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Augmentation of Paddy Plant Health through Nano-chitosan (NC) and Nano-chitosan Tricyclazole Conjugate (NCT) based Seed Treatments Approaches

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ABSTRACT

Paddy (*Oryza sativa* L.) is a staple crop that suffers significant yield losses due to blast disease caused by *Pyricularia oryzae*. Conventional fungicide-based management, particularly tricyclazole, poses environmental risks and promotes resistance in pathogen. In this study, synthesized nano-chitosan (NC) and nano-chitosan-tricyclazole conjugate (NCT) were evaluated for their impact on seed germination, seedling vigour and disease suppression through seed treatment. Different concentrations (100, 250, 500 and 750 ppm) were tested using blotter paper and pot studies under greenhouse conditions. NCT at 500 ppm demonstrated the highest germination percentage (100%), seedling vigour and disease resistance, significantly outperforming conventional tricyclazole treatment (3 g/kg seeds). NC at 500 ppm also improved germination and seedling growth but was less effective than NCT. The results suggest that nano-based formulations enhance bioavailability and controlled release, making NCT a promising eco-friendly alternative for paddy blast management and robust early-stage development.

Keywords : Conjugation, Disease incidence, Nano particles, Seedling emergence, Vigour index

PADDY (*Oryza sativa* L.) is a staple food for more than half of the world's population and its productivity is significantly affected by various biotic and abiotic stresses (Gopi *et al.*, 2022). India is the second-largest producer of rice, cultivating over 44 million hectares and producing around 130 million tonnes annually (FAOSTAT, 2023). Among the various biotic constraints, paddy blast, caused by *Pyricularia oryzae*, is one of the most devastating fungal diseases, leading to yield losses ranging from 10% to as high as 50% under severe epidemics (Dean *et al.*, 2012 and Prabhu *et al.*, 2003). This disease affects all above-ground parts of the plant and is prevalent in almost all major rice-growing regions of India, including West Bengal, Odisha, Andhra Pradesh, Chhattisgarh and Tamil Nadu, with sporadic outbreaks also reported from northern states. It is a well-studied disease in the country, with numerous efforts focused on resistance breeding and chemical control, yet effective and sustainable management remains a challenge. Paddy blast disease is seed-borne in nature and severely affects rice during the nursery stage, making early protection crucial for healthy crop establishment (Hulbert *et al.*, 2001).

Botanicals and biopesticides have demonstrated moderate effectiveness against rice blast fungus under field conditions (Kiran and Shankar, 2017). In contrast, conventional management practices largely depend on synthetic fungicides. However, their overuse raises significant concerns related to environmental toxicity, chemical residues, and the emergence of fungicide-resistant pathogen strains (Ortuno *et al.,* 2008). A similar study reported diminishing efficacy of systemic fungicides in managing rice blast, underscoring the urgent need for alternative disease management strategies. Comparative trials by Sahana and Ramesh (2016) revealed that although systemic fungicides provide initial control, their effectiveness declines over time, likely due to resistance development, highlighting the importance of adopting controlled-release formulations or integrated disease management approaches

Integrated disease management approaches involving eco-friendly agents have shown promise in earlier studies. Chandrashekar and Jayalakshmi (2017) emphasized the importance of combining biological agents and resistant varieties with minimal chemical inputs to effectively manage rice blast. Their advocacy for sustainable alternatives aligns well with our approach, utilizing nano-chitosan-based conjugates. Further more, recent reports of *Magnaporthe oryzae* developing resistance to tricyclazole in Karnataka's rice-growing regions (Lakshmi and Puttaswamy, 2023) highlight the urgent need for nano-encapsulation strategies to enhance fungicide efficacy. These authors documented increasing resistance and recommended chitosan-based integrated management solutions, directly supporting the relevance of our nano-encapsulation approach. This underscores the necessity of exploring sustainable, eco-friendly alternatives that ensure effective disease control without adverse environmental effects.

Nanotechnology-based agricultural inputs have gained significant attention for their potential to enhance nutrient uptake, strengthen plant defense mechanisms and enable controlled suppression of pathogens (Khan *et al.*, 2019). Chitosan, a biopolymer derived from chitin, has been recognized for its antifungal, elicitor and plant growth-promoting properties (El Hadrami *et al.*, 2010). Its nano formulation, known as nano-chitosan (NC), offers superior bioavailability, increased surface area and improved plant uptake compared to conventional chitosan (Fellet *et al.*, 2021). Furthermore, chitosan-based nano composites have been investigated for controlled fungicide delivery, providing prolonged efficacy with minimal environmental impact (Elsherbiny *et al.*, 2023). Shanthala and Suresh (2019) highlighted the potential of nano material-based seed coatings as a sustainable approach for enhancing seed performance. Their work demonstrated that such coatings can improve germination rates, early seedling vigor and crop establishment, while simultaneously reducing input usage and mitigating environmental risks.

Tricyclazole, a systemic fungicide widely used for paddy blast management, inhibits melanin biosynthesis in *M. oryzae*, preventing fungal penetration and infection (Narayanasamy and Narayanasamy, 2008). However, its direct application, excessive improper dosing and frequent use can contribute to environmental contamination and the development of fungicide resistance (Kunova *et al*., 2014). Encapsulation of tricyclazole with nano-chitosan (NCT) offers a novel approach to controlled fungicide release, reducing chemical dosage while enhancing plant defense responses (Maluin and Hussein, 2020).

Seed treatment is a vital strategy in crop protection, promoting early-stage plant health and resilience against pathogens (Biswas *et al.*, 2008). As the paddy blast pathogen is seed-borne and primarily affects plants during the nursery stage, it is essential to assess the effectiveness of nano-chitosan and its tricyclazole conjugate as seed treatments to prevent initial infection. Nano-chitosan seed priming has been reported to enhance germination, seedling vigor and stress tolerance by modulating the plant's biochemical and physiological responses (Ayub *et al.*, 2022).

Moreover, seed priming with biocontrol agents and botanicals has also been shown to improve seed vigor under stress conditions. Prakash and Harish (2015) reported increased germination, vigor and stress tolerance in rice following such treatments. Similarly, Sudharshana and Shylaja (2021) observed effective blast suppression using chitosan-based nanoemulsions, attributing their efficacy to both direct antifungal activity and the induction of systemic resistance. This study aimed to evaluate the impact of seed treatment with synthesized nano-chitosan (NC) and nano-chitosan-tricyclazole conjugate (NCT), applied at varying concentrations, on enhancing rice plant health and suppressing early-stage blast infection.

Material and Methods

Synthesis of Nano-chitosan (NC) and Nano-chitosan-Tricyclazole Conjugate (NCT)

Nano-chitosan particles (NC) were synthesized *via* the ionic gelation method as described by Calvo *et al.* (1997), by dissolving chitosan in acetic acid, adjusting pH to 4.6-4.8 and adding Tween-80 as a stabilizer. Sodium tripolyphosphate (TPP) was added as a crosslinker under stirring and nano particles were recovered by centrifugation. For conjugation, tricyclazole (2 mg/mL) was incorporated into the optimized chitosan-TPP mixture to form CNPT with enhanced stability and fungicidal potential.

Effect of Synthesized Nano-chitosan (NC) and Nano-chitosan-Tricyclazole Conjugate (NCT) through Seed Treatment

To determine the optimal dosage that effectively suppresses pathogens while minimizing adverse effects on plant growth and development, the effects of NC and NCT were evaluated at various concentrations as mentioned in Table 1 through seed treatment using the blotter paper method and a pot study under greenhouse conditions. The experiment was conducted under *in-vitro* conditions using the susceptible rice variety MTU-1001 to evaluate the protective efficacy of synthesized NC and NCT treatments. No artificial inoculation of *Pyricularia oryzae* (blast pathogen) was carried out, as paddy blast is a seed-borne and air-borne disease; the natural infection process was relied upon to assess resistance (Rani *et al.*, 2021).

Table 1

Treatment details of NC and NCT for phytotoxicity study through seed treatment

Treat. No.	Treatment details
T1	ST with NC at 100 ppm
T2	ST with NC at 250 ppm
T3	ST with NC at 500 ppm
T4	ST with NC at 750 ppm
T5	ST with NCT at 100 ppm
T6	ST with NCT at 250 ppm
T7	ST with NCT at 500 ppm
T8	ST with NCT at 750 ppm
T9	ST with Chitosan (3 mg/mL)
T10	ST with Tricyclazole with concentration (3 gm/kg seeds)
T11	Control

*ST-Seed treatment, NC- Chitosan nano-particles, NCT- Nano-chitosan tricyclazole conjugate

Surface Sterilization of Seeds

Paddy seeds were surface sterilized by immersing them in a 2 per cent sodium hypochlorite solution for 1 minute with continuous shaking. The treated seeds were then rinsed three times with sterilized distilled water for 1 minute each to remove residual sterilizing agents.

A. Assessment of Seed Treatment Efficacy using the Blotter Paper Method

Surface-sterilized seeds were soaked for 1 hour in different concentrations of nano-chitosan (NC) and nano-chitosan-tricyclazole conjugate (NCT) solutions (100, 250, 500 and 750 ppm). Positive controls included seeds treated with chitosan (3 mg/mL) and tricyclazole (3 g/kg), while seeds soaked in distilled water served as negative controls. After treatment, seeds were shade-dried and subjected to a standard germination test using the 'between paper' method as per ISTA rules (ISTA, 2023).

Fifty seeds per treatment were placed between two moistened germination papers (blotters), ensuring even spacing. These papers were gently rolled, secured with rubber bands, and placed vertically in germination trays. The rolls were maintained at 25 ± 1 °C and 95 per cent relative humidity in a germination chamber. The test was conducted in a completely randomized design (CRD) with four replications. Germinated seeds were evaluated at 5 and 7 days to determine parameters such as germination percentage, germination rate, and seedling vigour, following ISTA criteria for normal and abnormal seedling classification.

Impact of Seed Treatment on Germination Performance Indicators

Plant growth-promoting properties were assessed based on germination performance indicators, including germination energy (GE), germination rate (GR), germination index (GI) and seedling vigour index (Vi). The GE, GR and GI were determined by measuring the shoot and root lengths of seedlings, by following the formula as given below. Germination percentage (GP) was to enable comparative analysis, the relative vigour index (RVi) was also calculated, providing a relative measure of seedling vigour in comparison to a control. Vi and RVi were calculated using the equations, as described by Sadjad *et al.* (1999) as follows:

Vi = (RL + SL) x GP

Where; Vi = vigour index, RL = root length (cm), SL = shoot length (cm) and GP = germination percentage

$$\text{Relative Vigour index (\%)} = \frac{\text{Vigour index of the seeds} - \text{Vigour index of the control}}{\text{Vigour of the control}} \times 100$$

GE, GR, and GI were calculated using the methodologies described by Saeed *et al*., (2022) as follows:

GE (%) = (Number of germinated seeds on the 5th day)/ total number of seeds) ß 100

GR (%) = (Number of germinated seeds on the 7th day)/ total number of seeds) ß 100

GI = '[(Days of germination)/(corresponding number of germinated seeds)]'

GP calculated using the methodologies described (Gholami *et al.,* 2009) as follows :

$$\text{Germination percentage} = \frac{\text{Total of normal shoots seven days germination}}{\text{Total number of seeds}} \times 100$$

B. Greenhouse Study of Seed Treatments on Paddy Growth and Blast Incidence

Overnight-soaked and surface-sterilized paddy seeds were treated for one hour with different concentrations of nano-chitosan (NC) and nano-chitosan–tricyclazole conjugate (NCT), along with positive and negative controls, as detailed in Table 1. Following treatment, the seeds were shade-dried to remove excess moisture and facilitate better absorption of the treatment. Sterilized soil was filled to three-fourths of each pot, and the treated seeds were sown at a rate of 10 seeds per pot. The experiment was laid out in a Completely Randomized Design (CRD) with five replications. Pots were maintained under greenhouse conditions at a temperature of 26/ °C to 30/ °C and relative humidity of 75% to 90%, with regular watering.

Seedling Emergence, Growth Parameters and Paddy Blast Disease Incidence

Seedling emergence was assessed two weeks after sowing, based on the presence of above-ground hypocotyls. Observations on blast disease incidence and various growth parameters including plant height, dry weight, stem circumference (measured using Vernier calipers), and chlorophyll content were recorded 35 days after sowing. Chlorophyll content of the youngest fully expanded leaves was measured non-destructively using a SPAD-501 portable chlorophyll meter. The leaf area of the youngest fully expanded leaf was measured from three randomly selected seedlings per pot using a leaf area meter (LI-3100C, LI-COR, Inc.).

Good seedling quality (vigor) was measured in terms of Substantiality as it is essential for early establishment and subsequent growth, contributing to improved yield components and resilience against environmental stresses. Hence, substantiality was calculated using the following formula.

Substantiality = shoot dry weight/plant height

The percent disease incidence (PDI) of paddy blast was calculated as follows:

$$\text{Percent disease incidance} = \frac{\text{Number of diseased seedlings}}{\text{Total number of inspected seedlings}} \times 100$$

Statistical Analysis

A Completely Randomized Design (CRD) was used for the *in-vitro* study at a 1 per cent significance level (P = 0.01). Data were analyzed for the coefficient of deviation, and treatment means were compared for efficacy.

Results and Discussion

The germination test, conducted using the blotter paper method under *in-vitro* conditions, demonstrated the effectiveness of Nano-chitosan (NC) and Nano-chitosan-Tricyclazole conjugate (NCT) in enhancing early growth parameters of rice seedlings. Treatments significantly influenced germination percentage, root length, coleoptile length, fresh weight and dry weight (Table 2). Overall, NCT treatments out performed NC alone across all measured parameters. Among the treatments, NCT at 500 ppm (T7) was the most effective, recording the highest values across key traits, followed by NCT at 750 ppm (T8). Specifically, T8 achieved a germination percentage of 98.67 per cent at 14 days after incubation (DAI), with notable improvements in root length (13.72 cm), coleoptile length (9.16 cm), fresh weight (770.20 mg) and dry weight (162.03 mg).

Among NC treatments, 500 ppm (T3) showed the best response, achieving 97.33 per cent germination, a root length of 13.34 cm and a fresh weight of 624.40 mg. However, these values remained lower compared to the NCT treatments, highlighting the superior efficacy of the conjugate formulation over NC alone.

At higher concentrations (750 ppm), both NC and NCT showed a slight decline in growth performance compared to their respective 500 ppm counterparts, suggesting the existence of a concentration threshold beyond which further increases may not confer additional benefits and could even inhibit growth. This reduction is likely due to osmotic stress or interference with nutrient uptake. Similar inhibitory effects of excessive nano-chitosan concentrations on enzyme activity and nutrient absorption were previously reported by Ashraf *et al.* (2022).

When compared with traditional chitosan treatment (T9) and tricyclazole alone (T10), NCT treatments significantly enhanced seedling development. Treatments T9 and T10 recorded lower germination rates (82.67 and 90.67%, respectively), shorter root lengths (8.12 cm and 10.51 cm) and reduced fresh weights (357.80 mg and 445.40 mg, respectively). The untreated control (T11) exhibited the poorest performance, with a germination rate of 77.33 per cent, root length of 6.20 cm and fresh weight of 313.60 mg. These results further reinforce the importance of NCT as a potent seed treatment agent for enhancing seedling vigour.

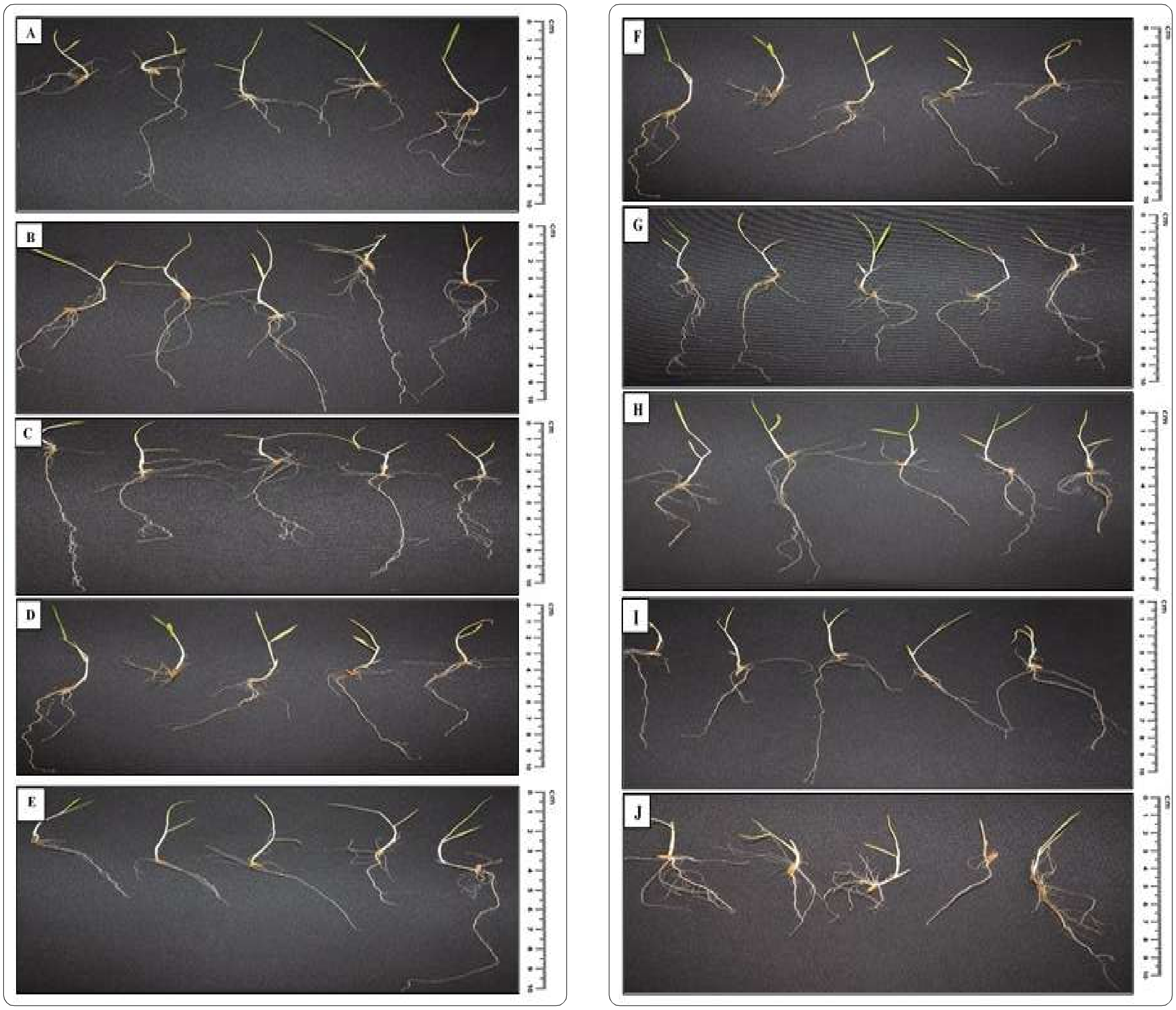
The findings clearly demonstrate that NCT, particularly at 500 ppm, is an optimal formulation for improving germination, coleoptile and root growth and overall biomass accumulation in rice seedlings. The superior performance of NCT over NC underscores the benefits of integrating nano-chitosan with tricyclazole, establishing it as a promising approach for enhancing early seedling establishment and vigour in rice cultivation. These observations are further supported by earlier findings from Reddy and Somashekar (2020), who reported improved seedling vigour and establishment

Table 2

Effect of seed treatment with nano-chitosan (NC) and nano-chitosan-tricyclazole conjugate (NCT) on growth parameters during germination test using the blotter paper method under *in-vitro* conditions

Treat. No.	Treatment details	Germination percentage (%)		Root length (cm)		Coleoptile length (cm)		Fresh weight (mg)	Dry weight (mg)
		7 DAI	14 DAI	7 DAI	14 DAI	7 DAI	14 DAI	14 DAI (10 germinated seeds)	
T1	ST with NC at 100 ppm	73.33 (58.91) *	83.33 (65.91)	6.10	9.52	3.68	6.88	244.20	98.12
T2	ST with NC at 250 ppm	80.67 (63.92)	86.67 (68.58)	8.50	12.21	4.14	7.04	332.60	112.56
T3	ST with NC at 500 ppm	86.67 (68.58)	97.33 (80.60)	10.54	13.34	6.84	10.18	624.40	156.62
T4	ST with NC at 750 ppm	81.33 (64.40)	96.67 (79.48)	8.94	12.82	5.24	8.56	489.60	126.45
T5	ST with NCT at 100 ppm	77.33 (61.57)	79.33 (62.96)	7.30	11.18	4.08	7.82	417.80	102.71
T6	ST with NCT at 250 ppm	81.33 (64.40)	91.33 (72.88)	8.68	12.88	5.28	9.06	497.40	136.11
T7	ST with NCT at 500 ppm	96.67 (79.48)	100.00 (90.00)	12.20	17.85	6.24	11.58	911.20	178.13
T8	ST with NCT at 750 ppm	90.67 (72.21)	98.67 (83.37)	9.18	13.72	5.8	9.16	770.20	162.03
T9	ST with Chitosan (3 mg/mL)	71.33 (57.63)	82.67 (65.40)	5.02	8.12	3.12	5.24	357.80	42.63
T10	ST with Tricyclazole with concentration (3 gm/kg seeds)	78.67 (62.49)	90.67 (72.21)	6.82	10.51	4.16	6.64	445.40	66.12
T11	Control	68.67 (55.96)	77.33 (61.57)	4.22	6.20	1.82	4.22	313.60	36.32
	SE (m) ±	0.77	1.02	0.15	0.19	0.08	0.12	-	-
	C.D. @ p = 0.01	2.28	3.01	0.42	0.54	0.24	0.34	-	-

*Arcsine transformed values; ST- Seed treatment; NC- Chitosan nano-particles; NCT- Nano-chitosan tricyclazole conjugate



A. NC 100 ppm; B. NC 250 ppm; C. NC 500 ppm; D. NC 750 ppm; E. NCT 100 ppm, F. NCT 250 ppm; G. NCT, 500 ppm; H. NCT 750 ppm; I. Positive control (Tricyclazole); J. Negative control (distilled water)

Plate 1 : Effect of chitosan nano-particles (NC) and nano-chitosan tricyclazole conjugate (NCT) on germination through seed treatment

in rice following seed treatments with chitosan and microbial inoculants. Their results align with our findings, suggesting that nano-chitosan promotes seed metabolic activation and improves germination efficiency.

Impact of Seed Treatment with NC and NCT on Germination Vigour Parameters in Paddy

Expanding on these findings, the study further evaluated the influence of NC and NCT on seed vigor in paddy by assessing germination energy (GE), germination rate (GR), germination index (Gi), vigor index (Vi) and relative vigor index (RVi) using standard formulas outlined in the materials and methods. This detailed analysis demonstrated how Nano-encapsulated Tricyclazole enhances germination efficiency, uniformity and seedling strength, reinforcing its role in improving seed quality and establishment. The results, graphically represented in Fig. 1 and Fig. 2, revealed a significant enhancement in seed germination and seedling vigour under NCT and NC treatments

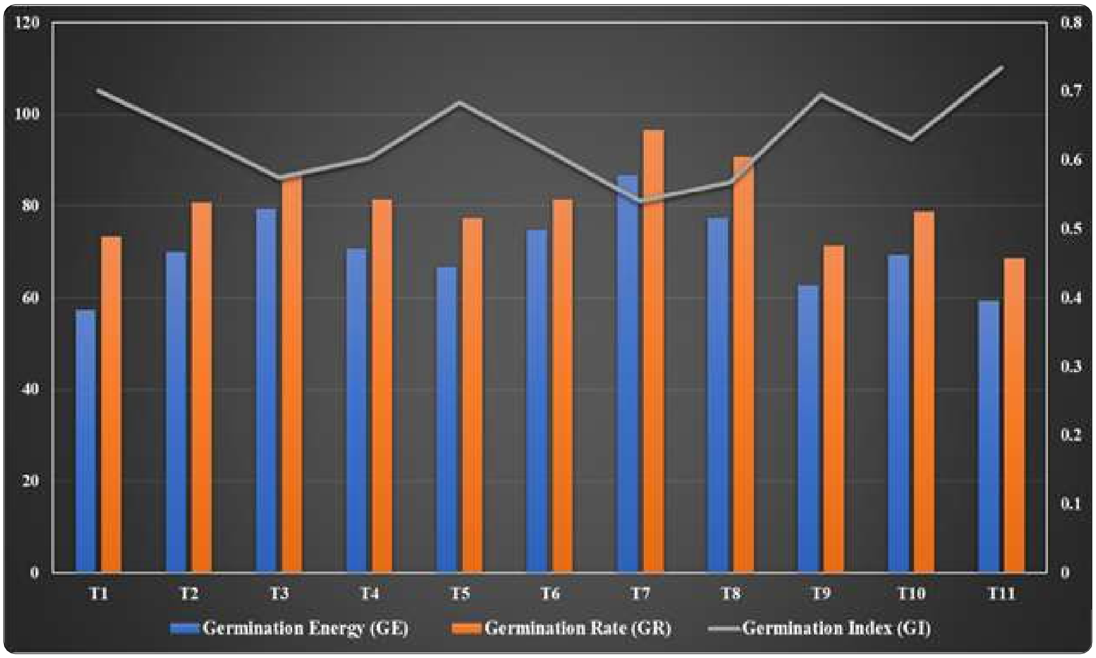
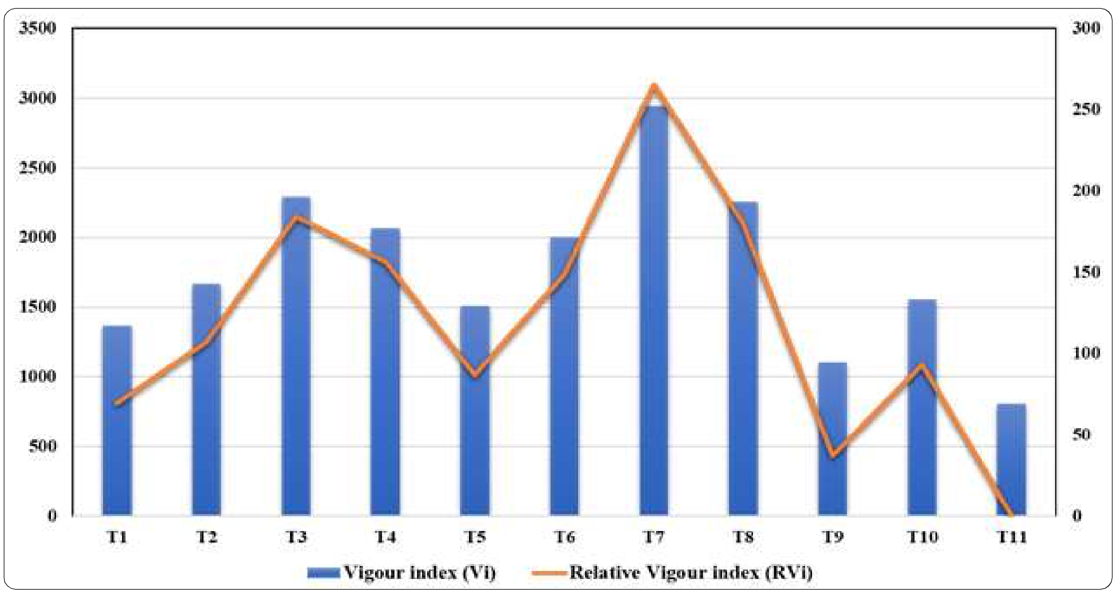


Fig. 1 : Impact of Chitosan Nano-particles (NC) and Nano-chitosan Tricyclazole Conjugate (NCT) on Germination Energy (GE), Germination Rate (GR) and Germination Index (GI) in treated seeds



Treatment details : T_1 - ST with NC at 100 ppm; T_2 - ST with NC at 250 ppm; T_3 - ST with NC at 500 ppm; T_4 - ST with NC at 750 ppm; T_5 - ST with NCT at 100 ppm; T_6 - ST with NCT at 250 ppm; T_7 - ST with NCT at 500 ppm; T_8 - ST with NCT at 750 ppm; T_9 - ST with Chitosan (3 mg/mL); T_{10} - ST with Tricyclazole with concentration (3 gm/kg seeds); T_{11} - Control.

Fig. 2 : Impact of chitosan nano-particles (NC) and nano-chitosan tricyclazole conjugate (NCT) seed treatment on vigour index (VI) and relative vigour index (RVI) of germinated seedlings

compared to the control, chitosan and tricyclazole alone. Among all treatments, seed treatment with NCT at 500 ppm (T7) exhibited the highest effectiveness, recording the highest germination energy (86.67%), germination rate (96.67%), vigour index (2943.40) and relative vigour index (265.27). These results indicate that Nano-encapsulation of Tricyclazole with chitosan significantly enhances seed germination and early seedling development compared to other treatments.

A comparative analysis of NC and NCT treatments at different concentrations revealed distinct patterns in their effectiveness. At 500 ppm, both NCT (T7) and NC (T3) significantly enhanced seed germination and seedling vigour, with NCT outperforming NC. NCT at 500 ppm recorded a vigour index of 2943.40 and a relative vigour index of 265.27, whereas NC at the same concentration resulted in a vigour index of 2289.28 and a relative vigour index of 184.10. These findings suggest that the conjugation of Nano-chitosan with Tricyclazole improves its efficacy in promoting germination and early seedling establishment. However, at 750 ppm, a decline in performance was observed in both NCT (T8) and NC (T4) compared to their 500 ppm counterparts. NCT at 750 ppm resulted in a vigour index of 2257.89 and a relative vigour index of 180.20, while NC at the same concentration recorded a vigour index of 2066.93 and a relative vigour index of 156.50. These findings indicate that higher concentrations might induce mild stress, leading to reduced germination performance.

At lower concentrations, NCT consistently outperformed NC. At 250 ppm, NCT (T6) showed a vigour index of 2003.85 and a relative vigour index of 148.67, while NC (T2) recorded a vigour index of 1667.99 and a relative vigour index of 106.99. Similarly, at 100 ppm, NCT (T5) had a vigour index of 1507.33 and a relative vigour index of 87.06, whereas NC (T1) recorded a vigour index of 1366.67 and a relative vigour index of 69.60. These results suggest that while both Nano-chitosan and Nano-chitosan-Tricyclazole conjugates promote germination, NCT offers superior benefits even at lower concentrations.

A comparison with bulk Chitosan and Tricyclazole alone further highlighted the advantages of nano-based formulations. Bulk chitosan (T9) exhibited the lowest seedling vigour (vigour index: 1104.43, relative vigour index: 37.06) among all chitosan-based treatments, demonstrating that nano-formulation enhances bioavailability and effectiveness. Similarly, Tricyclazole alone (T10) resulted in a lower vigour index (1554.57) and relative vigour index (92.92) compared to NCT treatments, confirming that nano-encapsulation improves the efficiency of the fungicide. The control treatment (T11) exhibited the lowest germination performance (vigour index: 805.81, relative vigour index: 0.00), highlighting the significance of Nano-chitosan-based seed treatments in improving germination and seedling growth.

These results demonstrates that Nano-chitosan tricyclazole conjugate (NCT) at 500 ppm was the most effective treatment, significantly enhancing seed germination, root development and seedling vigour compared to all other treatments. Nano-chitosan (NC) at 500 ppm also showed promising effects, but NCT outperformed it across all evaluated parameters. Increasing the concentration to 750 ppm did not provide additional benefits, confirming that 500 ppm is the optimal dosage for maximizing germination efficiency.

The study by Divya *et al*. (2019) demonstrated the efficacy and safety of chitosan nanoparticles (ChNP) as a seed treatment for enhancing rice germination and early seedling growth. Their findings confirmed that ChNP is non-toxic and promotes seedling vigor, with the optimal treatment observed at 1 mg/mL for 120 minutes. Furthermore, ChNP retained its bioactivity for up to seven months at room temperature, underscoring its stability and potential as a reliable seed treatment for improving early plant development.

Similar results have been reported by Ravikumar and Shailaja (2018), where nanoparticle seed coating significantly improved seedling vigour and germination in paddy by enhancing water uptake, promoting uniform emergence and improving the metabolic activation of seeds during early growth stages. Their study also emphasized the role of nanoparticle-mediated treatments in increasing enzymatic activity and root development, which are critical for better establishment under nursery conditions.

TABLE 3

Impact of seed treatment with Nano-chitosan (NC) and Nano-chitosan tricyclazole conjugate (NCT) seedling emergence, leaf area, chlorophyll content and growth parameters in paddy under greenhouse conditions

Treat No.	Treatment details	Seedling Seedling emergence (2 weeks)		No. green leaves	Area of green leaves/ seedling (cm^2)	Chlorophyll content (SPAD Units)
		Mean	(%)			
T1	ST with NC at 100 ppm	16.00	80	5.33	5.60	2.06
T2	ST with NC at 250 ppm	16.60	83	5.86	5.82	2.21
T3	ST with NC at 500 ppm	18.40	92	7.23	6.22	2.54
T4	ST with NC at 750 ppm	17.40	87	6.36	6.12	2.41
T5	ST with NCT at 100 ppm	17.00	85	6.36	5.92	2.41
T6	ST with NCT at 250 ppm	18.60	93	7.88	6.82	2.66
T7	ST with NCT at 500 ppm	19.00	95	8.12	7.14	2.84
T8	ST with NCT at 750 ppm	18.00	90	6.81	6.16	2.43
T9	ST with Chitosan (3 mg/mL)	15.00	75	4.37	4.58	1.73
T10	ST with Tricyclazole with concentration (3 gm/kg seeds)	15.60	78	4.72	5.22	2.05
T11	Control	14.00	70	4.18	4.24	1.64
	SE (m) ±	0.316		0.051	0.046	0.010
	C.D @ p = 0.01	0.904		0.147	0.132	0.027

ST- seed treatment ; NC- Nano-chitosan; NCT- Nano-chitosan tricyclazole conjugate

TABLE 4

Effect of seed treatment with Nano-chitosan and Nano-chitosan tricyclazole conjugate on plant growth, dry weight, substantiality, and disease resistance in paddy under greenhouse conditions

Treat No.	Treatment details	Plant height (cm)	Stem circumference (mm)	Dry weight (g/20 plants)	Substantiality (g/cm)	PDI (%)
T1	ST with NC at 100 ppm	16.78	2.26	1.92	0.114	53.52 (47.02) *
T2	ST with NC at 250 ppm	17.16	2.41	2.04	0.119	47.92 (43.81)
T3	ST with NC at 500 ppm	20.22	2.92	2.63	0.120	31.20 (33.96)
T4	ST with NC at 750 ppm	19.63	2.86	2.23	0.114	36.78 (37.33)
T5	ST with NCT at 100 ppm	18.83	2.66	2.18	0.116	42.35 (40.60)
T6	ST with NCT at 250 ppm	21.91	3.32	2.64	0.119	20.05 (26.60)
T7	ST with NCT at 500 ppm	22.13	3.64	2.94	0.127	8.91 (17.37)
T8	ST with NCT at 750 ppm	23.17	2.91	2.36	0.117	14.48 (22.37)
T9	ST with Chitosan (3 mg/mL)	15.32	1.91	1.24	0.081	59.07 (50.23)
T10	ST with Tricyclazole with concentration (3 gm/kg seeds)	15.72	2.21	1.84	0.117	25.63 (30.42)
T11	Control	14.64	1.56	0.92	0.063	64.65 (53.52)
	SE (m)±	0.452	0.054	0.012	-	-
	C.D @ p=0.01	1.335	0.154	0.034	-	-

*Arcsine transformed values; ST- seed treatment; NC- Nano-chitosan; NCT- Nano-chitosan tricyclazole conjugate

Effect of NC and NCT Seed Treatment on Paddy Growth and Blast Disease Resistance under Greenhouse Conditions

The application of NC and NCT as seed treatments significantly influenced seedling emergence, growth parameters and blast disease resistance in paddy, as presented in Table 3, and Table 4.

Seedling Emergence and Establishment

Seed treatment significantly influenced seedling emergence, with the highest emergence recorded in T7 (NCT 500 ppm, 95 %), followed by T6 (NC 500 ppm, 93 %), compared to T11 (Control, 70 %) (Plate 2). The enhanced emergence in NCT- and NC-treated seeds suggests improved seed vigour, which aligns with previous reports indicating that nano- chitosan enhances water uptake, enzymatic activity and early seedling development (Sen and Das, 2024). Similar findings have been reported in wheat and paddy, where nano-chitosan treatment improved germination rate and seedling emergence (Sangwan *et al*., 2023).

Growth Dynamics and Biomass Accumulation

Seed treatment with NCT at 500 ppm (T7) demonstrated the most significant improvements in plant growth parameters. It recorded the highest plant height (22.13 cm), outperforming NC at 500 ppm (T6, 21.91 cm), while the untreated control (T11) exhibited the lowest height (14.64 cm). Similarly, the number of green leaves was highest in T7 (8.12), followed by T6 (7.88), indicating enhanced vegetative growth. The increased stem circumference in T7 (3.64 mm) and T6 (3.32 mm) suggests improved mechanical strength, a crucial factor for lodging resistance (Zhang *et al*., 2018).

Biomass accumulation was also significantly enhanced by nano-chitosan-based treatments. T7 (NCT at 500 ppm) recorded the highest dry biomass (7.14 g/20 plants), surpassing T6 (NC at 500 ppm, 6.82 g/20 plants), while the lowest dry biomass was observed in the untreated control (T11, 4.24 g/20 plants). Moreover, the substantiality index, a key parameter reflecting plant robustness, was highest in T7 (2.94 g/cm), followed by T6 (2.64 g/cm), confirming the superior structural integrity conferred by NCT. These findings align with previous reports suggesting that nano-chitosan enhances nutrient uptake, cell wall fortification and biomass accumulation in cereals (Saad *et al*., 2022). The superior performance of NCT at 500 ppm highlights its potential in promoting plant vigour, making it a promising seed treatment for improving rice growth and resilience.

Vinutha and Mahadevaiah (2022) reported enhanced plant growth in rice following the application of nano-fertilizers under aerobic conditions. They found that nano-fertilizers improved shoot growth and chlorophyll content in rice, demonstrating the broad

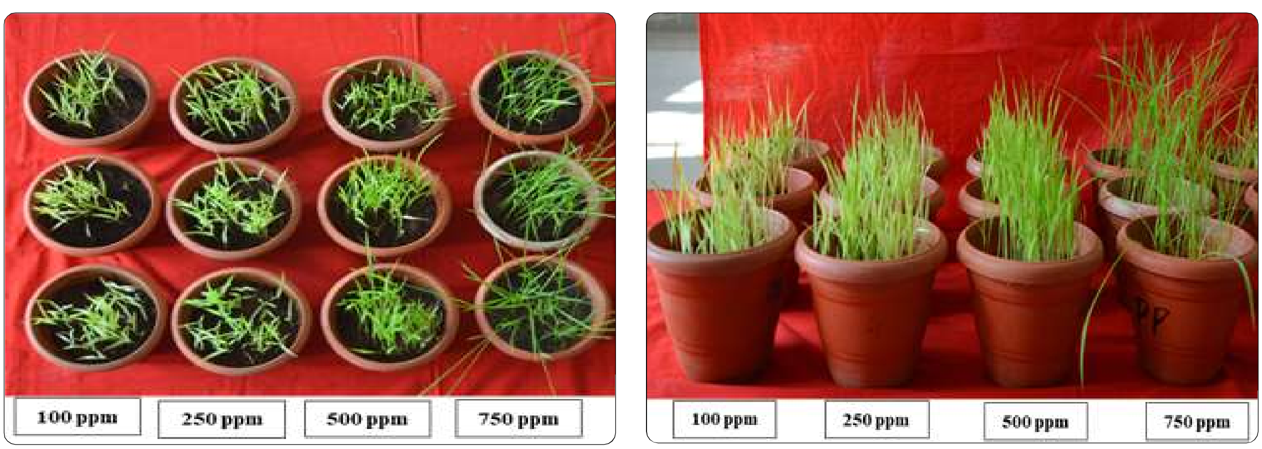


Plate 2 : Impact of Nano-chitosan tricyclazole conjugate (NCT) on seedling emergence and growth of paddy seedling under greenhouse conditions

applicability of nanoformulations across different cultivation systems. This aligns with our findings, further highlighting the potential of nano-based treatments to enhance plant performance.

Physiological Attributes and Chlorophyll Content

Chlorophyll content, measured in SPAD units, was significantly enhanced in nano- chitosan-treated plants, indicating improved photosynthetic efficiency. Seed treatment with NCT at 500 ppm (T7) recorded the highest chlorophyll content (2.84 SPAD units), followed by NC at 500 ppm (T6, 2.66 SPAD units), whereas the control (T11) exhibited the lowest value (1.64 SPAD units). This increase suggests that NCT enhances chlorophyll biosynthesis, leading to better light absorption and prolonged photosynthetic activity. Previous studies have also reported that nano-chitosan promotes chlorophyll accumulation and delays leaf senescence, contributing to sustained plant growth and productivity (Kumaraswamy *et al*., 2021). These findings highlight the superior potential of NCT over conventional treatments in enhancing photosynthetic efficiency, which is critical for improved biomass accumulation and overall plant vigour.

These findings align with Manjunatha and Nandini (2020), who demonstrated that chitosan formulations promote plant height and biomass accumulation in rice. They found that chitosan-based formulations significantly improved rice growth and grain yield, attributing this to enhanced nutrient uptake, elicitation of plant defense pathways and improved physiological responses. These results align with our findings on NCT treatment enhancing biomass and chlorophyll content.

Increased physiological efficiency, including chlorophyll content, in nano-treated paddy was similarly reported by Bharathi and Kumar (2018). They observed that nano-bioformulations significantly enhanced physiological traits such as SPAD values and dry matter accumulation in paddy, while also providing notable suppression of blast disease. These findings further support the physiological and protective benefits observed in NCT-treated seedlings.

Blast disease incidence (PDI %)

The percentage disease incidence (PDI%) recorded at 35 days after sowing was significantly lower in plants raised from seeds treated with nano-chitosan formulations, underscoring their effectiveness in enhancing blast resistance. Notably, seed treatment with NCT at 500 ppm (T7) recorded the lowest PDI of 8.91 per cent, followed by NCT at 250 ppm (T8) with 14.48 per cent, while the untreated control (T11) showed a markedly higher PDI of 64.65 per cent. This enhanced disease suppression indicates that the conjugation of tricyclazole with nano-chitosan not only provides a sustained fungicidal effect but also more effectively prevents *Pyricularia oryzae* infection compared to conventional treatments, corroborating previous findings on the antifungal efficacy and systemic resistance induced by nano-chitosan formulations (Parthasarathy *et al.*, 2023).

These results demonstrate that seed treatment with both NC and NCT significantly enhances seedling emergence, growth dynamics, physiological efficiency and blast disease resistance in paddy. Among these, NCT at 500 ppm emerged as the most effective treatment, promoting superior plant vigour while conferring exceptional disease suppression, thereby offering a promising strategy for integrated disease management and improved crop performance.

Soni *et al.* (2023) demonstrated that seed priming with chitosan nanoparticles (NC) and chitosan solution (CS) at 50 µg/mL significantly enhanced rice germination, seedling growth, and biochemical responses compared to water-primed controls. Notably, seedlings from NC- and CS-primed seeds exhibited improved tolerance to salt stress, reflected in enhanced physiological performance and stronger antioxidant defense mechanisms. These findings underscore the potential of chitosan-based nano-priming as a promising approach to accelerate early seedling establishment and strengthen stress resilience in rice cultivation.

Similar trends in blast suppression were observed with chitosan-based nanoemulsions, as reported by Sudharshana and Shylaja (2021). They demonstrated that chitosan-based nanoemulsions significantly reduced rice blast incidence under field conditions, attributing the effect to both direct antifungal activity and the induction of systemic resistance. These findings align with the enhanced disease resistance observed in our NCT seed treatments.

The present study identifies nano-chitosan–tricyclazole conjugate (NCT) at 500 ppm as the most effective seed treatment for enhancing seed germination, seedling vigor, and blast disease resistance in paddy. NCT consistently outperformed nano-chitosan (NC) alone across all evaluated parameters, indicating that tricyclazole conjugation significantly improves the bio-efficacy of nano-chitosan. While 500 ppm emerged as the optimal concentration for both NC and NCT, a slight reduction in performance at 750 ppm suggests a threshold beyond which further increases may lead to stress-induced inhibition.

Compared to bulk chitosan and conventional tricyclazole applications, nano-formulated treatments demonstrated markedly superior results, highlighting the advantages of nanotechnology in seed treatment. Notably, NCT at 500 ppm not only enhanced early plant growth but also provided strong protection against blast disease, positioning it as a promising tool for integrated disease management.

These findings highlight the potential of nano-chitosan-based seed treatments as sustainable, eco-friendly solutions to improve seedling establishment, enhance stress tolerance and ultimately contribute to increased productivity in paddy cultivation.

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