

# Invigouration of Three Photosensitive Rice Varieties by Cinnamon-Immersion Water: A Comparative Study

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## Abstract

Maintenance of seed health during storage conditions is essential before sowing seeds in the field. A robust seed coat is essential for good seed quality because it shields the seed from imbibition-related nutrient leakage and guards against any kind of infections that might further degrade seed quality. Among various techniques and treatments related to seed invigouration, our study showed that cinnamon-immersed water can improve seed coat health and invigourate photosensitive rice seeds of three varieties when seeds were pretreated with this water. Our findings were proved by studying germination percentage, T50 of germination, growth of seedlings, and dry weight accumulation of both treated and untreated seeds. Data were further supported by examining the biochemical assays of the electrolytes leached out from both treated and untreated seeds. This study undoubtedly aids farmers in increasing the germination percentage of seeds with lower germination rates and enhancing the growth and vigour of seedlings by boosting the seed coat health.

**Keywords:** Invigouration, seed coat, pretreatment, germination, growth, vigour, seed electrolyte

## Introduction

Rice is the most desired cereal crop to satisfy the world's indiscriminate food needs. It requires storage for one or more planting seasons before cultivation. Researchers have long worked to introduce better tools and techniques for maintaining seeds' health while they are being stored [1-3]. More recently, the infusion of fungicides, growth regulators, pesticides, bio-products, bio-ingredients, agro-chemicals, herbicides, and nanomaterials into the seeds before germination is reported to alleviate the impact of adverse factors on seed quality and performance [4-7]. Seeds invigouration implies an improvement in seed performance by any post-harvest treatment such as soaking-drying with chemicals, growth regulators, etc, resulting in an improvement in germination percentage, greater storability, high yielding capability, and high vigour of plants than the corresponding untreated seeds [8]. Cell membrane injury may arise from a decrease in the water status of plant tissue [9]. Tissue permeability is increased by hydrogen peroxide ( $H_2O_2$ ), a harmful plant molecule that builds up in water-stressed tissue. According to reports, ascorbic acid lowers  $H_2O_2$  levels and controls membrane integrity in tissue under water stress [10]. Another interesting technique in nano-based agriculture is nanopriming with micronutrients. Introducing nanoparticles in rice fields also proves better crop productivity [11]. To maintain the seed health so far, many tools and techniques have been introduced by researchers, including seed coatings, seed hydration, pre-soaking, and seed priming techniques [12-16]. The impact of seed invigoration on variously aged bhendi and lab-lab seeds under various soil conditions was documented by Satha Celine Mary et al., 1994 [17]. Priming rice seeds with  $KH_2PO_4$  increased the germination rate and vigour of

seedlings, according to Borgohain and Phukon, 1994 [18]. To increase the vigour of rice seeds, a potassium salt concentration of 4% produced the greatest results. Another experiment revealed that the germination percentage of six-month-old IR-8 rice seeds rose when they were treated in several compounds [19]. The highest percentage of germination (76%) was seen in seeds treated with 0.5%  $NaCl$  out of all the compounds [20]. According to Chen et al. (2005), treating rice seed with glycine betaine and gibberellic acid increased the vigour of the rice seedlings at low temperatures [21]. While other rice cultivars like "Ratna" and "Suakalma" showed a significant loss in germinability, earlier research showed that the photosensitive varieties of rice "Jaya" and "Pankaj" were viable for longer periods under ambient circumstances [21].

Excessive dryness in stored seeds has also been observed to shorten seed lifetime, even while high temperatures, high humidity, and subsequently high seed moisture content effect the quick loss of seed viability [22]. An effort has been undertaken to track the deteriorating changes in the seeds of two popular photosensitive rice cultivars (IET-4786; IR-64) in light of the findings revealed in this study. Both seed kinds were prepared with certain inorganic, organic, and fungicidal-like substances ( $CuSO_4$ ,  $HgCl_2$ ,  $NaH_2PO_4$ ,  $Na_2HPO_4$ , and ascorbic acid) following seven months of storage [21].

Keeping in view the reported observations, an attempt has been made to follow the deteriorative changes in seeds of three hybrid varieties of rice, namely Rajdeep, Gosaba 5, and Sukumar. After 7 months of preservation,

both the seed varieties were pretreated with cinnamon immersed water (CIW). Cinnamon bark obtained from *Cinnamomum cassia* significantly exhibits antibacterial, antifungal, and anti-inflammatory properties due to the presence of a high concentration of cinnamaldehyde [23]. The viability of rice seeds in terms of germination was more or less high in all the treated seeds. The effectiveness of cinnamon-soaked water during invigoration will be demonstrated by the comparative study of Rajdeep (R1), Goshaba 5 (R2), and Sukumar (R3).

## 2. Materials and Methods:

### 2.1 Seed collection:

Certified seeds of three rice varieties were collected from Rice Research Station, Chinsurah, West Bengal, Hooghly, India, in June (harvesting period). Seeds were repeatedly sundried and stored separately in perforated paper bags for 7 months under normal laboratory conditions. After that period, seeds were used for germination under different treatments. The germinability of the seeds before invigoration treatments was 70%, 75%, and 65% for R1, R2, and R3, respectively.

### 2.2 Methods of pretreatment

Seeds of all varieties were surface sterilized with 0.1%  $HgCl_2$  for 1 min and then subjected to the treatment. Treatments were adopted by using cinnamon bark immersed overnight and preparing three concentrations with distilled water. An appropriate amount of cinnamon bark was directly dissolved in double-distilled water for 12 hours. After that, the bark was removed and the water was collected for further use. Seeds of R1, R2, and R3 were soaked in CIW for 6 h. After completion of the treatments, the seeds were separately surface dried with blotting paper and dried back to their original weight under the sun. The control (i.e., untreated) seeds were soaked with distilled water (DW) and dried under the sun along with the other treated seeds. After completion of pretreatment, seeds were stored in perforated paper bags and used for the experiment after 15 days.

### 2.3 Germination technique

All seeds were surface sterilized by immersing them in a 10% sodium hypochlorite solution before to germination [24]. To determine the percentage of germination, 20 separate seed samples were placed in Petri plates with filter paper that had been moistened with 10 ml of distilled water or, if applicable, CIW solution. The International Rules for Seed Testing Association were followed, and germination data were collected every 24 hours [25]. Seeds were considered to be completely germinated when the radicle attained a length of 1 mm and the plumule had just unfolded [26]. All the experiments were repeated thrice with 6 replications in each case. Details of seeds treated in different concentrations of CIW are given below-

T0 – DW-treated seeds

T1 – 0.1 mg/ml concentration of CIW

T2 – 0.3 mg/ml concentration of CIW

T3 – 0.5 mg/ml concentration of CIW

T4 – 0.7 mg/ml concentration of CIW

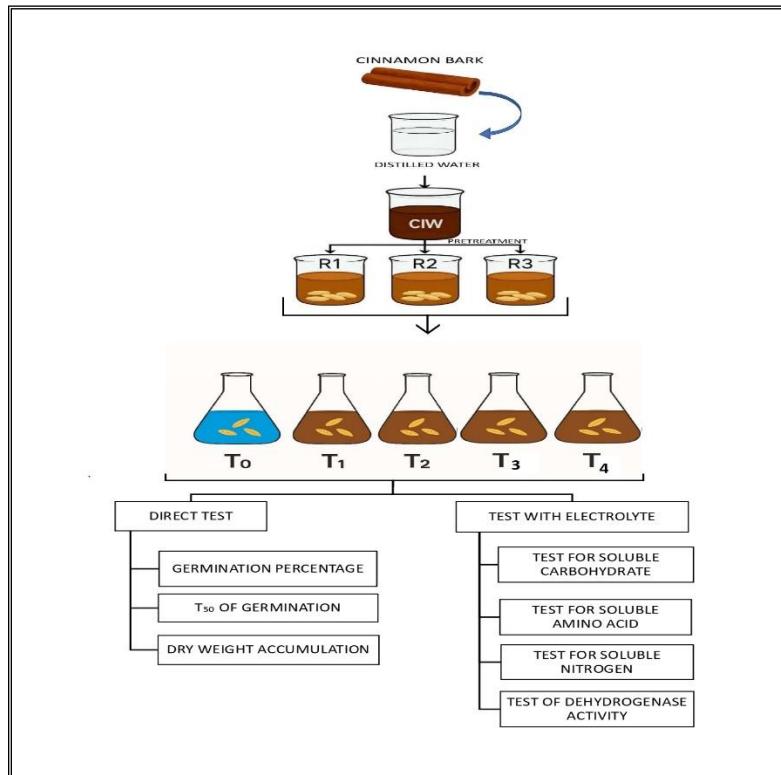
## 3. Method for measurement of other variables

The time required for 50% germination, or T50, was measured following three iterations of the germination process, each including six replications. After ten days of treatment, the dry weight accumulation of the rice seedlings was measured. 25 seeds, each with six duplicates, were placed in a 100 ml beaker filled with 25 ml of double-distilled deionized water at  $27 \pm 1^\circ C$  to quantify electrolyte leakage (EL). The seeds were taken out after a day.

A conductivity meter was used to directly measure the electrolytes' electrical conductivity in microSiemens ( $\mu S$ ) / 25 ml. The McCready et al., 1950 technique was used to determine the amount of soluble carbohydrates. A spectrophotometer was used to measure the green colour's intensity in terms of OD at 620 nm after 30 minutes [27]. A standard curve created by glucose was used as the basis for quantitative estimation.

The method of Moore and Stein (1948) was used to determine the soluble amino acids. Using glycine as the reference amino acid, the OD was measured at 580 nm and compared to the standard curve [28]. After determining the nitrogen content of the leachate using Vogel's 1965 technique, the OD was measured at 420 nm and compared to the standard curve created using various concentrations of  $NH_4Cl$ . Next, each case's percentage of N2 in the leachate was determined.

Twenty de-husked seeds were imbibed in distilled water with three duplicates for twenty-four hours at  $28 \pm 1^\circ C$  under sterile conditions to measure the dehydrogenase activity. Tetrazolium can be reduced to a red-colored formazan by the hydrogen atoms produced by the respiration-related dehydrogenase enzyme [29]. Following incubation, this formazan was extracted using 5 ml of 100% alcohol, and the solution's optical density (OD) values were noted at 520 nm. This approach was used with minor adjustments following Rudrapal and Basu (1979) [30]. After running each test three times with six replications in each case, we conducted a statistical analysis of the data from all of the aforementioned experiments. Using a one-way ANOVA and a suitable multiple comparison. Using a one-way ANOVA and a suitable multiple comparison test at  $p < 0.05$ , statistical significance was examined.



**Figure I: Diagrammatic representation of our experimental procedure**

## Results and Discussion:

### 4.1 Germination percentage:

From Table I, it was observed that the germination percentage was maximum in R2 for the treatment T2, while T4 showed the lowest germination percentage in R3. Germination percentage was gradually increased in the case of R2 in the T2, but it was further decreased in T4, indicating the detrimental effect of the treatment at higher concentrations. Values correspond to average  $\pm$  standard deviation obtained for all seeds from

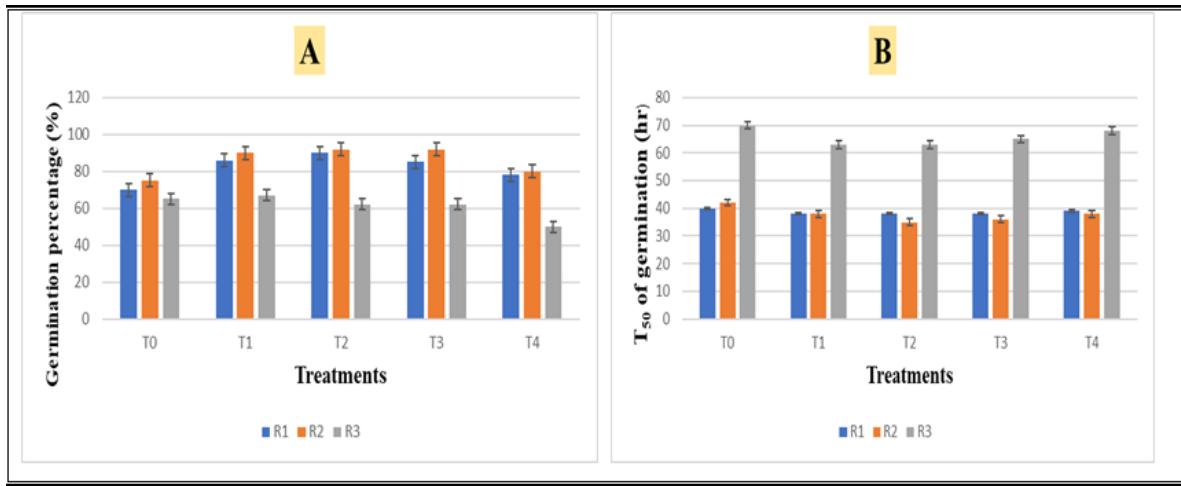
hexaplates where  $0.282 \leq p \leq 0.976$ ; # significant differences compared to control

### 4.2 T50 of germination:

The least T50 was observed in R2, i.e., 35 hrs in T2, and it was maximum, i.e., 68 hrs for R3 in T4 (Table I). In all the treatments, T50 was decreased in the case of R1 and R2. Values correspond to average  $\pm$  standard deviation obtained for all seeds from hexaplates\* where  $0.282 \leq p \leq 0.976$ ; # significant differences compared to control

Treatments	Germination percentage (%)			T50 of germination (hr)		
	R1 ( $\pm 4.67$ )	R2 ( $\pm 5.25$ )	R3 ( $\pm 5.07$ )	R1 ( $\pm 3.38$ )	R2 ( $\pm 4.60$ )	R3 ( $\pm 5.02$ )
T <sub>0</sub>	70	75	65	40	42	70
T <sub>1</sub>	86	90	67	38	38	63
T <sub>2</sub>	90	92	62	38	35	63
T <sub>3</sub>	85	92	62	38	36	65
T <sub>4</sub>	78	80	50	39	38	68

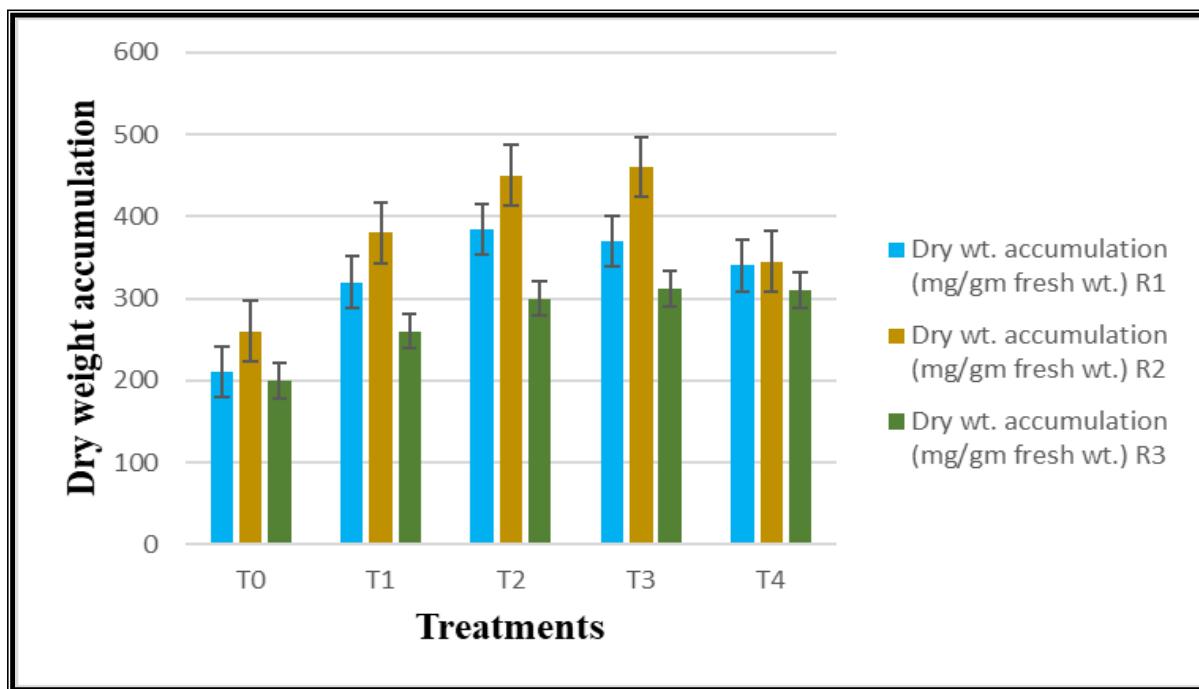
**Table I: Germination percentage and T50 of all the treatments in all the seed varieties**



**Figure 2: Bar diagram showing A, Germination percentage, and B, T<sub>50</sub> of germination of three rice varieties in prescribed treatments**

#### 4.3 Dry wt. Accumulation:

It was gradually increased in all the treatments in case of R1 and R2, but in R3, dry weight accumulation was decreased than the control, and it was the lowest in case of R3 in the T4.



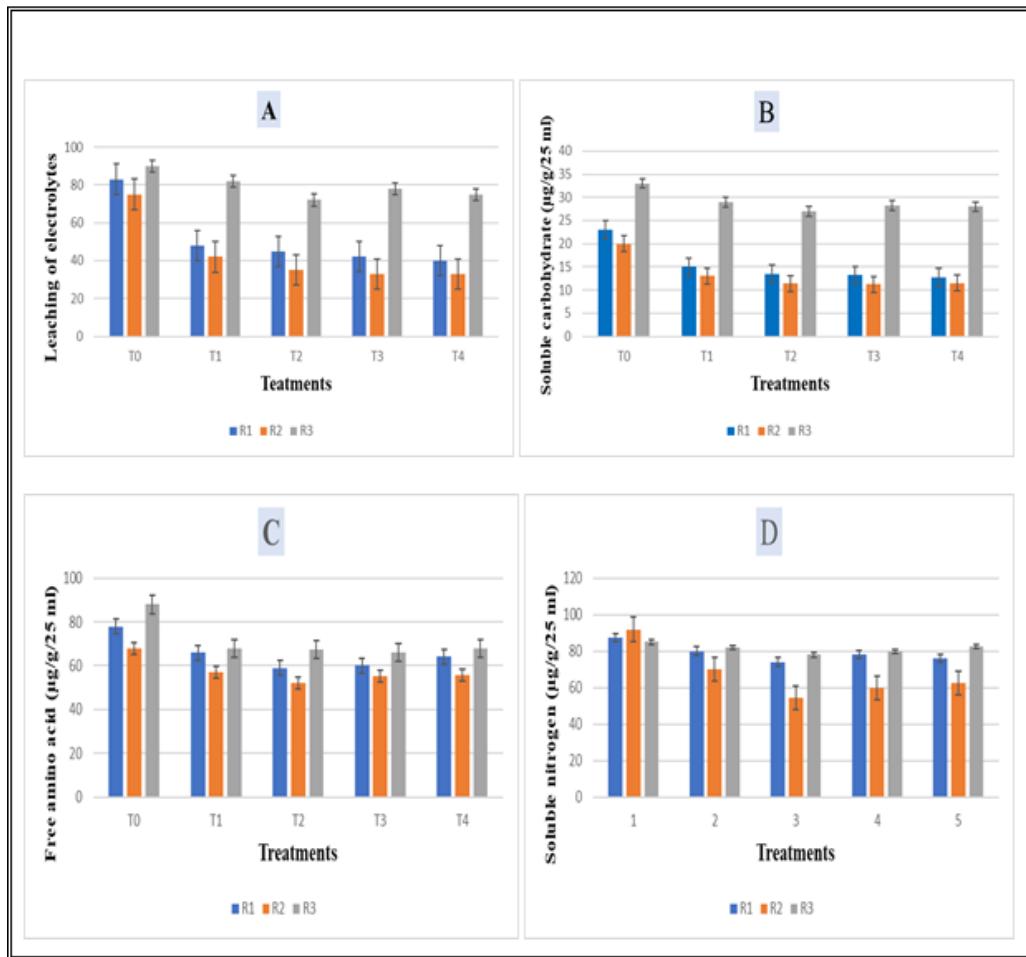
**Figure 3: Bar diagram showing dry weight accumulation of three rice seed varieties in different treatments**

#### 4.4 Conductivity test

Conductivity of leachates of 24 h was maximum for R3 in T0 that was reduced little in T1, T2 and T3 but leaching of electrolytes was maximally reduced than T0 in case of T2 for R2. In all treated cases conductivity of electrolytes reduced than T0.

#### 4.5 Test for soluble carbohydrate, free amino acid and soluble nitrogen:

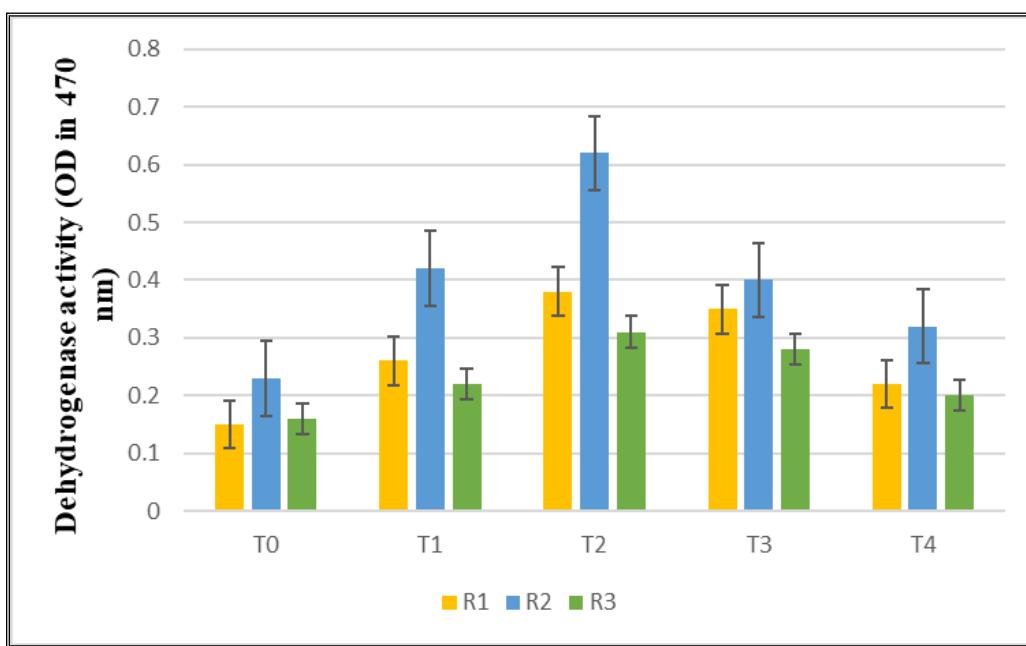
Leached out amount of soluble carbohydrate was reduced in all the treatments compare to the control. It was minimum i.e. 11.4 µg/g/25 ml in T2 in case of R2 and for R1 it was 13.5 µg/g/25 ml. Free amino acid and soluble nitrogen leached out through the seed coat were minimum in case of R2 for the treatment T2 and for R3 leached out nutrient content was significantly higher than other two varieties and it was more all less similar like control.



**Figure 4:** Bar diagram showing A, Leaching of electrolytes; B, Soluble carbohydrates; C, Free amino acids and D, soluble nitrogen present in the electrolytes in R1, R2 and R3 in different treatments

#### 4.6 Test of dehydrogenase activity:

It was clear from the fig 4 that for all R1, R2, and R3, T0 showed the lowest dehydrogenase activity over all the treatments. OD values of the red formazan formation, when recorded at 470 nm it was increased in all other treatments and the maximum OD was recorded in R3 for the T4 condition.



**Figure 5:** Bar diagram showing dehydrogenase activity of R1, R2 and R3 in different treatments

#### 4. Discussion:

We know that a seed's membrane is its most crucial component, and it seems that high temperatures and high moisture storage conditions have the biggest impact on it. It may be argued that every substance that has been shown to impact seed viability also affects membrane integrity. The increase in seed germination percentage and decrease in T50 observed in our study demonstrate the advantageous impact of pretreated seeds with the chosen compounds. The aforementioned pattern also supports seedling vigour as measured by dry weight gain. It is evident from the study that certain compounds exhibit superior benefits at lower dosages, while others do so at larger dosages. It was proposed that the leaching of toxic metabolites from the seeds made the positive effects of chemical pretreatments feasible [31, 32].

Literature review on invigoration study gave us clear data that different types of pretreatments are reported to successfully enhance seed viability. Treatments with antioxidants, salts, organic acids, essential oils, plant growth regulators, nanoparticles, etc. can influence the viability of seeds [33-35].

Additionally, it was demonstrated that the conductivity of the pooled-out electrolyte increased in tandem with the degree of membrane degradation [25]. Since the invigoration results from our research were positive, membrane permeability was used to assess the relative efficiencies of our findings. Deterioration of the seed membrane is indicated by increased leaching of electrolytes with other metabolites from seeds, which was seen in T0 of all three kinds. The fact that pretreatment with CIW stops leaching from seeds (Fig. 3) suggests that CIW prevents membrane deterioration, which in turn lessens the membrane's harmful effects. Due to membrane degradation, internal seed components such as potassium, phosphate, sugar, amino acids, etc., leak out when seeds are submerged in water (Tajbakhsh, 2000).

The investigation on solute leaching that coincides with a decline in germinability and viability has supported the idea that a loss of membrane integrity and the development of membrane lesions may be important factors in the degeneration of seeds (Bhattacharjee et al., 2006). For more than a century, it has been understood that when plant tissues are submerged in a solution, the electrical conductivity of the solution increases [36]. Numerous researchers concluded that greater membrane permeability, which permits

more leaching of carbohydrates, amino acids, soluble nitrogen, and salts from seeds, was the reason for the imbibing solution's decreased resistance [37, 38]. In the current investigation, the pretreated seeds showed a more noticeable halt of the leaching of soluble chemicals than T0. The aquaporin in the seed membrane is responsible for the increase in water absorption [39]. Additionally, aquaporins can control the electrochemical potential of membranes and significantly decrease the flow of ions across them [40]. It is anticipated that this aquaporin characteristic would boost the germination rate and vigor of every treated seed [41].

Numerous variables, including pH, heavy metal concentrations, high osmotic pressure, salinity, and water channel expression genes like PIP (Plasma membrane Intrinsic Protein) [42] and SIP (Small basic Intrinsic Protein) [43], affect how aquaporins function. The significance of PIP genes in rice seed germination was detailed by Liu et al. in 2007 [38].

CIW has strong anti-inflammatory activity with antimicrobial and anticancerous properties that prevent microbial deterioration of the rice seed coat. Cinnamon also contains essential minerals like calcium, magnesium, iron, fibre etc. that help to rejuvenate seed coat health by supplying nutrients [44, 45].

#### 5. Conclusion:

From our overall study, we can conclude that CIW has enough potency to rejuvenate seed coat health if it deteriorates during storage conditions. Rice seeds older than one year may also be sown in the fields after treating them in CIW water. Our study showed that 0.3mg/ml concentration of CIW i.e. T2 exhibited best effect on Gosaba 5 i.e. on R2 and the application of higher dose on all R1, R2 and R3 showed toxicity which was recorded by the data of T4 on all three. Moreover, the antiinfection effect of CIW may exhibited additional advantage on deteriorative seeds by nullifying any kind of antibacterial and antifungal issues. Finally, CIW pretreated seeds also boost up their nutritional content with the microelements present in the cinnamon bark.

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