



Pre-sowing seed treatment utilizing radiation-processed sodium alginate (ISA) and chitosan (ICH) enhances seed germination and embryonic seedling establishment of the chili pepper (*Capsicum annum* L.).

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Received: 10 July 2025, Revised: 15 September 2025, Accepted: 05 December 2025, Available Online: 15 December, 2025

<https://doi.org/10.5281/zenodo.19680293>

Abstract

Chili pepper (*Capsicum annum* L.) is a crucial plant in both culinary and therapeutic applications, surrounding ayurvedic and allopathic therapies for various health issues. Chili seeds have factually been allotted with physiologically or internally generated state of dorm which effects their germination potential. Problems related with insufficient seed germination rates and vigor growth may result in unfavorable growing patterns of seedlings and reduced agricultural productivity. The ultimate goal of this study endeavor was to evaluate the role of two radiation-treated natural polysaccharides, sodium alginate (ISA) and chitosan (ICH), on the germination and embryonic seedling progress of chili. **Method:** Seeds were immersed in water for 12 hours in containing unirradiated sodium alginate (USA) at a rate of 40 mg per L, and unirradiated chitosan (UCH) at a rate of 40 mg per L, which were employed as controls as well. Furthermore, numerous doses of ICH and ISA (40, 80, 120, and 160 mg L⁻¹) were used separately as treatments for both experiments. **Result:** The findings indicate that of the employed concentrations, 120 mg L⁻¹ of ISA and 80 mg L⁻¹ of ICH emerged as the most effective, leading to significant improvement of all evaluated parameters, viz., seed germination percentage, germination index, seed vigor index I, and seed vigor index II, as well as early seedling growth, in terms of shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight compared to control treatments (DDW). However, the respective treatments shortened the mean germination time compared to the control. Seed-soaking treatments with ISA and ICH also led to an increase in the activities of enzymes such as α -amylase, β -amylase, and protease, as well as the leaf content of photosynthetic pigments, chlorophyll, and carotenoids, respectively, as compared to the control

(water-soaking treatment). **Conclusion:** Conclusively, the administration of ISA and ICH at the recommended doses is an efficient and secure approach to increase the germination of seeds as well as early seedling development in chili.

Keywords:

Capsicum annum, Irradiated sodium alginate, Irradiated chitosan, Natural polysaccharides, seed germination, seedling growth.

1. Introduction

India stands as the world's leading producer of chilies, contributing nearly 41.95% of the total global output. Andhra Pradesh, Karnataka, Madhya Pradesh, and Telangana appear as the leading Indian states for the cultivation of chili peppers (Anonymous, 2018). Chili is an significant plant and spice crop and holds extensive economic significance as a chief cash crop in the Indian subcontinent. Chili accomplishes diverse roles, being used as a vegetable, spice, and condiment, while also contributing significantly to culinary applications and nutritional value. The biochemical compounds such as capsorubin and capsanthin, along with the capsaicin, play a crucial role in providing the distinctive red color and strong aroma of fully ripened chilies (Mathew, 2002). The chili fruit primarily serves as an enhancement in a range of culinary applications. This method is frequently utilized in traditional medical practices for various human conditions (Prathibha *et al.*, 2013; Olatunji & Afolayan, 2018).

The primary goal of farmers is to attain improved seed germination rates and robust seedling growth throughout the growing season. This suggests that the seeds demonstrate outstanding quality and are likely to produce the best possible crop outcomes. Thorough studies have been conducted in the past to achieve these goals. Chili seeds often display physiological or spontaneous dormancy, which complicates their germination (Alcalá-Rico *et al.*, 2019). Seed dormancy is the temporary condition in which viable seeds cannot germinate, even when favorable climatic conditions are present. This phenomenon can be linked to the inherent traits of the seeds that hinder the germination process, leading to a postponement in emergence (Baskin & Baskin, 2004; ElKeblawy, 2017).

Sodium alginate is a naturally occurring biopolymer derived from brown algae of the class *Phaeophyceae*. It is a linear polysaccharide composed of two anionic monosaccharides, α -L-guluronic acid (G) and β -D-mannuronic acid (M). These residues are arranged within the polymer chain as homopolymeric G-blocks, homopolymeric M-blocks, and heteropolymeric MG-blocks (Fig. 1). Gamma irradiation leads to the depolymerization of sodium alginate into its low-molecular-weight oligomers. These irradiated sodium alginate (ISA) oligomers have been widely documented as effective plant growth-promoting agents. Previous studies have demonstrated that ISA application enhances a range of biological and physiological processes in plants, including induction of phytoalexins, stimulation of vegetative growth, improvement of seed germination and flower development, antimicrobial activity, and alleviation of heavy-metal stress (Hein *et al.*, 2000; Kume *et al.*, 2002; Hu *et al.*, 2004; Hegazy *et al.*, 2009; Aftab *et al.*, 2011; Idrees *et al.*, 2011; Naeem *et al.*, 2021).

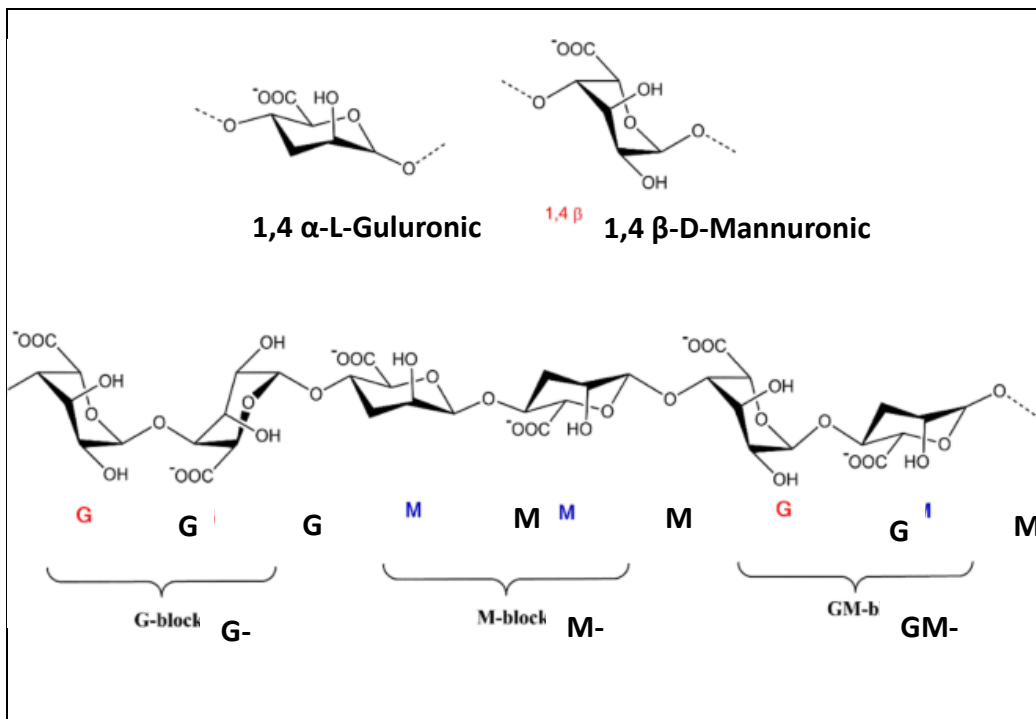


Figure.1. Chemical structure of Sodium alginate. With the curtesy of Abasalizadeh *et al.*, 2020

Chitosan is another naturally occurring polysaccharide of significant scientific interest, obtained primarily from fungal cell walls and the exoskeletons of crustaceans, insects, and shrimp. It consists of β -(1 \rightarrow 4)-linked D-glucosamine (deacetylated) and N-acetyl-D-glucosamine (acetylated) units distributed randomly along the polymer backbone (Fig. 2) (Shahidi *et al.*, 1999). Gamma irradiation induces the degradation of chitosan into low-molecular-weight, water-soluble oligomers, which exhibit physicochemical and biological properties distinct from those of the native polymer. These transformed forms of chitosan have revealed better bioactivity, especially in agricultural and biological applications (Naeem *et al.*, 2021).

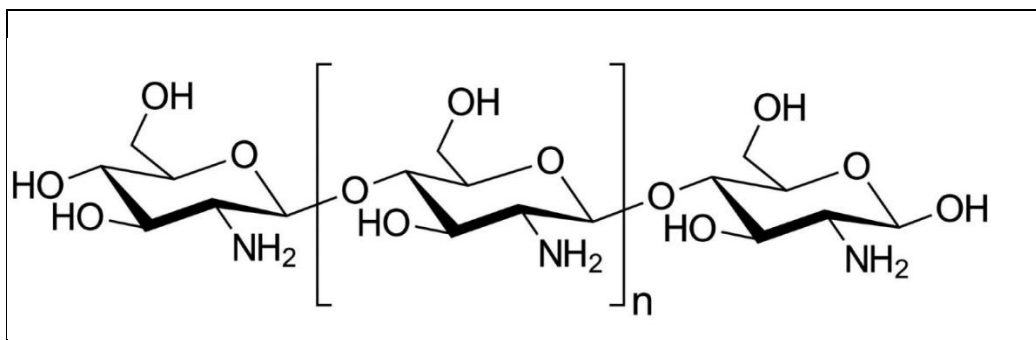


Figure.2. Chemical structure of chitosan (Image credit: grebeshkovmaxim/Shutterstock.com)

Previous research has proved that the use of gamma-irradiated chitosan and its oligomer products has a beneficial effect in several plant biological and physiological reactions. The effects that have been reported include improved seed germination (Hein *et al.*, 2000; Batool Mahdavi & Asghar Rahimi, 2013; Behboud *et al.*, 2020; Ali *et al.*, 2022), accelerated shoot development (Hein *et al.*,

2000; Batool Mahdavi & Asghar Rahimi, 2013; Behboud *et al.*, 2020; Ali *et al.*, 2022) and increased root development (Iwasaki & Matsubara, 2000; Batool Mahdavi & Asghar Rahimi, 2013; Martins *et al.*, 2018). This study investigated the physiological responses of chili pepper to seed-soaking treatments utilizing different concentrations of ISA and ICH, focusing on germination behavior and initial seedling development.

Materials and method

1.1. Plant material and radiation processing

Seeds of chili pepper (*Capsicum annuum* L. var. Pusa Sadabahar) were procured from the Indian Agricultural Research Institute (IARI), New Delhi, India. Gamma irradiation of sodium alginate and chitosan was carried out using a cobalt-60 (^{60}Co) source at dose rates of 2.4 kGy h^{-1} and 520 kGy h^{-1} , respectively, in the Gamma Radiation Chamber at the Bhabha Atomic Research Centre (BARC), Mumbai, India.

1.2. Preparation of aqueous solutions of ISA and ICH

Aqueous solutions of gamma-irradiated chitosan (ICH) and gamma-irradiated sodium alginate (ISA) were prepared at concentrations of 0, 40, 80, 120, and 160 mg L^{-1} using double-distilled water for Experiments 1 and 2, respectively. Prior to solution preparation, ICH was first dissolved in 10% (v/v) acetic acid to ensure complete solubilization. In both experiments, distilled water was used as the absolute control. Additionally, a concentration of 40 mg L^{-1} of unirradiated sodium alginate (USA) and unirradiated chitosan (UCH) was included as the respective polymer controls for Experiment 1 and 2.

Detailed scheme of foliar-spray treatments involving varying concentrations of γ -irradiated forms of chitosan (ICH) and sodium alginate (ISA) and chitosan (ICH) is given in **Tables 1 (A and B)**.

Table 1 (A) Treatment scheme of sodium alginate application (Experiments 1).

	Treatments	Explanation of foliar-spray treatments
1	Control (DW)	Seed-soaking using distilled water
2	USA-40	Seed-soaking using USA at 40 mg/L
3	ISA-40	Seed-soaking using ISA at 40 mg/L
4	ISA- 80	Seed-soaking using ISA at 80 mg/L
5	ISA-120	Seed-soaking using ISA at 120 mg/L
6	ISA-160	Seed-soaking using ISA at 160 mg/L

USA – Unirradiated sodium alginate; ISA – Irradiated sodium alginate; DW – Distilled water

Table 1 (B) Treatment scheme of chitosan application (Experiments 2).

S. No.	Treatments	Explanation of foliar-spray treatments
1	Control (DW)	Seed-soaking using distilled water
2	UCH-40	Seed-soaking using UCH at 40 mg/L
3	ICH-40	Seed-soaking using ICH at 40 mg/L
4	ICH- 80	Seed-soaking using ICH at 80 mg/L
5	ICH-120	Seed-soaking using ICH at 120 mg/L
6	(ICH-160	Seed-soaking using ICH at 160 mg/L

UCH – Unirradiated chitosan, ICH – Irradiated chitosan, DW – Distilled water

1.3. Experiment setup

Two independent experiments were conducted simultaneously at the Department of Botany, Aligarh Muslim University, Aligarh, Uttar Pradesh, India, to see the effects of different doses of ISA and ICH on seed germination and initial seedling development of chili pepper. Petri dishes were thoroughly cleaned and sterilized in a hot air oven at 160 °C for an h prior to use. Uniform, healthy, and fully developed chili seeds were disinfected by dipping in 1% (w/v) mercuric chloride (HgCl₂) solution for a min, followed by repeated rinsing with DDW to eliminate residual disinfectant. The seeds were air-dried under sterile conditions and placed on sterile filter paper. Seeds were initially soaked in DDW for 12 h. Treatments included DDW (absolute control), unirradiated sodium alginate (USA; 40 mg L⁻¹), unirradiated chitosan (UCH; 40 mg L⁻¹), and gamma-irradiated sodium alginate (ISA) or gamma-irradiated chitosan (ICH) applied separately at concentrations of 40, 80, 120, and 160 mg L⁻¹. Following the soaking process, seeds were uniformly placed in sterilized Petri dishes lined with Whatman No. 1 filter paper and were moistened with the appropriate treatment solutions.

The experiment employed a completely randomized design, facilitating five replicates for each treatment, with each replicate consisting of 15 seeds placed in a Petri dish. Germination and early seedling development were maintained in the Petri dishes for 15 days under laboratory conditions. To achieve a favorable moisture condition, filter papers were re-moistened with the correct treatment solutions at two-day intervals. Seed germination was followed once both radicle and coleoptile had reached 2–3 mm in length. For germinated seeds, they were counted daily for 10 days after sowing to obtain germination percentage (GP), germination index (GI), and mean germination time (MGT). Activities of α -amylase (EC 3.2.1.1), β -amylase (EC 3.2.1.2), and protease (EC 3.4.21) were also determined during the germination phase. Seedling growth factors (shoot and root length, fresh and dry biomass, and seed vigor indices) were obtained at 15 days after germination. Leaf photosynthetic pigments, namely chlorophylls and carotenoids, were also quantified at this time.

1.4. Seed germination percentage

The seed germination percentage (G.P.) was calculated by using the following formula.

$$GP = \frac{\text{No. of germinated seeds per treatment on the final day}}{\text{Total number of seeds sown per treatment}} \times 100$$

1.5. Seed germination index

The seed germination index (SGI) is an integrated parameter that reflects both the percentage and rate of seed germination. Higher SGI values indicate faster and more uniform germination of the seed lot. The SGI was calculated using the following formula:

$$\text{SGI} = \frac{\text{No. of germinated seeds}}{\text{Day of the first count}} + \dots + \frac{\text{No. of germinated seeds}}{\text{Day of the final count}}$$

1.5. Mean germination time

Mean germination time (MGT) is a measure of the average time required for seeds to germinate, with emphasis on the period during which the majority of seeds germinate. MGT was calculated according to the equation proposed by Ellis and Roberts (1981):

$$\text{MGT} = \frac{\sum(D \times n)}{\sum n}$$

where n is the number of seeds germinated on day D , and D is the number of days from the beginning of the germination experiment.

1.6. The activity of germination-related enzymes

Uniformly germinated seeds (2 mg) from each treatment were homogenized in five volumes (1:5, w/v) of ice-cold saline in a pre-chilled mortar and pestle. The crude extract was passed through microfiltration and subsequently centrifuged at 10,000 rpm for about 10 min at 4 °C. Resulting soluble fraction was collected and used as the raw enzyme extract. Activities of α -amylase (EC 3.2.1.1), β -amylase (EC 3.2.1.2), and protease were assayed by following the method described by Das and Sen-Mandi (1992). All enzyme activity measurements were performed in triplicate to ensure analytical accuracy and reproducibility.

1.7. Determination of photosynthetic pigments

Total leaf chlorophyll and carotenoid contents were quantified using the methods described in Lichtenthaler and Buschmann (2001). With pure acetone and grinding, newly collected interveinal leaf tissue was meticulously homogenized. The optical density (OD) of the extract was determined using a single-beam spectrophotometer (Shimadzu UV-1700, Tokyo, Japan) at 662, 645, and 480 nm to quantify the content of chlorophyll a, chlorophyll b, and total carotenoids, respectively. The quantities of chlorophyll a and chlorophyll b were added to determine the overall chlorophyll content in leaves. The levels of photosynthetic pigments—in particular chlorophylls and carotenoids—were weighed in milligrams per gram fresh leaf weight (mg g^{-1} F.W).

1.8. Seedling growth

On day 15, five seedlings were collected from each treatment group: ISA, ICH, and the control. The growth parameters of the seedlings, specifically shoot and root length, were recorded by measuring the thread length with a meter scale. The seedlings were subsequently surface-dried with an absorbent, and the fresh weight of both the shoot and root was checked through a computerized scale. Thereafter, the seedling samples were dehydrated in a hot air oven at 60°C for 48 hours to ascertain the dry weight of the shoot and root individually.

1.9. Seed vigor index

The International Seed Testing Association (ISTA) characterizes seed vigor as "the collective attributes of seeds that influence the degree of activity and performance within a seed lot during germination and seedling emergence." The seed vigor index (SVI) is computed following the ISTA principles first set forth in 1976.

SVI (I) is calculated using the subsequent formula:

$$\text{SVI (I)} = [\text{Seedling length (cm)} \times \text{Germination percentage}]$$

SVI (II) is calculated using the subsequent formula:

$$\text{SVI (II)} = [\text{Seedling dry mass (mg)} \times \text{germination percentage}]$$

2. Statistical analysis

There were five replicates in each treatment; a Petri dish was considered a single replicate. Dataset were statistically assessed through SPSS software version 22. Means of treatment were compared statistically with Duncan's Multiple Range Test (DMRT) to a significance level of $p \leq 0.05$. Means \pm SE (Standard Error) were the reported values to indicate differences among means of treatment.

3. Result

For both experiments, DDW treatment was the control treatment and it presented statistically similar values as that of unirradiated sodium alginate in Experiment 1 and unirradiated chitosan in Experiment 2 (Tables 1 and 2). Pre-sowing seed soaking with ISA and ICH at different concentrations resulted in significant improvements in seed germination related attributes such as germination percentage, germination index, seed vigor indices, and early seedling growth parameters in comparison to the DDW control.

Moreover, ISA and ICH treatments markedly enhanced the rate of germination-associated enzymes (α -amylase, β -amylase, and protease), along with increased accumulation of leaf photosynthetic pigments. All measured parameters showed a progressive increase with rising concentrations of ISA and ICH up to 120 mg L⁻¹ and 80 mg L⁻¹, respectively, followed by a significant decline at higher doses. The maximum enhancement of germination, enzymatic activity, and seedling growth was observed at 120 mg L⁻¹ ISA and 80 mg L⁻¹ ICH, identifying these concentrations as the optimal doses under the experimental conditions of the present study (Tables 1 and 2; Figures 3–5).

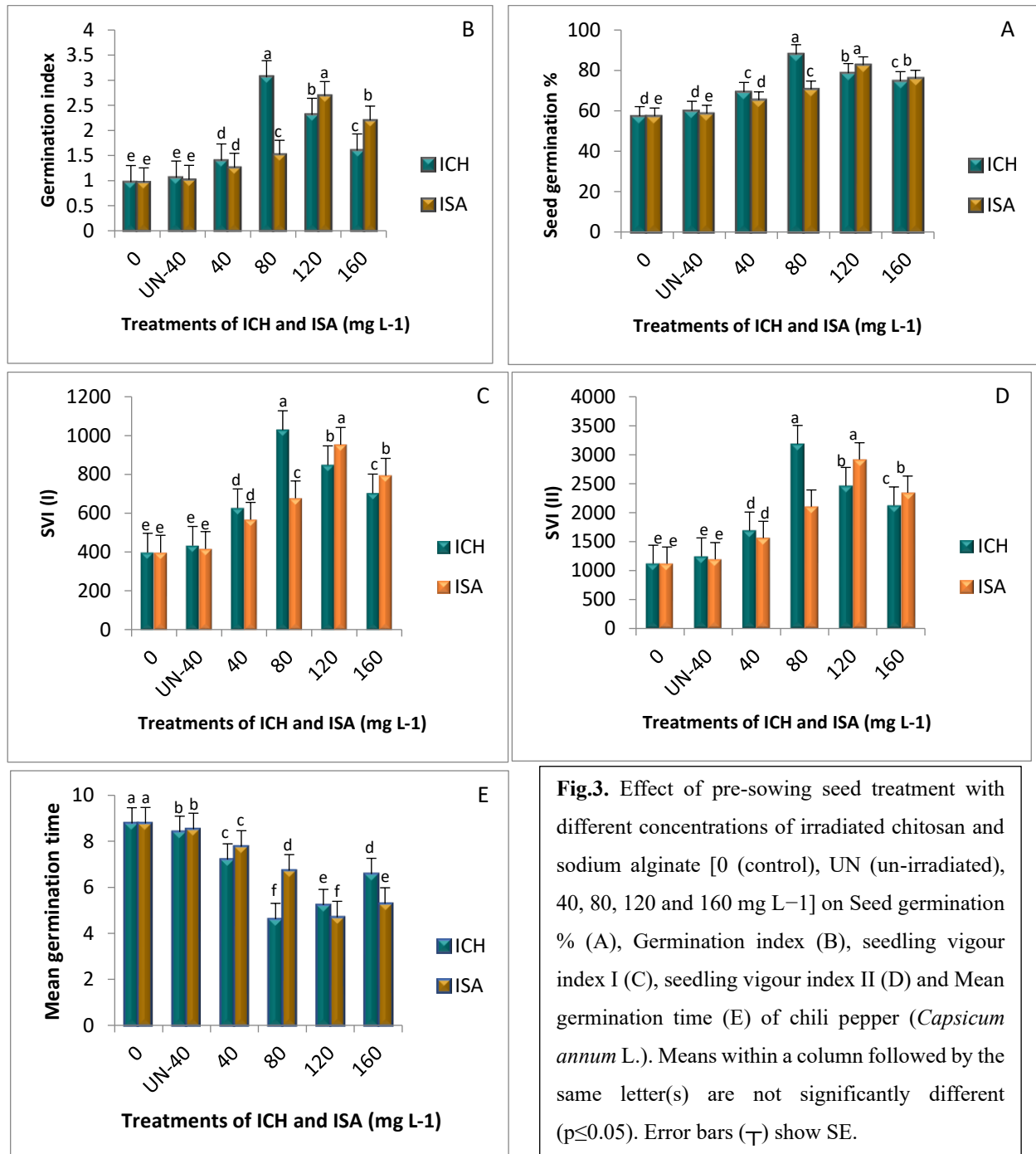
3.1. Seed germination parameters

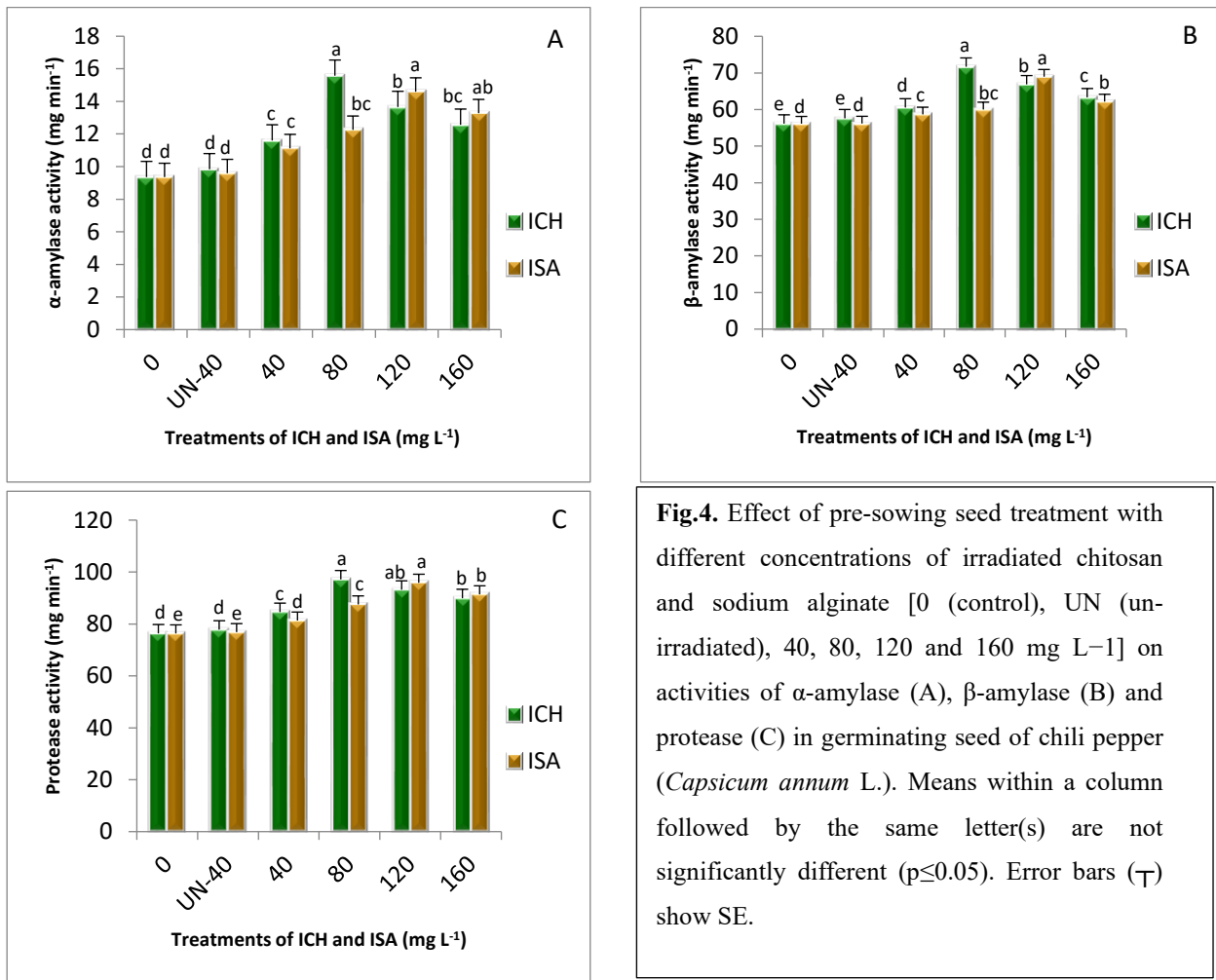
ISA at a concentration of 120 mg L⁻¹ and ICH at 80 mg L⁻¹ maximally increased the percentage of seed germination by (44.1% and 53.4%), germination index by (177.3% and 215.4%), seed vigor index I by (139.9% and 159.0%) and seed vigor index II by (161.0% and 184.8%) compared to control treatments (DDW). However, respective treatments shortened mean germination time by 46.4% and 47.3% compared to the control (**Fig.3A-3E**).

3.2. The activity of germination-related enzymes

Seed-soaking treatments with ISA and ICH significantly enhanced the activities of germination-related enzymes, including α -amylase, β -amylase, and protease, compared with the control (DDW). Maximum enzyme activities were recorded at 120 mg L⁻¹ ISA and 80 mg L⁻¹ ICH. At these optimal concentrations, α -amylase activity increased by 55.9% and 66.4%, β -amylase by

22.8% and 27.7%, and protease by 25.5% and 27.2% under ISA and ICH treatments, respectively, relative to the control (Figs. 4A–C).





3.3.Growth characteristics

The respective treatments (ISA at 120 mg L⁻¹ and ICH at 80 mg L⁻¹) enhanced seedling growth in terms of Shoot length by 56.22% for ISA and 57.60% for ICH; root length grew by 82.30% for ISA and 86.92% for ICH; shoot fresh weight rose by 56.98% for ISA and 58.72% for ICH; shoot dry weight increased by 74.90% for ISA and 78.03% for ICH; root fresh weight improved by 84.38% for ISA and 92% for ICH; and root dry weight increased by 86.69% for ISA and 94.95% for ICH, relative to control treatments (Tables 2 A & 2B).

Table.2 (A) The influence of pre-sowing seed treatment utilizing irradiated sodium alginate (ISA) on growth of chili pepper (*Capsicum annum* L.) seedlings.

Parameters	Controls		ISA concentrations (mg L ⁻¹)			
	Control 1 (DDW)	Control 2 (USA-40)	40	80	120	160
SL (cm)	4.34±0.12 ^d	4.46±0.12 ^d	5.28±0.12 ^c	5.76±0.10 ^{bc}	6.78±0.13 ^a	6.22±0.33 ^b
RL (cm)	2.60±0.15 ^c	2.62±0.15 ^c	3.38±0.10 ^d	3.82±0.14 ^c	4.74±0.14 ^a	4.24±0.10 ^b
SFM (mg)	51.80±1.43 ^e	53.24±1.31 ^e	63.24±1.50 ^d	69.12±1.26 ^c	81.32±1.55 ^a	75.14±1.97 ^b
RFM (mg)	15.50±0.89 ^c	15.66±0.96 ^c	20.08±0.59 ^d	23.10±0.75 ^c	28.58±0.90 ^a	25.52±0.65 ^b
SDM (mg)	15.30±0.73 ^d	15.88±0.22 ^d	18.28±0.28 ^e	22.46±0.74 ^b	26.76±0.65 ^a	23.68±0.47 ^b
RDM (mg)	4.36±0.31 ^c	4.46±0.23 ^c	5.42±0.20 ^d	6.64±0.14 ^c	8.14±0.11 ^a	7.44±0.10 ^b

Each value represents the mean of 5 replicates with \pm S.E. Means within a column, followed by the same letter(s), are not significantly different ($p \leq 0.05$). **DDW**: double distilled water (control 1); **USA-40**: 40 mg L⁻¹ of unirradiated sodium alginate (Control 2); **ISA**: Irradiated sodium alginate; **SL**: Shoot length; **RL**: Root length; **SFM**: Shoot fresh mass; **RFM**: Root fresh mass; **SDM**: Shoot dry mass; **RDM**: Root dry mass.

Table.2 (B) The influence of pre-sowing seed treatment utilizing irradiated chitosan (ICH) on the growth of chili pepper (*Capsicum annum* L.) seedlings.

Parameters	Controls		ICH concentrations (mg L ⁻¹)			
	Control 1 (DDW)	Control 2 (UCH-40)	40	80	120	160
SL (cm)	4.34 \pm 0.12 ^c	4.58 \pm 0.13 ^c	5.48 \pm 0.10 ^d	6.84 \pm 0.10 ^a	6.42 \pm 0.13 ^b	5.88 \pm 0.13 ^c
RL (cm)	2.60 \pm 0.15 ^d	2.64 \pm 0.13 ^d	3.56 \pm 0.16 ^c	4.86 \pm 0.17 ^a	4.36 \pm 0.15 ^b	3.72 \pm 0.12 ^c
SFM (mg)	51.80 \pm 1.43 ^d	54.84 \pm 1.35 ^d	65.68 \pm 1.25 ^c	82.22 \pm 1.18 ^a	77.04 \pm 1.28 ^b	68.18 \pm 1.62 ^c
RFM (mg)	15.50 \pm 0.89 ^e	16.32 \pm 0.53 ^e	21.52 \pm 0.87 ^d	29.76 \pm 1.08 ^a	26.52 \pm 0.67 ^b	24.02 \pm 0.64 ^c
SDM (mg)	15.30 \pm 0.73 ^e	16.18 \pm 0.23 ^e	18.54 \pm 0.30 ^d	27.24 \pm 0.47 ^a	24.20 \pm 0.30 ^b	22.24 \pm 0.76 ^b
RDM (mg)	4.36 \pm 0.31 ^e	4.56 \pm 0.17 ^e	5.82 \pm 0.20 ^d	8.50 \pm 0.20 ^a	7.62 \pm 0.28 ^b	6.64 \pm 0.32 ^c

Each value represents the mean of 5 replicates with \pm S.E. Means within a column, followed by the same letter(s), are not significantly different ($p \leq 0.05$). **DDW**: double distilled water (control 1); **UCH-40**: 40 mg L⁻¹ of unirradiated chitosan (Control 2); **ICH**: Irradiated chitosan; **SL**: Shoot length; **RL**: Root length; **SFM**: Shoot fresh mass; **RFM**: Root fresh mass; **SDM**: Shoot dry mass; **RDM**: Root dry mass.

3.4. Photosynthetic pigments

The levels of photosynthesis-related pigments in the leaves (total chlorophyll and carotenoids) progressively increased to 120 mg L⁻¹ of ISA (Experiment 1) and 80 mg L⁻¹ of ICH (Experiment 2), respectively. Subsequently, an unforeseen decline in the levels was noted (Figs. 3A and 3B). Therefore, the levels of photosynthetic pigments were measured at 120 mg L⁻¹ of ISA (Experiment 1) and 80 mg L⁻¹ of ICH (Experiment 2), resulting in an increase in total chlorophyll content by 27.77% and 30.15%, respectively, and an increase in carotenoids by 40% and 43.17%, respectively, with respect to the control (water-soaking treatment) (Figs. 5A and 5B).

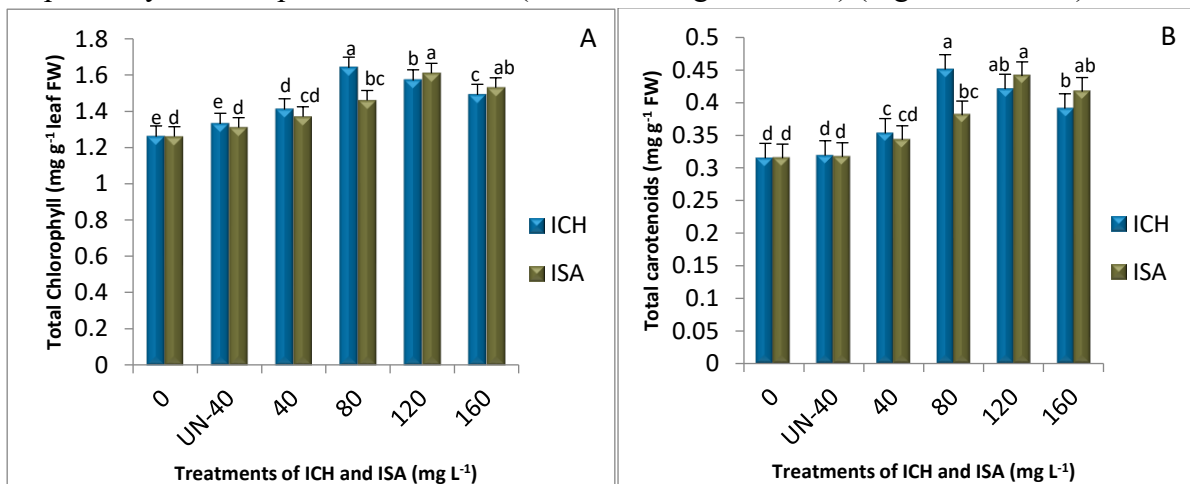


Fig.5. Effect of pre-sowing seed treatment with different concentrations of irradiated chitosan and sodium alginate [0 (control), UN (un-irradiated), 40, 80, 120 and 160 mg L⁻¹] on leaf-contents of chlorophyll (A), - carotenoids (B) of chili pepper (*Capsicum annum* L.). Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). Error bars ($\bar{\tau}$) show SE.

4. Discussion

Germination of seedlings is a key phase of the growth cycle for higher plants, providing the infrastructure (Bewley, 1997) for seedling establishment and plant growth. Efficient and synchronized germination is the precondition for vigorous seedling development (and as a result greater plant productivity) (Foolad *et al.*, 2007). Indeed, this biotic process which is tightly regulated is a result of a combination of both internal and external factors (Qu *et al.*, 2008; Weitbrecht *et al.*, 2011; Cho *et al.*, 2012; Miransari and Smith, 2014). It is a series of well orchestrated cellular events including storage reserve mobilization, enzyme activation, signal transmission, and metabolic pathways controlled operation. Seeds, prior to germination, were treated with γ -irradiated sodium alginate and γ -irradiated chitosan, which the present study has demonstrated markedly improved germination performance. Significant changes occurred in the germination percentage, germination index, and seed vigor indices. These responses were associated with faster germination and acceleration in early seedling proliferation, as attested by a shorter mean germination time, elongated shoots, and augmented fresh and dry biomass. The highest effect size on germination was observed at doses of 120 mg L⁻¹ ISA and 80 mg L⁻¹ ICH. This showed significant improvement in seed germination and seedling establishment compared to the control (DDW). The stimulatory effects of ISA and ICH were directly correlated with increased activity levels of germination linked hydrolytic enzymes such as α -amylase, β -amylase and protease in the context of seed germination. Germination and early seedling development rely on a key energy source: endosperm starch degradation, facilitated by α -amylase, β -amylase, debranching enzymes, and α -glucosidase. α -Amylase acts as a hydrolytic enzyme, and hydrolyzes the internal α -1–4-glycosidic bonds in starch granules to produce maltose and glucose (Pujadas and Palau, 2001) while β -amylase methodically liberates maltose units by breaking down the non-reducing termini of starch chains (Yamaguchi *et al.*, 1999). These enhanced activities of these enzymes in ISA- and ICH-treated seeds imply greater rapid and efficient mobilization of stored reserves for faster and more uniform germination.

Results from Experiment 1 are compatible with previous studies establishing the promotive activity of alginate-derived oligosaccharides in seed germination and seedling vigor. For instance, maize (Hu *et al.*, 2004), fennel (Idrees *et al.*, 2012), and *Eucalyptus citriodora* (Ali *et al.*, 2018) have enhanced germination upon alginate oligosaccharide treatment. We find that the gamma-irradiated sodium alginate oligomers act as endogenous growth regulators capable of activating critical metabolic enzymes and modulating genes important for growth and development (Ma *et al.*, 2010). Moreover, the upregulation of α - and β -amylase activities observed in the present study reinforces the hypothesis that ISA promotes germination by both metabolic and transcriptional regulation (Farmer *et al.*, 1991; John *et al.*, 1997).

Similarly, the enhancement of germination characteristics and enzyme activities seen in Experiment 2 after ICH treatment corresponds to the observations observed with other plant species. The agronomic impact of chitosan on germination percentage, germination rate, and early seedling growth has been documented under stress conditions especially under stressed conditions in maize (Batool Mahdavi and Rahimi, 2013; Martins *et al.*, 2018).

Optimised effect of an irradiated chitosan on seed germination and seedling quality has also been reported in *Eucalyptus citriodora* (Ali *et al.*, 2022), *Platycodon grandiflorus* (Liu *et al.*, 2022),

white clover (Ling *et al.*, 2022) and maize in response to osmotic stress (Behboud *et al.*, 2020). Chitosan is recognized as an agent promoting plant growth that affects physiological processes, production reactions, defense dynamics and enzyme activities (Darvill *et al.*, 1992), these effects are accelerated after gamma irradiation by modification of molecular arrangement of chitosan and bioactivity of chitosan (El-Rehim, 2006). Furthermore, chitosan, a nitrogenous compound, may favor an increased enzyme synthesis and metabolic activity at germination (Ohta *et al.*, 1999).

In addition to improving germination and enzymatic regulation, leaf photosynthetic pigment content, especially chlorophylls and carotenoids, significantly increased following treatment with ISA and ICH, with maximal increase observed at 120 mg L⁻¹ for ISA and 80 mg L⁻¹ for ICH. Chlorophyll plays a central role in photosynthesis and directly affects plant growth and biomass accumulation. Ascending of photosynthetic pigment can be due to better chloroplast development, chloroplast construction, increased chloroplast number and size, stimulation of chlorophyll biosynthesis, promotion of chlorophyll biosynthesis, and proper grana formation in the surface of the chlorophyll generation systems (Akimoto *et al.*, 1999). The aforementioned outcomes align with previous reports showing that there was a similar improvement in increase in photosynthetic efficacy and pigment building up under ISA and ICH application (Ahmed *et al.*, 2020, 2022; Moussa *et al.*, 2023).

5. Conclusion

Overall, the present study demonstrates that pre-sowing seed soaking with optimal concentrations of γ -irradiated sodium alginate and chitosan effectively enhances seed germination, early seedling growth, enzymatic mobilization of reserves, and photosynthetic pigment accumulation. Overall, these changes highlight the suitability of ISA and ICH as eco-friendly seed-priming modes for increasing crop productivity. Forthcoming molecular-level research is necessary to explore the specific signaling cascades and mechanisms of gene regulation that modulate these beneficial effects.

Acknowledgments

The author shows thankfulness to the University Grants Commission (UGC) in New Delhi, India, for supporting a PhD scholarship. Furthermore, the author extends thanks to the Bhabha Atomic Research Centre (BARC) in Maharashtra, India, for providing gamma irradiation facilities for the natural polysaccharides, chitosan, and sodium alginate used in present examination.

CRedit authorship contribution statement

Sangram Singh: Methodological approach, Investigative approach, Analytical assessment, Visual illustration, Composition—the original draft. **Moin Uddin:** Oversight, methodological framework, Analytical rigor, Writing—review and refinement. **Sarika Singh:** Analytical examination, Visual illustrations. **Urooj Hassan Bhat:** A meticulous examination and representation of data. **M. Masroor A. Khan:** Oversight, Ideation, Resource Allocation, Methodological Framework, Analytical Evaluation, and Validation Processes. **Akshay Chauhan:** Rigorous examination, Visualization. **Rajat Kumar Gautam:** Rigorous examination, Illustrative visualization.

Conflict of interest

The authors of the manuscript have stated that there are no competing interests related to the publication of the aforementioned research.

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