. Humidity over time period ranges from 75-96 per cent.

3.3 Seed source

Freshly harvested seeds of germination percentage 94.5% of the high yielding rice variety Jyothy were collected from the Department of Seed Science and Technology, College of Agriculture, Vellanikkara.

3.4 Description of variety

Jyothy (PTB-39) is commonly known as vadi rice and is locally described as Palakkadanmatta. It was released from RARS, Pattambi, KAU (1974). It is a red kernelled variety suitable for direct seeding, transplanting and special systems of Kole and Kuttanad, suitable for direct sowing. The duration of the variety is 110-125 days. It is cultivated largely in Kerala and Karnataka. In Kerala, it is mainly cultivated in the fields of Palakkad and Kuttanad.

Table I: Monthly meteorological data from December 2020 to August 2021

Month	Temperature (°C)		Rainfall (mm)	Rainy days	RH (%)
	Mean max	Mean min			
December	32.0	21.9	7.7	1	75
January	32.3	21.3	45.7	1	78
February	34.6	21.6	0.0	0	70
March	36.8	23.0	31.8	1	84
April	34.9	23.6	72.4	4	89
May	32.7	22.9	550.5	16	94
June	31.2	23.7	473.0	21	94
July	29.8	23.5	626.9	22	96
August	30.2	23.4	409.1	22	96
September	30.7	23.9	291.7	14	83

3.5 Experiment material

The study consisted of two experiments:

Experiment I: Effect of biological primers on seed quality and shelf life

Experiment II: Accelerated ageing experiment

3.5.1 Layout

Design : Completely Randomized Design (CRD)

Replications : 3

Variety : Jyothy

Treatments : 9

3.5.2 Treatment (T) details

Sl. No	Treatment	Dosage/Kg of Seed
T ₁	<i>Pseudomonas fluorescens</i>	10g
T ₂	<i>Trichoderma viride</i>	4g
T ₃	Coconut water	75%
T ₄	<i>P. fluorescens</i> + <i>T. viride</i>	10g + 4g
T ₅	<i>P. fluorescens</i> + coconut water	10g + 75%
T ₆	<i>T. viride</i> + coconut water	4g + 75%
T ₇	<i>P. fluorescens</i> + <i>T. viride</i> + coconut water	10g + 4g + 75%
T ₈	Hydro priming	Water
T ₉	Control	Untreated seeds



Trichoderma viride
talc powder (4g/kg)

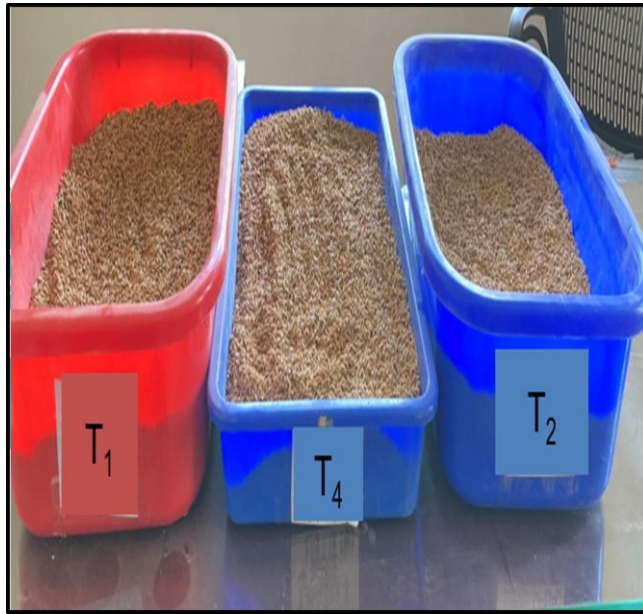


Pseudomonas fluorescens
talc powder (10g/kg)

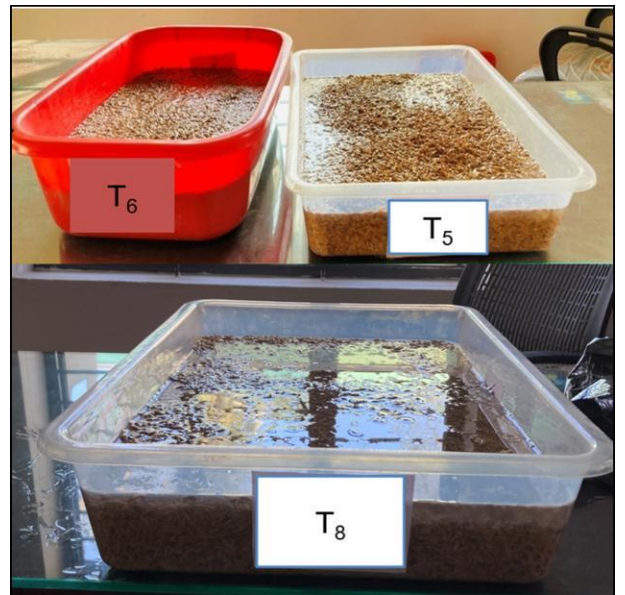


Coconut water (75%)

Plate-1: Seed priming treatments in rice



Dry treatment



Wet treatment

Plate- 2: Dry and wet method of imposition of seed priming



Plate-3: Polyethylene (700 gauge) packaging of primed seeds

3.5.3 Imposition of treatments

T₁: *Pseudomonas fluorescens*

Seeds dried to a moisture content of < 10 per cent were dry dressed with the talc-based formulation of *P. fluorescens* @10g/kg of seed (Nithya and Geetha, 2017).

T₂: *Trichoderma viride*

Seeds were dried to a moisture content of less than 10 per cent treated with *Trichoderma* @ 4g/kg and packed (Pan *et al.*, 2011).

T₃: Coconut water

Seeds were soaked in coconut water such that seeds were fully immersed in solution for a period of 16hours. Drained seeds were kept for shade drying and moisture brought down to < 10 per cent and packed (Lopez, 1997).

T₄: *P. fluorescens* + *T. viride*

Seeds were dry dressed with the combination of *Pseudomonas* @ 10g/kg and *Trichoderma* @ 4g/Kg of seed and packed.

T₅: *P. fluorescens* + coconut water

Seeds were wet dressed with combination of *Pseudomonas* @10g/kg and coconut water @ 75per cent. The seeds were soaked for 16 hours with a seed/solution ratio of 1:1.5 (weight:volume) and was re-dried to the original moisture content and stored at room temperature (Black, M and Bewley, 2000).

T₆: *T. viride* + coconut water

Seeds were wet dressed with combination of *Trichoderma* @4g/kg and coconut water @ 75per cent such that seeds were fully immersed in solution up to 16hours and then they were kept for shade drying till the moisture of seeds reach down to less than 10 per cent

T₇: *P. fluorescens* + *T. viride* + coconut water

Seeds were wet dressed with combination of *Pseudomonas* @10g/kg, *Trichoderma* @4g/kg and coconut water @ 75per cent. The seeds were soaked for 16 hours with a seed/solution ratio of 1:1.5 (weight: volume) and was redried to the original moisture content and stored at room temperature.

T₈: Hydro priming

Seeds are soaked in tap water for 16 hours. The seed/solution ratio was 1:1.5 (weight: volume). The treated seeds (5 kg/repeat) were dried in the shade to less than 10 per cent moisture content (Mondo *et al.*, 2016)

3.5.4 Packaging of Seeds

The dried seeds were portioned to small parts and were placed in 700-gauge polythene bags and sealed air tight and were stored at ambient conditions.

3.6. Artificial ageing

The treated seeds were packed in butter paper bags with pin holes to absorb moisture for ageing and placed in the wire gauge / mesh above the water well such that seeds are not in touch with water inside BOD incubator from 0 to 7 days and received a temperature of 40 ± 1°C and a relative humidity of 98%. Accelerated aged samples were taken at daily intervals and received further tests to determine a variety of quality parameters (Delouche and Baskin, 1973).

3.7 Observations recorded

3.7.1 Seed moisture content

The moisture content of the seeds was estimated as per the procedure of high constant temperature protocol of ISTA (2010). Four replicates of 5g seeds from each replication of each treatment were weighed and ground to a coarse powder. The powdered seed material

was placed in, a weighed moisture aluminum cup and after removing the lid, kept in a hot air oven maintained at $103 \pm 2^\circ\text{C}$ for 17 ± 1 hour and were allowed to dry. The aluminum cup was closed using the lid on removal from hot air oven and allowed to cool in a desiccator for half an hour. Later, the aluminum cup along with the lid and seed was weighed using an electronic balance. The moisture content was calculated as per the equation below and expressed as per cent

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

Where, M1: Weight of the moisture cup alone

M2: Weight of the moisture cup + sample before drying

M3: Weight of the moisture cup + sample after drying

3.7.2 Germination (ISTA, 2001)

The germination test was performed using four replicates, 100 seeds which is repeated thrice representing 3 replicates for each treatment in paper medium (Roll towel method) in the nursery chamber, maintained at $25 \pm 1^\circ\text{C}$ and $90 \pm 2\%$ RH. At the end of the fourteenth day, the number of normal plants in each propagation was counted and the average germination percentage was calculated.

$$\text{Germination (\%)} = \frac{\text{Number of seed germinated}}{\text{Total number of seeds}} \times 100$$

3.7.3 Speed of germination (Maguire, 1962)

Seeds germinated on a paper medium with four replicas of 100 seeds each which is repeated thrice representing 3 replications for each treatment (Top of paper method). The number of germinated seeds was recorded daily until the last day of counting. The germination rate was calculated according to the following formula.

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

Where, X_n = Number of seeds germinated at n^{th} count

Y_n = Number of days from sowing to n^{th} count

3.7.4 Seedling root length (cm)

Ten normal plants were randomly selected from each replicate of a treatment at the end of the germination trial and root length was measured from the apical to terminal primary root zone. The average length of the roots is expressed in centimeters (cm).

3.7.5 Seedling shoot length (cm)

Ten normal plants used to measure root length were used to measure shoot length. Shoot length was measured from the base of the primary leaf to the neck region. The average shoot length is expressed in centimeter (cm).

3.7.6 Seedling dry weight (g/10 seedlings)

Ten normal seedlings were placed in butter paper and dried in a hot air oven maintained at $70 \pm 2^\circ\text{C}$ for 24 h. Then the plants were removed and allowed to cool in a desiccator for 30 min before being weighed in an electronic balance. The average weight was calculated and expressed as dry weight of the plants in mg of seedlings (10) (ISTA, 2010).

3.7.7 Mean germination time (MGT)

The seeds kept for germination on paper medium started to germinate and were checked daily at about the same time. Normal seedlings were removed from the test the radicle protruded approximately 2mm in length and the same was continued until all the normal seedlings germinate as prescribed size and mean germination time is calculated as following

Ellis and Robert (1981) formulated the equation of Mean Germination Time (MGT) as

$$\text{Mean germination time (MGT)} = \frac{\sum Dn}{\sum n}$$

Where, n = number of D

D = day after planting / number of days counted from the beginning of germination

3.7.8 Time taken for 50% germination (T_{50})

The seeds kept on paper medium that started to germinate were checked daily at about the same time. Normal seedlings were removed from the test when they reached a predetermined size. This procedure was continued until all the seeds capable of producing a normal seedling germinated.

Coolbear *et al.* (1984) formulated the 50% germination time equation (T_{50}) as modified by Farooq *et al.* (2006c) like

$$T_{50} = t_i + \left[\frac{\left[\frac{(N+1)}{2} - n_j \right]}{n_j - n_i} \right] \times (t_j - t_i)$$

Where, N = Final number of seeds germinate

n_i and n_j = Total number of seeds germinated by adjacent counts at times t_i and t_j

while $n_i < N/2 < n_j$

3.7.9 Vigour index

This is a secondary trait calculated using recorded data on germination (%) and seedling dry weight (g) or shoot length (cm)

3.7.9.1 Vigour index- I (VI-I)

The seedling viability index I was calculated according to the formula given by Abdul Baki and Anderson (1973).

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

3.7.9.2 Vigour index- II (VI II)

The seedling viability index II was calculated according to the formula given by Bewly and Black (1994).

Vigour index- II = Germination (%) x seedling dry weight (mg)

3.7.10 Dehydrogenase enzyme activity (Kittock and Law, 1968)

Seeds were preconditioned by soaking in water for 24 h and 25 seeds in four replicates were taken at random and the seeds were unhusked. The unhusked seeds were soaked in 0.5% solution of 2,3,5-triphenyl tetrazolium chloride and kept in the dark for 4 h at 40°C for colouring of living tissues. After staining, the excess solution was decanted and washed thoroughly with distilled water. The colour was then extracted from the stained embryos by soaking in 2 ml of methyl cello solve for 4- 6 h. Colour intensity was read in a spectrophotometer at 470 nm. The OD value has been reported as the enzyme dehydrogenase activity.

3.7.11 Super Oxide Dismutase enzyme activity (Dhindsa *et al.*, 1981)

The activity of SOD was tested by measuring the photochemical inhibition of Nitro Blue Tetrazolium (NBT) reduction by the method of Beauchamp and Fridovich (1971). The reaction mixture of 3ml contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA and 0.50 µl enzyme extract. Riboflavin was added last and the tubes were shaken and placed 30 cm below a light bank consisting of two 15W fluorescent lamps. The reaction was started by switching on the

light and was allowed to run for 10 min during which time it was found earlier to be linear. The reaction was stopped by switching off the light and the tubes were kept in dark. The absorbance by the reaction mixture at 560 nm was read. The unsorted reaction mixture did not develop colour and was used as a control. There was no measurable effect of the diffused room light. The reaction mixture lacking enzyme developed the maximum colour and this decreased with increasing volume of enzyme extract added. Log A560 was plotted as a function of the volume of enzyme extract used in the reaction mixture (Giannopolitis and Ries, 1977). From the resultant graph the volume of enzyme extract corresponding to 50% inhibition of the reaction was read and was considered as one enzyme unit (Beauchamp and Fridovich, 1971).

$$\text{SOD (U/ mg Protein)} = \frac{\text{Blank} - \text{Sample}}{\text{Blank}/2}$$

3.7.12 Electrical conductivity (ISTA, 2010)

Conductivity was measured in four replicates of five grams of grains from each treatment weighed to two decimal places. The seeds were then soaked in 25 ml of distilled water and incubated at a constant temperature of $25 \pm 1^\circ\text{C}$ for 24 h. The electrical conductivity of the leachate from the grains was measured with a digital conductivity meter. After subtracting the conductivity of distilled water from the value obtained from the leachate of the grains, the actual conductivity due to the electrolyte is measured and expressed in μScm^{-1} at $25 \pm 1^\circ\text{C}$.

3.7.13 Seed microflora (%)

Seeds were tested for fungal infection using the standard moist blotter method and the agar plate method at the starting and end of the storage period, as recommended by ISTA (1999).

3.7.13.1 Blotter paper method

Particle microbiological detection was performed using the standard ISTA blotter test described by Neergaard (1979).

Sterilized Petri dishes were lined with three layers of sterilized blotting paper. Sterilized water was added to the plate just sufficient to soak the filter paper. Twentyfive seeds were placed equidistantly on the moistened blotter paper in and incubated for seven days at 20°C for an alternate cycle of 12hours of light and for remaining twelve hours in the dark. On the eighth day, the seeds were examined under a stereomicroscope for the presence of any seed-borne fungi. The number of infected seeds were counted and expressed in percent. Fungal bodies were also identified on the basis of the morphological characteristics of sporophores, conidia and fruiting bodies by making identical slides followed by examination under compound microscope (Olympus CH₂O; BIMF)

3.7.13.2 Agar plate method

In agar plate method seeds were surface sterilized using 0.1% sodium hypochlorite and then washed with sterile distilled water thrice. The washed seed is then placed on sterile filter paper to remove excess water from its surface. The seeds were kept equidistant on potato dextrose agar medium (PDA) under aseptic conditions. The petri plates were incubated for few days and the fungal growth was examined under a stereo binocular microscope. The identification of infectious pathogens is also achieved through the preparation of microscopic slide mounts of samples.

3.7 Statistical analysis

3.7.1 Analysis of data

Statistical analysis of the data on various seed quality parameters was performed following the Completely Randomized Design (CRD) with three replications using OPSTAT, a Statistical software package developed by Department of Mathematical statistics, CCS

HAU, Hisar with various seed treatments, as per Fisher's method of analysis of variance (Gomez and Gomez, 1976). Significant test was done by using Duncan's Multiple Range Test (DMRT) using Grapes Stat tool developed by KAU, Kerala.

3.7.1.1 ANOVA for Completely Randomized Design

The data recorded in the experiment was analyzed using ANOVA (CRD) so as to test the differences between two or more independent groups. The mean squares due to different sources of variation were worked out using the following analysis of variance (Gomez and Gomez, 1976).

Source of variation	Degree of freedom (df)	Sum of squares (SS)	Mean square MS = SS/df	Computed F
Treatment	$t - 1$	TrSS	TrSS	TrSS/EMS
Error	$n - t$	ESS	EMS	
Total	$n - 1$	TSS		

Where,

r = No. of replications

t = No. of treatments

n = Total number of observations

TrSS = Sum of squares of treatment

ESS = Sum of squares of error

TSS = Sum of squares of total

EMS = Mean square of error

3.4.3. Pair wise comparison using DMRT test

Duncan's multiple range test (DMRT) has been used for experiments requiring the evaluation of all possible pairs of processing agents, especially when the total number of treatments is large.

The calculation of the numerical limits that allow to classify the difference between two treatments or agents as significant or not significant is performed. Unlike, LSD tests where only one value is required for each pair comparison at a prescribed level of significance, DMRT requires the calculation of a set of values, each corresponding to a specific set of pair comparisons (Gomez and Gomez, 1976). The test has been conducted using Grapes stat tool developed by KAU, Kerala.

Step 1: List all treatment agents in descending (or ascending) order. It is customary to arrange the treatment means in the order of preference.

Step 2: The standard error of the mean sum of squares of errors (SEm) was calculated using the formula

$$SEm = \sqrt{\frac{2EMS}{r}} \times 100$$

where, „EMS“ is the error mean sum of squares and „r“ is the number of replications.

Step 3: Compute the (t - 1) values of the shortest significant ranges as:

$$Rp = \frac{(rp)(SEm)}{\sqrt{2}}$$

For p = 2, 3, ..., t

Where, „t“ is the total number of treatments, „s“ is the standard error of the mean difference computed in step 2, „r“ values are the tabular values of the significant ranges, and „p“ is the

distance in rank between the pairs of treatment means to be compared (i.e., $p = 2$ for the two means with consecutive rankings and $p = t$ for the highest and lowest means).

Step 4: Identify and group all treatments that are not significantly different from each other.

Step 5: Use the alphabetical notation in order of precedence to represent the test results.

3.4.4. Ranking and scoring

In order to identify treatments that are effective for improvement of seed yield and quality, scoring and ranking were carried out for all the characters studied under experiment I and in experiment II. Based on DMRT, the treatments were ranked in descending order for all the characters. The score for each treatment was calculated by adding up the ranks obtained in all the thirteen characters under consideration.

Results

IV. RESULTS

The results obtained from the study "Impact of biopriming on seed quality and longevity in rice" (*Oryza sativa* L.) is presented hereunder.

4.1 Quality of seeds before storage

4.1.1 Quality of seeds before seed treatment

Seed quality parameters were assessed prior to the seed treatment, variety Jyothy and enumerated in Table 2.

The seed lot had 94.32 per cent germination and a seed moisture content of 13.40 per cent. The seedlings' average root length, shoot length and seedling dry weight, were 13.95cm, 10.39cm, and 0.23 g, respectively. The vigour index I was 2006, and the vigour index II was 2194. The mean germination time, time taken for 50% germination and speed of germination were 3.08, 2.03 and 41.68 respectively. Seed were found to be free from pathogen infection.

Table 2: Quality of seeds before treatment in rice variety Jyothy

Sl. No	Parameters	
1	Seed germination (%)	94.32
2	Seed moisture content (%)	13.4
3	Seedling root length (cm)	10.39
4	Seedling shoot length (cm)	13.95
5	Seedling dry weight (g/10 seedlings)	0.233
6	Vigour index-I	2006
7	Vigour index-II	2194
8	EC leachate (μScm^{-1})	33.5

9	Speed of germination	22.68
10	Mean Germination Time (MGT)	3.08
11	Time taken for 50% germination (T ₅₀)	2.03
12	Seed microflora (%)	0.00

4.1.2. Initial quality of treated seeds

Prior to the start of the storage study, the initial seed quality characteristics were examined and presented in Table 3a and 3b.

4.1.2.1. Germination (%)

There were significant changes in germination percent between the treatments. It ranged between 87 per cent (T₉- control) and 92.6 per cent (T₇- *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%))

4.1.2.2. Seedling shoot length (cm)

There were substantial differences in the parameter between the treatments. The seedling shoot length varied from 9.79 cm (T₄) to 5.41 cm (T₉). The highest value was recorded in T₄- *P. fluorescens* @10g/kg + *T. viride* @4g/kg (9.79 cm) which was followed by T₁-*P.fluorescens*@10g/kg (9.37), T₈-Hydro priming (9.14), T₂-*T.viride*@4g/kg (9.10).T₅-*P.fluorescens* (10g/kg) + coconut water (75%)(6.50 cm) and T₇-*P.fluorescens* (10g/kg) + *T. viride* (4g/kg)+ coconut water (75%)(6.47 cm) were statistically on par to each other with the least values after control (T₉- 5.41 cm).

4.1.2.3. Seedling root length (cm)

The average seedling root length varied greatly ranging from 17.21 cm (T₃- Coconut water (75%)) to 11.57 cm (T₁-*P. fluorescens* (10g/kg)) among the treatments. Least value was recorded in T₉-Control (11.25 cm).

4.1.2.4. Seedling dry weight (mg)

The effects of the treatments on the initial mean seedling dry weight were found to be significant and it ranged from 0.214g (T₉- control) to 0.255g (T₃-coconut water (75%)). Mean seedling dry weight of T₇ (*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%)) and T₈ (hydro priming) were 0.240g and 0.236g respectively.

4.1.2.5. Vigour index I

Vigour index ranged from 1449 (T₉) to 2205 (T₃). Highest value was recorded in T₃-coconut water (75%) (2205) followed by T₄- *P. fluorescens* @10g/kg + *T. viride*@4g/kg (2145). Control recorded the least value (1449) which was on par with T₁-*P. fluorescens* @10g/kg (1886).

4.1.2.6. Vigour index II

The treatments exhibit significant differences for vigour index II varying from 1859 (T₉) to 2351 (T₃). T₃-coconut water (75%) recorded the highest value (2351) which was followed by T₇-*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (2238) and T₈-hydro priming (2152). Control recorded the lowest vigour index (1859) and it was followed by T₄- *P. fluorescens*@10g/kg + *T. viride*@4g/kg (1908).

4.1.2.7. Electrical conductivity of seed leachates (μScm^{-1})

The electrical conductivity of seed leachates. ranged from 21.06 μScm^{-1} (T₁) to 28.15 μScm^{-1} (T₉). Least electrical conductivity was recorded in T₁-*P. fluorescens*@10g/kg (21.06 μScm^{-1}) which was followed by T₈- hydro priming (21.18 μScm^{-1}) and T₂-*T. viride* @4g/kg(23.21 μScm^{-1}) whereas control which recorded the highest value (28.15 μScm^{-1}).

4.1.2.8. Moisture content (%)

Significant differences were observed among the treatments for seed moisture content. It varied from 7.2 per cent (T₃-Coconut water (75%)) to 8.1 per cent (T₉-control). Least value was recorded in T₃-Coconut water (75%) and T₂-*T.*

viride@4g/kg as 7.2 per cent and 7.4 per cent respectively which was on par with T₅-*P. fluorescens* (10g/kg) + Coconut water (75%) (7.5 per cent) whereas control recorded the highest value as 8.1 per cent.

4.1.2.9. Speed of germination

speed of germination (42.56) was highest in T₁-*P. fluorescens* @10g/kg followed by T₈-hydro priming (42.28) and least of 38.46 in T₉ (Control).

4.1.2.10 Mean Germination Time

Least mean germination time was registered in T₅- *P. fluorescens* (10g/kg) + coconut water (75%) as 2.76 followed by T₈-hydro priming (2.78) and T₆ - *T. viride* (4g/kg) + coconut water (75%) (2.81), whereas T₃ - coconut water (75%) recorded the highest value of 3.00.

4.1.2.11 Time taken for 50% germination

T₅- *P. fluorescens* (10g/kg) + coconut water (75%) registered a value of 1.96 followed by T₇-*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) and T₆- *T. viride* (4g/kg) + coconut water (75%). T₂-*T. viride* @4g/kg and T₄- *P. fluorescens*@10g/kg + *T. viride* @4g/kg took 2.25 days to reach 50 % germination.

4.1.2.12. Seed infection (%)

Existence of significant differences in seed infection was observed among the treatments. In agar plate method, the mean seed infection varied from 1.33 (T₁) to 5.66 per cent (T₉). T₁-*P. fluorescens*@10g/kg seed recorded least infection (1.33%) and was on par with T₇-*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) and T₂-*T. viride*@4g/kg (2.33%) whereas higher seed infection was observed in T₉-Control (5.66%) and it was on par with T₈ (5.00%). In blotter paper method, T₈-hydro priming had recorded the highest seed infection (14.33%) while least infection was observed in T₃-coconut water (75%) (2.66%).

Table 3a. Impact of biopriming on seed quality parameters in rice

Treatments	Seed initial quality							
	Germination (%)	Seedling shoot length (cm)	Seedling root length (cm)	Seedling dry weight (g/10 seedlings)	Seedling vigour index-1	Seedling vigour index-II	Seed moisture content (%)	Electrical conductivity (μScm^{-1})
T ₁ : <i>Pseudomonas fluorescens</i>	90.53 ^b	9.37 ^b	11.57 ⁱ	0.217 ^c	1886 ^{gi}	1954 ^f	8.0 ^a	21.06 ^h
T ₂ : <i>Trichoderma viride</i>	91.71 ^{ab}	9.10 ^d	12.7 ^h	0.232 ^b	1940 ^g	2090 ^{cd}	7.4 ^c	23.21 ^f
T ₃ : Coconut water	92.25 ^a	6.76 ^f	17.21 ^a	0.255 ^a	2205 ^a	2351 ^a	7.2 ^c	25.14 ^d
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	92.54 ^a	9.79 ^a	13.52 ^f	0.207 ^c	2145 ^b	1908 ^g	7.9 ^a	24.17 ^e
T ₅ : <i>P. fluorescens</i> + coconut water	90.75 ^b	6.50 ^g	15.73 ^b	0.234 ^b	2002 ^e	2107 ^e	7.5 ^{bc}	24.16 ^e
T ₆ : <i>T. viride</i> + coconut water	90.57 ^b	6.91 ^e	14.12 ^e	0.234 ^b	1893 ^h	2113 ^d	7.8 ^{ab}	26.17 ^c
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	92.60 ^a	6.47 ^h	14.96 ^c	0.240 ^b	1993 ^f	2238 ^b	7.8 ^{ab}	26.22 ^b
T ₈ : Hydro priming	91.51 ^{ab}	9.14 ^c	13.39 ^g	0.236 ^b	2050 ^c	2152 ^c	7.9 ^a	21.18 ^g
T ₉ : Control	87.63 ^c	5.41 ⁱ	11.25 ⁱ	0.214 ^c	1449 ⁱ	1859 ^h	8.1 ^a	28.15 ^a
C.D	1.663	0.037	0.120	0.012	3.568	3.376	0.346	0.035
SE(m)	0.556	0.012	0.040	0.004	1.192	1.128	0.115	0.012

Table 3b. Impact of biopriming on seed quality parameters in rice

Treatments	Seed initial quality						
	Speed of germination	Mean germination time	Time taken for 50% germination	Micro flora Blotter paper method		Micro flora Agar plate method	
				With sterilization	Without sterilization	With sterilization	Without sterilization
T ₁ : <i>Pseudomonas fluorescens</i>	22.56 ^a	2.84 ^{cd}	2.08 ^d	9.00 ^d	9.33 ^b	4.00 ^{cd}	1.33 ^d
T ₂ : <i>Trichoderma viride</i>	20.96 ^c	2.83 ^{cd}	2.25 ^a	12.33 ^{cd}	7.66 ^{bcd}	3.00 ^d	2.33 ^{cd}
T ₃ : Coconut water	19.46 ^f	3.00 ^a	2.18 ^b	12.00 ^{cd}	2.66 ^e	4.00 ^{cd}	3.00 ^{cd}
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	20.17 ^e	2.95 ^b	2.25 ^a	13.00 ^c	6.33 ^{cd}	5.00 ^{bcd}	3.33 ^{bc}
T ₅ : <i>P. fluorescens</i> + coconut water	20.21 ^d	2.76 ^f	1.96 ^f	12.33 ^{cd}	5.00 ^{de}	7.00 ^b	4.00 ^{abc}
T ₆ : <i>T. viride</i> + coconut water	18.56 ^h	2.81 ^{de}	2.05 ^d	20.66 ^{ab}	6.00 ^{cd}	9.33 ^a	4.00 ^{abc}
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	19.41 ^g	2.86 ^c	2.00 ^e	13.33 ^c	8.33 ^{bc}	5.33 ^{bcd}	2.33 ^{cd}
T ₈ : Hydro priming	22.28 ^b	2.78 ^{ef}	2.12 ^c	22.00 ^a	14.33 ^a	5.33 ^{bcd}	5.00 ^{ab}
T ₉ : Control	38.46 ⁱ	2.96 ^b	2.12 ^c	18.00 ^b	6.00 ^{cd}	6.33 ^{bc}	5.66 ^a
C.D	0.028	0.035	0.033	0.393	0.427	0.428	0.403
SE(m)	0.009	0.012	0.011	0.131	0.143	0.143	0.135

4.2 Seed quality assessment during storage

4.2.1 Analysis of Variance

Analysis of variance revealed the existence of significant differences in the impact of various seed treatments on seed quality parameters like germination, moisture content, seedling dry weight, vigour index I and II, throughout the storage period. Significant differences in Super oxide dismutase (SOD), Dehydrogenase enzyme activity, Mean Germination Time (MGT), Time taken for 50% germination (T_{50}) among the treatments, over the storage period, were also evident.

4.2.2.1. Germination (%)

The effect of bioprimering treatments on germination per cent during the course of storage is presented in Table 5. Significant differences were observed among the treatments for germination per cent from the first month of storage. The treatments had recorded gradual decrease in the mean germination per cent with the advancement of storage period. Highest germination per cent was observed in T_1 -*P. fluorescens*@10g/kg (90.08%) and T_4 - *P. fluorescens*@10g/kg + *T. viride*@4g/kg (89.88) during the fifth month of storage and the least germination per cent was recorded in T_8 -Hydro priming (80.83%).

During the sixth month, germination recorded by T_1 -*P. fluorescens*@10g/kg seeds (90.08%) was followed by T_2 -*T.viride*@4g/kg (88.88%) found to be on par with T_7 -*P. fluorescens* (10g/kg)+ *T. viride* (4g/kg)+ coconut water (75%) (88.58%). Lower germination was recorded in T_8 -Hydro priming (80.83%) which was followed by T_6 - *T. viride* (4g/kg) + coconut water (75%) (81.36%).

At the ninth month of storage, T_7 -*P. fluorescens* (10g/kg)+ *T. viride* (4g/kg)+ coconut water (75%) recorded higher germination per cent (83.69%) and they were found statistically on par with T_1 -*P. fluorescens*@10g/kg seeds and T_4 - *P. fluorescens*@10g/kg + *T.viride*@4g/kg (83.23 and 83.15 respectively). T_8 -hydro priming recorded a lower value (77.85%) followed by T_6 - *T. viride* (4g/kg) + coconut water (75%) (79.08%) and the least germination was recorded in control (77.62%).

All treatments maintained the MSCS (Minimum Seed Certification Standard) of 80 per cent germination till the end of six months of storage and at the end of storage (9 months) germination ranged between 77.62 per cent (T₉) and 83.69 per cent (T₇).

4.2.2.2. Shoot length (cm)

Seed treatment using biopriming had a noticeable effect on seedling shoot length (Table 6). The treatments were found to be significantly different from each other. Over the period of storage, a gradual decrease was observed in seedling shoot length.

In the sixth month, T₇ *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) and T₂ *T. viride* @4g/kg had recorded the highest value (9.12cm and 9.14 cm respectively). It was followed by T₆- *T. viride* (4g/kg) + coconut water (75%) (8.83 cm). T₃-coconut water (75%) and T₄- *P. fluorescens*@10g/kg + *T. viride*@4g/kg recorded a lower value (8.21 cm and 8.17 cm respectively) and it is on par with T₅- *P. fluorescens* (10g/kg) + coconut water (75%) (8.24 cm).

After 9 MAS, T₆- *T. viride* (4g/kg) + coconut water (75%) (8.37 cm) produced longer shoots followed by T₇-*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (8.21 cm). Among the treated seeds, T₄- *P. fluorescens*@10g/kg + *T. viride*@4g/kg and T₁-*P. fluorescens*@10g/kg seeds recorded shorter shoot length (7.38 cm and 7.95 cm respectively).

4.2.2.2. Root length (cm)

Seedling root length differed significantly among the treatments during the period of study (Table 7). The mean seedling root length showed gradual decline with advancement of storage irrespective of the treatments. The highest value was recorded by T₇-*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) and T₄- *P. fluorescens*@10g/kg + *T. viride*@4g/kg in the third month (17.27 cm and 17.26 cm respectively) and the least value was observed in control at the end of storage (10.29 cm).



Roll towel method of germination



Top paper method of germination



**10 seedlings selected for measuring
shoot and root length**

Plate-4: Germination tests for evaluating seed quality

In the sixth month of storage, higher seedling root length was recorded by T₁-*P.fluorescens*@10g/kg seeds (13.85 cm) which was on par with T₇-*P. fluorescens* (10g/kg)+ *T. viride* (4g/kg)+ coconut water (75%) (13.65 cm) followed by T₄- *P. fluorescens*@10g/kg + *T. viride*@4g/kg (13.38 cm), while T₈-hydro priming recorded the least value (10.76 cm).

At the end of storage, the mean seedling root length was observed to be higher in T₇-*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (11.44 cm), T₃-coconut water (75%) (11.29 cm), T₁-*P. fluorescens* @10g/kg seeds (11.26 cm) respectively. Control recorded the lowest value (10.29 cm) and it was on par with T₆-*T. viride* (4g/kg) + coconut water (75%) (10.52 cm).

4.2.2.4. Seedling dry weight (mg)

Seedling dry weight differed significantly throughout the storage period irrespective of seed treatments in a declining trend with the advancement of the period of storage (Table 8).

At 9 MAS, seedling dry weight was high in T₇-*P. fluorescens* (10g/kg) + *T.viride* (4g/kg)+ coconut water (75%) (0.242 g) which was on par with T₂-*T.viride*@4g/kg (0.240 g). The lowest seedling dry weight was recorded in control (0.223 g) and T₈-Hydro priming (0.222 g).

4.2.2.5. Vigour index I

From the data on vigour index, I presented in Table 9; it is clearly evident that the treatments significantly differed for the parameter throughout the course of storage. The mean vigour index I declined gradually to the end of storage after attaining a peak in the second month (2528).

Over the months of storage, vigour index I was found to be significantly higher in T₇-*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (1645) followed by T₃-coconut water (75%) (1561), T₁ - *P. fluorescens* @10g/kg seeds (1553), T₅- *P. fluorescens* (10g/kg) + coconut water (75%) (1541),

Table 4: Impact of bioprimering on seed germination (%) of rice

Treatments	MAS1	MAS2	MAS3	MAS4	MAS5	MAS6	MAS7	MAS8	MAS9
T ₁ : <i>Pseudomonas fluorescens</i>	93.30 ^a	94.99 ^a	90.38 ^a	91.08 ^a	90.78 ^a	90.08 ^a	84.36 ^c	83.75 ^{bc}	83.23 ^{ab}
T ₂ : <i>Trichoderma viride</i>	90.58 ^d	90.97 ^{cd}	89.19 ^{bc}	90.50 ^{abc}	89.88 ^b	88.88 ^b	85.61 ^{ab}	84.97 ^a	82.89 ^b
T ₃ : Coconut water	89.80 ^d	89.71 ^{ef}	89.69 ^{ab}	89.77 ^c	89.11 ^{cd}	88.11 ^{cd}	84.16 ^c	83.91 ^{abc}	81.84 ^c
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	91.47 ^c	94.38 ^c	85.88 ^e	91.19 ^a	90.88 ^a	89.88 ^a	86.61 ^a	83.38 ^c	83.15 ^{ab}
T ₅ : <i>P. fluorescens</i> + coconut water	89.88 ^d	89.96 ^b	89.77 ^{ab}	90.77 ^{ab}	84.08 ^e	83.08 ^e	82.69 ^d	81.88 ^d	81.1 ^d
T ₆ : <i>T. viride</i> + coconut water	91.55 ^c	90.18 ^f	84.97 ^f	86.69 ^e	82.36 ^f	81.36 ^f	81.19 ^e	79.27 ^e	79.08 ^e
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	92.08 ^{bc}	91.69 ^{de}	88.58 ^c	90.08 ^{bc}	89.58 ^{bc}	88.58 ^{bc}	84.88 ^{bc}	84.69 ^{ab}	83.69 ^a
T ₈ : Hydro priming	86.27 ^e	85.87 ^g	83.19 ^g	85.97 ^e	80.83 ^g	79.83 ^g	79.58 ^f	77.97 ^f	77.85 ^f
T ₉ : Control	92.41 ^b	90.58 ^{de}	87.27 ^d	88.77 ^d	87.88 ^d	85.88 ^d	84.58 ^{bc}	81.19 ^d	77.62 ^e
C.D	0.771	2.265	1.147	0.807	0.668	0.613	1.098	1.136	0.702
SE(m)	0.258	0.757	0.383	0.27	0.223	0.205	0.367	0.379	0.234

Table 5: Impact of biopriming on seedling shoot length (cm) of rice

Treatments	MAS1	MAS2	MAS3	MAS4	MAS5	MAS6	MAS7	MAS8	MAS9
T ₁ : <i>Pseudomonas fluorescens</i>	8.438 ^c	10.532 ^{ab}	9.081 ^d	9.432 ^c	9.017 ^b	8.429 ^c	7.423 ^e	7.484 ^e	7.395 ^f
T ₂ : <i>Trichoderma viride</i>	9.008 ^a	10.767 ^a	9.761 ^{bc}	10.001 ^a	9.725 ^a	9.141 ^a	8.399 ^{bc}	8.224 ^b	7.905 ^d
T ₃ : Coconut water	7.922 ^d	10.438 ^b	8.527 ^e	8.520 ^e	8.290 ^d	8.218 ^e	8.347 ^c	7.975 ^c	7.780 ^e
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	8.277 ^c	9.632 ^d	10.053 ^a	10.020 ^a	9.172 ^b	8.173 ^e	8.035 ^d	7.583 ^e	7.380 ^f
T ₅ : <i>P. fluorescens</i> + coconut water	8.677 ^b	9.341 ^e	9.713 ^c	9.377 ^c	8.246 ^d	8.249 ^{de}	8.049 ^d	7.987 ^c	7.997 ^c
T ₆ : <i>T. viride</i> + coconut water	8.738 ^b	8.298 ^g	8.186 ^f	8.305 ^f	8.684 ^c	8.834 ^b	8.644 ^a	8.405 ^a	8.375 ^a
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	8.369 ^c	8.711 ^f	8.015 ^g	8.728 ^d	9.010 ^b	9.121 ^a	8.558 ^{ab}	8.290 ^b	8.215 ^b
T ₈ : Hydro priming	8.418 ^c	10.490 ^{ab}	9.862 ^b	9.666 ^b	9.057 ^b	8.491 ^c	8.406 ^{bc}	8.080 ^c	8.040 ^c
T ₉ : Control	8.258 ^c	9.943 ^c	9.718 ^c	9.254 ^c	8.442 ^d	8.404 ^{cd}	8.289 ^c	7.844 ^d	7.730 ^e
C.D	0.176	0.277	0.119	0.19	0.198	0.166	0.17	0.114	0.085
SE(m)	0.059	0.092	0.04	0.063	0.066	0.056	0.057	0.038	0.028

T₂ *T. viride* @ 4g/kg (1537). Lower value for the parameter was recorded in T₈ hydro priming (1452), whereas control recorded the least value (1417)

4.2.2.6. Vigour index II

Effect of biopriming on vigour index II during the storage period is presented in Table 10. There existed significant variations among the treatments with a decline for the parameter throughout the storage period.

In the last month of storage, highest value for vigour index II was observed in T₇ *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (2029) which was followed by T₂ *T. viride*@4g/kg (1989). Least was recorded in T₈ hydro priming (1724) found to be on par with the lower value of control (1737).

4.2.2.7. Speed of germination

There was no significant difference initially among the treatments (Table 11) and statistically highest speed of germination was recorded in T₁ (21.04) at the second month of storage. Whereas, lowest (18.45) was recorded in (T₈).

At the end of the storage period speed of germination was high in T₄- *P. fluorescens*@10g/kg + *T. viride*@4g/kg (19.02), T₇ -*P. fluorescens* (10g/kg)+ *T. viride* (4g/kg)+ coconut water (75%) (18.99), T₁-*P. fluorescens*@10g/kg seeds (18.89). While T₈-hydro priming recorded the least value of 15.80.

4.2.2.8. Mean germination time

The results obtained on mean germination time were significant and has increased during the storage period (Table 12). The mean germination time was initially low in T₈ (hydro priming) with 2.53 days while, it increased towards the end of storage reaching 5.36 days in control (T₉).

Seeds treated with (T₇) *P. fluorescens* (10g/kg)+ *T. viride* (4g/kg)+ coconut water (75%) and (T₅) *P. fluorescens* (10g/kg) + coconut water (75%) recorded 5.08 respectively, followed by (T₄) *P. fluorescens* @10g/kg + *T. viride* @4g/kg and (T₃) coconut water (75%) (5.13 and 5.15 days respectively) recorded the least value for mean germination time and was found to be superior over all other treatments at the

end of storage. The highest mean germination time of 5.36 days at the end of the storage period was recorded under the treatment, T₉ - Control followed by T₁-*P. fluorescens* @10g/kg seeds (5.22 days).

4.2.2.9. Time taken for 50% germination

The results recorded for time taken for 50% germination as influenced by the priming treatments during the storage period are enumerated in Table 13.

Treatments T₇ - *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%), T₈-hydro priming, T₁ - *P. fluorescens*@10g/kg seeds recorded the least value as 2.05, 2.07 and 2.07 respectively and found to be significantly superior over the other treatments during the initial month of storage. The highest time taken for 50% germination of 5.29 days was recorded by control at the end of the storage period.

Time taken for 50% germination was initially low in T₇-*P. fluorescens* (10g/kg)+ *T. viride* (4g/kg)+ coconut water (75%) (2.05 days) and increased towards the end of storage reaching, 4.42 days in T₄- *P. fluorescens*@10g/kg + *T. viride*@4g/kg was followed by T₅- *P. fluorescens* (10g/kg) + coconut water (75%) and T₂-*T. viride*@4g/kg (4.54 and 4.56 days respectively) and highest time was recorded by control (T₉) as 5.29 days was on par with T₆- *T. viride* (4g/kg)+ coconut water (75%) (5.24 days).

4.2.2.10. Electrical conductivity (EC) of seed leachates (μScm^{-1})

Electrical conductivity of seed leachates (Table 14) varied significantly among the treatments throughout the storage period. The conductivity gradually increased over the months of storage irrespective of the treatments. Lowest mean electrical conductivity was recorded in the first month (19.23 μScm^{-1}) and the highest towards the end of storage (38.86 μScm^{-1}).

Table 6: Impact of biopriming on seedling root length (cm) of rice

Treatments	MAS1	MAS2	MAS3	MAS4	MAS5	MAS6	MAS7	MAS8	MAS9
T ₁ : <i>Pseudomonas fluorescens</i>	15.675 ^c	16.088 ^b	14.706 ^c	15.339 ^c	5.070 ^{cd}	3.852 ^a	2.931 ^a	2.232 ^a	1.265 ^a
T ₂ : <i>Trichoderma viride</i>	16.321 ^b	16.856 ^a	15.844 ^b	15.833 ^b	5.528 ^b	2.815 ^e	2.070 ^b	1.222 ^{cd}	0.650 ^b
T ₃ : Coconut water	14.481 ^e	15.499 ^{bc}	16.248 ^b	15.946 ^b	5.283 ^{bc}	2.995 ^{de}	2.099 ^b	1.570 ^{bc}	1.295 ^a
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	16.120 ^b	13.437 ^d	17.264 ^a	15.741 ^b	4.874 ^{de}	3.388 ^{bc}	2.147 ^b	1.100 ^{de}	0.715 ^b
T ₅ : <i>P. fluorescens</i> + coconut water	17.121 ^a	12.193 ^e	15.979 ^b	15.169 ^c	4.729 ^e	3.210 ^{cd}	1.971 ^b	1.107 ^{de}	0.545 ^b
T ₆ : <i>T. viride</i> + coconut water	14.928 ^d	12.874 ^{de}	13.938 ^d	13.991 ^d	3.015 ^f	2.082 ^f	1.895 ^b	1.078 ^{de}	0.520 ^{bc}
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	13.761 ^f	15.044 ^c	17.279 ^a	16.876 ^a	5.888 ^a	3.657 ^{ab}	2.668 ^a	1.924 ^{ab}	1.445 ^a
T ₈ : Hydro priming	16.369 ^b	15.472 ^{bc}	13.364 ^e	13.711 ^d	2.165 ^g	0.764 ^h	0.925 ^c	0.782 ^{de}	0.615 ^b
T ₉ : Control	15.248 ^d	14.971 ^c	16.178 ^b	15.801 ^b	13.115 ^f	11.282 ^g	11.049 ^c	10.682 ^e	10.295 ^c
C.D	0.342	0.742	0.463	0.331	0.313	0.273	0.271	0.411	0.238
SE(m)	0.114	0.248	0.155	0.11	0.105	0.091	0.09	0.137	0.079

Table 7: Impact of biopriming on seedling dry weight (g) of rice

Treatments	MAS1	MAS2	MAS3	MAS4	MAS5	MAS6	MAS7	MAS8	MAS9
T ₁ : <i>Pseudomonas fluorescens</i>	0.228 ^b	0.228 ^c	0.249 ^a	0.236 ^{ab}	0.238 ^{bc}	0.239 ^b	0.230 ^d	0.239 ^{cd}	0.229 ^c
T ₂ : <i>Trichoderma viride</i>	0.226 ^{bc}	0.226 ^d	0.223 ^{de}	0.227 ^{de}	0.235 ^{cd}	0.239 ^b	0.241 ^a	0.249 ^{ab}	0.240 ^{ab}
T ₃ : Coconut water	0.240 ^a	0.240 ^a	0.222 ^{de}	0.227 ^{de}	0.231 ^{de}	0.233 ^c	0.231 ^d	0.235 ^d	0.231 ^c
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	0.225 ^{bc}	0.225 ^e	0.221 ^e	0.226 ^e	0.226 ^f	0.228 ^d	0.233 ^{cd}	0.237 ^d	0.230 ^c
T ₅ : <i>P. fluorescens</i> + coconut water	0.235 ^a	0.235 ^b	0.237 ^b	0.230 ^{cd}	0.233 ^d	0.231 ^{cd}	0.238 ^{abc}	0.237 ^d	0.228 ^c
T ₆ : <i>T. viride</i> + coconut water	0.219 ^d	0.219 ^g	0.232 ^{bc}	0.233 ^{bc}	0.239 ^b	0.240 ^b	0.239 ^{ab}	0.238 ^{cd}	0.232 ^c
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	0.213 ^e	0.213 ^h	0.235 ^b	0.240 ^a	0.244 ^a	0.249 ^a	0.242 ^a	0.250 ^a	0.242 ^a
T ₈ : Hydro priming	0.222 ^{cd}	0.222 ^f	0.231 ^{bc}	0.220 ^f	0.229 ^{ef}	0.230 ^{cd}	0.224 ^e	0.225 ^e	0.222 ^d
T ₉ : Control	0.226 ^{bc}	0.225 ^d	0.228 ^{cd}	0.223 ^{ef}	0.220 ^g	0.215 ^e	0.234 ^{bcd}	0.233 ^c	0.223 ^d
C.D	0.005	0	0.006	0.004	0.004	0.004	0.005	0.006	0.004
SE(m)	0.002	0	0.002	0.001	0.001	0.001	0.002	0.002	0.001

Table 8: Impact of biopriming on seedling vigour index-I in rice

Treatments	MAS1	MAS2	MAS3	MAS4	MAS5	MAS6	MAS7	MAS8	MAS9
T ₁ : <i>Pseudomonas fluorescens</i>	2249 ^b	2528 ^a	2243 ^{ef}	2256 ^{bc}	2177 ^b	2007 ^a	1717 ^b	1651 ^b	1553 ^b
T ₂ : <i>Trichoderma viride</i>	2294 ^a	2512 ^a	2311 ^{cd}	2337 ^a	2252 ^a	1951 ^b	1752 ^b	1652 ^b	1537 ^b
T ₃ : Coconut water	2011 ^d	2326 ^b	2202 ^f	2196 ^d	2114 ^c	1869 ^c	1720 ^b	1640 ^{bc}	1561 ^b
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	2231 ^b	2176 ^c	2494 ^a	2349 ^a	2065 ^d	1791 ^d	1669 ^c	1529 ^e	1467 ^{cd}
T ₅ : <i>P. fluorescens</i> + coconut water	2319 ^a	1936 ^d	2378 ^b	2228 ^{cd}	2062 ^d	1928 ^b	1734 ^b	1592 ^{cd}	1541 ^b
T ₆ : <i>T. viride</i> + coconut water	2166 ^c	1909 ^d	1945 ^g	1933 ^f	1843 ^e	1701 ^e	1667 ^c	1544 ^{de}	1494 ^c
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	2037 ^d	2178 ^c	2275 ^{de}	2306 ^{ab}	2205 ^b	2017 ^a	1801 ^a	1711 ^a	1645 ^a
T ₈ : Hydro priming	2138 ^c	2229 ^c	1969 ^g	2009 ^e	1765 ^f	1537 ^f	1538 ^d	1470 ^f	1452 ^d
T ₉ : Control	2172 ^c	2256 ^{bc}	2323 ^c	2224 ^{cd}	1881 ^e	1730 ^e	1635 ^c	1522 ^e	1417 ^e
C.D	43.593	77.273	44.884	55.751	41.85	33.952	47.004	51.655	30.636
SE(m)	14.559	25.808	14.991	18.62	13.977	11.339	15.699	17.252	10.232

Table 9: Impact of biopriming on seedling vigour index- II in rice

Treatments	MAS1	MAS2	MAS3	MAS4	MAS5	MAS6	MAS7	MAS8	MAS9
T ₁ : <i>Pseudomonas fluorescens</i>	2130 ^{ab}	2167 ^a	2350 ^a	2148 ^a	2149 ^a	2149 ^b	1939 ^b	2002 ^b	1906 ^c
T ₂ : <i>Trichoderma viride</i>	2045 ^{de}	2054 ^b	2016 ^{de}	2055 ^{bc}	2092 ^b	2120 ^b	2060 ^a	2112 ^a	1989 ^b
T ₃ : Coconut water	2151 ^a	2152 ^a	1976 ^e	2038 ^c	2071 ^b	2053 ^c	1940 ^b	1975 ^{bc}	1890 ^c
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	2057 ^{cde}	2123 ^a	2017 ^{de}	2056 ^{bc}	1944 ^d	1896 ^e	1927 ^b	1939 ^{cd}	1865 ^{cd}
T ₅ : <i>P. fluorescens</i> + coconut water	2112 ^{abc}	2115 ^a	2190 ^b	2089 ^b	2093 ^b	2075 ^c	2061 ^a	1978 ^{bc}	1891 ^c
T ₆ : <i>T. viride</i> + coconut water	2008 ^{ef}	1978 ^c	2043 ^d	2018 ^c	2032 ^c	1950 ^d	1937 ^b	1884 ^d	1834 ^d
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	1957 ^{fg}	1950 ^{cd}	2111 ^c	2162 ^a	2162 ^a	2204 ^a	2052 ^a	2117 ^a	2029 ^a
T ₈ : Hydro priming	1912 ^g	1903 ^d	1961 ^e	1889 ^e	1903 ^e	1836 ^f	1780 ^c	1754 ^e	1724 ^e
T ₉ : Control	2084 ^{bcd}	2042 ^b	2045 ^d	1956 ^d	1920 ^{de}	1889 ^e	1975 ^b	2000 ^{bc}	1867 ^{cd}
C.D	52.79	50.79	57.86	47.33	38.57	39.39	59.42	56.91	38.652
SE(m)	17.63	16.96	19.32	15.80	12.88	13.15	19.84	19.00	12.909

In the first month, electrical conductivity of seed leachate was observed highest in control ($36.44 \mu\text{Scm}^{-1}$) followed by T₆ *T. viride* (4g/kg) + coconut water (75%) ($36.14 \mu\text{Scm}^{-1}$). They were on par with each other. The least value for EC of seed leachate ($21.33 \mu\text{Scm}^{-1}$) was recorded in T₇ *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) was followed by T₈ hydro priming ($21.95 \mu\text{Scm}^{-1}$).

At the end of storage, T₉ control had recorded the highest electrical conductivity ($38.86 \mu\text{Scm}^{-1}$) followed by T₄ *P. fluorescens* @10g/kg + *T. viride* @4g/kg ($37.85 \mu\text{Scm}^{-1}$) whereas T₅ *P. fluorescens* (10g/kg) + coconut water (75%) recorded the least value ($30.83 \mu\text{Scm}^{-1}$). T₅ was followed with T₁-*P. fluorescens*@10g/kg seeds ($32.60 \mu\text{Scm}^{-1}$).

4.2.2.11. Seed moisture content (%)

Seed moisture content at the end of storage is presented in Table 15. The treatments exhibited significant variations for seed moisture content at the end of storage. However, there was a marginal increase in seed moisture content in all the treatments.

At first month, seed moisture content varied between 7.23 per cent in T₃-coconut water (75%) and 8.24 per cent in T₁-*P. fluorescens*@10g/kg seeds. All other treatments were found to be on par with each other including control except T₆-*T. viride* (4g/kg) + Coconut water (75%) (7.77%) and T₄-*P. fluorescens* @10g/kg + *T. viride* @4g/kg (7.95%).

At the end of the storage period, relatively low moisture content was recorded in seeds treated with T₄ (*P. fluorescens* @10g/kg + *T. viride* @4g/kg:10.61%), it was followed by T₁ (*P. fluorescens*@10g/kg: 10.71%) and T₂ (*T. viride* @4g/kg: 10.91), while it was high in seed treatment T₈ (Hydro priming: 11.55%) and control T₉ (11.62%).

Table 10: Impact of biopriming on speed of germination in rice

Treatments	MAS1	MAS2	MAS3	MAS4	MAS5	MAS6	MAS7	MAS8	MAS9
T ₁ : <i>Pseudomonas fluorescens</i>	20.71	21.04 ^a	20.77 ^a	20.40 ^a	20.42 ^a	20.34 ^a	19.88 ^a	19.21 ^{ab}	18.89 ^a
T ₂ : <i>Trichoderma viride</i>	20.75	20.11 ^{ab}	19.63 ^c	19.42 ^{cd}	19.25 ^{cde}	19.21 ^b	18.05 ^b	18.23 ^{cd}	17.61 ^{cd}
T ₃ : Coconut water	19.99	19.85 ^{ab}	19.86 ^{bc}	19.49 ^{bcd}	19.10 ^{de}	19.09 ^b	18.83 ^{ab}	18.49 ^{bcd}	18.3 ^b
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	20.57	20.47 ^{ab}	20.45 ^{ab}	20.21 ^a	20.07 ^{ab}	20.34 ^a	19.69 ^a	19.45 ^a	19.02 ^a
T ₅ : <i>P. fluorescens</i> + coconut water	20.38	19.76 ^{abc}	19.28 ^{cd}	19.81 ^{abc}	19.42 ^{bcd}	19.35 ^b	19.16 ^{ab}	17.92 ^d	17.52 ^{cd}
T ₆ : <i>T. viride</i> + coconut water	21.76	19.52 ^{bc}	19.42 ^{cd}	20.07 ^{ab}	19.46 ^{bcd}	19.30 ^b	18.02 ^b	16.95 ^e	16.49 ^d
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	21.06	19.64 ^{abc}	20.35 ^{ab}	20.35 ^{abc}	19.97 ^{abc}	20.04 ^a	19.44 ^a	18.94 ^{abc}	18.99 ^a
T ₈ : Hydro priming	20.18	18.45 ^c	18.88 ^d	18.88 ^d	18.67 ^e	17.64 ^c	15.97 ^c	16.13 ^f	15.80 ^e
T ₉ : Control	20.47	20.16 ^{ab}	20.35 ^{ab}	20.35 ^{abc}	19.90 ^{abc}	20.23 ^a	19.16 ^{ab}	18.69 ^{bc}	17.84 ^c
C.D	N/A	2.564	1.218	1.163	1.375	1.236	2.364	1.39	0.38
SE(m)	0.784	0.856	0.407	0.389	0.459	0.413	0.79	0.464	0.127

Table 11: Impact of biopriming on mean germination time in rice

Treatments	MAS1	MAS2	MAS3	MAS4	MAS5	MAS6	MAS7	MAS8	MAS9
T ₁ : <i>Pseudomonas fluorescens</i>	2.77 ^a	2.86 ^c	3.05 ^{ab}	3.08 ^a	3.10 ^a	3.11 ^a	4.11 ^a	4.15	5.22 ^b
T ₂ : <i>Trichoderma viride</i>	2.80 ^a	2.91 ^{bc}	2.97 ^{abc}	2.93 ^{bc}	2.93 ^c	3.00 ^{ab}	3.99 ^{ab}	4.01	5.19 ^{bc}
T ₃ : Coconut water	2.80 ^a	2.85 ^c	2.91 ^{bc}	2.82 ^d	2.82 ^d	2.99 ^{ab}	4.03 ^{ab}	4.02	5.15 ^d
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	2.84 ^a	3.04 ^a	2.99 ^{abc}	3.00 ^{ab}	3.08 ^a	2.88 ^{bc}	4.05 ^{ab}	4.05	5.13 ^d
T ₅ : <i>P. fluorescens</i> + coconut water	2.81 ^a	2.87 ^c	2.87 ^c	2.96 ^{bc}	2.94 ^{bc}	2.87 ^{bc}	3.96 ^{ab}	4.00	5.08 ^e
T ₆ : <i>T. viride</i> + coconut water	2.77 ^a	2.87 ^c	2.98 ^{abc}	2.88 ^{cd}	2.90 ^{cd}	2.88 ^{bc}	3.94 ^{ab}	4.07	5.19 ^{bc}
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	2.95 ^a	2.99 ^{ab}	3.10 ^a	3.00 ^{ab}	3.05 ^a	3.13 ^a	3.91 ^b	4.08	5.08 ^e
T ₈ : Hydro priming	2.53 ^b	2.60 ^d	2.71 ^d	2.59 ^e	2.58 ^e	2.79 ^c	3.69 ^c	4.00	5.18 ^{bc}
T ₉ : Control	2.82 ^a	2.95 ^{abc}	2.99 ^{abc}	2.98 ^{abc}	3.03 ^{ab}	3.04 ^{ab}	3.89 ^{ab}	4.18	5.36 ^a
C.D	0.169	0.106	0.152	0.092	0.096	0.155	0.161	NS	0.044
SE(m)	0.057	0.036	0.051	0.031	0.032	0.052	0.054	0.036	0.015

Table 12: Impact of biopriming on time taken for 50% germination in rice

Treatments	MAS1	MAS2	MAS3	MAS4	MAS5	MAS6	MAS7	MAS8	MAS9
T ₁ : <i>Pseudomonas fluorescens</i>	2.07 ^c	1.46 ^{de}	1.55 ^e	2.11 ^d	3.3 ^d	4.23 ^b	4.44 ^e	4.65 ^e	4.70 ^d
T ₂ : <i>Trichoderma viride</i>	2.19 ^a	1.49 ^d	2.11 ^c	2.06 ^e	3.43 ^c	4.20 ^b	4.41 ^e	4.53 ^f	4.56 ^e
T ₃ : Coconut water	2.10 ^{bc}	1.46 ^{de}	2.08 ^{cd}	2.09 ^{de}	3.48 ^c	4.24 ^b	4.42 ^e	4.80 ^d	4.9 ^c
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	2.09 ^{bc}	1.43 ^e	2.05 ^d	2.12 ^d	3.35 ^d	4.24 ^b	4.38 ^{ef}	4.36 ^g	4.42 ^f
T ₅ : <i>P. fluorescens</i> + coconut water	2.15 ^{ab}	2.09 ^b	2.09 ^{cd}	2.05 ^e	4.11 ^{ab}	4.06 ^c	4.32 ^f	4.49 ^f	4.54 ^e
T ₆ : <i>T. viride</i> + coconut water	2.08 ^{bc}	2.18 ^a	2.38 ^b	2.50 ^b	4.14 ^a	4.80 ^a	4.62 ^d	5.26 ^b	5.24 ^a
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	2.05 ^c	2.05 ^c	2.38 ^b	2.38 ^c	4.05 ^b	4.77 ^a	4.72 ^c	4.94 ^c	5.00 ^b
T ₈ : Hydro priming	2.07 ^c	2.10 ^b	2.45 ^a	2.68 ^a	4.12 ^a	4.78 ^a	4.83 ^b	4.93 ^c	5.01 ^b
T ₉ : Control	2.21 ^a	2.20 ^a	2.42 ^{ab}	2.50 ^b	4.14 ^a	4.82 ^a	5.23 ^a	5.32 ^a	5.29 ^a
C.D	0.073	0.04	0.052	0.044	0.065	0.067	0.076	0.053	0.058
SE(m)	0.024	0.013	0.017	0.015	0.022	0.023	0.025	0.018	0.019

4.2.2.12. Dehydrogenase enzyme activity (OD value)

Dehydrogenase enzyme activity was maximum in T₁ (*P. fluorescens*@10g/kg: 0.795 OD value) at 1MAS followed by T₇ (*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%): 0.768 OD value) respectively. The minimum value (0.511 OD value) was registered in T₃ Coconut water (75%).

Dehydrogenase enzyme activity OD value decreased from 0.795 OD value at initial month of storage observation to 0.246 after 9 months of storage indicating the results in Table 16.

So also, at the end of storage period, T₇ -*P. fluorescens* (10g/ kg) + *T. viride* (4g/kg) + coconut water (75%) registered maximum dehydrogenase enzyme activity of 0.357 OD value followed by T₁ (*P. fluorescens*@10g/kg: 0.353 OD value) and T₄ (*P. fluorescens* @10g/kg + *T. viride* @4g/kg:0.342 OD value) respectively. Whereas, minimum was registered in T₅- *P. fluorescens* (10g/kg) + coconut water (75%) (0.246 OD value).

4.2.2.13. Super oxide dismutase enzyme activity (U/mg protein)

Super oxide dismutase enzyme activity decreased from 112.22 U/mg protein (T₇ -*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%)) at 1MAS to 27.23 U/mg protein (T₉-Control) after 9 months of storage (Table 16).

SOD enzyme activity was maximum in T₇ (*P. fluorescens* (10g/ kg) + *T. viride* (4g/kg) + Coconut water (75%):112.22 U/mg protein) at initial month of observation was followed by T₅ (*P. fluorescens* (10g/kg) + coconut water (75%):104.63 U/mg protein) which was on par with T₁ (*P. fluorescens*@10g/kg: 102.5 U/mgprotein). The minimum value (67.33 U/mg protein) was registered in T₉-Control.

Also, at the end of storage period, T₇ *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) registered maximum SOD enzyme activity of 64.29 U/mg protein and was followed by T₁ (*P. fluorescens*@10g/kg:58.13 U/mg protein) and T₅ (*P. fluorescens* @10g/kg + coconut water (75%): 57.14 U/mg protein) respectively. Whereas, minimum was registered in control (T₉) as 27.23 U/mg protein.

4.2.2.14. Seed infection (%)

The data on mean seed infection percent in each treatment at the end of storage is presented in Table 18. The parameter showed significant variations among the treatments.

In agar plate method lowest seed infection was observed in T₁ *P. fluorescens* @10g/kg (7.00%) which was on par with T₇ *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (8.00%) followed by T₂ *T. viride* @4g/kg and T₃ coconut water (75%) recorded as 10.33%. Control (22.33%) and T₄ *P. fluorescens* @10g/kg + *T. viride* @4g/kg (18.00%) were found inferior with significantly higher seed infection.

Similar observations were recorded in blotter paper method. Lower seed infection was observed in T₁ *P. fluorescens* @10g/kg (10.66%), T₂ *T. viride* @4g/kg (11.33%), T₇ *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (11.66%) These were on par with T₅ *P. fluorescens* @10g/kg + coconut water (75%) (12.33%) while T₉ control recorded highest seed infection (22.00%). T₉ was followed by T₄ *P. fluorescens* @10g/kg + *T. viride* @4g/kg (18.66%), T₆ *T. viride* (4g/kg) + coconut water (75%) (18.00%) and T₈ Hydro priming (17.66%).

Aspergillus spp. was observed in all the treatments. In addition, *Rhizopus* spp. was observed in treatments T₄, T₆, T₈, T₉ *Trichoderma* spp. was observed in T₃, T₆, T₇, T₈ and T₉. In addition to these, seed borne pathogens of rice like *Helminthosporium oryzae* and *Rhizoctonia solani*, saprophytic fungi like *Pencillium* spp., *Mucor* spp., *Curvularia* spp., *Syncephalastrum*, were also observed

Table 13: Impact of biopriming on electrical conductivity (μScm^{-1}) of seed leachates in rice

Treatments	MAS1	MAS2	MAS3	MAS4	MAS5	MAS6	MAS7	MAS8	MAS9
T ₁ : <i>Pseudomonas fluorescens</i>	27.13 ^f	19.23 ⁱ	30.17 ^c	27.54 ^e	30.28 ^d	29.65 ^h	31.25 ^h	32.25 ^h	32.60 ^g
T ₂ : <i>Trichoderma viride</i>	25.46 ^g	24.94 ^d	31.70 ^b	32.09 ^b	29.53 ^e	31.55 ^d	32.15 ^e	36.14 ^c	36.28 ^c
T ₃ : Coconut water	24.16 ^d	22.65 ^g	29.82 ^d	31.24 ^c	30.82 ^c	30.34 ^g	32.41 ^d	33.56 ^g	33.94 ^e
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	25.36 ^c	24.82 ^e	25.37 ^e	29.44 ^d	33.24 ^a	36.46 ^a	35.61 ^b	37.34 ^b	37.85 ^b
T ₅ : <i>P. fluorescens</i> + coconut water	28.43 ^e	22.85 ^f	19.93 ^h	22.54 ⁱ	26.76 ⁱ	28.18 ⁱ	29.32 ⁱ	30.41 ⁱ	30.83 ^h
T ₆ : <i>T. viride</i> + coconut water	26.14 ^b	26.09 ^c	22.40 ^g	26.87 ^f	28.94 ^f	30.85 ^f	31.43 ^g	33.62 ^f	33.70 ^f
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	21.33 ⁱ	26.66 ^b	22.92 ^f	25.65 ^h	27.95 ^h	32.66 ^c	32.84 ^c	34.51 ^d	34.73 ^d
T ₈ : Hydro priming	21.95 ^h	22.43 ^h	22.55 ^g	25.80 ^g	28.44 ^g	31.24 ^e	31.63 ^f	33.81 ^e	33.99 ^e
T ₉ : Control	28.44 ^a	29.51 ^a	32.32 ^a	32.65 ^a	32.44 ^b	34.32 ^b	36.51 ^a	38.33 ^a	38.86 ^a
C.D	0.062	0.051	0.178	0.082	0.069	0.045	0.029	0.035	0.144
SE(m)	0.021	0.017	0.06	0.027	0.023	0.015	0.01	0.012	0.048

Table 14: Impact of biopriming on moisture content (%) of seeds in rice

Treatments	MAS1	MAS2	MAS3	MAS4	MAS5	MAS6	MAS7	MAS8	MAS9
T ₁ : <i>Pseudomonas fluorescens</i>	8.24 ^a	8.32 ^c	8.54 ^d	8.83 ^e	9.13 ^f	9.53 ^f	10.32 ^c	10.51 ^e	10.71 ^g
T ₂ : <i>Trichoderma viride</i>	7.41 ^e	7.63 ^e	7.87 ^f	8.23 ^g	8.53 ^h	8.81 ^g	9.52 ^e	10.61 ^d	10.91 ^f
T ₃ : Coconut water	7.23 ^f	7.52 ^f	8.14 ^e	8.84 ^e	9.56 ^c	9.72 ^d	10.22 ^d	10.62 ^d	11.02 ^e
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	7.95 ^b	8.23 ^d	8.53 ^d	8.76 ^f	8.93 ^g	9.62 ^e	10.23 ^d	10.51 ^e	10.61 ^h
T ₅ : <i>P. fluorescens</i> + coconut water	7.82 ^c	8.32 ^{bc}	9.06 ^b	9.24 ^c	9.51 ^d	9.81 ^c	10.33 ^c	10.73 ^c	11.22 ^d
T ₆ : <i>T. viride</i> + coconut water	7.77 ^d	8.67 ^a	9.61 ^a	9.71 ^a	9.92 ^a	10.23 ^b	10.54 ^a	10.82 ^b	11.32 ^c
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	7.84 ^c	8.23 ^d	8.54 ^d	9.04 ^d	9.22 ^e	9.53 ^f	10.23 ^d	10.74 ^c	11.02 ^e
T ₈ : Hydro priming	7.86 ^c	8.35 ^b	8.82 ^c	9.56 ^b	9.84 ^b	10.33 ^a	10.42 ^b	10.73 ^c	11.55 ^b
T ₉ : Control	7.82 ^c	8.22 ^d	8.54 ^d	8.85 ^e	9.93 ^a	10.31 ^a	10.53 ^a	11.24 ^a	11.62 ^a
C.D	0.043	0.03	0.026	0.022	0.025	0.017	0.087	0.048	0.027
SE(m)	0.014	0.01	0.009	0.007	0.008	0.006	0.029	0.016	0.009

Table 15: Impact of biopriming on dehydrogenase enzyme activity (OD value) of seeds in rice

Treatments	MAS6	MAS7	MAS8	MAS9
T ₁ : <i>Pseudomonas fluorescens</i>	0.795 ^a	0.653 ^a	0.453 ^a	0.353 ^b
T ₂ : <i>Trichoderma viride</i>	0.672 ^f	0.576 ^e	0.377 ^f	0.278 ^g
T ₃ : Coconut water	0.511 ^h	0.565 ^f	0.365 ^g	0.268 ^h
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	0.577 ^g	0.541 ^g	0.441 ^b	0.342 ^c
T ₅ : <i>P. fluorescens</i> + coconut water	0.578 ^g	0.542 ^g	0.342 ^h	0.246 ⁱ
T ₆ : <i>T. viride</i> + coconut water	0.719 ^d	0.618 ^b	0.418 ^c	0.323 ^d
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	0.768 ^b	0.652 ^a	0.452 ^a	0.357 ^a
T ₈ : Hydro priming	0.683 ^e	0.597 ^d	0.397 ^e	0.294 ^f
T ₉ : Control	0.741 ^c	0.614 ^c	0.414 ^d	0.309 ^e
C.D	0.004	0.004	0.004	0.004
SE(m)	0.001	0.001	0.001	0.001

Table 16: Impact of biopriming on super oxide dismutase enzyme activity (U/mg protein) of seeds in rice

Treatments	MAS6	MAS7	MAS8	MAS9
T ₁ : <i>Pseudomonas fluorescens</i>	102.50 ^{bc}	88.13 ^{ab}	68.10 ^b	58.13 ^b
T ₂ : <i>Trichoderma viride</i>	95.74 ^e	84.45 ^{cd}	55.44 ^d	47.36 ^f
T ₃ : Coconut water	98.5d ^e	81.32 ^d	55.73 ^d	48.20 ^e
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	100.45 ^{cd}	86.38 ^{bc}	61.13 ^c	53.10 ^d
T ₅ : <i>P. fluorescens</i> + coconut water	104.63 ^b	87.25 ^{bc}	65.54 ^b	57.14 ^c
T ₆ : <i>T. viride</i> + coconut water	75.05 ^g	61.31 ^e	43.71 ^e	35.76 ^h
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	112.22 ^a	91.45 ^a	72.73 ^a	64.29 ^a
T ₈ : Hydro priming	86.71 ^f	63.67 ^e	46.33 ^e	36.16 ^g
T ₉ : Control	63.66 ^h	55.20 ^f	33.08 ^f	27.23 ⁱ
C.D	3.457	3.385	3.547	0.061
SE(m)	1.155	1.131	1.185	0.02

Table 17: Impact of biopriming on seed infection (%) of seeds in rice

Treatments	Seed infection (%)				Fungi observed
	Blotter paper method		Agar plate method		
	Without sterilization	With sterilization	Without sterilization	With sterilization	
T ₁ : <i>Pseudomonas fluorescens</i>	24.0 ^c	10.66 ^d	13.33 ^c	7.00 ^f	<i>Aspergillus</i> spp., <i>Mucor</i> spp., <i>Syncephalastrum</i> spp.
T ₂ : <i>Trichoderma viride</i>	18.33 ^d	11.33 ^d	13.66 ^c	10.33 ^e	<i>Alternaria</i> spp., <i>Penicillium</i> spp., <i>Helminthosporium oryzae</i>
T ₃ : Coconut water	22.00 ^{cd}	15.00 ^{bc}	15.00 ^c	10.33 ^e	<i>Aspergillus</i> spp., <i>Trichoderma</i> spp.
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	37.33 ^a	18.66 ^b	22.00 ^b	18.00 ^b	<i>Aspergillus</i> spp., <i>Rhizopus</i> spp., <i>Curvularia</i> spp.,
T ₅ : <i>P. fluorescens</i> + coconut water	18.00 ^d	12.33 ^{cd}	21.00 ^b	10.66 ^{de}	<i>Aspergillus</i> spp.
T ₆ : <i>T. viride</i> + coconut water	37.33 ^a	18.00 ^b	21.66 ^b	14.33 ^c	<i>Trichoderma</i> spp., <i>Rhizopus</i> spp.
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	18.33 ^d	11.66 ^d	15.66 ^c	8.00 ^{ef}	<i>Aspergillus</i> spp., <i>Trichoderma</i> spp., <i>Curvularia</i> spp.
T ₈ : Hydro priming	30.00 ^b	17.66 ^b	22.00 ^b	13.00 ^{cd}	<i>Aspergillus</i> spp., <i>Trichoderma</i> spp., <i>Rhizopus</i> spp., <i>Rhizoctonia solani</i>
T ₉ : Control	30.66 ^b	22.00 ^a	25.66 ^a	22.33 ^a	<i>Aspergillus</i> spp., <i>Mucor</i> spp., <i>Rhizopus</i> spp., <i>Trichoderma</i> spp.
C.D	0.398	0.393	0.277	0.358	-
SE(m)	0.133	0.131	0.093	0.119	-



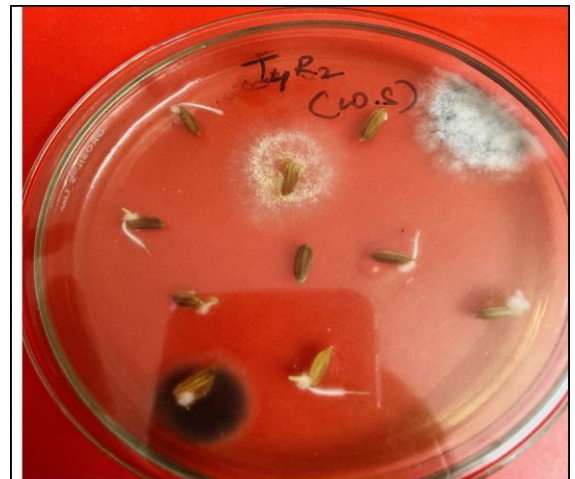
Blotter paper method



Agar plate method



Externally seed borne



Internally seed borne

Plate-5: Tests for identifying seed infection (%)

Table 18: Ranking of priming treatments based on seed quality parameters after storage

Treatments	T₁	T₂	T₃	T₄	T₅	T₆	T₇	T₈	T₉
Seed quality parameters	Ranks								
Seed germination (%)	1	2	3	1	4	5	1	6	5
Seed moisture content (%)	2	3	4	1	5	6	4	7	8
Seedling root length(cm)	1	2	1	2	2	2	1	2	3
Seedling shoot length(cm)	6	4	5	6	3	1	2	3	5
Seedling dry weight(g)	3	1	3	3	3	3	1	4	4
Vigour index-I	2	2	2	3	2	3	1	4	5
Vigour index-II	3	2	3	3	3	4	1	5	4
EC leachate (μScm^{-1})	2	6	4	7	1	3	5	4	8
Speed of germination	1	3	2	1	3	4	1	5	3
Mean Germination Time (MGT)	4	3	2	2	1	3	1	3	5
Time taken for 50% germination (T ₅₀)	3	2	4	1	2	6	5	5	6
Seed infection (%)	1	2	2	5	3	4	2	3	6
Dehydrogenase enzyme activity (nm)	2	7	8	3	9	4	1	6	5
Superoxide dismutase enzyme activity ($\text{mg}^{-1}/\text{protein}$)	2	6	5	4	3	8	1	7	9
Total score	33	45	48	42	44	56	27	64	76
Final Ranks	2	5	6	3	4	7	1	8	9

4.3 Artificial ageing (Delouche and Baskin, 1973)

The treated seeds were packed in butter paper bags with pin holes to absorb moisture for ageing and placed in the wire gauge / mesh above the water well so that seeds were not in contact with water inside the BOD incubator from 0 to 7 days at a temperature of $40\pm 1^{\circ}\text{C}$ and a relative humidity (RH) of 98 percent. At daily intervals, accelerated aged samples were gathered and tested to evaluate a range of quality characteristics.

4.3.1. Germination (%)

At initial stage all the treatments were on par with each other with the highest record in T₁*P. fluorescens* @10g/kg (89.56%), while minimum was recorded in T₈ hydro priming (79.95 %).

Irrespective of treatments as enumerated in Table 20, germination per cent was high at initial stage (89.56%) and decreased to 72.55 per cent at the end of artificial ageing.

At the end of artificial ageing, T₁ *P. fluorescens* @10g/kg and T₂ *T. viride* @4g/kg recorded maximum germination (83.11% and 83.58%), which was on par with T₄*P. fluorescens* @10g/kg + *T. viride* @ 4g/kg (82.93%) and minimum of 72.55 percentage was recorded in T₉-control.

4.3.2. Seedling shoot length (cm)

The treatment T₈ Hydro priming produced longest shoot of 9.17 cm, which was on par with T₂*T. viride* @4g/kg (8.69 cm), whereas the shortest shoot of 7.29 cm was produced by T₆*T. viride* (4g/kg) + coconut water (75%) at initial stage.

Decreasing trend of seedling shoot length was observed from 9.17cm at initial day of ageing to 6.18 cm at the end of artificial ageing as enumerated in Table 21.

At the end of ageing period, T₂ *T. viride* @4g/kg recorded maximum shoot length (8.73 cm) and was followed by T₁-*P. fluorescens*@10g/kg and T₈-hydro

priming (8.41 and 8.38 respectively). minimum seedling shoot length of 6.18 and 6.45 was recorded in T₃coconut water (75%) and T₉ control respectively.

4.3.3. Seedling root length (cm)

The treatment T₁*P. fluorescens*@10g/kg produced longest root of 15.65 cm, which was followed by T₂*T. viride* @4g/kg (14.15 cm) and T₈Hydro priming (13.99 cm). These were on par with each other, whereas the shortest root of 12.57 cm was produced by seeds treated with (T₅) *P. fluorescens* @10g/kg + coconut water (75%) at initial stage.

Decline in seedling root length was observed from 15.65 cm at day one of ageing to 9.57 cm at the end of artificial ageing as enumerated in Table 22.

At the end of ageing period, T₃ -Coconut water (75%) and T₇ -*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) recorded highest root length (14.12 cm and 14.09 cm respectively) and was on par with T₂-*T. viride* @4g/kg (13.78 cm). Whereas, minimum seedling root length of 9.57 cm was recorded in T₆-*T. viride* (4g/kg) + coconut water (75%) followed by T₉-control (11.18 cm).

4.3.4. Seedling dry weight (g)

Seedling dry weight differed significantly initially during the ageing period irrespective of seed treatments in a declining trend with the advancement of the ageing period and was non-significant at the end (Table 23).

At initial, maximum dry matter production was recorded in T₁-*P. fluorescens*@10g/kg of seed (0.249 g) and was on par with T₃, T₄, T₅, T₆, T₇, T₈, T₉ (0.248 g). Least was recorded in T₂-*T. viride* @4g/kg (0.226 g). At the end of ageing period treatments were non-significant recording a dry weight of 0.237 g to 0.243 g respectively.

Table 19: Impact of biopriming on germination (%) of rice seeds after artificial ageing

Treatments	D1	D2	D3	D4	D5	D6	D7
T ₁ : <i>Pseudomonas fluorescens</i>	89.56 ^a	86.69 ^{ab}	86.69 ^c	86.63 ^b	85.69 ^a	84.65 ^{ab}	83.11 ^a
T ₂ : <i>Trichoderma viride</i>	86.67 ^a	88.62 ^{ab}	88.26 ^a	87.26 ^a	86.81 ^a	85.97 ^a	83.58 ^a
T ₃ : Coconut water	86.72 ^a	85.45 ^b	84.77 ^d	82.70 ^{de}	80.43 ^d	75.64 ^{ef}	73.15 ^d
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	89.26 ^a	88.67 ^a	87.08 ^b	85.58 ^{bc}	85.16 ^b	83.98 ^b	82.93 ^{ab}
T ₅ : <i>P. fluorescens</i> + coconut water	85.61 ^a	85.25 ^b	84.68 ^d	82.37 ^{bc}	79.64 ^d	77.13 ^e	74.69 ^{cd}
T ₆ : <i>T. viride</i> + coconut water	85.24 ^a	83.27 ^c	82.58 ^e	81.31 ^e	79.72 ^d	79.03 ^d	76.62 ^c
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	88.55 ^a	86.07 ^b	85.76 ^{cd}	84.16 ^{cd}	83.09 ^c	81.07 ^d	81.06 ^b
T ₈ : Hydro priming	79.95 ^b	79.77 ^d	79.47 ^f	78.60 ^f	77.55 ^e	74.16 ^f	73.24 ^d
T ₉ : Control	86.72 ^a	85.1 ^b	82.61 ^e	81.58 ^e	80.06 ^c	76.09 ^c	72.55 ^e
C.D	3.91	3.03	2.12	1.54	1.51	1.72	2.11
SE(m)	1.30	1.01	0.71	0.51	0.50	0.57	0.70

Table 20: Impact of biopriming on seedling shoot length (cm) of rice seeds after artificial ageing

Treatments	D1	D2	D3	D4	D5	D6	D7
T ₁ : <i>Pseudomonas fluorescens</i>	8.20 ^b	6.89 ^e	8.27 ^{bc}	8.13 ^{de}	7.35 ^b	8.29 ^{cd}	8.41 ^b
T ₂ : <i>Trichoderma viride</i>	8.69 ^{ab}	7.77 ^{abc}	8.59 ^{bc}	8.39 ^{bc}	7.93 ^{ab}	8.41 ^c	8.73 ^a
T ₃ : Coconut water	7.55 ^{cd}	7.47 ^{cd}	7.61 ^d	7.92 ^{ef}	7.26 ^b	7.97 ^f	6.18 ^g
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	7.83 ^c	7.61 ^{cd}	8.68 ^b	8.77 ^a	8.41 ^a	8.87 ^a	7.90 ^d
T ₅ : <i>P. fluorescens</i> + coconut water	7.70 ^c	7.96 ^{ab}	6.83 ^e	7.72 ^f	7.14 ^b	7.53 ^h	6.57 ^f
T ₆ : <i>T. viride</i> + coconut water	7.29 ^d	6.91 ^e	9.69 ^a	8.61 ^{ab}	7.37 ^b	7.78 ^g	6.93 ^e
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	8.34 ^b	7.33 ^d	8.16 ^c	8.25 ^{cd}	8.01 ^{ab}	8.06 ^{ef}	8.09 ^c
T ₈ : Hydro priming	9.17 ^a	8.05 ^a	8.44 ^{bc}	8.29 ^{cd}	8.31 ^a	8.22 ^{de}	8.38 ^b
T ₉ : Control	8.29 ^b	7.70 ^{bc}	8.78 ^b	8.54 ^{ab}	7.18 ^b	6.60 ^g	6.45 ^g
C.D	0.36	0.31	0.47	0.22	0.78	0.18	0.15
SE(m)	0.12	0.10	0.15	0.07	0.26	0.06	0.05

Table 21: Impact of bioprimering on seedling root length (cm) of rice seeds after artificial ageing

Treatments	D1	D2	D3	D4	D5	D6	D7
T ₁ : <i>Pseudomonas fluorescens</i>	15.65 ^a	16.50 ^a	13.11 ^d	12.03 ^e	10.21 ^d	10.82 ^f	11.75 ^{cd}
T ₂ : <i>Trichoderma viride</i>	14.15 ^b	16.66 ^a	13.18 ^{cd}	13.26 ^{cd}	13.37 ^a	13.59 ^{ab}	13.78 ^{ab}
T ₃ : Coconut water	13.52 ^{cd}	14.95 ^c	13.03 ^d	13.11 ^d	12.70 ^{ab}	13.96 ^a	14.12 ^a
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	13.37 ^d	16.08 ^{ab}	17.08 ^a	14.71 ^a	12.03 ^{abc}	12.38 ^{de}	13.22 ^b
T ₅ : <i>P. fluorescens</i> + coconut water	12.57 ^e	16.28 ^{ab}	11.22 ^e	11.33 ^f	11.42 ^{bcd}	11.78 ^e	13.31 ^b
T ₆ : <i>T. viride</i> + coconut water	13.22 ^d	17.06 ^a	14.35 ^{bcd}	11.76 ^e	11.13 ^{cd}	10.56 ^f	9.57 ^e
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	13.09 ^d	15.44 ^{bc}	15.18 ^b	14.17 ^b	10.73 ^{cd}	12.87 ^{cd}	14.09 ^a
T ₈ : Hydro priming	13.99 ^{bc}	15.32 ^{bc}	14.89 ^b	13.26 ^{cd}	10.55 ^{cd}	10.56 ^f	12.17 ^c
T ₉ : Control	13.59 ^{cd}	13.34 ^d	14.58 ^{bc}	13.56 ^c	11.24 ^{cd}	11.14 ^{bc}	11.18 ^d
C.D	0.50	1.04	1.37	0.36	1.34	0.64	0.60
SE(m)	0.16	0.34	0.45	0.12	0.44	0.21	0.20

Table 22: Impact of biopriming on seedling dry weight (g) of rice seeds after artificial ageing

Treatments	D1	D2	D3	D4	D5	D6	D7
T ₁ : <i>Pseudomonas fluorescens</i>	0.249 ^a	0.247 ^{cd}	0.247 ^a	0.240 ^d	0.233 ^c	0.234	0.23
T ₂ : <i>Trichoderma viride</i>	0.226 ^b	0.258 ^a	0.233 ^d	0.249 ^b	0.253 ^a	0.242	0.24
T ₃ : Coconut water	0.248 ^a	0.232 ^e	0.227 ^e	0.233 ^e	0.239 ^{bc}	0.24	0.243
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	0.243 ^a	0.243 ^d	0.237 ^{bc}	0.242 ^{cd}	0.241 ^{bc}	0.242	0.241
T ₅ : <i>P. fluorescens</i> + coconut water	0.248 ^a	0.250 ^{bc}	0.247 ^a	0.234 ^e	0.245 ^{ab}	0.231	0.231
T ₆ : <i>T. viride</i> + coconut water	0.248 ^a	0.245 ^d	0.234 ^{cd}	0.244 ^c	0.240 ^{bc}	0.243	0.242
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	0.248 ^a	0.260 ^a	0.227 ^e	0.256 ^a	0.245 ^{ab}	0.239	0.233
T ₈ : Hydro priming	0.248 ^a	0.245 ^d	0.236 ^{bc}	0.233 ^e	0.237 ^{bc}	0.234	0.234
T ₉ : Control	0.248 ^a	0.253 ^b	0.238 ^b	0.243 ^{cd}	0.246 ^{ab}	0.244	0.237
C.D	0.006	0.004	0.003	0.003	0.009	N/A	N/A
SE(m)	0.002	0.001	0.001	0.001	0.003	0.004	0.005

4.3.5. Seedling vigour index I

Among the treatments, T₁*P. fluorescens* @10g/kg showed maximum seedling vigour index (2136) and it was followed by T₇*P. fluorescens* (10g/kg)+ *T. viride* (4g/kg)+ coconut water (75%) (1997), T₄*P. fluorescens* @10g/kg + *T. viride* @4g/kg (1892), T₉control (1897) respectively, while, T₆ *T. viride* (4g/kg) + coconut water (75%) and T₅*P. fluorescens* @10g/kg + coconut water (75%) registered minimum value (1748 and 1735) respectively.(Table 24)

At the end of ageing period, T₇*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) and T₄*P. fluorescens* @10g/kg + *T. viride* @4g/kg (1646 and 1634 respectively) recorded maximum seedling vigour It was on par with T₂*T. viride* @4g/kg (1631). minimum seedling vigour index was noticed in T₉ -control (1393) was followed by T₈-Hydro priming (1404).

4.3.6. Vigour index II

Significant differences due to seed treatments in vigour index II was observed during the ageing period and it declined during the advancement of ageing (Table 25). Seeds treated with T₂-*T. viride* @4g/kg (2005) which was followed by T₄-*P. fluorescens* @10g/kg + *T. viride* @4g/kg (1994) and T₇-*P. fluorescens* (10g/ kg) + *T. viride* (4g/kg) + coconut water (75%) (1888) retained superiority in maintaining vigour index II. Among the seed treatments T₈-Hydro priming (1713) exhibited lowest vigour index II.

4.3.7. Speed of germination

Among the treatments, early germination was observed in T₁ -*P. fluorescens*@10g/kg (18.50) which was on par with T₃-coconut water (75%) (18.38). Whereas, delayed germination was observed in T₈-hydro priming and T₅-*P. fluorescens* @10g/kg + coconut water (75%) (16.20 and 16.71) respectively at initial stage.

Irrespective of treatments, the early germination T_1 *P. fluorescens*@10g/kg (18.50) was recorded at initial day of ageing and rate of germination was late after 7 days of ageing (11.76) in T_9 Control as indicated in Table 26.

At the end of artificial ageing T_4 *P. fluorescens* @10g/kg + *T. viride* @4g/kg, T_7 *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) and T_1 *P. fluorescens*@10g/kg recorded highest speed of germination (15.04, 14.94 and 14.69) respectively and was followed by T_3 Coconut water (75%) (14.32). While, T_9 Control recorded lowest speed of germination (11.76) was on par with T_8 Hydro priming (11.83).

4.3.8. Mean germination time

The results obtained on mean germination time were significant and has increased during the storage period are presented in Table 27. The mean germination time was initially low in T_8 (hydro priming) with 2.59 days while, it increased towards the end of artificial ageing period reaching 6.52 days in control (T_9).

Among the treatments, mean germination time was observed least in T_8 -Hydro priming and T_6 - *T. viride* (4g/kg) + coconut water (75%) (2.59 d and 2.63 d) respectively. Whereas, highest mean germination time was observed in T_4 -*P. fluorescens* @10g/kg + *T. viride* @4g/kg and T_7 -*P. fluorescens* (10g/ kg) + *T. viride* (4g/kg) + coconut water (75%) (3.01d and 3.03 d) respectively at initial stage.

At the end of artificial ageing T_8 -hydro priming (6.04 d) followed by T_3 -coconut water (75%) (6.22 d) recorded least mean germination time. While, T_9 -Control and T_1 -*P. fluorescens*@10g/kg recorded highest mean germination time (6.52 d and 6.42 d) was on par with T_2 -*T. viride* @4g/kg (6.37 d)

4.3.9. Time taken for 50% germination

The results recorded on time taken for 50% germination as influenced by the priming treatments during the ageing period are enumerated in Table 28. The time taken for 50% germination was initially low in T_8 (Hydro priming) with 3.67 days

while, it increased towards the end of artificial ageing period reaching 7.54 days in control (T₉).

Time taken for 50% germination was initially low in T₈ hydro priming with 3.67 days was on par with control (3.7 days) and increased towards the end of storage it became nonsignificant and highest time was recorded by control (T₉) as 7.54 days. This was on par with other treatments T₁, T₂, T₃, T₄, T₅, T₆, T₇, T₈ recorded as 7.35, 7.18, 7.47, 7.14, 7.03, 7.14, 7.03, 7.25 respectively.

4.3.10 Electrical conductivity (μScm^{-1})

The electrical conductivity of seed leachates was maximum in T₉ Control ($31.25 \mu\text{Scm}^{-1}$). Whereas, minimum was recorded in T coconut water (75%) ($22.95 \mu\text{Scm}^{-1}$) and was on par with T *P. fluorescens*@10g/kg ($25.85 \mu\text{Scm}^{-1}$) at initial stage.

The increasing trend in electrical conductivity of seed leachates was observed with increase in days of ageing ($22.95 \mu\text{Scm}^{-1}$ at initial day to $68.65 \mu\text{Scm}^{-1}$ after 7 days of ageing) as enumerated in Table 29.

At the end of artificial ageing, highest electrical conductivity of seed leachates was noticed in treatment T₉ Control ($68.65 \mu\text{Scm}^{-1}$) whereas, lowest in treatment, T₄ *P. fluorescens* @10g/kg + *T. viride* @4g/kg ($43.45 \mu\text{Scm}^{-1}$) and T₂ *T. viride* @4g/kg ($44.55 \mu\text{Scm}^{-1}$) respectively.

4.3.11 Moisture content (%)

The seed moisture content was maximum in T₄ *P. fluorescens* @10g/kg + *T. viride* @4g/kg (10.50%). Whereas, minimum was recorded in T₂ *T. viride* @4g/kg (8.74%) and was on par with T₆ *T. viride* (4g/kg) + coconut water (75%) and T₅ *P. fluorescens* @10g/kg + coconut water (75%) (8.90%) at initial stage.

Table 23: Impact of bioprimering on seedling vigour index I of rice seeds after artificial ageing

Treatments	D1	D2	D3	D4	D5	D6	D7
T ₁ : <i>Pseudomonas fluorescens</i>	2136 ^a	1874 ^{abc}	1741 ^c	1746 ^{bc}	1621 ^c	1558 ^c	1516 ^b
T ₂ : <i>Trichoderma viride</i>	1806 ^{bc}	1803 ^a	1740 ^{cd}	1733 ^{cd}	1709 ^{bc}	1671 ^b	1631 ^{ab}
T ₃ : Coconut water	1827 ^{bc}	1762 ^{cde}	1750 ^{cd}	1740 ^{bc}	1606 ^{cd}	1559 ^c	1485 ^d
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	1892 ^b	1972 ^a	1871 ^b	1776 ^b	1740 ^b	1685 ^b	1634 ^a
T ₅ : <i>P. fluorescens</i> + coconut water	1735 ^c	1964 ^a	1833 ^{bc}	1626 ^e	1578 ^{cd}	1489 ^d	1485 ^d
T ₆ : <i>T. viride</i> + coconut water	1748 ^c	1706 ^{bcd}	1651 ^c	1558 ^{ef}	1475 ^e	1449 ^{de}	1434 ^e
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	1997 ^b	1916 ^{ab}	1902 ^a	1886 ^a	1857 ^a	1776 ^a	1646 ^a
T ₈ : Hydro priming	1840 ^{bc}	1711 ^{de}	1704 ^{ef}	1694 ^e	1463 ^e	1413 ^e	1404 ^e
T ₉ : Control	1897 ^b	1654 ^e	1630 ^{cde}	1603 ^e	1530 ^d	1443 ^{de}	1393 ^f
C.D	127.48	133.51	129.37	53.54	94.59	89.46	71.73
SE(m)	42.57	44.59	43.21	17.88	31.59	29.88	23.95

Table 24: Impact of biopriming on seedling vigour index II of rice seeds after artificial ageing

Treatments	D1	D2	D3	D4	D5	D6	D7
T ₁ : <i>Pseudomonas fluorescens</i>	2158 ^a	2145 ^a	2083	2059 ^{bc}	2042 ^a	1972 ^{bc}	1911 ^{bc}
T ₂ : <i>Trichoderma viride</i>	2202 ^a	2195 ^a	2080	2058 ^{bc}	2042 ^a	2009 ^a	2005 ^a
T ₃ : Coconut water	2119 ^a	2107 ^a	2034	1927 ^e	1876 ^e	1807 ^{de}	1777 ^{cd}
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	2154 ^a	2073 ^b	2100	2071 ^b	2028 ^b	1998 ^b	1994 ^b
T ₅ : <i>P. fluorescens</i> + coconut water	2114 ^a	2025 ^{bc}	1993	1978 ^{cd}	1943 ^c	1893 ^{cd}	1725 ^{cd}
T ₆ : <i>T. viride</i> + coconut water	2065 ^a	1997 ^{de}	1957	1945 ^d	1903 ^d	1896 ^{cd}	1854 ^{cd}
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	2167 ^a	2156 ^a	2140	2150 ^a	2045 ^a	1962 ^{bc}	1888 ^c
T ₈ : Hydro priming	1978 ^b	1903 ^{ef}	1859	1855 ^e	1831 ^f	1757 ^e	1713 ^d
T ₉ : Control	2110 ^a	2097 ^{bc}	2055	2015 ^c	1982 ^{bc}	1819 ^d	1766 ^{cd}
C.D	114.43	71.88	N/A	90.30	50.05	99.91	66.17
SE(m)	38.22	24.01	71.74	30.16	16.71	33.36	22.1

Table 25: Impact of biopriming on speed of germination of rice seeds after artificial ageing

Treatments	D1	D2	D3	D4	D5	D6	D7
T ₁ : <i>Pseudomonas fluorescens</i>	18.50 ^a	17.79 ^a	17.34 ^a	17.27 ^a	16.90 ^a	15.89 ^a	14.69 ^a
T ₂ : <i>Trichoderma viride</i>	17.87 ^{bc}	17.37 ^b	17.18 ^a	17.23 ^a	17.20 ^a	14.61 ^{cd}	13.31 ^{cd}
T ₃ : Coconut water	18.38 ^{ab}	16.60 ^c	15.08 ^c	15.08 ^{cd}	14.90 ^c	15.3 ^b	14.32 ^b
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	17.69 ^{cd}	17.01 ^{bc}	16.18 ^b	16.42 ^b	16.40 ^{ab}	16.02 ^a	15.04 ^a
T ₅ : <i>P. fluorescens</i> + coconut water	16.71 ^e	17.17 ^b	17.21 ^a	15.50 ^c	15.10 ^c	14.52 ^{cd}	13.32 ^{cd}
T ₆ : <i>T. viride</i> + coconut water	17.29 ^d	16.17 ^d	15.04 ^c	14.68 ^{de}	15.30 ^c	13.49 ^d	12.37 ^d
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	17.37 ^{cd}	17.36 ^b	17.01 ^a	16.37 ^b	15.70 ^{bc}	15.99 ^a	14.94 ^a
T ₈ : Hydro priming	16.20 ^e	15.13 ^e	14.54 ^c	14.05 ^e	12.98 ^d	12.8 ^e	11.83 ^e
T ₉ : Control	17.88 ^{bc}	17.17 ^b	16.11 ^{bc}	15.07 ^{cd}	13.70 ^{cd}	12.84 ^e	11.76 ^e
C.D	0.531	0.418	0.621	0.71	0.851	0.38	0.26
SE(m)	0.177	0.139	0.207	0.237	0.284	0.127	0.124

Table 26: Impact of biopriming on mean germination time of rice seeds after artificial ageing

Treatments	D1	D2	D3	D4	D5	D6	D7
T ₁ : <i>Pseudomonas fluorescens</i>	2.95 ^{ab}	3.00 ^a	3.05 ^a	3.74 ^a	4.77 ^a	5.32 ^a	6.42 ^a
T ₂ : <i>Trichoderma viride</i>	2.93 ^{ab}	2.92 ^{ab}	2.95 ^{ab}	3.79 ^a	4.79 ^a	5.3 ^a	6.37 ^{ab}
T ₃ : Coconut water	2.85 ^{bc}	3.00 ^a	2.90 ^b	3.41 ^c	4.46 ^c	4.96 ^{cd}	6.22 ^c
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	3.01 ^a	2.90 ^{bc}	2.90 ^b	3.66 ^{ab}	4.69 ^{ab}	5.14 ^b	6.27 ^{cde}
T ₅ : <i>P. fluorescens</i> + coconut water	2.80 ^c	2.72 ^d	3.05 ^a	3.45 ^c	4.64 ^b	5.3 ^a	6.29 ^{cd}
T ₆ : <i>T. viride</i> + coconut water	2.63 ^d	2.88 ^{bc}	2.88 ^b	3.48 ^c	4.46 ^c	4.95 ^d	6.24 ^{de}
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	3.03 ^a	2.82 ^c	2.87 ^b	3.54 ^{abc}	4.66 ^b	5.27 ^a	6.32 ^{bc}
T ₈ : Hydro priming	2.59 ^d	2.63 ^d	2.88 ^b	3.17 ^d	4.4 ^c	5.03 ^c	6.04 ^f
T ₉ : Control	2.95 ^{ab}	2.92 ^{ab}	3.10 ^a	3.71 ^a	4.72 ^{ab}	5.29 ^a	6.52 ^a
C.D	0.1	0.088	0.101	0.143	0.099	0.078	0.057
SE(m)	0.034	0.03	0.034	0.048	0.033	0.026	0.019

Table 27: Impact of bioprimering on time taken for 50% germination of rice seeds after artificial ageing (days)

Treatments	D1	D2	D3	D4	D5	D6	D7
T ₁ : <i>Pseudomonas fluorescens</i>	4.12 ^a	5.35 ^a	6.06 ^c	6.66	6.98	7 ^b	7.35
T ₂ : <i>Trichoderma viride</i>	4.16 ^a	5.35 ^a	6.09 ^c	6.65	7.04	7.04 ^b	7.18
T ₃ : Coconut water	4.06 ^a	5.35 ^a	6.1 ^c	6.57	7.01	7.02 ^b	7.47
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	4.00 ^a	4.85 ^b	6.02 ^c	6.75	6.99	7.07 ^b	7.14
T ₅ : <i>P. fluorescens</i> + coconut water	4.04 ^a	5.1 ^{ab}	6.48 ^a	6.57	6.97	7.05 ^b	7.03
T ₆ : <i>T. viride</i> + coconut water	4.02 ^a	4.97 ^{ab}	6.46 ^a	6.8	6.97	7.01 ^b	7.14
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	3.95 ^{ab}	5.22 ^{ab}	6.42 ^{ab}	6.67	7.00	7.02 ^b	7.03
T ₈ : Hydro priming	3.67 ^c	5.22 ^{ab}	6.31 ^b	6.75	7.01	7.19 ^a	7.25
T ₉ : Control	3.7 ^{bc}	5.35 ^a	6.41 ^{ab}	6.75	6.96	7.07 ^b	7.54
C.D	0.266	0.345	0.135	N/A	N/A	0.081	N/A
SE(m)	0.089	0.115	0.045	0.076	0.072	0.027	0.122

The increasing trend in seed moisture content observed with increase in days of ageing (8.74 per cent at initial day to 19.4 per cent after 7 days of ageing) as enumerated in Table 30.

At the end of artificial ageing, highest moisture content was noticed in treatment T₉Control (19.4%) whereas, lowest in treatment, T₂*T. viride* @4g/kg and T₆ *T. viride* (4g/kg) + coconut water (75%) (14.25%) which on par with T₄-*P. fluorescens* @10g/kg + *T. viride* @4g/kg (14.35) and T₇*P. fluorescens* (10g/kg)+ *T. viride* (4g/kg)+ coconut water(75%) (14.5%) respectively.

4.3.12 Dehydrogenase enzyme activity (OD value)

T₁*P. fluorescens* @10g/kg and T₇*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) registered maximum dehydrogenase enzyme activity (0.775 and 0.741 OD value) at initial stage. The minimum dehydrogenase enzyme activity (0.518 OD value) was registered in T₃ Coconut water (75%) which is on par with T₅ *P. fluorescens* @10g/kg + coconut water (75%) and T₄*P. fluorescens* @10g/kg + *T. viride* @4g/kg (0.555 and 0.566 OD values) respectively.

The mean dehydrogenase enzyme activity decreased with increase in days of ageing from 0.775 OD value at initial day to 0.112 OD value after 7 days of ageing irrespective of the treatments as indicated in Table 31.

At the end of ageing period, T₇ *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) registered maximum dehydrogenase enzyme activity of 0.268 OD value and was on par with T₁*P. fluorescens* @10g/kg (0.206 OD value). Whereas, minimum was registered in T₅*P. fluorescens* @10g/kg + coconut water (75%) (0.112 OD value).

4.3.13 Super oxide dismutase enzyme activity (mg¹/protein)

Super oxide dismutase enzyme activity has been decreased from 97.26 U/mg protein recorded in T₇ *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water

(75%) at initial months of storage observation to 25.51 U/mg protein in T₄*P. fluorescens* @10g/kg + *T. viride* @4g/kg indicating the results in Table 32.

SOD enzyme activity was maximum in T₇ (*P. fluorescens* (10g/ kg) + *T. viride* (4g/kg) + Coconut water (75%):97.25 U/mg protein) at initial was followed by T₁ (*P. fluorescens*@10g/kg: 92.65 U/mg protein). The minimum value (53.67 U/mgprotein) was registered in T₉Control.

Also, at the end of ageing period, the treatments became non significant with T₇ *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) registered maximum SOD enzyme activity of 27.17 U/mg protein and was on par with T₁, T₂, T₃, T₄, T₅, T₆, T₈, T₉ recorded as 23.73, 24.25, 21.58, 22.45, 23.23, 23.6, 24.66, 21.65 respectively.

4.3.14. Seed infection (%)

The data on mean seed infection per cent in each treatment is presented in Table 33a and 33b. The parameter showed significant variations among the treatments. Least seed infection is seen in T₁-*P. fluorescens*@10g/kg (1.33 per cent Agar plate with surface sterilization) and highest seed infection is observed in T₉-Control (64 per cent in blotter method without sterilization).

In agar plate method significantly lower seed infection was observed in T₁-*P. fluorescens*@10g/kg (1.33%) which was on par with T₇-*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) and T₂-*T. viride* @4g/kg and T₃-coconut water (75%) recorded as 2.33%. Control (21.33%) and T₄-*P. fluorescens* @10g/kg + *T. viride* @4g/kg (19.33%) were found inferior with significantly higher seed infection.

Similar observations were recorded in blotter paper method. Lower seed infection was observed in T₃-coconut water (75%) (2.66%). This is on par with T₅-*P. fluorescens* @10g/kg + coconut water (75%) (5.00%) while T₉- control recorded highest seed infection (26.00%).

Table 28: Impact of bioprimering on electrical conductivity (μScm^{-1}) of rice seeds after artificial ageing

Treatments	D1	D2	D3	D4	D5	D6	D7
T ₁ : <i>Pseudomonas fluorescens</i>	25.85 ^{de}	27.8	30.20 ^{cd}	41.15 ^f	43.15 ^e	44.15 ^e	47.95 ^d
T ₂ : <i>Trichoderma viride</i>	27.05 ^{cd}	27.85	35.50 ^{ab}	42.25 ^e	40.80 ^c	42.80 ^e	44.55 ^f
T ₃ : Coconut water	22.95 ^e	24.5	34.30 ^{bcd}	48.35 ^c	46.55 ^b	49.55 ^b	54.65 ^b
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	27.65 ^{cd}	29.5	29.90 ^d	41.25 ^f	43.35 ^e	44.35 ^e	46.45 ^f
T ₅ : <i>P. fluorescens</i> + coconut water	26.45 ^{cd}	29.4	34.75 ^{bc}	51.65 ^b	45.60 ^d	45.60 ^d	51.50 ^c
T ₆ : <i>T. viride</i> + coconut water	29.35 ^{bc}	31.25	34.35 ^{bcd}	42.90 ^e	47.05 ^c	48.05 ^c	48.15 ^e
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	29.25 ^{bc}	30.35	35.20 ^b	39.55 ^g	44.35 ^d	46.35 ^d	46.65 ^{de}
T ₈ : Hydro priming	24.85 ^{de}	28.45	36.20 ^{ab}	55.15 ^a	42.85 ^e	42.85 ^e	53.40 ^{bc}
T ₉ : Control	31.25 ^a	32.75	40.050 ^a	46.20 ^d	48.10 ^a	52.10 ^a	68.65 ^a
C.D	3.149	N/A	4.391	1.218	1.032	0.881	1.594
SE(m)	1.052	1.996	1.467	0.407	0.345	0.294	0.533

Table 29: Impact of biopriming on seed moisture content (%) of rice seeds after artificial ageing

Treatments	D1	D2	D3	D4	D5	D6	D7
T ₁ : <i>Pseudomonas fluorescens</i>	9.60 ^{bc}	11.35 ^{bc}	13.30 ^a	13.40 ^{ab}	13.90 ^a	14.35 ^c	14.75 ^d
T ₂ : <i>Trichoderma viride</i>	8.74 ^d	10.00 ^f	12.30 ^{cd}	12.55 ^d	12.90 ^{bc}	13.65 ^{de}	14.25 ^e
T ₃ : Coconut water	9.75 ^{ab}	10.35 ^{ef}	11.40 ^e	11.90 ^e	12.25 ^d	12.75 ^f	15.25 ^c
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	10.50 ^a	10.80 ^d	11.25 ^e	12.05 ^e	12.55 ^{cd}	13.25 ^e	14.35 ^{de}
T ₅ : <i>P. fluorescens</i> + coconut water	8.90 ^{cd}	11.65 ^b	12.35 ^{cd}	12.70 ^d	12.80 ^c	13.75 ^d	14.75 ^d
T ₆ : <i>T. viride</i> + coconut water	8.90 ^{cd}	10.70 ^{de}	11.30 ^e	11.70 ^e	12.25 ^d	13.50 ^{de}	14.25 ^e
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	9.10 ^{bc}	11.20 ^c	12.25 ^d	13.15 ^{bc}	12.90 ^{bc}	13.25 ^e	14.50 ^{de}
T ₈ : Hydro priming	9.90 ^{ab}	11.65 ^b	12.65 ^{bc}	12.75 ^{cd}	13.25 ^b	17.25 ^a	18.35 ^b
T ₉ : Control	9.15 ^{bc}	12.25 ^a	12.75 ^b	13.75 ^a	14.25 ^a	16.25 ^b	19.40 ^a
C.D	0.733	0.388	0.366	0.417	0.356	0.412	0.453
SE(m)	0.245	0.129	0.122	0.139	0.119	0.137	0.151

Table 30: Impact of biopriming on dehydrogenase enzyme activity (OD value) of rice seeds after artificial ageing

Treatments	D1	D2	D3	D4	D5	D6	D7
T ₁ : <i>Pseudomonas fluorescens</i>	0.775 ^a	0.706 ^{ab}	0.667 ^{ab}	0.639 ^a	0.440 ^{ab}	0.330 ^{ab}	0.206 ^{ab}
T ₂ : <i>Trichoderma viride</i>	0.669 ^{bc}	0.635 ^d	0.618 ^c	0.577 ^c	0.422 ^{ab}	0.274 ^{de}	0.180 ^b
T ₃ : Coconut water	0.518 ^d	0.494 ^f	0.470 ^d	0.435 ^d	0.406 ^b	0.258 ^e	0.152 ^{bc}
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	0.566 ^d	0.539 ^e	0.500 ^d	0.451 ^d	0.432 ^{ab}	0.339 ^a	0.171 ^{bc}
T ₅ : <i>P. fluorescens</i> + coconut water	0.555 ^d	0.520 ^{ef}	0.505 ^d	0.466 ^d	0.355 ^c	0.222 ^f	0.112 ^d
T ₆ : <i>T. viride</i> + coconut water	0.682 ^{bc}	0.676 ^{abc}	0.627 ^{bc}	0.561 ^c	0.444 ^{ab}	0.302 ^c	0.160 ^{bc}
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	0.741 ^a	0.711 ^a	0.678 ^a	0.644 ^a	0.467 ^a	0.337 ^a	0.268 ^a
T ₈ : Hydro priming	0.693 ^b	0.667 ^{bcd}	0.657 ^{abc}	0.623 ^{ab}	0.436 ^{ab}	0.306 ^{bc}	0.157 ^{bc}
T ₉ : Control	0.637 ^c	0.648 ^{cd}	0.637 ^{abc}	0.594 ^{bc}	0.398 ^{bc}	0.290 ^{cd}	0.136 ^{cd}
C.D	0.047	0.036	0.045	0.042	0.049	0.024	0.033
SE(m)	0.016	0.012	0.015	0.014	0.016	0.008	0.011

Table 31: Impact of biopriming on super oxide dismutase enzyme activity (U/mg protein) of rice seeds after artificial ageing

Treatments	D1	D2	D3	D4	D5	D6	D7
T ₁ : <i>Pseudomonas fluorescens</i>	92.65 ^b	83.45 ^a	72.04 ^a	66.76 ^a	41.60 ^b	26.51 ^c	23.73
T ₂ : <i>Trichoderma viride</i>	75.80 ^e	64.33 ^d	53.31 ^{de}	44.37 ^d	38.59 ^{bc}	35.48 ^a	24.25
T ₃ : Coconut water	78.67 ^e	67.88 ^c	56.73 ^{cd}	48.16 ^c	39.18 ^{bc}	28.18 ^{bc}	21.58
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	84.52 ^d	69.22 ^c	58.23 ^c	49.31 ^c	37.14 ^c	25.51 ^c	22.45
T ₅ : <i>P. fluorescens</i> + coconut water	89.21 ^c	78.22 ^b	68.21 ^b	57.16 ^b	39.99 ^{bc}	29.29 ^{bc}	23.23
T ₆ : <i>T. viride</i> + coconut water	65.11 ^f	54.30 ^e	49.26 ^f	43.19 ^d	37.56 ^c	30.47 ^b	23.6
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	97.26 ^a	86.27 ^a	75.35 ^a	67.96 ^a	45.24 ^a	36.62 ^a	27.17
T ₈ : Hydro priming	66.51 ^f	55.28 ^e	50.28 ^{ef}	44.73 ^d	38.14 ^{bc}	29.25 ^{bc}	24.66
T ₉ : Control	53.67 ^g	43.66 ^f	40.28 ^g	37.30 ^e	33.25 ^d	28.36 ^{bc}	21.65
C.D	3.386	3.486	3.732	3.42	3.315	3.42	N/A
SE(m)	1.131	1.164	1.246	1.142	1.107	1.142	1.092

Aspergillus spp. were observed in all the treatments. In addition, *Rhizopus* spp. was observed in treatments T₁, T₃, T₄, T₆, T₈, T₉. *Trichoderma* spp. was observed in T₂, T₇, T₈, T₉. In addition to these seed borne pathogens of rice like *Helminthosporium oryzae* and *Rhizoctonia solani*, saprophytic fungi like *Curvularia* spp., *Syncephalastrum* spp., *Pencillium* spp., *Mucor* spp., etc were also observed.

Table 32: Fungi observed in infected seeds after accelerated ageing

Treatments	Fungi observed
T ₁ <i>Pseudomonas fluorescens</i>	<i>Aspergillus</i> spp., <i>Mucor</i> spp., <i>Syncephalastrum</i> spp., <i>Rhizopus</i> spp.
T ₂ <i>Trichoderma viride</i>	<i>Alternaria</i> spp., <i>Pencillium</i> spp., <i>Helminthosporium oryzae</i> , <i>Trichoderma</i> spp.
T ₃ Coconut water	<i>Aspergillus</i> spp., <i>Rhizopus</i> spp.
T ₄ <i>P. fluorescens</i> + <i>T. viride</i>	<i>Aspergillus</i> spp., <i>Rhizopus</i> spp., <i>Pencillium</i> spp.
T ₅ <i>P. fluorescens</i> + coconut water	<i>Aspergillus</i> spp.
T ₆ <i>T. viride</i> + coconut water	<i>Aspergillus</i> spp., <i>Rhizopus</i> spp., <i>Trichoderma</i> spp.
T ₇ <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	<i>Aspergillus</i> spp., <i>Trichoderma</i> spp.,
T ₈ Hydro priming	<i>Aspergillus</i> spp., <i>Trichoderma</i> spp., <i>Rhizopus</i> spp. <i>Rhizoctonia solani</i> , <i>Pencillium</i> spp.
T ₉ Control	<i>Aspergillus</i> spp., <i>Mucor</i> spp., <i>Rhizopus</i> spp., <i>Trichoderma</i> spp.

Table 33a: Impact of biopriming on Seed infection (%) of rice seeds after artificial ageing

Treatments	Blotter paper method (per cent seed infection)													
	D1		D2		D3		D4		D5		D6		D7	
	W.S	S	W.S	S	W.S	S	W.S	S	W.S	S	W.S	S	W.S	S
T ₁ : <i>Pseudomonas fluorescens</i>	9.00 ^c	8.33 ^{ab}	12.33 ^d	8.66 ^{bc}	21.00 ^{bcd}	11.66 ^{cd}	25.00 ^b	14.00 ^{cd}	32.00 ^c	14.33 ^{ef}	34.33 ^c	15.66 ^e	40.33 ^c	15.33 ^e
T ₂ : <i>Trichoderma viride</i>	12.33 ^b	7.66 ^{bc}	18.00 ^b	9.66 ^{ab}	22.00 ^{bc}	12.66 ^{bc}	25.00 ^b	14.66 ^{bc}	31.00 ^c	16.66 ^c	28.00 ^d	18.66 ^c	30.33 ^d	16.66 ^d
T ₃ : Coconut water	12.00 ^b	2.66 ^d	4.00 ^{bcd}	5.66 ^e	18.00 ^{de}	9.66 ^e	21.33 ^c	12.66 ^{de}	21.0 ^e	13.66 ^f	25.00 ^{ef}	15.66 ^e	28.00 ^d	17.66 ^c
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	13.00 ^b	6.33 ^c	4.00 ^{bcd}	9.66 ^{ab}	17.66 ^{de}	10.66 ^{de}	20.00 ^c	12.66 ^{de}	21.00 ^e	5.66 ^{cde}	24.00 ^{ef}	17.66 ^{cd}	22.66 ^f	18.66 ^c
T ₅ : <i>P. fluorescens</i> +coconut water	11.66 ^b	5.00 ^{cd}	13.00 ^{cd}	8.66 ^{bc}	16.66 ^e	10.66 ^{de}	21.00 ^c	11.66 ^e	20.00 ^e	4.66 ^{def}	23.00 ^f	16.66 ^{de}	25.66 ^e	15.66 ^e
T ₆ : <i>T. viride</i> + coconut water	12.33 ^b	5.66 ^c	17.00 ^{bc}	7.00 ^{de}	19.00 ^{cde}	11.00 ^{de}	21.00 ^c	14.00 ^{cd}	23.00 ^d	16.00 ^{cd}	25.66 ^e	18.33 ^c	28.66 ^d	22.66 ^b
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	13.33	6.33 ^c	5.66 ^{bcd}	9.00 ^{bc}	17.33 ^{de}	13.00 ^{bc}	19.00 ^c	13.00 ^{de}	21.00 ^e	5.00 ^{def}	23.00 ^f	19.00 ^c	25.00 ^e	18.00 ^{cd}

T ₈ : Hydro priming	18.00	11.3 ^a	22.33 ^a	11.0 ^a	24.00 ^b	13.33 ^b	26.00 ^b	16.00 ^b	36.00 ^b	19.00 ^b	40.66 ^b	22.00 ^b	54.33 ^b	25.00 ^a
T ₉ : Control	17.33	5.66 ^c	22.00 ^a	8.00 ^{cd}	28.00 ^a	15.00 ^a	35.66 ^a	18.00 ^a	41.66 ^a	22.00 ^a	43.66 ^a	24.00 ^a	64.00 ^a	26.00 ^a
C.D	3.629	2.743	3.865	1.372	3.409	1.288	3.343	1.45	1.852	1.488	2.182	1.288	2.305	1.525
SE(m)	1.212	0.916	1.291	0.458	1.139	0.43	1.117	0.484	0.619	0.497	0.729	0.43	0.77	0.509

Table 33b: Impact of bioprimer on Seed infection (%) of rice seeds after artificial ageing

Treatments	Agar plate method (per cent seed infection)													
	D1		D2		D3		D4		D5		D6		D7	
	W. S	S	W. S	S	W. S	S	W. S	S	W. S	S	W. S	S	W. S	S
T ₁ : <i>Pseudomonas fluorescens</i>	4.21	1.33 ^d	5.3 ^{def}	3.00 ^c	7.12 ^e	5.00 ^d	8.00 ^d	5.33 ^{de}	8.00 ^f	6.00 ^e	11.6 ^e	8.00 ^e	15.00 ^c	11.00 ^d
T ₂ : <i>Trichoderma viride</i>	3.12	2.33 ^{cd}	4.00 ^f	3.00 ^c	6.14 ^e	3.33 ^e	9.11 ^d	5.00 ^e	13.12 ^e	6.00 ^e	16.21 ^d	9.00 ^e	15.60 ^c	14.0 ^{cd}
T ₃ : Coconut water	3.30	3.00 ^c	5.00 ^{ef}	4.00 ^{bc}	11.15 ^c	5.00 ^d	13.12 ^b	7.00 ^{cd}	15.13 ^{cd}	8.00 ^d	16.14 ^d	11.00 ^d	16.60 ^c	15.00 ^c
T ₄ : <i>P. fluorescens</i> + <i>T. viride</i>	4.12	3.33 ^{bc}	8.00 ^{bc}	6.00 ^{ab}	9.13 ^d	7.00 ^{bc}	12.13 ^c	8.00 ^{bc}	16.14 ^{bc}	14.00 ^a	18.15 ^{bc}	16.00 ^b	23.60 ^b	19.33 ^{ab}

T ₅ : <i>P. fluorescens</i> + coconut water	6.14	3.66 ^{bc}	9.00 ^b	6.00 ^{ab}	13.15 ^{ab}	8.00 ^{ab}	16.12 ^a	9.00 ^b	17.16 ^{ab}	9.00 ^d	19.15 ^b	12.00 ^{cd}	22.30 ^b	16.66 ^{bc}
T ₆ : <i>T. viride</i> + coconut water	7.30	3.66 ^{bc}	9.00 ^b	5.00 ^{abc}	12.14 ^{bc}	8.00 ^{ab}	14.3 ^{ab}	9.33 ^b	16.11 ^{bc}	11.00 ^c	18.16 ^b	13.00 ^c	23.00 ^b	14.00 ^{cd}
T ₇ : <i>P. fluorescens</i> + <i>T. viride</i> + coconut water	5.00	2.33 ^{cd}	6.00 ^{de}	4.00 ^{bc}	9.15 ^d	6.00 ^{cd}	13.14 ^b	7.0 ^{cd}	14.11 ^{de}	9.00 ^d	17.16 ^c	12.00 ^{cd}	17.30 ^c	14.00 ^{cd}
T ₈ : Hydro priming	5.3	4.66 ^{ab}	7.00 ^{cd}	5.00 ^{abc}	11.17 ^c	6.66 ^{bc}	13.11 ^b	7.66 ^{bc}	12.6 ^e	11.33 ^b	16.18 ^c	12.00 ^{cd}	23.30 ^b	14.66 ^c
T ₉ : Control	6.3	5.66 ^a	12.00 ^a	6.33 ^a	14.17 ^a	8.66 ^a	15.12 ^a	12.66 ^a	18.09 ^a	14.66 ^a	22.12 ^a	17.66 ^a	27.30 ^a	21.33 ^a
C.D	N/A	1.488	1.852	1.94	1.729	1.525	1.663	1.852	1.852	1.596	1.663	1.663	2.598	3.067
SE(m)	0.93	0.497	0.619	0.648	0.577	0.509	0.556	0.619	0.619	0.533	0.556	0.556	0.868	1.024

Table 34: Ranking of priming treatments based on seed quality parameters after accelerated ageing

Treatments	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
Seed quality parameters	Ranks								
Seed germination (%)	1	1	4	2	3	3	2	4	5
Seed moisture content (%)	2	1	3	2	2	1	2	4	5
Seedling shoot length(cm)	2	1	7	4	6	5	3	2	7
Seedling root length(cm)	4	2	1	2	2	4	1	3	5
Seedling dry weight(g)	1	1	1	1	1	1	1	1	1
Vigour index-I	2	2	3	1	3	4	1	5	6
Vigour index-II	3	1	4	2	4	4	3	4	4
EC leachate (μScm^{-1})	3	1	5	1	4	2	2	5	6
Speed of germination	1	3	2	1	3	4	1	5	6
Mean Germination Time (MGT)	6	5	2	3	3	2	4	1	6
Time taken for 50% germination (T50)	1	1	1	1	1	1	1	1	1
Seed infection (%)	1	2	3	5	4	2	2	3	6
Dehydrogenase enzyme activity(nm)	2	3	5	4	6	4	1	4	5
Superoxide dismutase enzyme activity ($\text{mg}^{-1}/\text{protein}$)	1	1	1	1	1	1	1	1	1
Total score	30	25	42	30	43	38	25	43	64
Final Ranks	2	1	4	2	5	3	1	5	6

Discussion

V. DISCUSSION

Seed is one of the most significant basic agricultural input which serves as a catalyst for maximizing the potential of all other agricultural inputs. Treating seeds with various agents-physical, chemical and biological enhances the seed quality and improves seed longevity. Seed priming is a type of seed treatment wherein the hydration-dehydration process, after priming is used to reduce the degree of moisture in seeds to levels compatible with storage while maintaining the beneficial effects of the treatment without quality loss due to rapid seed deterioration.

Priming with biological agents effectively improves seed health thereby controls disease in the plants, in addition to improving germination, uniformity of emergence, and decreasing the time to emergence like other priming methods. Organic seed treatments are easily accessible to farmers at low cost, with the added advantage of being ecofriendly is therefore gaining importance day by day.

In light of the foregoing, the findings of the current study, "Impact of biopriming on seed quality and longevity in rice" (*Oryza sativa* L.), is discussed here under.

5.1 Quality of seeds before seed treatment

The seed had a germination of 94.32 percent and a moisture content of 13.40 per cent. Hence the seed lot was considered of good quality suitable for the study as seeds longevity and storability have been found to be a consequence of the initial seed quality (Hatherley and Elmore, 2004).

5.2. Effect of seed priming on seed quality

Seed treatment had a favourable impact / modest increase in seed quality. Re-drying the treated seeds had ensured that the moisture level was conducive (<10.0%) for safe storage. Wang *et al.* (2018) reported that primed rice seeds stored below 10.00 and nine per cent moisture content respectively, retained seed quality over a period of 210 days.

The initial germination per cent ranged between 87 per cent (T₉ control) and 92.26 per cent [T₇-*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%)] similar to findings of Vinothini and Bhavyasree (2019) in ground nut.

The longest seedlings were produced from the bio primed treatment (*P. fluorescens* @10g/kg+ *T. viride* @4g/kg) which is in accordance with the findings of Rai *et al.* (2018) in chilli and Laxman and Ghodke (2018) in sunflower.

Seeds treated with coconut water exhibited longer roots and had higher seedling dry weight which according to Dunsin *et al* (2016) may be due to presence of cytokinin which induces cell division as of similar results reported by Origenes and Lapitan (2020) in kamagong This enhancement was reflected in the vigour indices wherein seeds treated with coconut water alone or in combination with bioagents exhibited higher vigour indices. Seeds treated with a combination of bioagents namely *P. fluorescens* and *T. virid e* also recorded higher seed vigour. Several workers have also reported similar results (Kumari *et al.* (2021), Bhavyasree and Vinothini (2019) and Reddy *et al* (2018)).

Least electrical conductivity was recorded in T₁ *P fluorescens*@10g/kg (21.06 μScm^{-1}) which was followed by T₈ Hydro priming (21.18 $\mu\text{S cm}^{-1}$) and 2T *T viride*@4g/kg (23.21 μScm^{-1}) This result is in accordance with the findings of Rinku *et al.* (2017) where hydropriming and control resulted in higher electrical conductivity value, when compared to biopriming.

Seeds primed with *P. fluorescens*@10g/kg and hydro primed seeds registered higher values for speed of germination while seeds primed with T₆ *T. viride* (4g/kg) + coconut water (75%) recorded least which were similar to the results of Sridevi and Manomani (2019) in barnyard millet. The primed seeds took less than three days to attain the mean germination time and around two days to attain 50% germination which is in concurrence with the findings of Mudi (2016) in rice.

In agar plate method seed infection varied from 1.33 (*P. fluorescens* @10g/kg) to 5.66 per cent (control). In blotter paper method, T₈-hydro priming had recorded the highest seed infection (14.33%) and least infection was observed in T₃-coconut water (75%) recorded as 2.66% which are co-related with findings of Nithya and Geetha (2017) in rice and Rajput *et al.* (2019) in tomato.

Mougy and Kader (2008) found that bioprimering faba bean seeds primed with either *T. viride* or *P. fluorescens* as efficient in protecting against root rot pathogen infection at both the pre-emergence and post-emergence stages

5.2 Seed quality assessment during storage

5.2.1 Analysis of Variance

During the storage period, analysis of variance revealed significant variations in the impact of treatments on seed quality measures such as germination, moisture content, seedling dry weight, seedling length, seed leachates, and vigour index I and II. SOD enzyme activity, dehydrogenase enzyme activity, and micro flora infection in seeds all showed significant variations.

5.2.2.1. Germination (%)

Significant differences were observed among the treatments for germination per cent from the initial month of storage. The treatments had recorded gradual decrease in the mean germination per cent with the advancement of storage period (Fig.1). Such gradual reduction in germination per cent with progress of seed ageing was earlier observed by Suganya (2013) in rice, Hussain *et al.* (2015), Nithya and Geetha (2017) and Kalaivani (2010) in maize.

Highest germination per cent was observed in *P. fluorescens* @10g/kg (90.08%) and combination of *P. fluorescens* @10g/kg + *T. viride* @4g/kg (89.88) during the fifth month of storage in concurrent with Prakash *et al.* (2021) in sorghum, indicating growth promoting activity of *Pseudomonas fluorescens*. Least germination per cent

was recorded in T₈ Hydro priming (80.83%) which is similar with the reports of Sridevi and Manomani (2019) in proso millet.

At the end of storage, the combined treatment of *P. fluorescens*, *T. viride* and coconut water recorded higher germination per cent (83.69%) which was in concurrence with the findings of Abdel Khader *et al.* (2012) in vegetables, Rosna (2019) in okra

All treatments maintained the MSCS (Minimum Seed Certification Standard) of 80 per cent germination up to six months of storage. At the end of the study period (nine months) the germination ranged between 77.62 per cent (T₉) and 83.69 per cent (T₇). Increase in storage duration depressed the longevity of primed seeds in addition to delayed and reduced germination as opined by McDonald (1999). He asserted that the frequency and severity of such detrimental effects increased with storage duration.

5.2.2.2. Seedling performance

Seed treatment with biopriming resulted in a significant increase in seedling shoot length however the effect decreased gradually over the period of storage,

In the sixth month, *T. viride* (4g/kg) treated individually and in combination recorded longest shoots (T₇, T₂) which is in accordance with the findings of Jaiman *et al.* (2020b) in brinjal and chilli, Karthika and Vangamudi (2013) in maize.

At the end of storage, the combination of *P. fluorescens*, *T. viride* and coconut water gave the longest shoots. According to Somasundaram and Bhaskaran (2017) *Pseudomonas* spp. produces gibberellins, auxins, and cytokinin, such as isopentenyladenosine (IPA), dihydroxy zeatin riboside (DHZR), and zeatin riboside (ZR), which help in stimulating the plant growth. These results were also in line with the findings of Dezfuli *et al.* (2008) in maize, Raj *et al.* (2004) in pearl millet.

The mean seedling root length showed gradual decline with advancement of storage irrespective of the treatments. The highest value was recorded by combinations

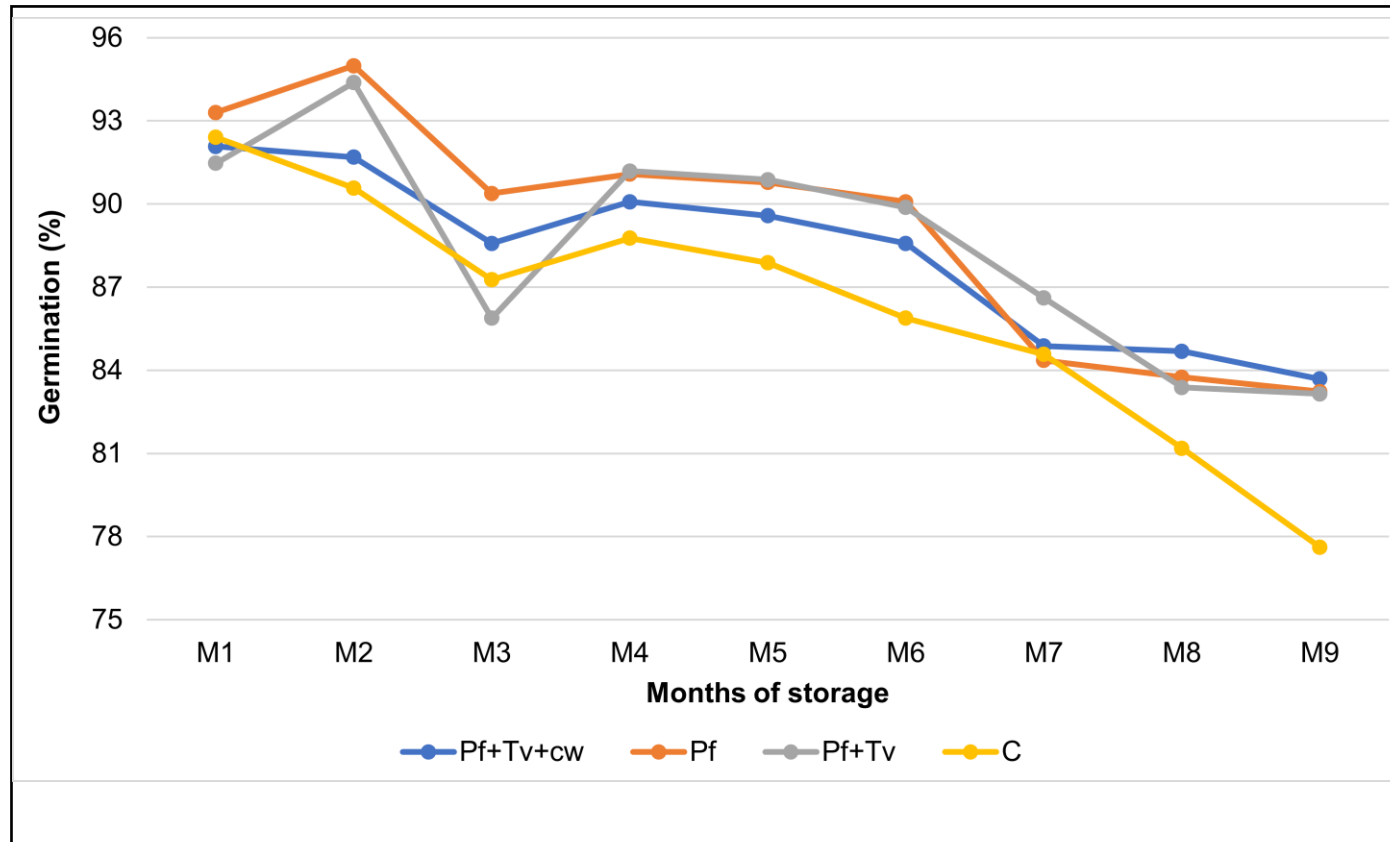


Fig.1. Germination of primed seeds of rice across the storage period

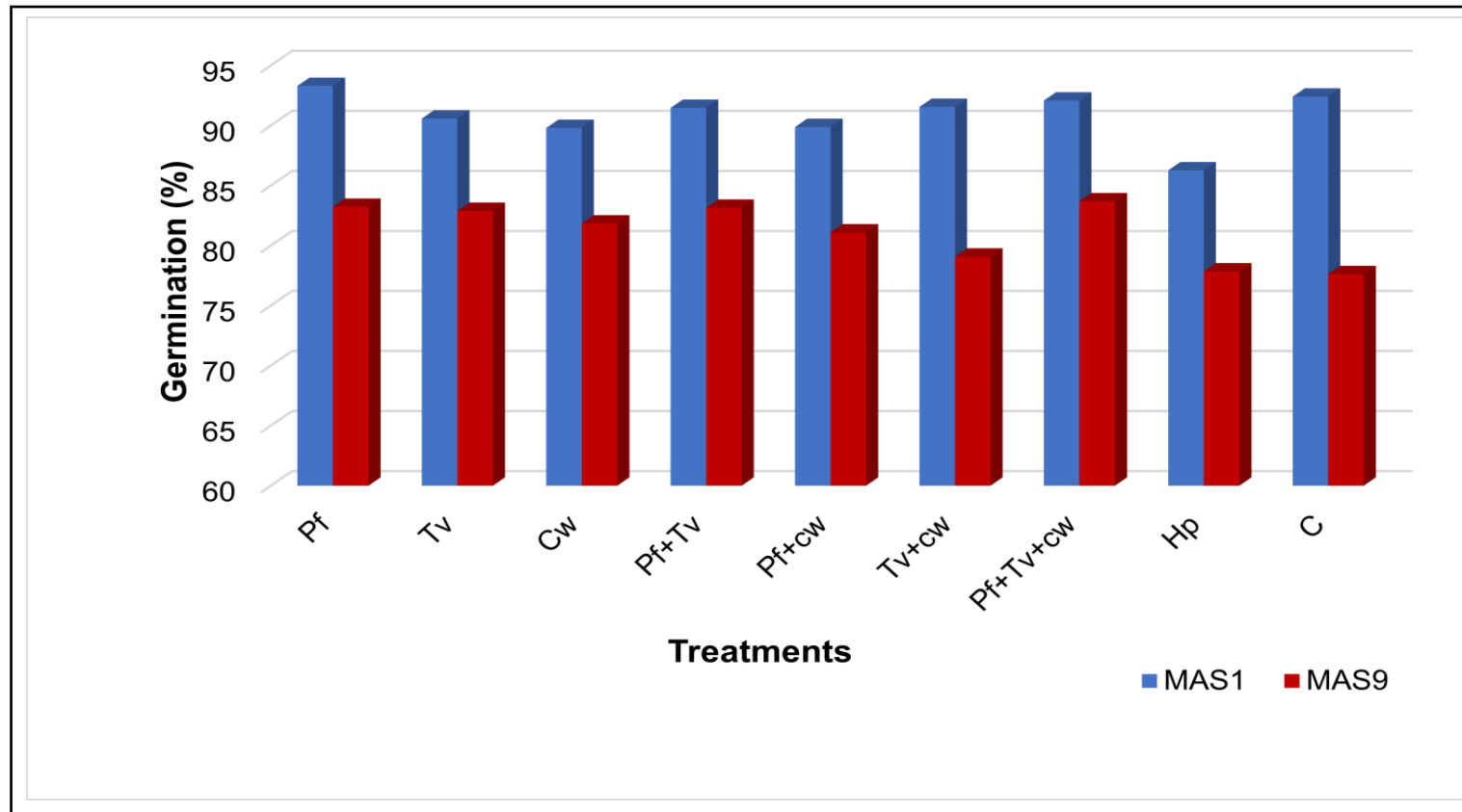


Fig.2. Impact of seed treatments on seed germination at 1MAS and 9MAS

of *P. fluorescens* and *T. viride* (T₇, T₄) in the third month (17.27 cm and 17.26 cm respectively)

The mean seedling root length was observed to be higher in treatments with coconut water individually and in combination T₇-*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (11.44 cm), T₃-coconut water (75%) (11.29 cm) which is due to the phytohormones auxins and cytokinin's produced by bio agents as well as coconut water have been able to induce better growth thereby retaining seed quality during storage. This result is in conformity with Zheng and Shetty (2000) in pea, Pradhan *et al.* 2017 in black gram.

5.2.2.3. Seedling dry weight (mg)

Regardless of seed treatments, seedling dry weight varied significantly throughout the storage period, with a decreasing tendency as the storage time progressed (fig. 4)

In the 9MAS, higher seedling dry weight was observed in T₇-*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (0.242 g) which was on par with T₂-*T. viride* @4g/kg (0.240 g. which was in line with the results of Karthika and Vanangamudi (2013) in maize, Kavitha (2011) in rice.

5.2.2.4. Vigour indices

Seed vigour is defined as "the sum of those seed qualities that govern the level of activity and performance of the seed or seed lot during germination and seedling emergence" (ISTA, 2001), as well as a measure of a seed lot's storage potential.

The treatments considerably differed for the parameter over the time of storage. After reaching a peak in the second month (2528), the mean vigour index I steadily decreased until the end of storage which is in concurrent with the results of Jaiman *et al.* (2020b) in vegetables, Moradi and Younasi (2009) in maize.

Over the months of storage, vigour index I was found to be significantly higher in bio primed treatments *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (1645), *P. fluorescens*@10g/kg (1553), *T. viride*@4g/kg (1537) individually as development promoting organisms like *Trichoderma* spp. and *Pseudomonas* spp., according to Diaz *et al.* (2001), improve plant growth and biomass production by boosting nutrient absorption (e.g., N, P, K) and providing hormones in the rhizosphere to encourage plant growth which was in line with the results. Control recorded the least value (1417). Similar results were also observed in findings of Murungu *et al.* (2004) in sorghum, Saglam *et al.* (2010) in lentil, Singh *et al.* (2011) in cowpea.

Vigour index II (Bewley and Black, 1994) is a function of seedling dry weight and germination per cent. Table 10 shows the effect of bioprimering on vigour index II during the storage period. There were substantial differences across the treatments, with the parameter declining over the storage time (fig.6).

At the end of storage, highest value for vigour index II was observed in T₇ *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (2029) which was followed by T₂ *T. viride* @4g/kg (1989). Least was recorded in control (1737). These results are in line with the research findings of Monalisa *et al.* (2017) in common bean, Yadav *et al.* (2013) in chick pea.

5.2.2.5. Speed of germination

There was no significant difference initially among the treatments as indicated in Table 11. Highest speed of germination was recorded in T₁ (21.04) whereas, lowest (18.45) was recorded in (T₈) which are in concurrent with findings of Sori (2014) in chickpea, Raja *et al.* (2017) in rice.

Enhanced seed germination by seed coating or seed inoculum is due to presence of cytokinin on germinating seeds (Holland and Polacco, 1994) was explained the highest speed of germination noticed in T₄ *P. fluorescens* @10g/kg + *T. viride* @4g/kg (19.02), T₇ *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (18.99), T₁ *P. fluorescens* @10g/kg seeds (18.89) whereas minimum was

noticed in T₈-hydro priming (15.80) which is in line with the results of Gajendra (2015) in rice.

5.2.2.6. Mean germination time

The average germination time is an important feature of vigour and can be used to determine the vigour of any seed lot. It is a method for calculating germination speed (Zhang *et al.*, 2014). The results obtained on mean germination time were significant and has increased during the storage period are presented in Table 12.

The mean germination time was initially low in T₈ (hydro priming) with 2.53 days while it increased towards the end of storage reaching 5.36 days in control (T₉). This significant increase in time was in line with the findings of Athulya (2019) and Nagendra *et al.* (2017) in oriental pickling melon.

The lower the MGT, the faster a population of seeds has germinated which has been observed in treatments with combination of bio primed and coconut water (T₇ and T₅) recorded 5.08 days reported as quickest, the highest mean germination time of 5.36 days at the end of the storage period was recorded under the treatment, T₉- Control.

5.2.2.7. Time taken for 50% germination

The time it takes for 50 per cent germination is inversely proportional to seedling vigour, which is determined by the time it takes to reach 50 per cent of germination with the argument method set to "cool bear," and is computed using the formula developed by Cool bear *et al.* (1984) and modified by Farooq *et al.* (2006c).

This germination indicator has the same pattern as the mean germination time. *P. fluorescens* (10g/kg) + *T. viride* (4g/kg)+ coconut water (75%) (2.05 days) found to be quickest and increased towards the end of storage reaching, 4.42 days in *P. fluorescens*@10g/kg + *T.viride* @4g/kg was followed by *P. fluorescens* (10g/kg) + coconut water (75%) and T₂-*T. viride* @4g/kg (4.54 and 4.56 days respectively) and highest time was recorded by control (T₉) as 5.29 days indicated *P. fluorescens* showed quickest germination in all combinations which was seen concurrent with the

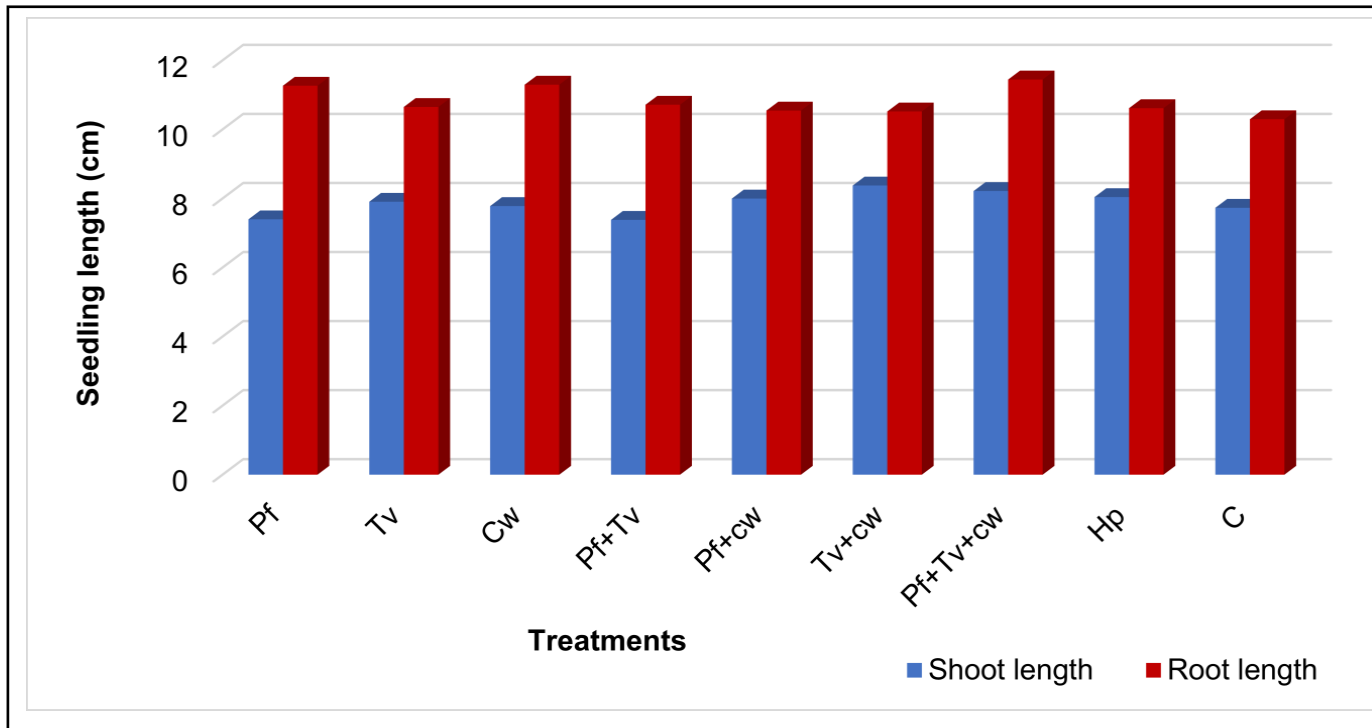


Fig.3. Impact of treatments on root length (cm) and shoot length (cm) of rice seedlings at 9MAS

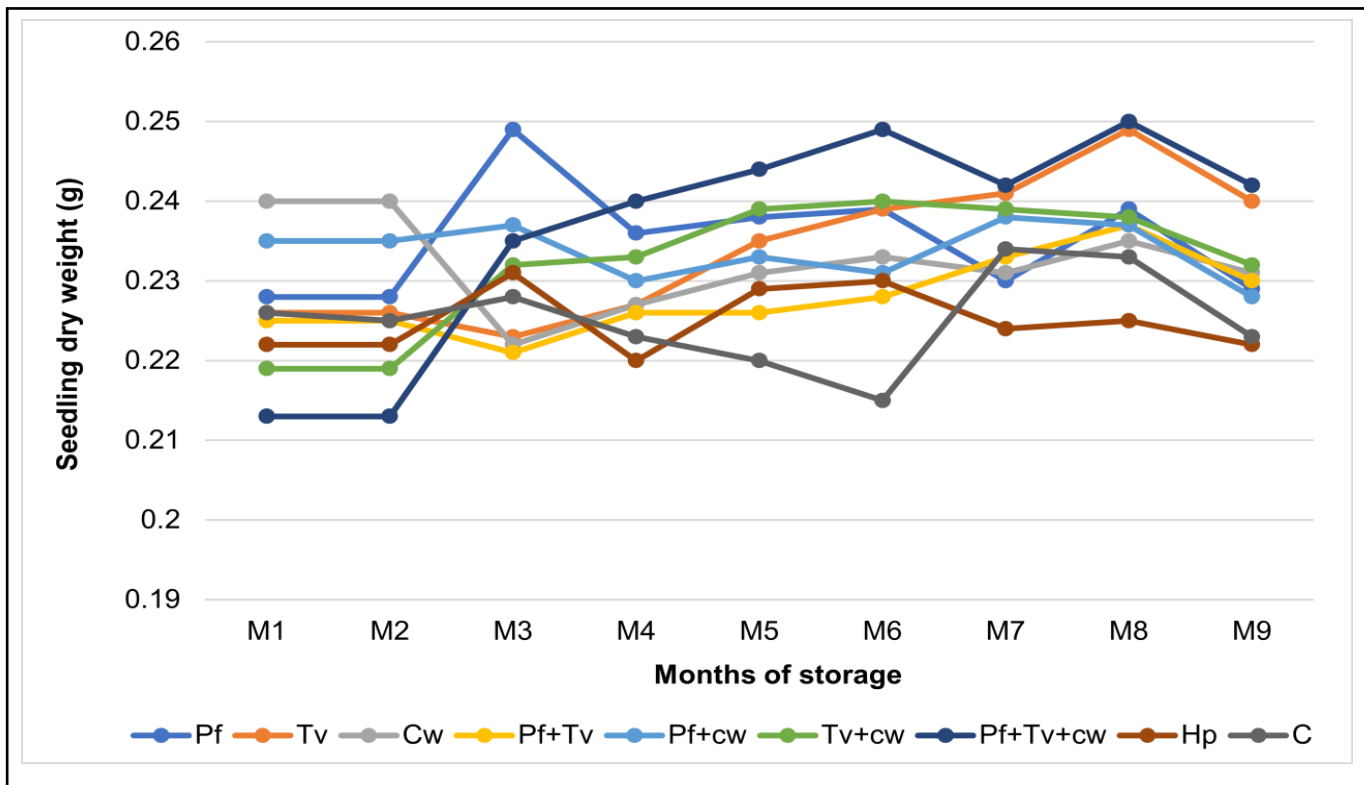


Fig. 4. Impact of treatments on dry weight of seedlings at 9 MAS

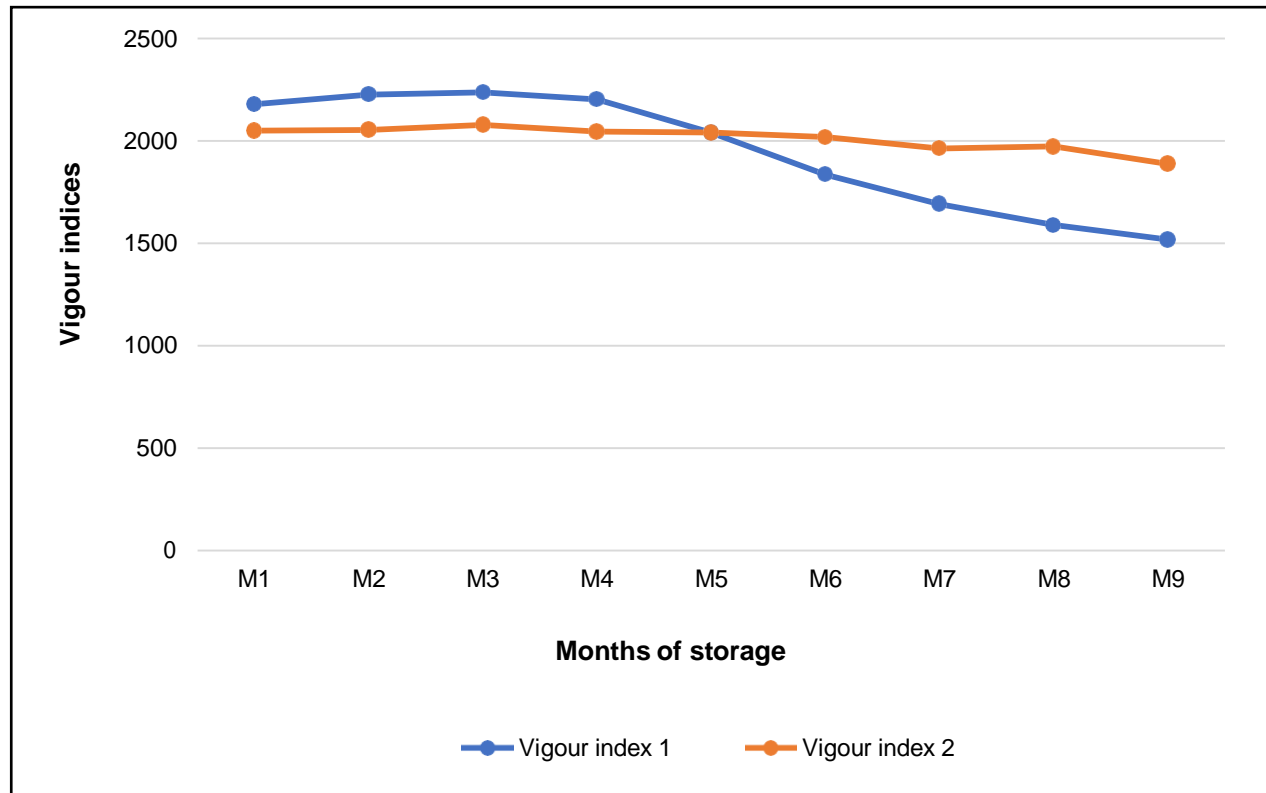


Fig. 5. Decline in seedling vigour I and II of rice over the storage period

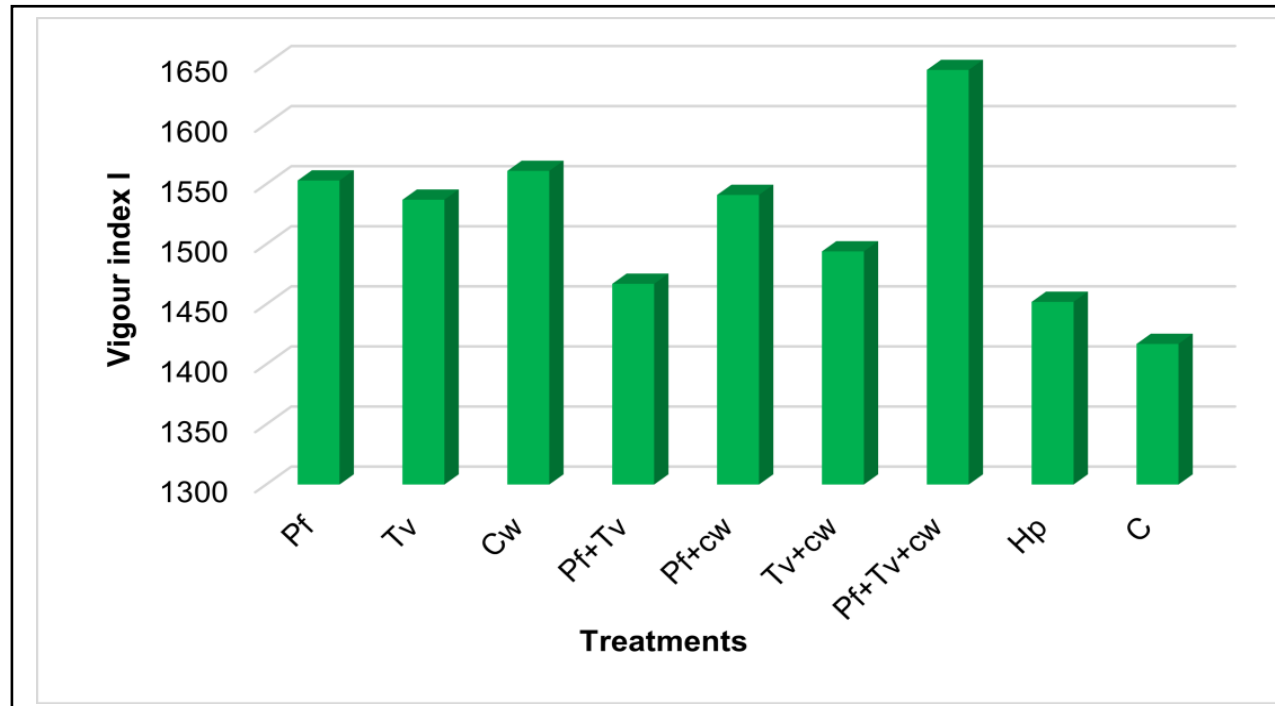


Fig. 6. Impact of treatments on seedling vigour index I at 9MAS

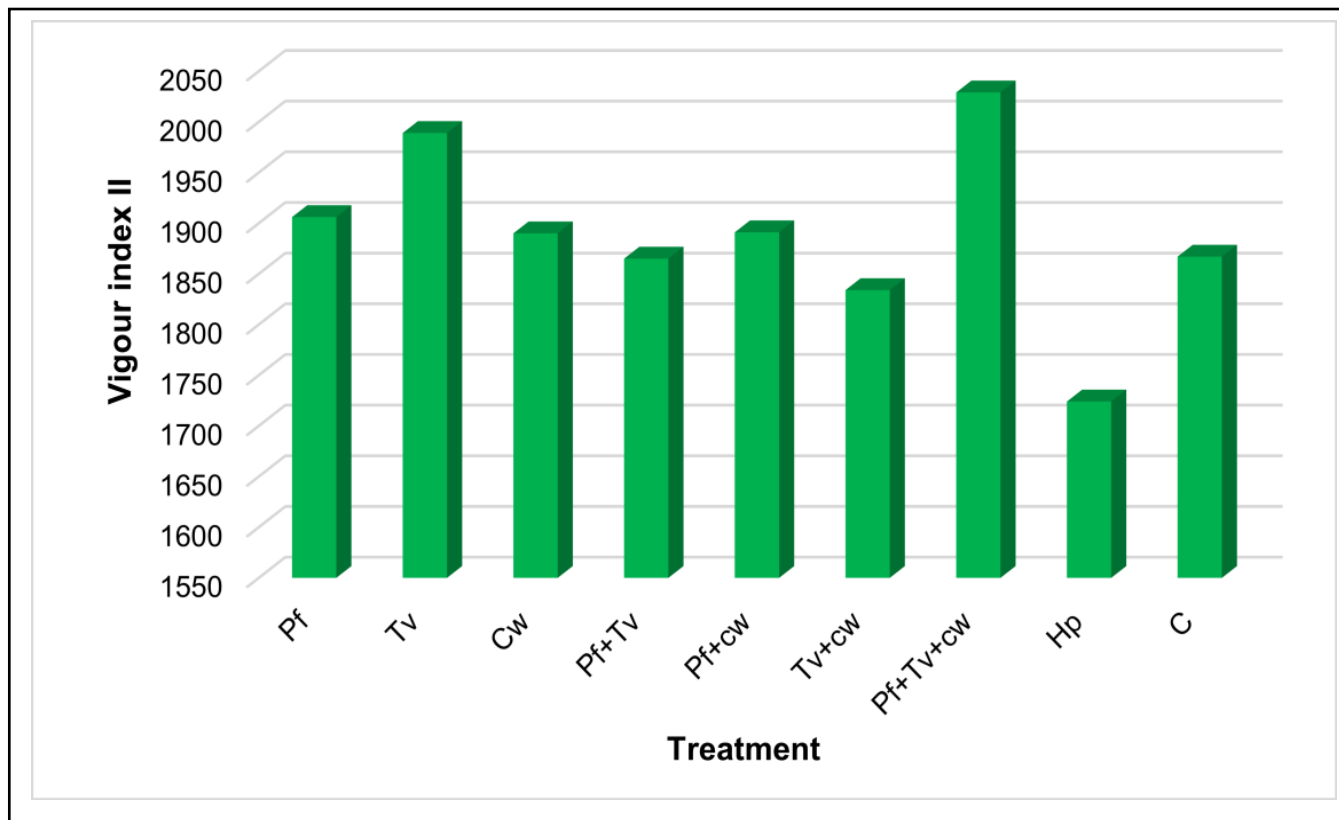


Fig. 7. Impact of treatments on seedling vigour index II at 9MAS

results of Jaiman *et al.* (2020b) in vegetables, Ananthi *et al.* (2014) in chilli, Reddy *et al.* (2011) in chick pea, Reshma (2018) in okra.

When tomato seeds were bio primed with *P. fluorescens*, Srivastava *et al.* (2010) discovered that germination was expedited by 2.0–2.5 days which was explained that production of endogenous plant growth regulators such as gibberellins, cytokinins, and/or indole acetic acid, have increased the availability of minerals and other ions, and/or helped in the increase of water uptake.

5.2.2.8. Electrical conductivity (EC) of seed leachates (μScm^{-1})

The electrical conductivity (EC) of seed leachate is a measure of the seed's vitality and vigour. More chemicals escape into the media as the membrane integrity of damaged seeds deteriorates. The severe mechanical injury, poor membrane structure, and leaky cells could all be to blame.

It also appears to be a determining factor for seed renewal time during storage in the eyes of retailers, and it can be used as a routine test for seed viability (Copeland and Mc Donald, 2001). Regardless of the treatments, the conductivity gradually increased throughout the storage period (fig.10).

In the first month, least value for EC of seed leachate ($21.33 \mu\text{Scm}^{-1}$) was recorded in T₇-*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) was followed by T₈-Hydro priming ($21.95 \mu\text{Scm}^{-1}$) and highest in control ($36.44 \mu\text{Scm}^{-1}$). This is consistent with the findings of Rinku *et al.* (2017), who found that biopriming had a lower electrical conductivity value than hydropriming and control.

At the end of storage, T₅ *P. fluorescens* (10g/kg) + coconut water (75%) recorded the least value ($30.83 \mu\text{Scm}^{-1}$) whereas, T₉ control had recorded the highest electrical conductivity ($38.86 \mu\text{Scm}^{-1}$) which are in concurrent with Chitra and Jijesh (2021) in eastern sandalwood, Parisa (2013) in chilli.

5.2.2.9. Seed moisture content (%)

At the end of storage, the seed moisture level varied significantly between treatments with a small rise in seed moisture content in all treatments (Table 15).

The meagre increase in moisture indicates the effectiveness of poly ethylene 700 gauge packaging and even under high humid conditions. The increase has been observed less in dry treatments than wet treatments which was in concurrent with research findings of Yildirim *et al.* (2021) in chilli, Mutai (2018) in soybean, Singh *et al.* (2014) in okra, Nataraj *et al.* (2011) in sunflower.

5.2.2.10. Dehydrogenase enzyme activity (OD value)

Initially dehydrogenase enzyme activity was high in T₁ (*P. fluorescens*@10g/kg: 0.795 OD value) and minimum value (0.511 OD value) was registered in T₃ Coconut water (75%) which followed a decreasing trend as enumerated in (fig.12)

At the end of storage period, bio primed treatments showed superior performance with T₇ registered maximum dehydrogenase enzyme activity of 0.357 OD value followed by T₁ (0.353 OD value) and T₄ (0.342 OD value) respectively. whereas, minimum was registered in *P. fluorescens* (10g/kg) + coconut water (75%) (0.246 OD value).

These results indicate dry dressed treatments of *P. fluorescens* gave highest enzyme activity which were similar to findings of Vaddinakatti (2014) in ground nut, Shakuntala *et al.* (2012) in paddy, Vasudevan *et al.* (2012) in groundnut and Reshma (2018) in okra.

5.2.2.13. Super oxide dismutase enzyme activity (mg⁻¹/protein)

SOD is an enzymatic antioxidant defense mechanism in plants that catalyses“ H₂O₂ and reactive oxygen species (ROS). When plants are exposed to oxidative stress, these free radicals cause damage, which leads to a reduction in physiological function

(Chatnaparat *et al.*, 2009; Hemsanit *et al.*, 2010). These antioxidants and protective enzymes play a crucial role in protecting plants from oxidative damage.

It has shown a decreasing trend throughout the storage period (fig.13) with initially maximum in T₇ (*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + Coconut water (75%):112.22 U/mg protein) and at the end of storage period, biopriming along with coconut water treatments T₇ (64.29 mg⁻¹protein) registered maximum SOD enzyme activity of and was followed by T₁ (58.13 U/mg protein) and T₅ (57.14 U/mg protein) respectively. Whereas, minimum was registered in control (T₉) as 27.23 U/mg protein. Prathuangwong *et al.* (2012) in rice and Wattanakulpakin *et al.* (2012) in maize have shown similar results as of present study.

5.2.2.14. Seed infection (%)

Seed treatment with root colonizing organisms like *Pseudomonads* and *Trichoderma* spp. is a possible control strategy to employ as rhizosphere resident microbial antagonists. Due to its outstanding root colonization ability, propensity to create a wide array of anti-microbial compounds, and induction of systemic resistance, *Pseudomonas fluorescens* and *Trichoderma viride* has attracted special attention among numerous biocontrol agents (Vanitha *et al.* ., 2009; Erdogan and Benlioglu, 2010).

In agar plate method lowest seed infection was observed towards the end of storage period in treatments primed with *P. fluorescens* (T₁ and T₇ which is in correlation with the findings of Shivalingaiah and Umesha (2013) in rice, Kumar *et al.* (2014) in chickpea, Sandhya, (2016) in chilli and Reshma, (2018) in okra.

Similar observations were also recorded in blotter paper method. Lower seed infection was observed in T₁ *P. fluorescens* @10g/kg (10.66%), T₂ *T. viride* @4g/kg (11.33%) and T₇ *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (11.66%). While, T₉ control recorded highest seed infection (22.00%) which are in line with the research results of Costa *et al.* (2013) in sorghum and Dorna *et al.* (2013) in onion.

Present study has observed hydro primed seeds with increased microbial infection towards the end of storage period which was supported by similar results observed for carrot hydro primed seeds, which demonstrated an increase in the *Alternaria radici* which is a parasitic fungus (Jensen *et al.*, 2004) with increasing soaking time, a considerable increase in seedborne fungus was also detected.

5.3 Artificial ageing

Seed deterioration is the decrease of quality, viability, and vigour of seeds as a result of ageing or adverse environmental influences. While ageing is defined as a gradual loss in biological capabilities that is followed by an increased risk of degenerative changes and death over time, with an increase in seed moisture content, storage length, or storage temperature, the rate of degradation accelerates (Sedghi *et al.*, 2011).

A number of metabolic abnormalities that accumulate in embryonic and non embryonic tissues have been linked to the loss of seed viability as seeds age. Seed ageing is linked to a variety of changes at the cellular level, including loss of membrane integrity, solute leakage, reduced energy metabolism, RNA (protein synthesis) impairment, and DNA degradation. Seed priming, which involves slowly absorbing and then re-drying seeds after soaking them in a low- water-potential solution, has been found to rejuvenate aged seeds (Arun *et al.*, 2021).

The present investigation shows the effect of accelerated ageing on seed quality parameters after ageing for seven days and readings were recorded on daily basis.

5.3.1. Germination (%)

Reduction in germination was noticed in accelerated aged seed (Fig. 16). Though initially all treatments are on par with each other there was a decreasing trend. Treatment *P. fluorescens*@10g/kg (89.56%) retained high value for germination per cent, while minimum was recorded in T₈ Hydro priming (79.95 %).

At the end, bioprimering treatments individually and in combination T₁ and T₂ recorded maximum germination (83.11% and 83.58%), which was on par with T₄ (82.93%) which indicates the superiority of dry treatments over wet treatments and minimum of 72.55 percentage was recorded in T₉ control similar to the results of Pandita *et al.* (2010) in okra, Ermis *et al.* (2016) in pepper.

5.3.2. Seedling shoot length (cm)

According to Ozbay (2018), seed aging may result in low seedling emergence and stand establishment. The treatment Hydro priming produced longest shoot of 9.17 cm, whereas the shortest shoot of 7.29 cm was produced by *T. viride* (4g/kg) + coconut water (75%) at initial stage which are in line with findings of Kairnat *et al.* (2015) in cucumber, broccoli (Bradford *et al.*, 1990; Jett *et al.*, 1996)

Decreasing trend of seedling shoot length was observed towards the end of ageing period (fig.16), bio primed treatments has shown superior results, *T. viride* @4g/kg recorded maximum shoot length (8.73 cm) and was followed by *P. fluorescens*@10g/kg and Hydro priming (8.41 and 8.38 respectively). Minimum seedling shoot length of 6.18 and 6.45 was recorded in control which was similar to findings of Saudi, (2017) in rice.

5.3.3. Seedling root length (cm)

Decline in seedling root length was observed from 15.65 cm at day one of ageing to 9.57 cm towards the end of artificial ageing (fig.18).

At the end of ageing period, coconut water (75%) and *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) recorded highest root length (14.12 cm and 14.09 cm respectively) and was on par with *T. viride* @4g/kg (13.78 cm) and minimum T₉ control (11.18 cm) similar results were also reported by Ramanujan *et al.* (2017) in black gram, Habib *et al.* (2010) in rice.

5.3.4. Seedling dry weight (g)

Seedling dry weight differed considerably at the start of the ageing period, regardless of seed treatments, and then declined in significance as the ageing period progressed, eventually becoming non significant at the end.

At the end of ageing period dry weight of 0.237 g to 0.243 g respectively was recorded which were in line with findings of Vijayan (2005) and Bijanzadeh *et al.* (2015) in rice.

5.3.5. Seedling vigour indices

Vigour indices has followed the same trend as germination percent of seeds T₁ showed maximum seedling vigour index (2136) and it was followed by T₇ (1997), T₄ (1892) respectively as indicated (fig.19).

At the end of ageing period, the combinations of biopriming and coconut water T₇ and T₄ (1646 and 1634 respectively) recorded high seedling vigour. While, minimum seedling vigour index was noticed in T₉ control (1393) which were concurrent with the findings of Vijayalakshmi (2012) in tomato and Saudi, (2017) in rice.

Significant differences due to seed treatments was observed in vigour index II during the ageing period and it declined with the advancement of ageing (Table 25). Zareian *et al.* (2013) has recorded similar results in wheat as biopriming treatments recorded highest as *T. viride* @4g/kg (2005) which was followed by T₄*P. fluorescens* @10g/kg + *T. viride* @4g/kg (1994) and T₇*P. fluorescens* (10g/kg)+ *T. viride* (4g/kg)+ coconut water (75%) (1888) retained superiority in maintaining vigour index II. Among the seed treatments hydro priming (1713) exhibited lowest vigour index II.

5.3.6. Speed of germination

Seed soaking treatments influenced significantly on germination speed index. Initially, early germination was observed in T₁ *P. fluorescens*@10g/kg (18.50) which

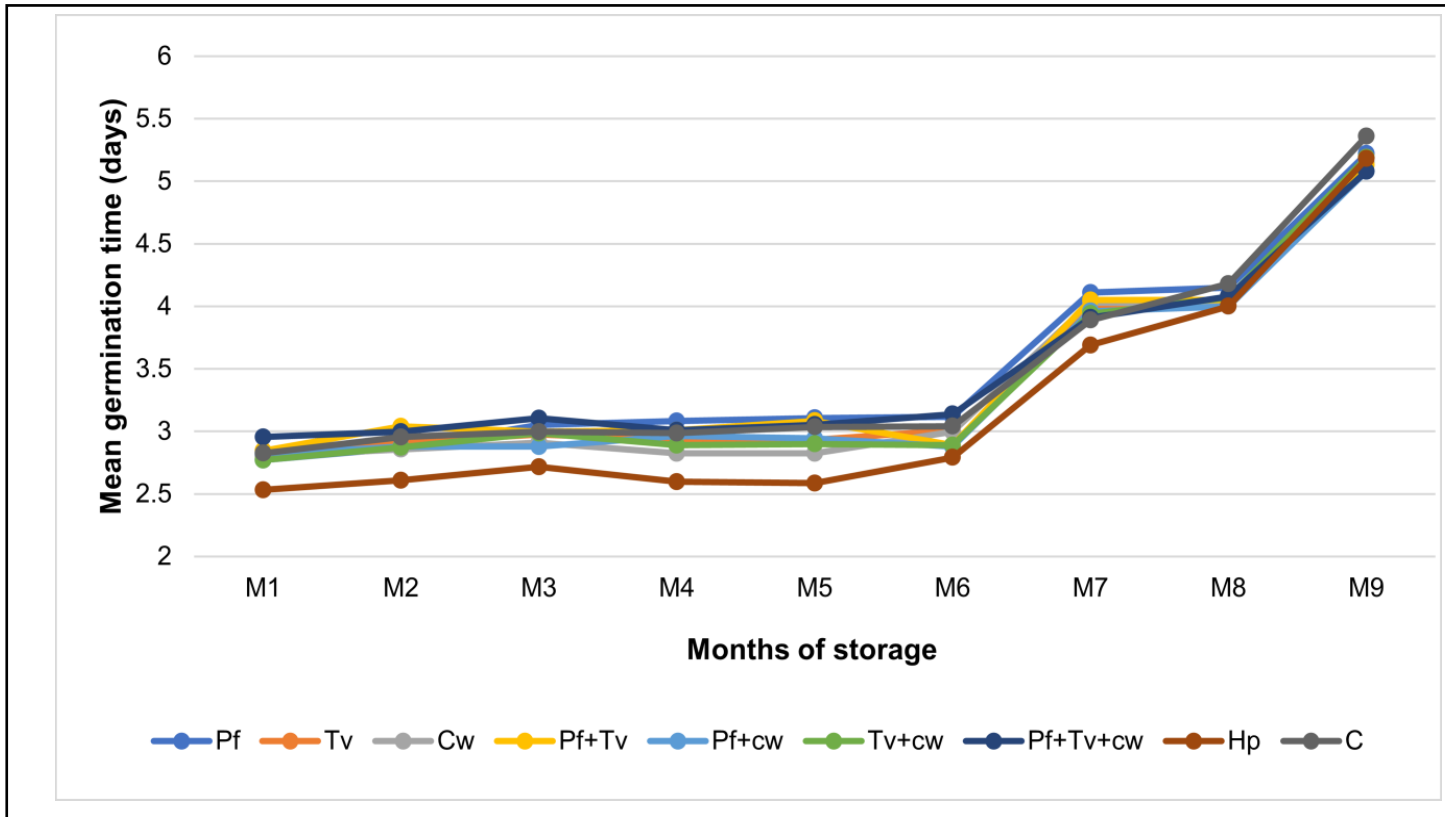


Fig 8: Impact of treatments on mean germination time (days) at 9 MAS in rice

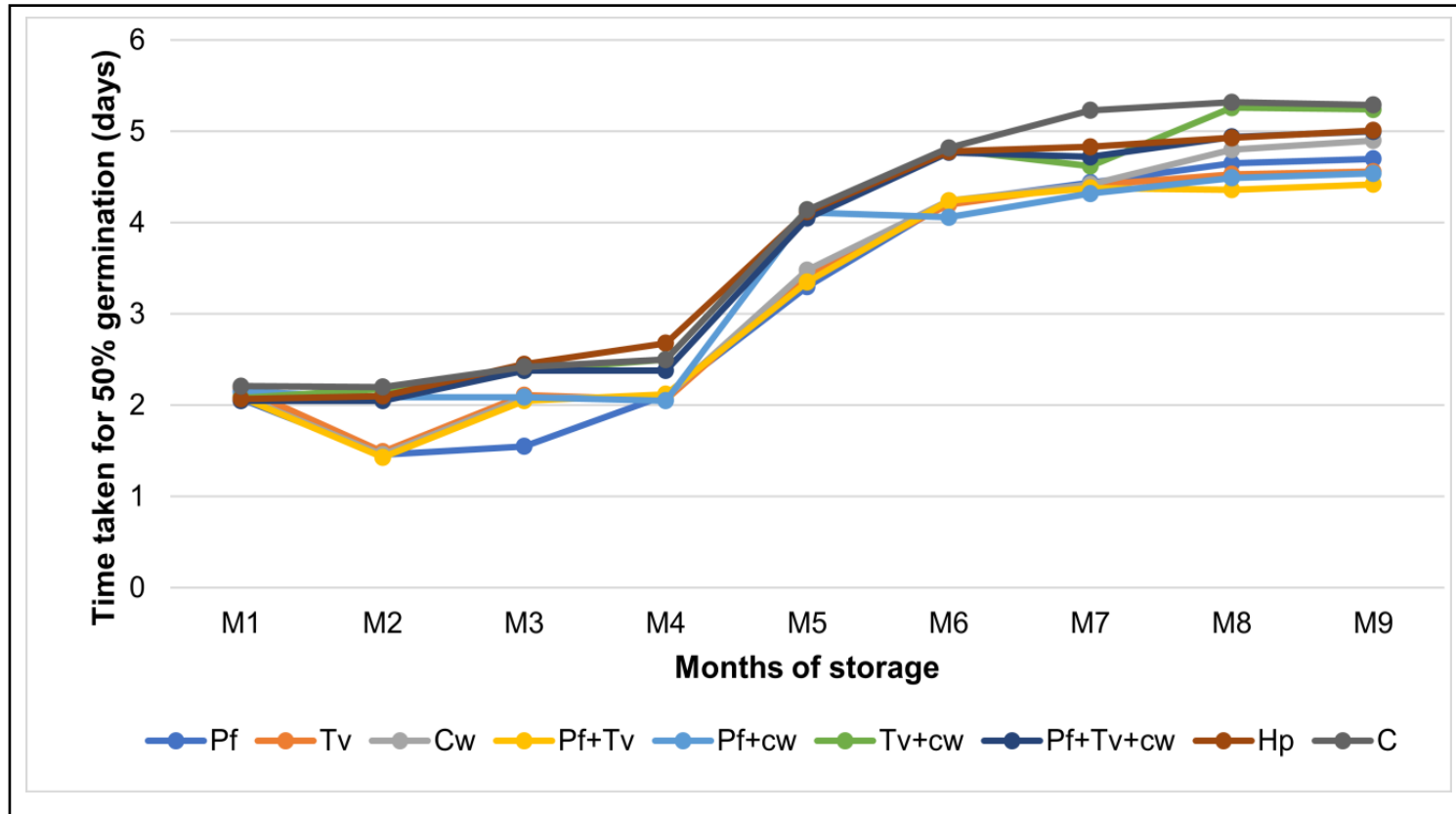


Fig 9: Impact of treatments on time taken for 50% germination (days) at 9 MAS

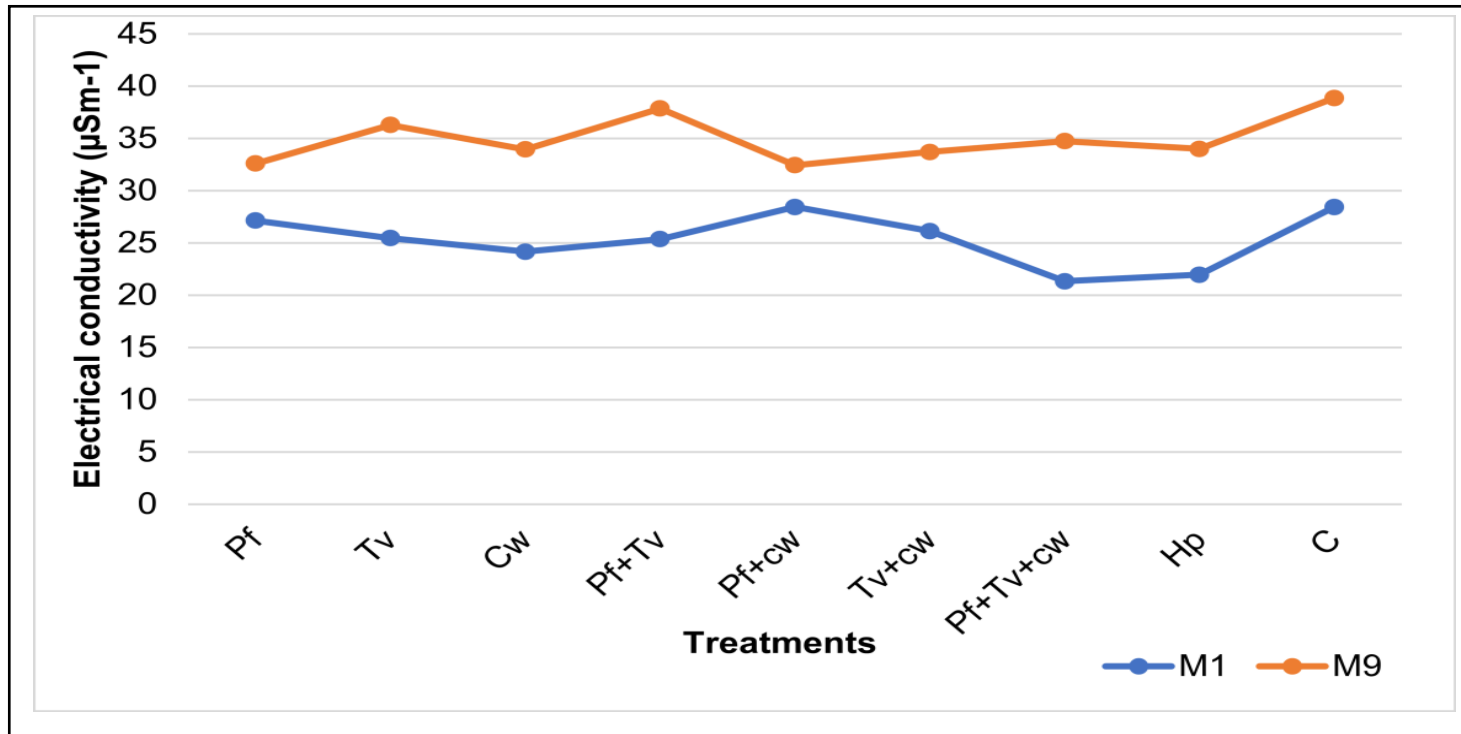


Fig 10: Impact of treatments on electrical conductivity of seed leachate (μScm^{-1}) at 1MAS and 9MAS

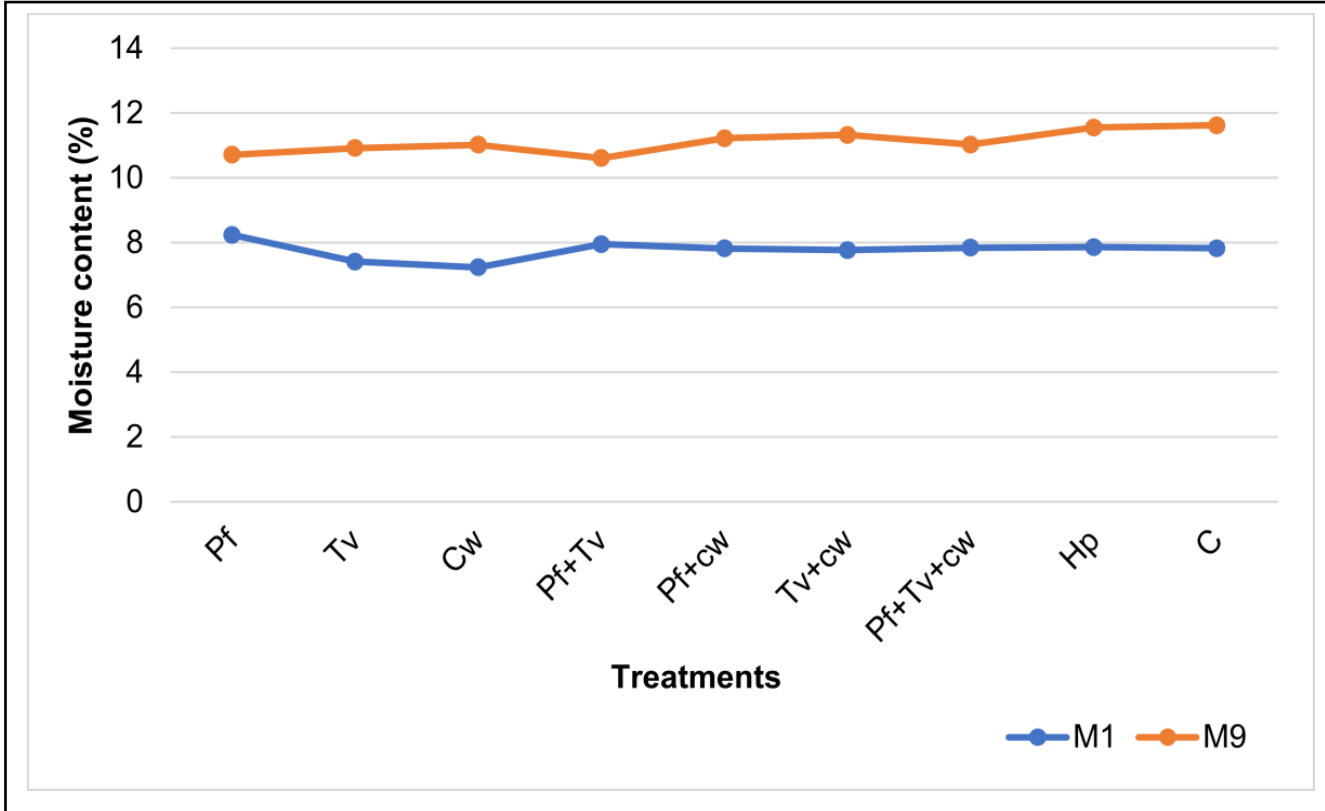


Fig 11: Impact of treatments on moisture content (%) at 1MAS and 9MAS

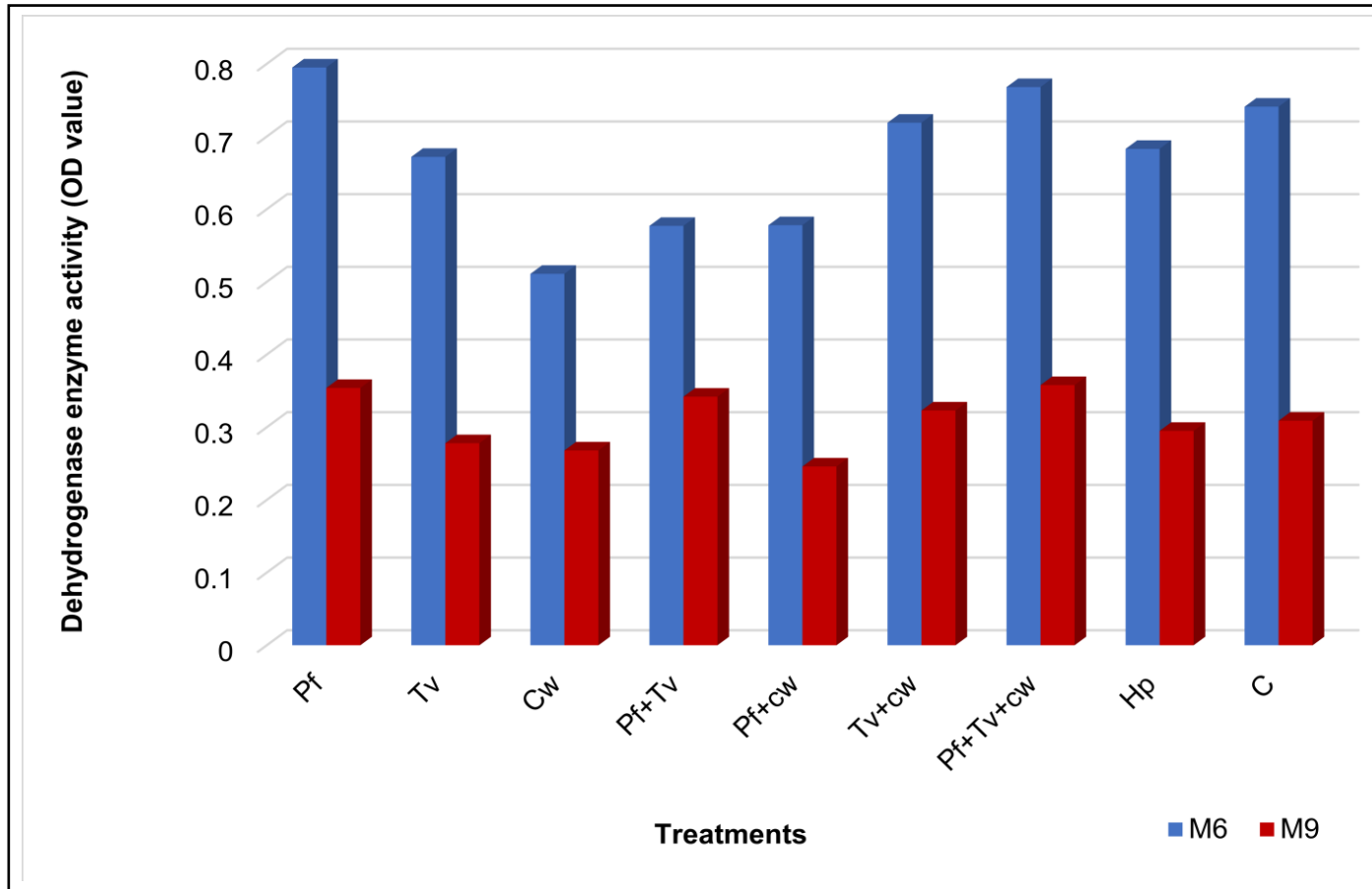


Fig.12. Impact of treatments on dehydrogenase enzyme activity at 6MAS and 9MAS

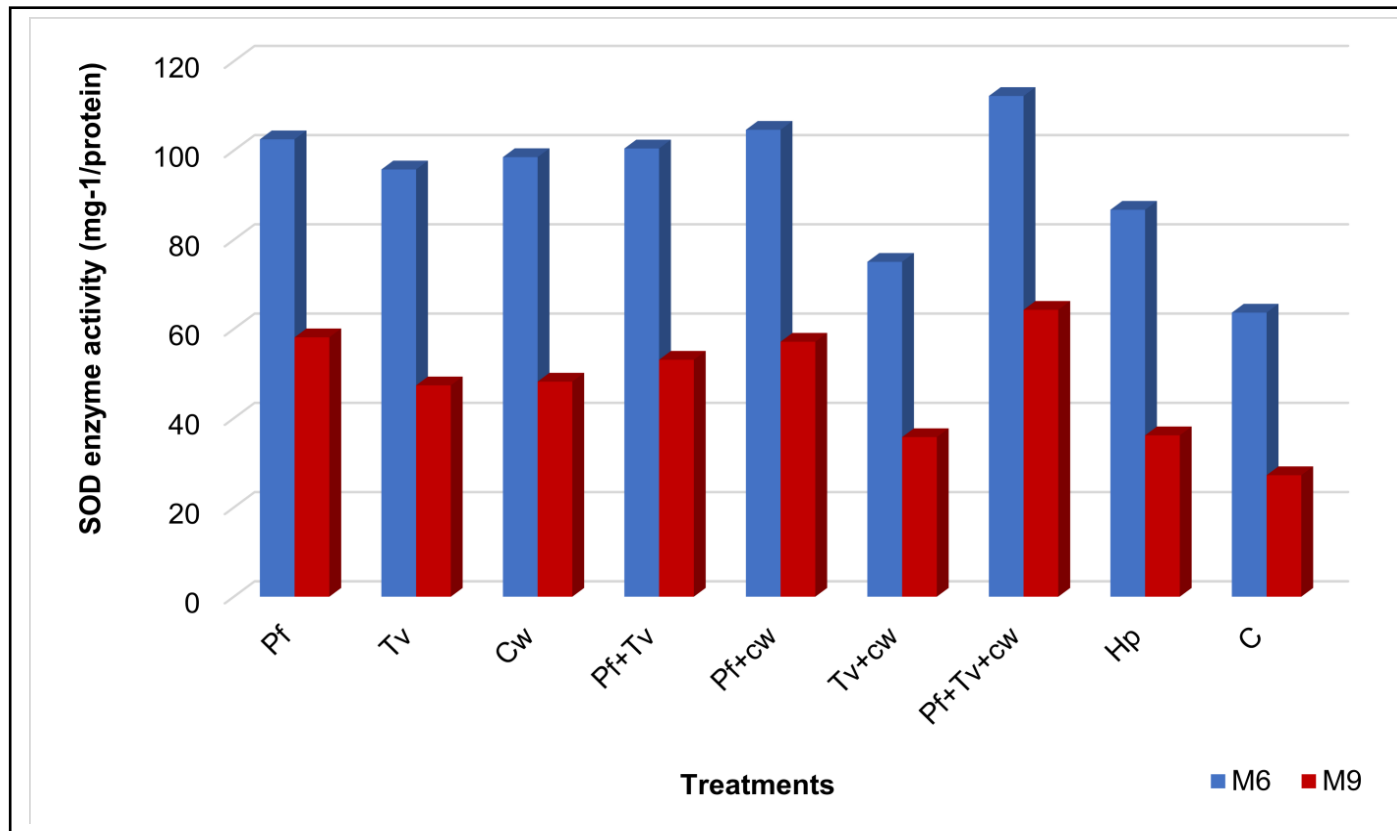


Fig.13. Impact of treatments on superoxide dismutase enzyme activity at 6MAS and 9MAS

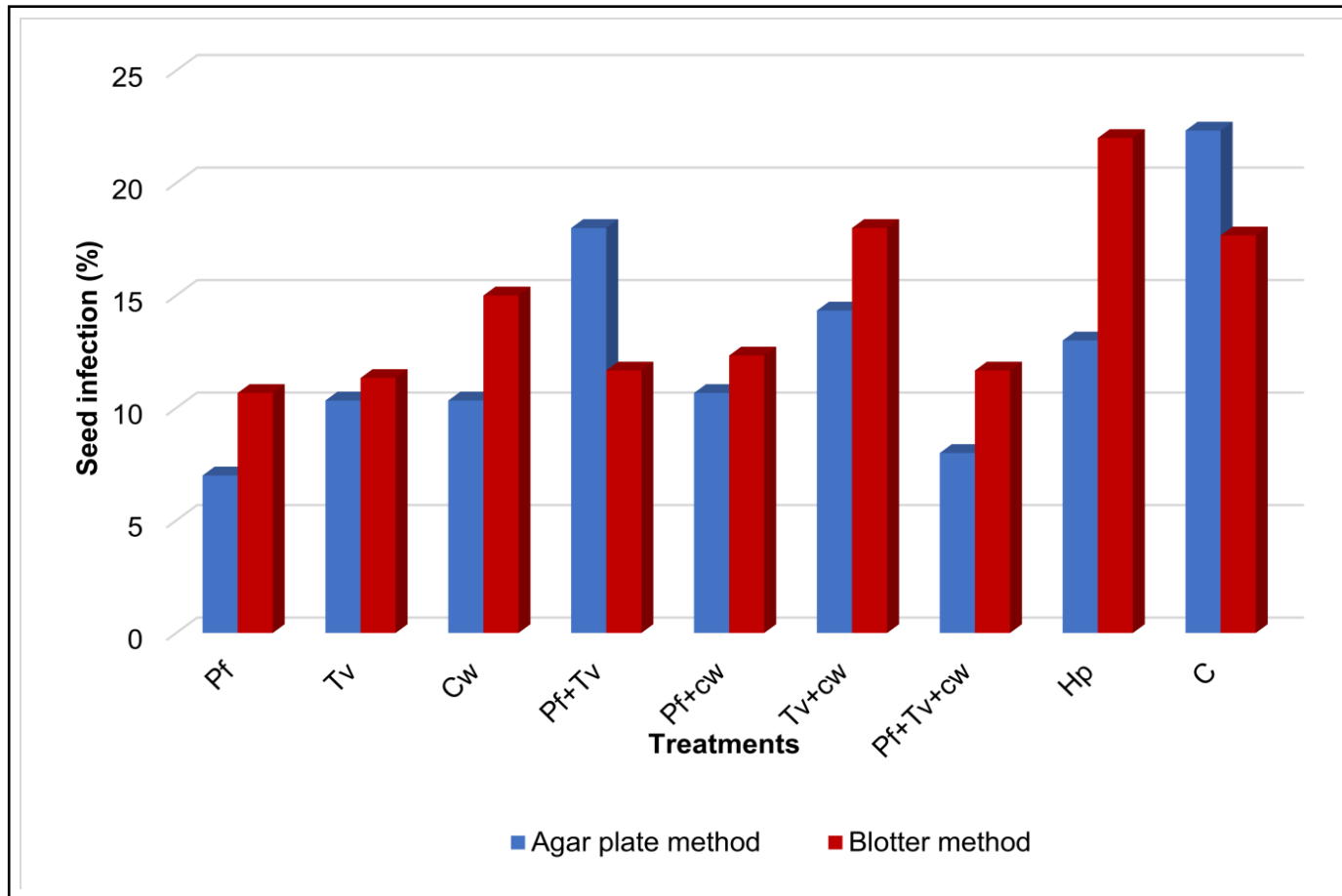


Fig.14. Impact of treatments on seed infection (%) with sterilization at 9MAS

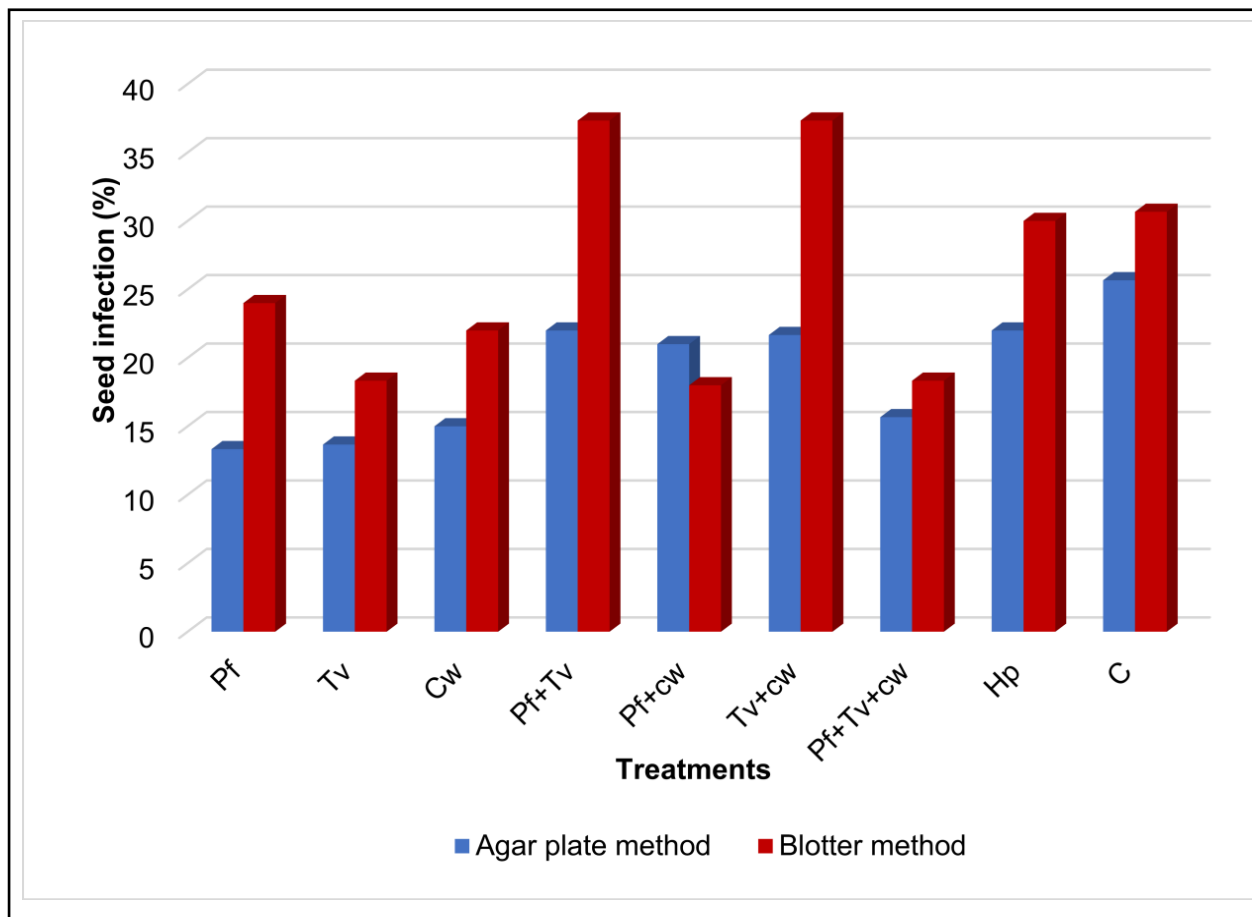


Fig.15. Impact of treatments on seed infection (%) with sterilization at 9MAS

was on par with T₃coconut water (75%) (18.38). Whereas, delayed germination was observed in T₈hydro priming and T₅*P. fluorescens* @10g/kg + coconut water (75%) (16.20 and 16.71) respectively at initial stage.

At the end of artificial ageing coconut water treatments (T₇ 14.94 and T₃14.32) have shown early emergence through its mediation in cloning RNA, cytokinin plays an essential role in coordinating seed germination, as well as increasing the permeability of seeds membranes and stimulating germination (Zare *et al.*, 2007). In addition, *P. fluorescens* treatments (T₄, T₁) also retained superiority (15.04 and 14.69) which were in similar trend to the research findings of Saudi, (2017) in rice.

5.3.8. Mean germination time

The average germination time was found to increase with increase in duration of accelerated ageing. Initially low in T₈ (hydro priming) with 2.59 days while, it increased towards the end of artificial ageing period reaching 6.52 days in control (T₉).

At the end of artificial ageing hydro priming (6.04 d) followed by coconut water (75%) (6.22 d) recorded least mean germination time, while control and T₁-*P. fluorescens* @10g/kg recorded highest mean germination time (6.52 d and 6.42 d) which explains superiority of hydropriming over biopriming in early emergence of seedlings as in with the findings of Ghahfarokhi *et al.* (2019) in corn, Yalamallae *et al.* (2019) in onion.

5.3.9. Time taken for 50% germination

The results recorded on time taken for 50% germination has followed the same trend as mean germination time during the ageing period. The time taken for 50% germination was initially low in T₈ (hydro priming) with 3.67 days while, it increased towards the end of artificial ageing period reaching 7.54 days in control (T₉).

Time taken for 50% germination was initially low in hydro primed seeds as Hsu *et al.* (2003) observed improved germination percent and mean emergence time of

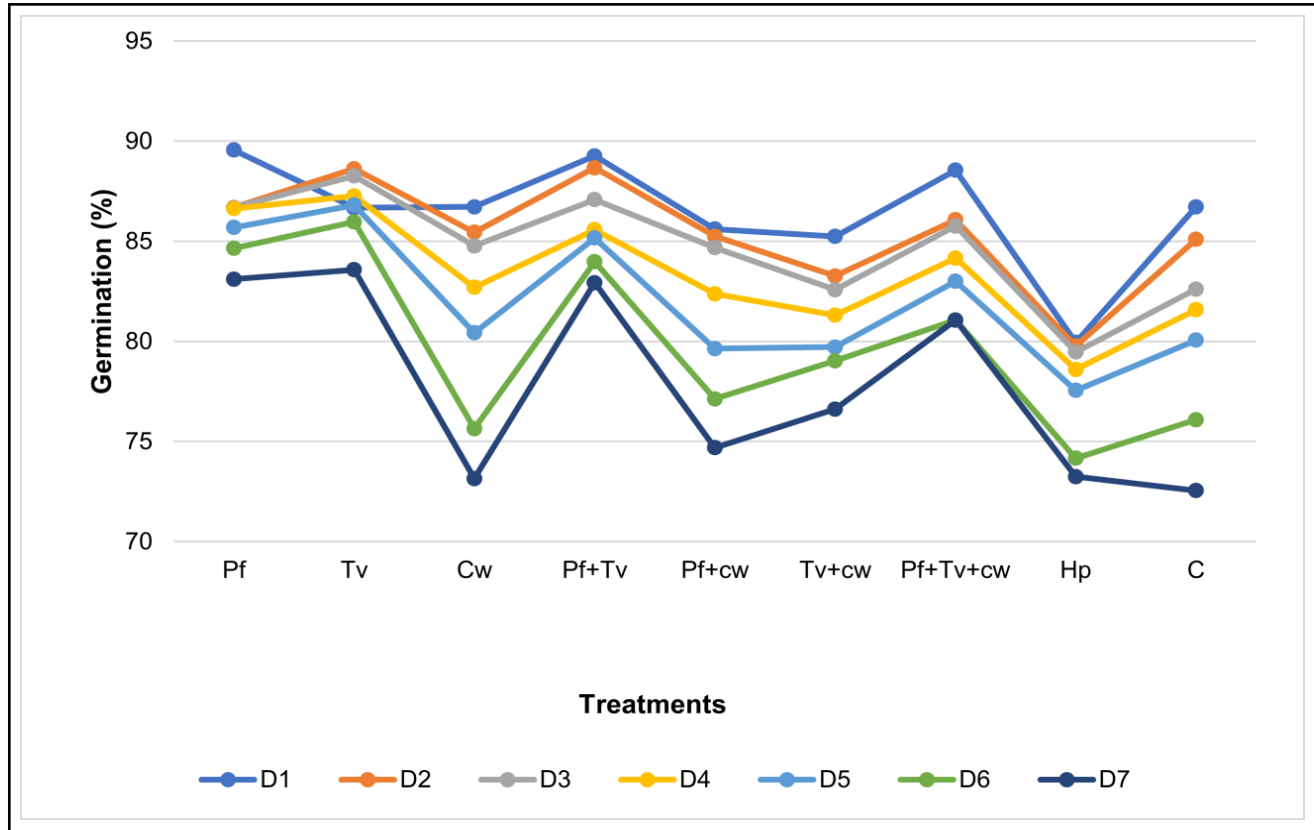


Fig.16. Germination of primed treatments across the ageing period

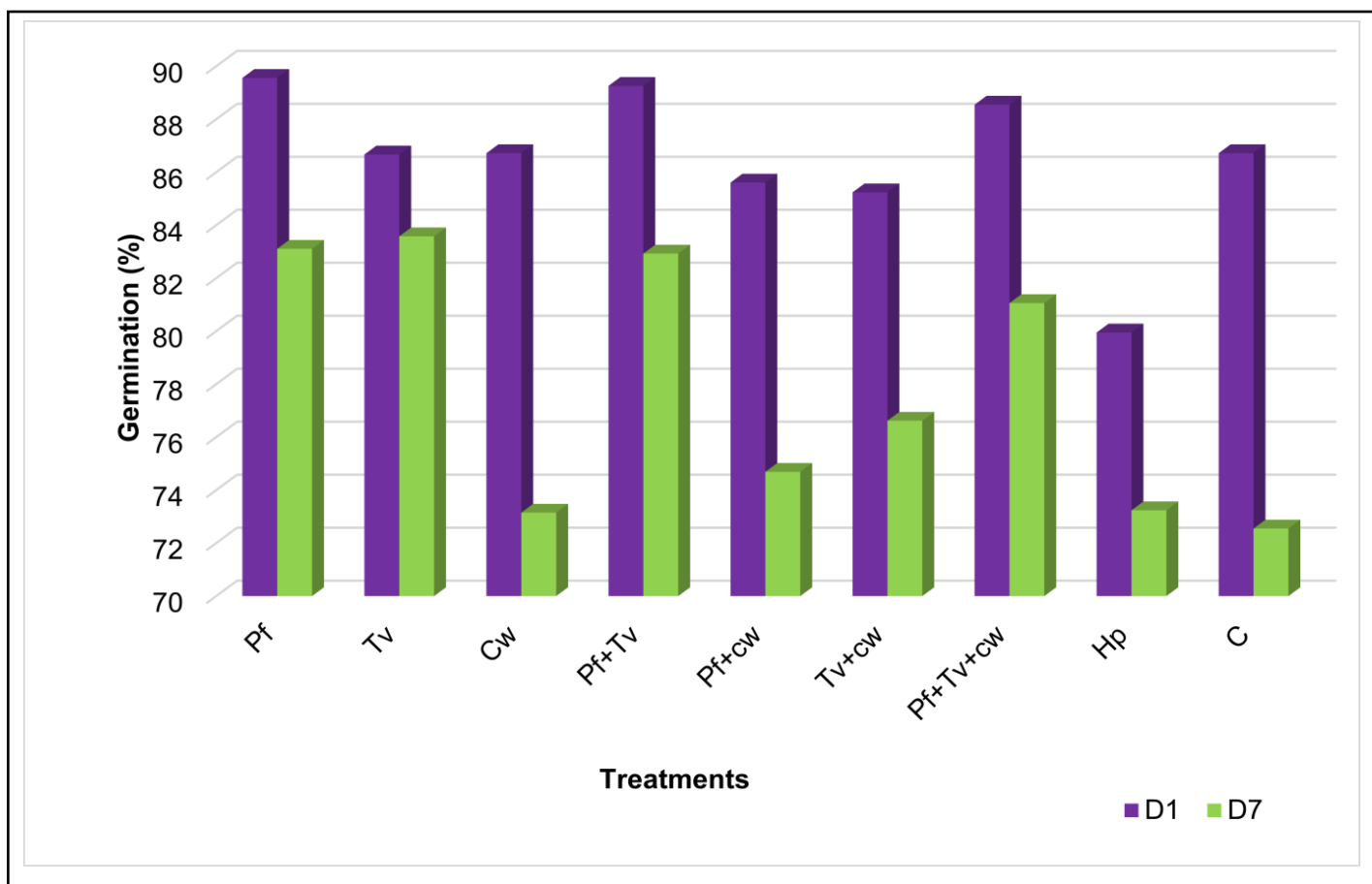


Fig.17. Germination of primed seeds at the start (D1) and end (D7) of ageing period

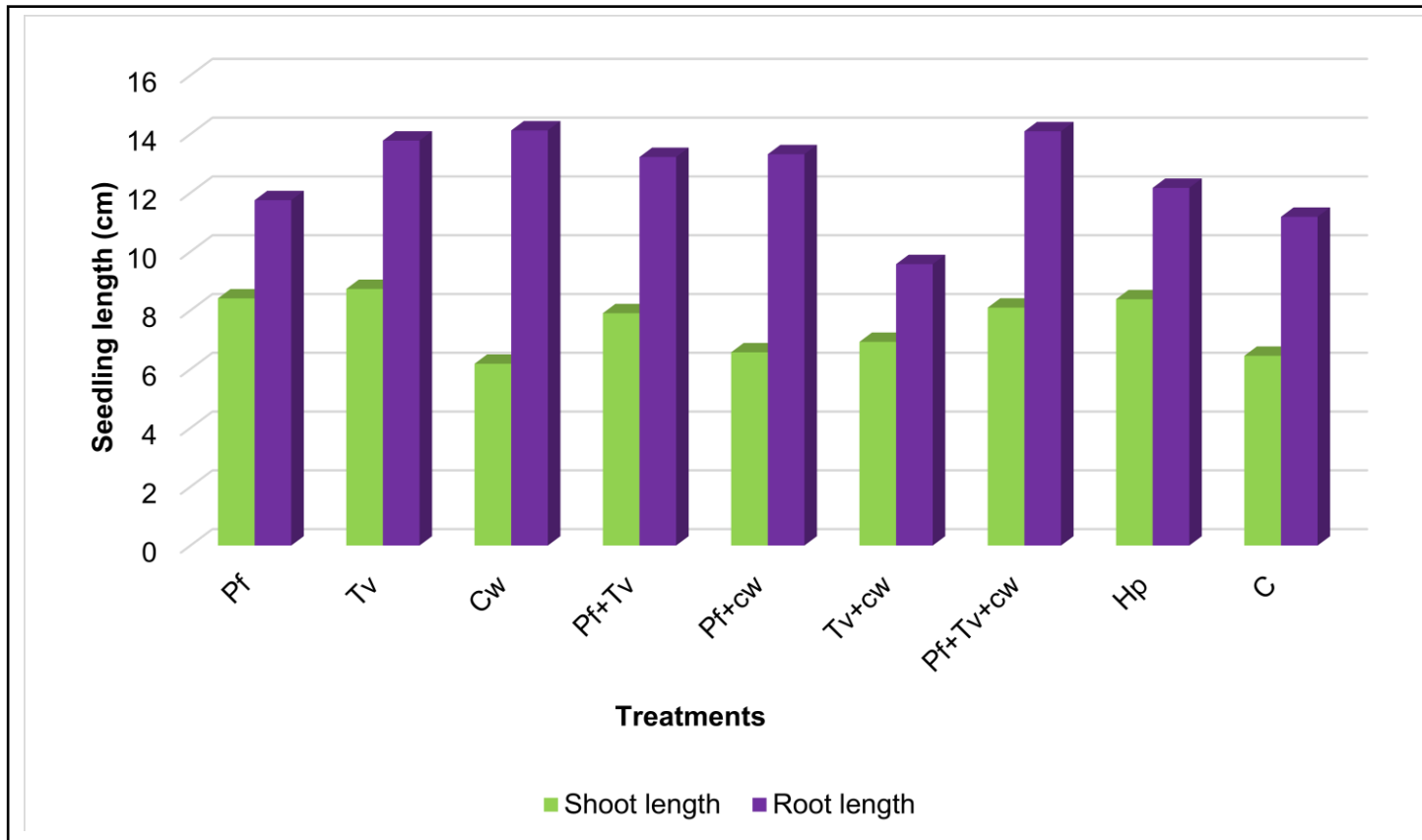


Fig.18. Impact of treatments on root and shoot length (cm) of rice seedlings after accelerated ageing

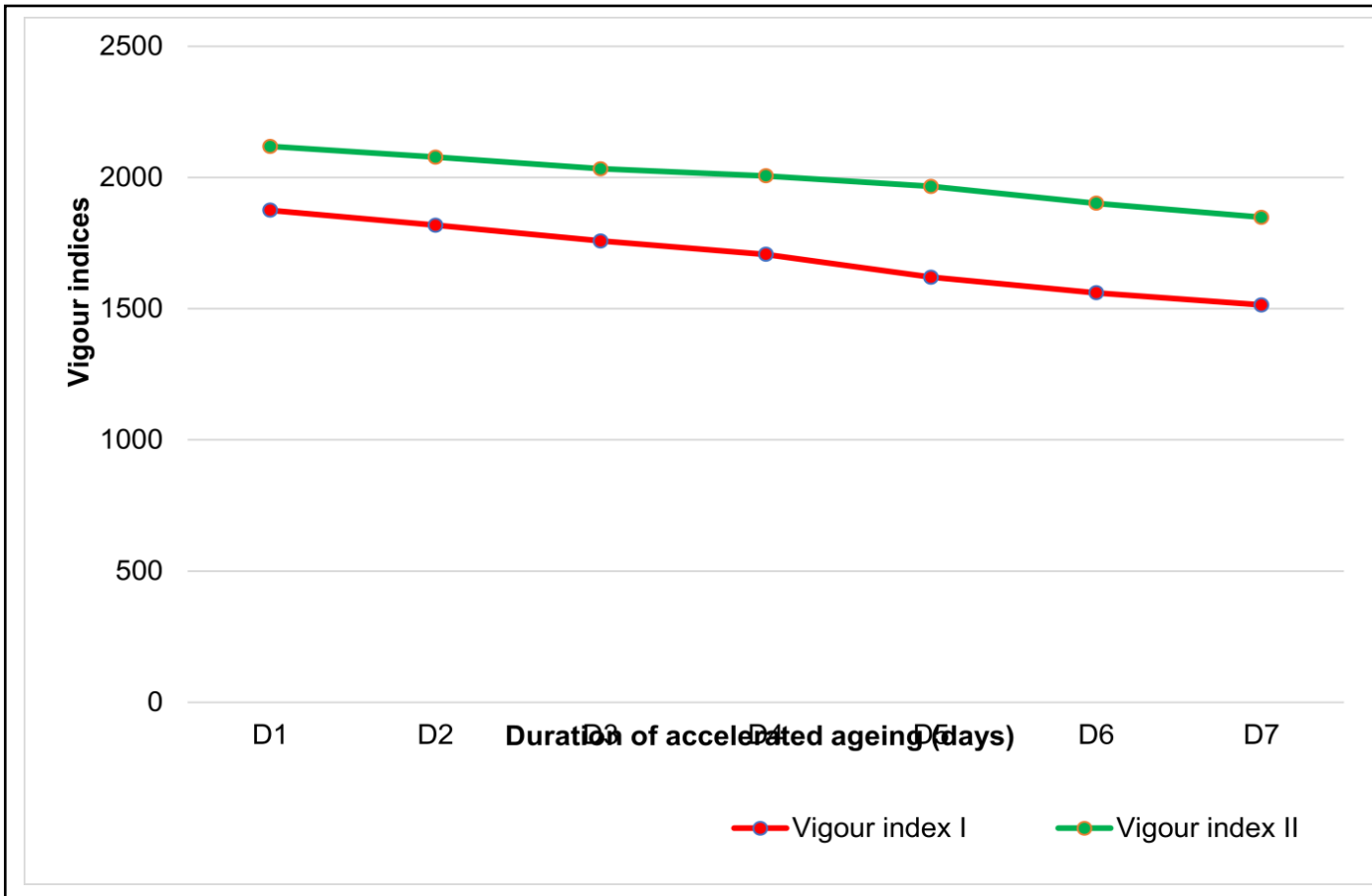


Fig.19. Decline in seedling vigour I and II across ageing period

bitter gourd seed. and highest time was recorded by control (T₉) as 7.54 days in line with findings of Bailey *et al.* (1998) in sunflower, Raj *et al.* (2013) in okra.

5.3.10 Electrical conductivity (μScm^{-1})

Measurement of seed solute leakage can be used to assess seed degradation. Biological membranes have been discovered to regulate the passage of materials into and out of the cell, implying that they play an important role in seed viability and vigour.

The increasing trend in electrical conductivity of seed leachates was observed with increase in days of ageing (22.95 μScm^{-1} at initial day to 68.65 μScm^{-1} after 7 days of ageing) as indicated in fig.22. which is due to the membrane reconfiguration period after rehydration, solute leakage from seeds occurs in tandem with seed imbibition. As a result, leakage rates depend on degree of cell membrane damage and repair as a function of age (Ferguson *et al.*, 1990; AlMaskri *et al.*, 2003).

At the end of artificial ageing, highest electrical conductivity of seed leachates was noticed in treatment T₉Control (68.65 μScm^{-1}) whereas, lowest in bio primed treatments T₄ (43.45 μScm^{-1}) and T₂ (44.55 μScm^{-1}) respectively which are in concurrent with the results of Khan *et al.* (2003) in pea, Bijanzadeh *et al.* (2015) in rice.

5.3.11 Moisture content (%)

According to Ellis *et al.* (1992), a rise in seed water content following ageing could be attributable to cell membrane instability.

According to Kapoor *et al.* (2011), prolonged seed ageing resulted in the disintegration of cell membranes and an increase in rice seed moisture which explains the increasing trend in seed moisture content observed with increase in days of ageing (8.74 per cent at initial day to 19.4 per cent after 7 days of ageing) as enumerated in fig.23.

At the end of artificial ageing, highest moisture content was noticed in treatment T₉ - control (19.4%) whereas, lowest in treatment, T₂ - *T. viride* @4g/kg and T₆ *T. viride* (4g/kg) + coconut water (75%) (14.25%) respectively which are similar with results of Bijanzadeh *et al.* (2015) in rice where seeds treated with *T. viride*.

5.3.12 Dehydrogenase enzyme activity (OD value)

The mean dehydrogenase enzyme activity decreased with increase in days of ageing from 0.775 OD value at initial day to 0.112 OD value after 7 days of ageing irrespective of the treatments as indicated in fig.24.

At the end of ageing period, superiority of *P. fluorescens* treatments T₇ registered maximum dehydrogenase enzyme activity of 0.268 OD value and was on par with T₁ (0.206 OD value) over other treatments can be observed which are in accordance to the results of Vijayan (2005) in paddy and Manimekalai (2006) in black gram.

5.3.13 Super oxide dismutase enzyme activity (mg¹/protein)

SOD is a powerful antioxidant that protects cells from oxidative damage. The ageing process has effect on SOD activity levels in rice seed (Fig. 25) explains steep decline in SOD enzyme activity from initial to the end of ageing period. Initially, maximum in T₇ (*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%):97.25 mg¹/protein).

There was an equivalent decline among the treatments towards the end of ageing period, showing that the seeds have equivalent ability to convert O₂ to H₂O₂.

5.3.14. Seed infection (%)

The data on mean seed infection percent has varied significantly among the treatments. Least seed infection was seen in T₁-*P. fluorescens*@10g/kg (1.33 per cent Agar plate with surface sterilization) and highest seed infection was observed in T₉ Control (64 percent in blotter method without sterilization).

Superiority of *P. fluorescens* over *T. viride* in controlling storage pathogens was observed in the study. Control recorded highest seed infection (26.00%). These results

found to be similar to Gajendra (2015) in rice, Kanwar *et al.* (2014) in bitter gourd, Aiyaz *et al.* (2015) in maize.

Based on the scores obtained by each treatment for seed quality parameters and enzyme activity analysis in natural ageing at nine months after storage (Table 19) T₇ *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) ranked the best. Treatments with followed by *P. fluorescens*@10g/kg, *P. fluorescens* @10g/kg + *T. viride* @4g/kg and *P. fluorescens* @10g/kg + coconut water (75%).

This implies the clear superiority of *P. fluorescens* individually or in combination with other bio control agents or organics can retain seed longevity and maintain seed quality for longer periods due to the endogenous plant growth regulators such as gibberellins, cytokinin, indole acetic acid and increased availability of minerals and other ions, have helped in the increase of water uptake.

Even coconut water is also a good supplement in increasing longevity and maintaining the quality of seeds due to cytokines identified such as N₆isopentenyl adenine, dihydrozeatin, trans zeatin, kinetin, orthotopolin, dihydrozeatin O glucoside, trans zeatin O glucoside, trans zeatin nucleoside, kinetin nucleoside and trans zeatin nucleoside 5 monophosphate (Yong *et al.* , 2009). In seed priming, the role of cytokinin is to mobilize the food reserve in the seed coat, causing the seed coat to absorb water which help in the increase of root shoot ratio eventually leading to better and uniform seedling growth.

Biological priming leads to biochemical changes, increased production of proteins, hormones, phenols and flavonoid compounds which contribute to better plant growth and better developmental performance. The percentage of soluble protein in seeds and seedlings that received biopriming was higher when compared to other types of priming. The microbial complex may also provide the ability to enhance resistance responses in plants against a variety of soil and seed pathogens. If the seeds need to be stored for a long time before sowing, it is best to re dry the primed seeds that can better establish a uniform support.

It can be suggested that the combined application of hydrolytic priming and seed coating with biological control agents would be a reasonable alternative to chemical seed treatment. Hence, the combination of *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) is suggested according to the results of natural ageing.

According to results of accelerated ageing there was a fore hand of dry treatments (T₁, T₂, T₄) over wet treatments (T₃, T₅, T₆, T₈) apart from the treatment *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (T₇) which has shown superiority in all seed quality parameters recorded which due to the high humidity conditions prevailing in Kerala as the storage of seeds was taken under ambient conditions.

Hence the combination of *P. fluorescens*, *T. viride*, coconut water can be suggested as alternative to chemical treatment as they farmer friendly with eco-friendly features without any side effects to the person who works with it. They are also cost friendly as talc powder of *P. fluorescens* (Rs 100/ kg) and *T. viride* (Rs 75/kg), coconut water is naturally obtained from plants and easily available to the farmers with help the farmers to make a move towards the organic farming without loss in productivity and also help in reducing in pollution caused by the excess use of Agro chemicals.

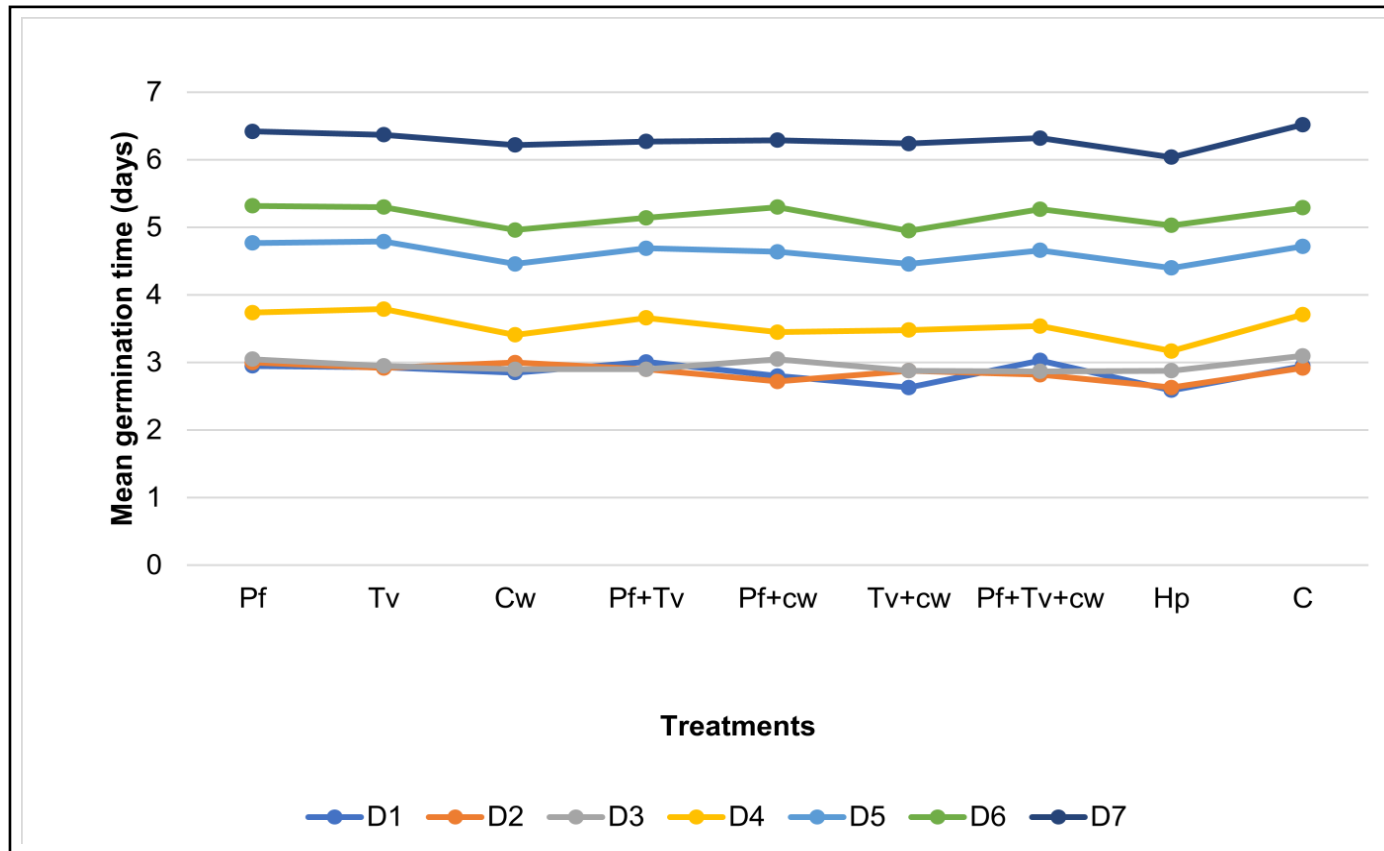


Fig 20: Impact of treatments on mean germination time (Days) over the ageing period

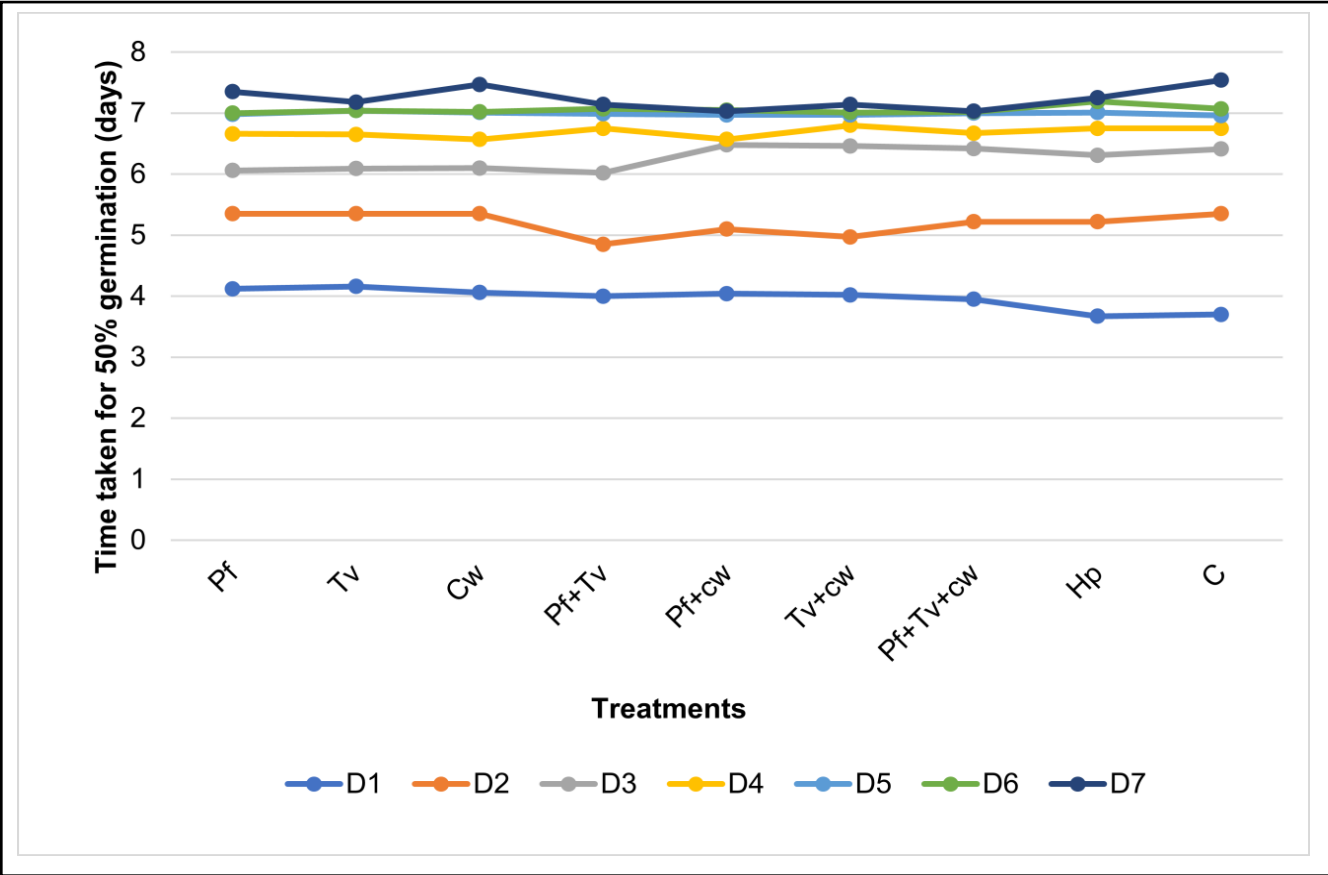


Fig 21: Impact of treatments on time taken for 50% germination (Days) over the ageing period ageing period in rice

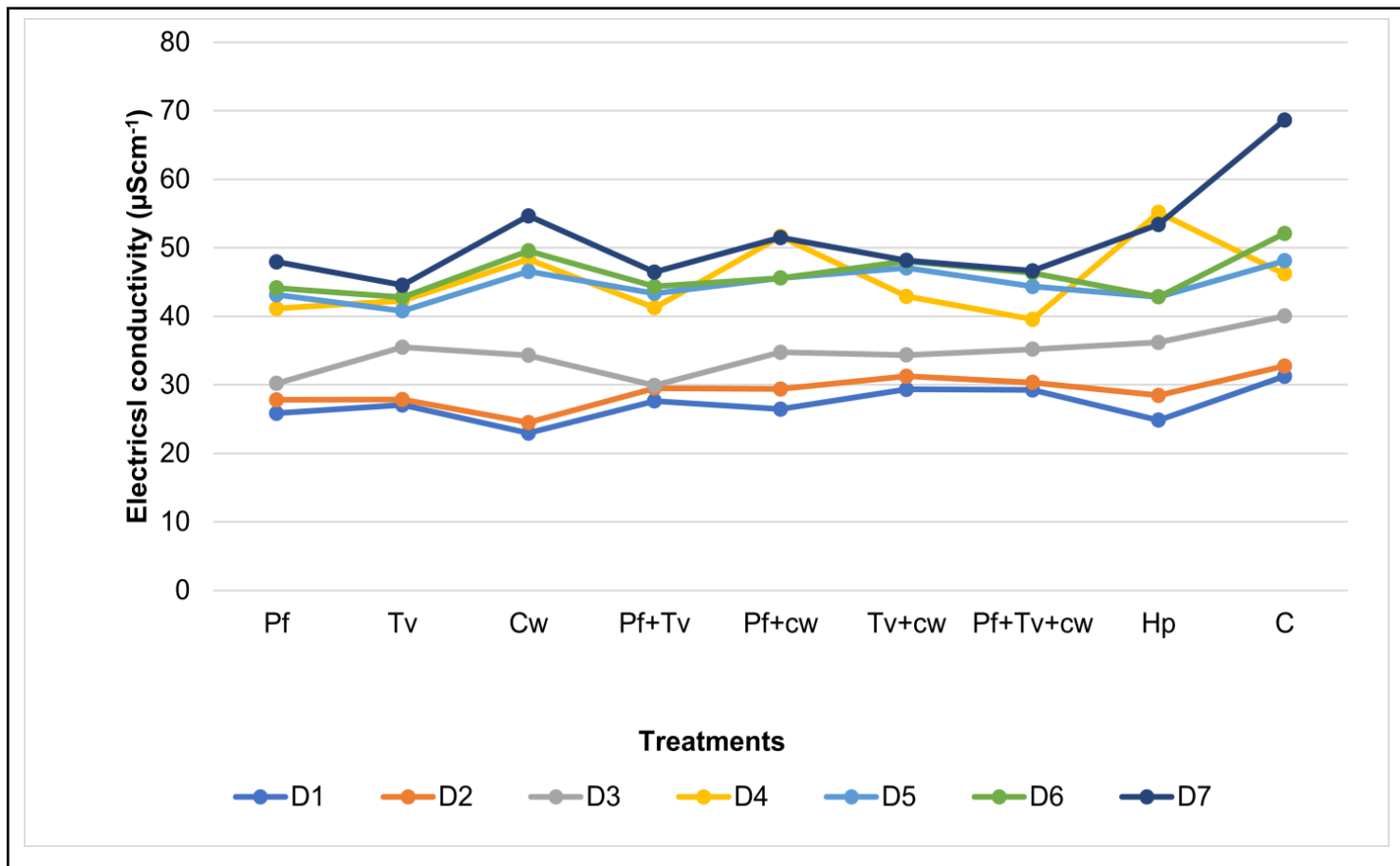


Fig 22: Impact of treatments on electrical conductivity of seed leachate ($\mu\text{S cm}^{-1}$) over the ageing period

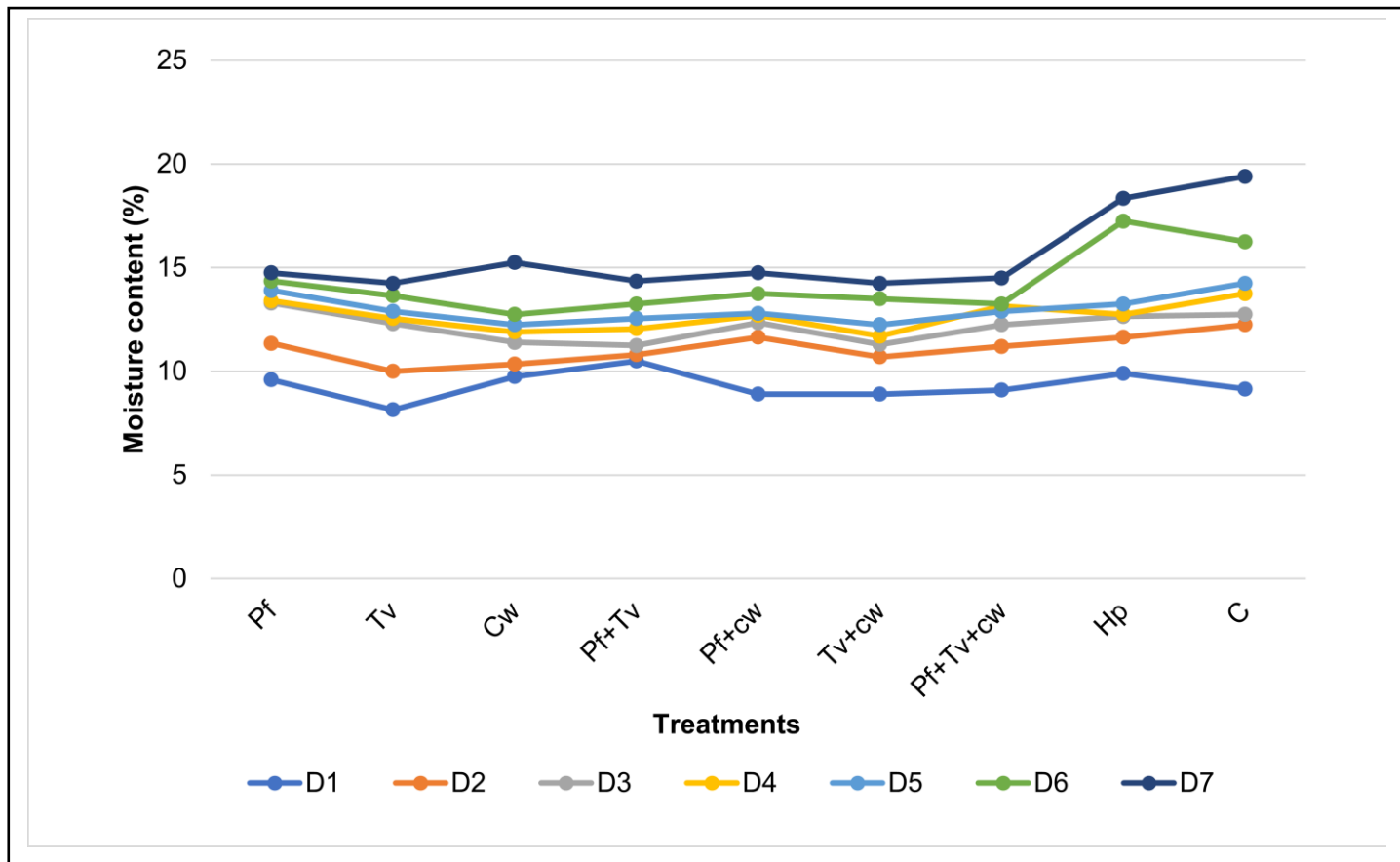


Fig 23: Impact of treatments on moisture content (%) over the ageing period

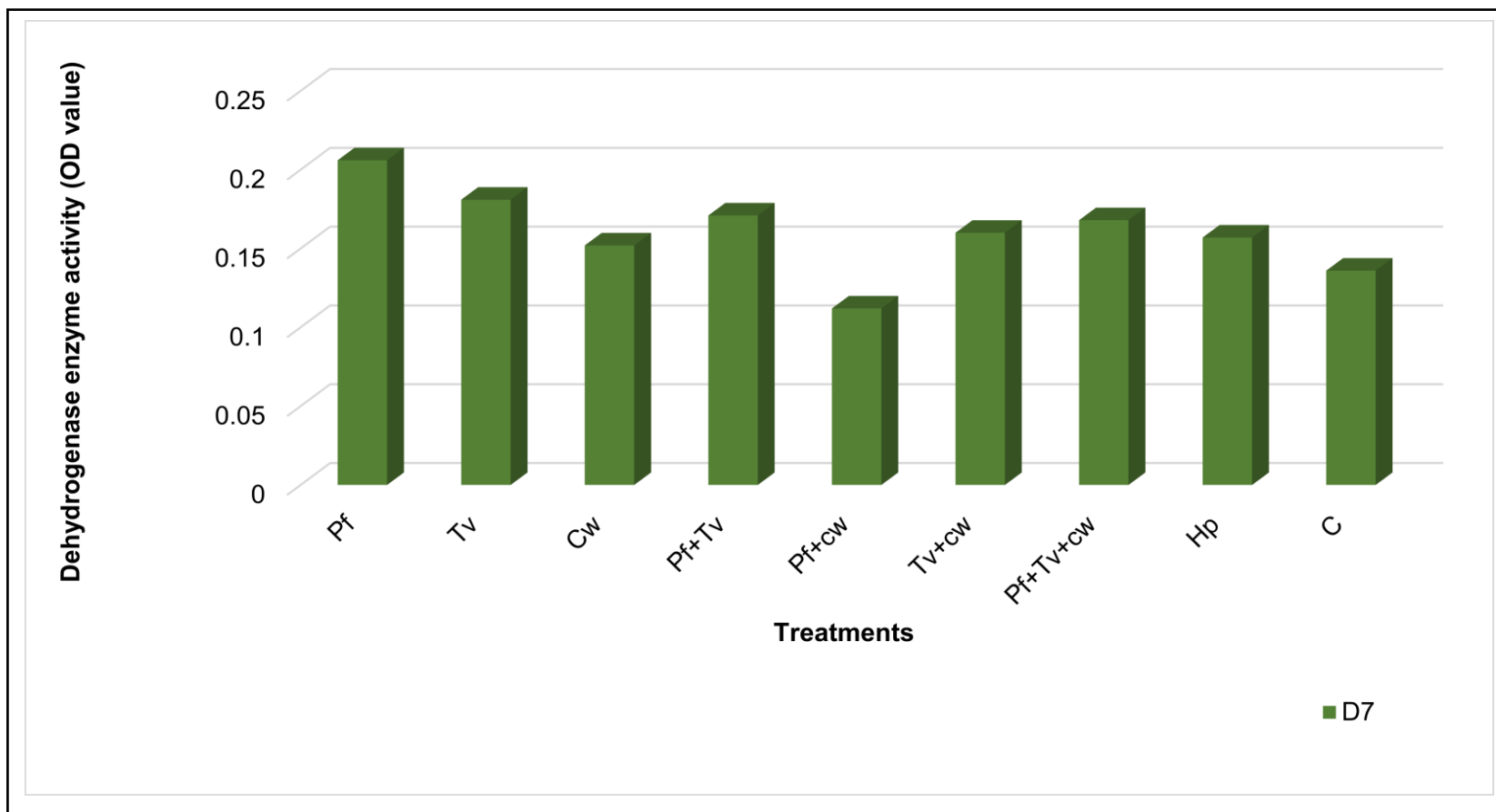


Fig.24. Impact of treatments on dehydrogenase enzyme activity after accelerated ageing in rice

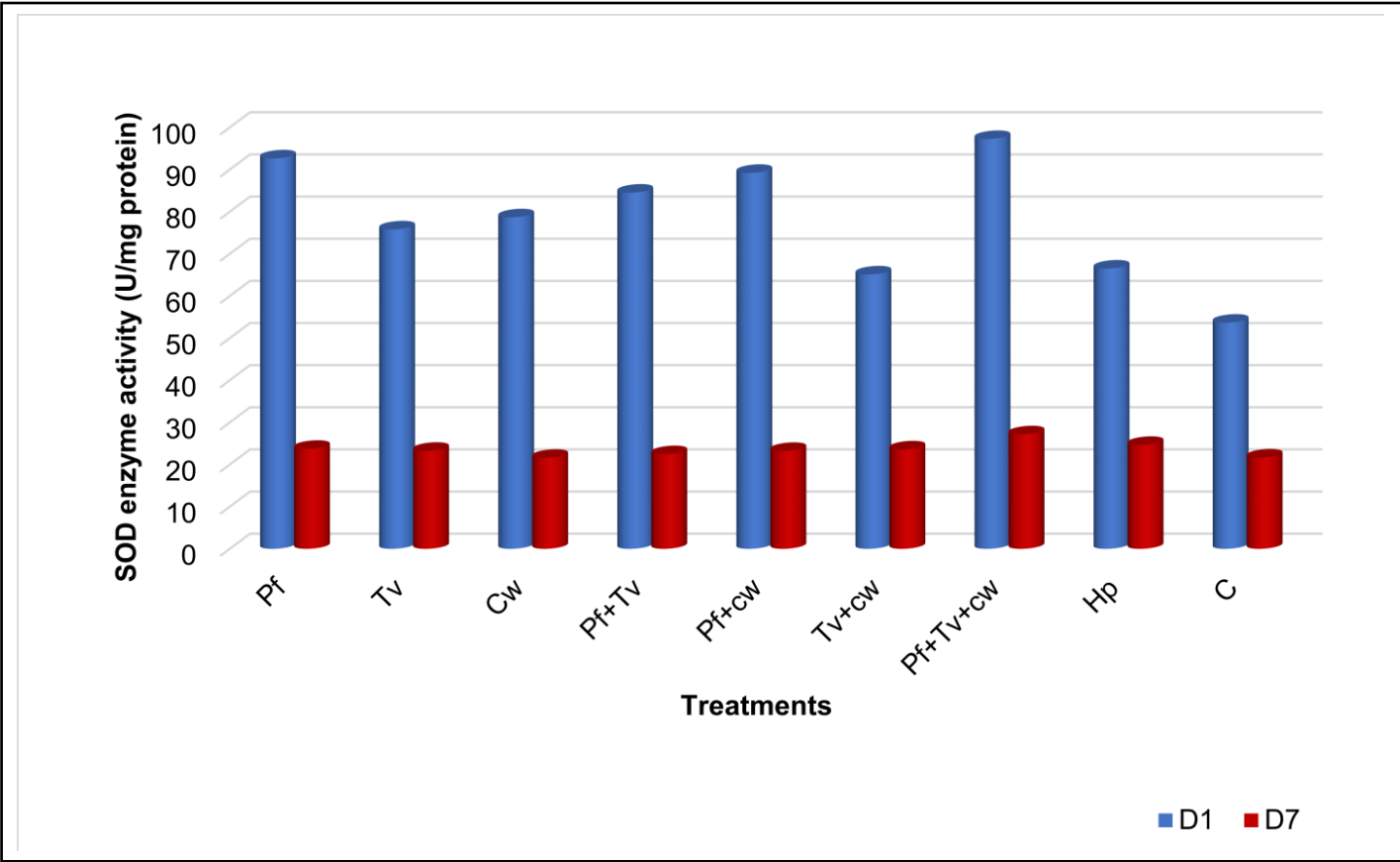


Fig.25. Impact of treatments on superoxide dismutase enzyme activity at start and end of ageing period

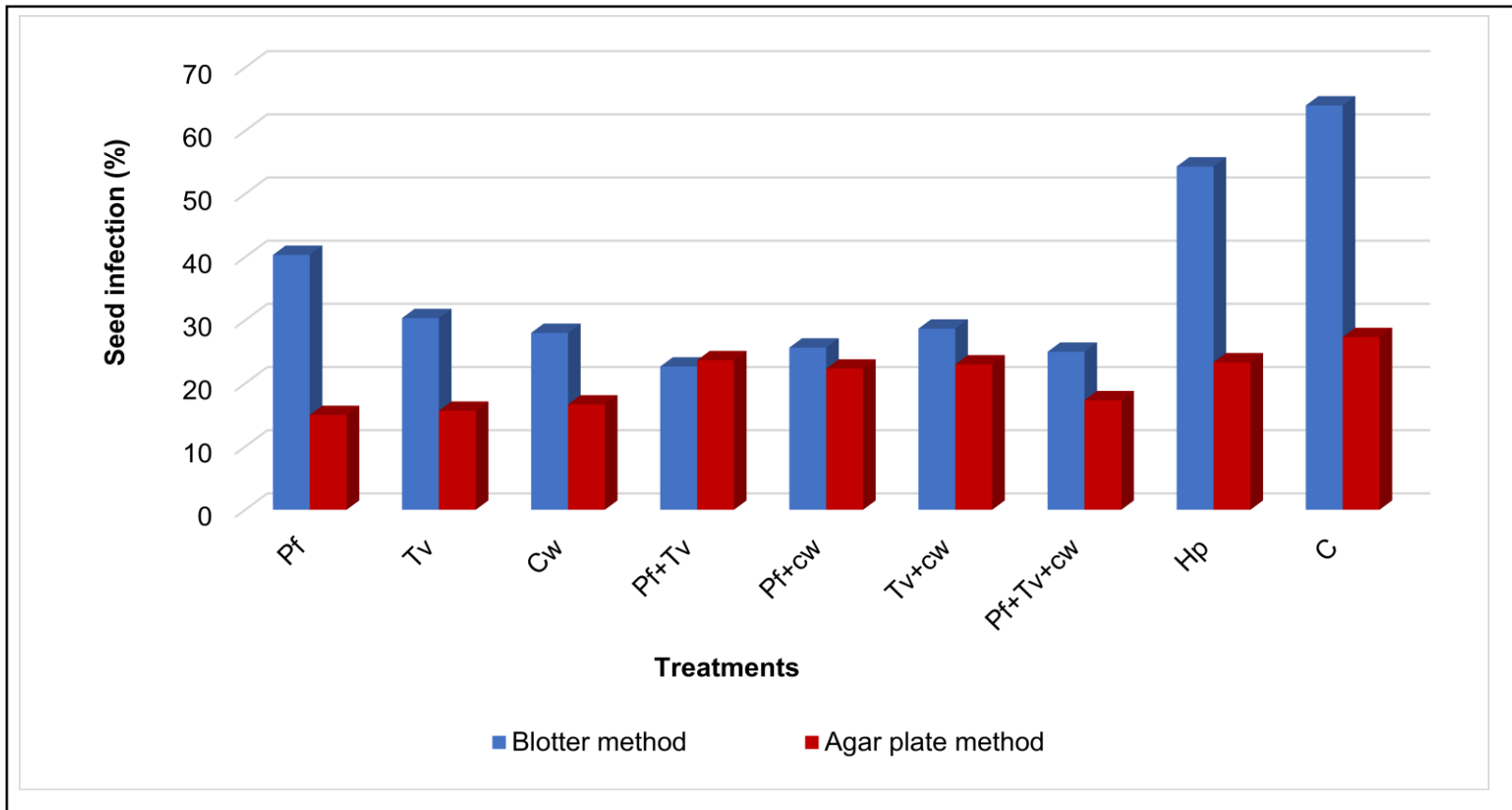


Fig.26. Impact of treatments on seed infection (%) with sterilization after accelerated ageing

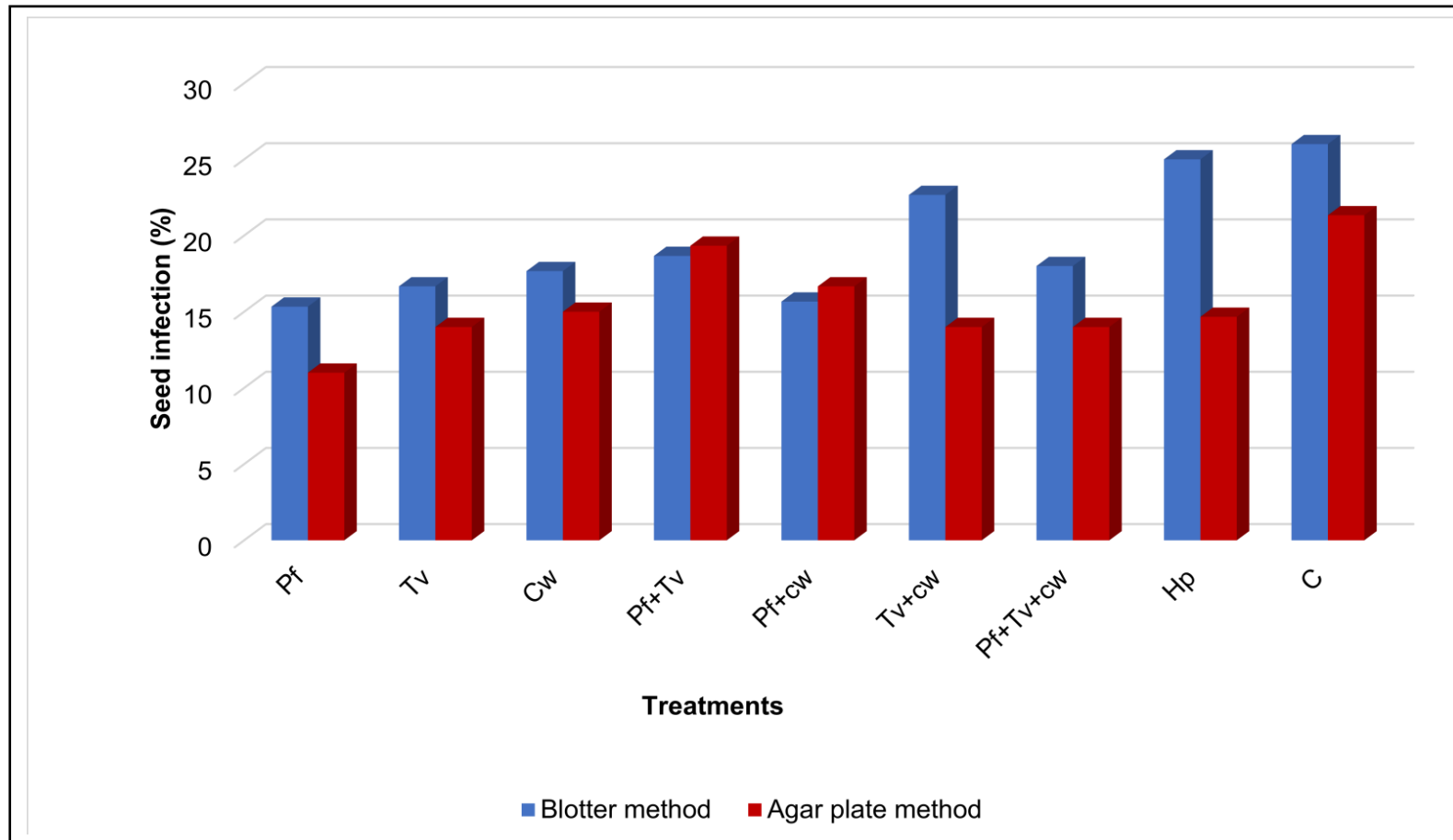


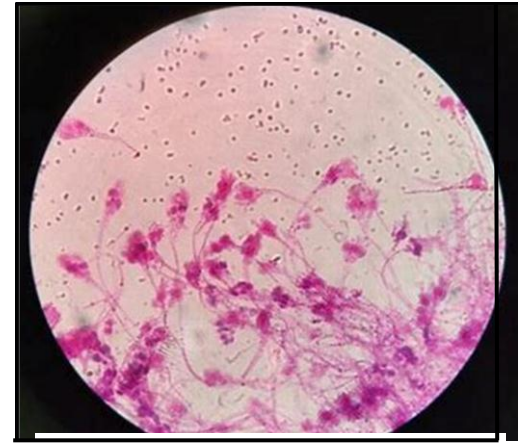
Fig.27. Impact of treatments on seed infection (%) without sterilization after accelerated ageing

FUTURE LINE OF WORK

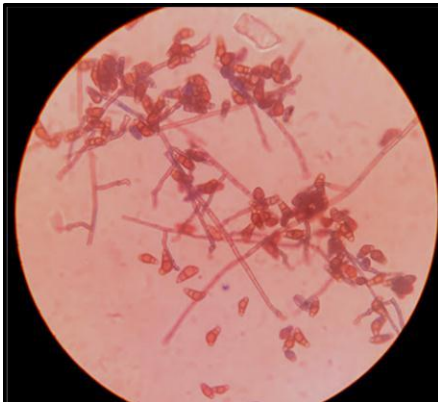
1. Field performance of present biopriming treatments is to be ascertained in order to study its performance on growth parameters.
2. Use of other organic primers like vermi wash, cow urine, custard leaf extract with the combination of present treatments can be employed in order to check their performance on seed quality parameters
3. Different crops can be researched to understand how individual and combined applications of bio primed seed treatment affect physiological quality and longevity.
4. For useful recommendations, the study might be expanded to determine the use of bio primers against pest and disease populations during storage.
5. The impact of bio primed treatments on biochemical parameters like lipid peroxidation, α amylase enzyme activity and malon dialdehyde content may also be assessed



***Aspergillus* spp.**



***Pencillium* spp.**



***Curvularia* sps.**



***Mucor* sps.**



***Rhizopus* spp.**

Plate-6: Microorganisms observed after accelerated ageing

Summary

VI. SUMMARY

Rice (*Oryza sativa* L.) is the most widely consumed staple food crop in the world. The safe storage of seeds for an extended period of time has always been a difficult task. Seed deterioration occurs during storage as seed is a living entity. Seed treatment with chemicals or organics helps in preserving the seed quality during storage. Use of bio control agents and organics is an environmentally safe technique. In the view of above, the present study “Impact of biopriming on seed quality and longevity in rice” (*Oryza sativa* L.) was undertaken at the Department of Seed Science and Technology, College of Agriculture, Vellanikkara from December 2020 to August 2021.

Rice seed cv. Jyothy was primed with the following treatment - *Pseudomonas fluorescens* (10g/kg) (T₁), *Trichoderma viride* (4g/kg) (T₂), Coconut water (75%) (T₃), *P. fluorescens* + *T. viride* (T₄), *P. fluorescens* + coconut water (T₅), *T. viride* + coconut water (T₆), *P. fluorescens* + *T. viride* + coconut water (T₇), Hydro priming (T₈) and Control (untreated) (T₉). Both treated and untreated seeds were dried to less than 8% moisture and packed in 700-gauge polyethylene bags and stored at room temperature. The seed quality criteria of the preserved seeds are summarized below:

I. Quality of seeds before storage

1. The seed lot used in the study was of good quality and suitable for storage studies, with quality parameters far exceeding the minimum standards recommended for rice. There was no seed microflora identified.
2. Seed quality characteristics was assessed after treatment and it was noticed that there were significant differences among the treatment with regard to seed quality.
3. Seeds treated with T₇ - *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) registered the highest germination (%), while, root length, seedling dry weight and seed vigour indices (VI-I and VI-II) was highest in T₃-coconut water (75%). Speed of germination and seed infection per cent was less in T₁ - *P. fluorescens* @10g/kg. Time taken for 50% germination and mean germination time

was less in T₅ - *P. fluorescens* (10g/kg) + coconut water (75%). The seed moisture content after treatment was brought to less than 8 % advocated for safe storage.

II. Impact of seed priming on seed quality under natural aging

1. The storage period had a substantial impact on the seed quality parameters, regardless of the seed treatment.
2. Seed quality was found to be declining in both treated and untreated seeds. A significant decrease in germination (%), seedling length (cm), dry weight (g), vigour indices (I and II), dehydrogenase enzyme activity and super oxide dismutase enzyme activity was evident with the increase in the storage duration, while the moisture content (%), electrical conductivity, speed of germination, mean germination time, time taken for 50 % germination and microflora (%) increased with the increase in the storage period.
3. Treated seeds retained germination above 80 % (IMSCS) even after 9 MAS with treatment T₇ *P. fluorescens* (10g/kg) + *T. viride* (4g/kg)+ coconut water (75%) recording the highest germination.
4. Vigour index I and II was found to be significantly higher in T₇ - *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (1645, 2029) followed by T₃- Coconut water (75%) (1561) in vigour index I and T₂ - *T. viride* @4g/kg (1989) in vigour index II at the end of storage.
5. Speed of germination and time taken for 50% germination was high in T₄ - *P. fluorescens* @10g/kg + *T. viride* @4g/kg recorded as 19.02, 4.42 days whereas, mean germination time and was found to be high in T₇ - *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) recorded as 5.08 days.
6. T₅- *P. fluorescens* (10g/kg) + coconut water (75%) (30.83 μScm^{-1}) followed with T₁ - *P. fluorescens* @10g/kg seeds (32.60 μScm^{-1}) recorded low levels of seed leachates at the end of storage period. Enzyme activities (dehydrogenase and super oxide dismutase) was found to be high in T₇ -*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%).

7. Seed infection percent at the end of storage was found to be minimum in the treatment T₁ - *P. fluorescens*@10g/kg in both agar plate and blotter method. Seed microflora observed were - *Aspergillus* spp. (storage fungus), *Mucor* spp. and *Syncephalastrum* spp. (saprophytic fungi), *Rhizopus* spp. (Parasitic fungi).
8. Seed treatments T₇ - *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) followed by T₁ - *P. fluorescens* @10g/kg were found to be superior in seed quality attributes.

III. Impact of seed priming on seed quality on accelerated aged seeds

Treated seeds were packed in butter paper bags with pin and placed inside the BOD incubator. For a period of seven days at a temperature of 40±1°C and a relative humidity (RH) of 98 per cent, the accelerated aged samples were collected and evaluated at daily intervals.

After artificial ageing, T₁ - *P. fluorescens* @10g/kg and T₂-*T. viride* @4g/kg recorded maximum germination (83.11% and 83.58%) respectively. Whereas, coconut water (75%) (T₃), *P. fluorescens* + coconut water (T₅), *T. viride* + coconut water (T₆), hydro priming (T₈), Control (T₉) resulted in the drop of germination below 80% (IMSCS) at the end of ageing period.

Seedling shoot length recorded longest in T₂-*T. viride* @ 4g/kg (8.73 cm) and T₃-Coconut water (75%) (14.12 cm) with longest root length, seedling dry weight is high in T₁ - *P. fluorescens*@10g/kg of seed (0.249 g) as recorded at the end of ageing period.

Vigour index I was recorded highest in the treatment T₇ - *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (1646) and vigour index II resulted highest in T₂ - *T. viride* @4g/kg (2005) at the end of the ageing period.

T₄ - *P. fluorescens* @10g/kg + *T. viride* @ 4g/kg recorded highest speed of germination as 15.04. while, mean germination time and time taken for 50% germination recorded least in T₈ - hydro priming as (6.04 d and 7.03d), respectively at the end of ageing period.

Electrical conductivity was recorded lowest in treatment, T₄ - *P. fluorescens* @10g/kg + *T. viride* @4g/kg (43.45 μScm^{-1}) and T₂-*T. viride* @4g/kg (44.55 μScm^{-1}) respectively with less seed leachates at the end of ageing.

An increasing trend in seed moisture content was observed with increase in days of ageing with 8.74 per cent at initial day to 19.4 per cent after 7 days of ageing. whereas, lowest was recorded in treatments, T₂ - *T. viride* @4g/kg and T₆ - *T. viride* (4g/kg) + coconut water (75%) (14.25%) at the end of ageing period.

Enzyme activities such as dehydrogenase and super oxide dismutase was found to be higher in T₇ -*P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) recorded as 0.268 OD value and 27.17 U/mg protein respectively towards the end of ageing period.

At the end of ageing period, lowest seed infection was recorded in T₁ - *P. fluorescens*@10g/kg both in agar plate method and blotter paper method as 11.03% and 15.33% respectively. *Aspergillus* spp., *Mucor* spp., *Syncephalastrum* spp. and *Rhizopus* spp. were observed in these plates.

According to the above findings of the accelerated ageing test, the treatments T₇ : *P. fluorescens* + *T. viride* + coconut water and T₂ : *Trichoderma viride* outperformed the other treatments in all seed quality parameters.

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Impact of biopriming on seed quality and longevity in rice (*Oryza sativa* L.)

by

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ABSTRACT OF THE THESIS

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ABSTRACT

The study "Impact of bio-priming on seed quality and longevity in rice" (*Oryza sativa* L.) was conducted at the Department of Seed Science and Technology, College of Agriculture, Vellanikkara to study the effect of different seed priming treatments on seed quality and seed longevity under natural as well as accelerated ageing. *Pseudomonas fluorescens*, *Trichoderma viride*, coconut water was used for seed priming rice variety Jyothy.

Seeds of rice variety Jyothy were treated with the following treatments: -, *Pseudomonas fluorescens* @ 10g/kg (T₁), *Trichoderma viride* @ 4g/kg (T₂), Coconut water @ 75% (T₃), *P. fluorescens* + *T. viride* (T₄), *P. fluorescens* + coconut water (T₅), *T. viride* + coconut water (T₆), *P. fluorescens* + *T. viride* + coconut water (T₇), Hydro priming (T₈), Control (untreated) (T₉). Both treated and untreated seeds were dried to <10 per cent moisture content and packed in Polyethylene bags (700 guage). The treated seeds were allowed to age both naturally and under accelerated ageing conditions.

There were significant differences among the treatments on seed quality parameters. Irrespective of the treatment, germination, seedling growth (dry weight, length of root and shoot), vigour index- I (VI-I), vigour index- II (VI-II), speed of germination, SOD enzyme activity, dehydrogenase enzyme activity decreased significantly over the storage period. A significant increase in seed moisture content, electrical conductivity, Mean Germination Time (MGT), Time taken for 50% germination (T₅₀) and seed microflora, was observed with increase in storage period.

Seed treatment with *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) retained a germination per cent of (83.69%) and higher vigour indices among the treatments after nine months of storage followed by *Pseudomonas fluorescens* @ 10g/kg (T₁) and *P. fluorescens* + *T. viride* (T₄) while treatment hydropriming (T₈) was found to be inferior in all quality parameters.

Coconut water treatments were found to be superior in increasing root and shoot length of the seedlings across the storage period which was reflected in the vigour indices. Bio priming with *P. fluorescens* resulted in early emergence of seedlings.

Seed microflora was found to increase significantly towards the end of storage period. This increase was least in *P fluorescens* @ 10g/kg (T₁), *Aspergillus* spp., *Pencillium* spp. were observed in the primed seeds. In present study it was found that among bio primed treatments *P fluorescens* treated seeds performed better than *T. viride* treated seeds in all quality parameters.

Accelerated aged seed samples were gathered and tested at daily intervals for 7 days to evaluate seed quality parameters. In general, dry priming performed better than wet primed seeds. Vigour indices was found to be highest in bio primed seeds (T₂, T₄, T₇) than other treatments

Seed longevity is found to be higher in bio primed seeds which can be evaluated from speed of germination (T₁, T₄, T₇), electrical conductivity (T₂, T₄), dehydrogenase and SOD enzyme activities. Seed infection (%) increased towards the end of ageing period and recorded least in *P fluorescens* treatments (T₁, T₇, T₅)

Seed treatments *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) was the best treatment followed by *P fluorescens* @ 10g/kg of seeds in improving seed quality as well as seed longevity. Treatments *P fluorescens* @ 10g/kg (T₁), *T viride* @ 4g/kg (T₂), *P. fluorescens* + *T. viride* (T₄), *P. fluorescens* + *T. viride* + coconut water (T₇) of seed may be used as seed treatments to enhance seed longevity in rice