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RESEARCH ARTICLE

Effect of bio-stimulant and nitrogen levels on growth and productivity of kharif rice

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Abstract

Kharif rice (*Oryza sativa* L.) production systems in India frequently encounter challenges, including erratic monsoon patterns, declining soil fertility and imbalanced nutrient management. This field experiment was carried out to evaluate the synergistic influence of foliar-applied seaweed-based bio-stimulant (*Kappaphycus alvarezii* sap) and graded nitrogen doses on the growth and productivity of kharif rice (cv. RNR-15048). The experiment was arranged in a split-plot design with three nitrogen doses (0, 60 and 120 kg ha) in main plots and four bio-stimulant doses (0, 5, 10 and 15 mL L^{-1}) as sub-plots which were replicated thrice. The results revealed significant improvements in growth attributes at different growth stages of rice. Yield components such as panicle bearing tillers m^{-2}, panicle length (cm), filled grains $panicle^{-1}$ and 1000-grain weight (g) were also significantly influenced due to the application of bio-stimulants and varied nitrogen levels. In case of bio-stimulants, the treatment with 15 mL L^{-1} *K. alvarezii* sap produced the highest grain and stover yield (3.8 t ha^{-1} and 4.8 t ha^{-1}, respectively) and with regards to nitrogen levels, application of 120 kg ha^{-1} of N recorded the maximum grain yield (4.1 t ha^{-1}) and stover yield (5.1 t ha^{-1}) of rice. The findings demonstrate that integrating bio-stimulant application at of 15 mL L^{-1} with an appropriate nitrogen level of 120 kg ha^{-1} can improve the growth and productivity of kharif rice.

Keywords: growth parameters; nitrogen management; rice; seaweed extract; yield

Introduction

Rice (*Oryza sativa* L.) is a staple food grain for more than half of the world's population, particularly in Asia, where it is cultivated under diverse agro-climatic conditions (1). In India, a significant portion of rice is cultivated during the kharif season, which primarily relies on the southwest monsoon and often faces challenges such as uneven rainfall, poor soil fertility, limited irrigation facilities and suboptimal crop management practices (2). Among these, nutrient management is a key strategy that significantly affects rice growth and yield (3). Among the primary nutrients, nitrogen promotes plant growth by increasing plant height, stimulating cell division, elongation and enhancing leaf area and dry matter production, resulting in taller and sturdier plants (4). Nitrogen is important chlorophyll synthesis, resulting in healthy and green leaves that enable plants to maximise photosynthetic activity, leading to higher rates of carbon dioxide fixation and photosynthate assimilation (1, 5, 6). Nitrogen is a key element for protein synthesis, various physiological and metabolic processes, including grain development, formation and the qualitative improvement of grains (4, 7, 8).

Along with optimal nitrogen application, to mitigate climate change, the use of bio-stimulants is becoming popular in crops to safeguard against various abiotic stresses, foster physiological and metabolic activities in plants and facilitate a higher nutrient uptake by crops (9). The use of bio-stimulants, especially seaweed extracts, has emerged as a promising approach for enhancing crop physiological performance and stress resilience (10, 11). Prior research has demonstrated that seaweed-based bio-stimulants enhanced growth and yield attributes in rice and other cereals by improving nutrient uptake, chlorophyll synthesis and enzymatic functions (12, 13). Moreover, the combined application of *Kappaphycus* sap and nitrogen creates a synergistic effect, improving nitrogen use efficiency (NUE) and sustaining plant growth during periods of abiotic stress, thereby improving the crop performance (14). However, empirical data on the combined impact of *Kappaphycus* sap and graded nitrogen doses on rice, particularly in Eastern India, remain scarce. The current study was, therefore, undertaken to assess the interactive influence of foliar-applied *Kappaphycus alvarezii* sap and nitrogen levels on growth, yield and nutrient content of kharif rice.

Materials and Methods

The experiment was conducted during the kharif season of 2024 at the P.G. Research Farm of Centurion University of Technology and Management, Paralakhemundi, Odisha. During the crop period, weekly maximum temperatures ranged from 31.2 °C to 34.3 °C, while minimum temperatures varied between 19.8 °C and 27.1 °C. The relative humidity recorded was between 83.8 % and 87.2 % (maximum) and 61.7 % to 81.8 % (minimum). The total rainfall received during the season was 826.7 mm. Weekly bright sunshine hours ranged from 4.5 to 8.8 hr per day throughout the crop growth period (Fig. 1). The soil belonged to the sandy clay loam category, consisting of 75 % sand, 10 % silt and 15 % clay, with a bulk density of 1.43 kg m^3. The chemical analysis indicated low fertility status, with organic carbon content at 0.5 %, available N, P and at 218, 12 and 142 kg ha^{-1}. The soil reaction was slightly acidic with a pH of 6.4 and the electrical conductivity was measured at 0.7 dS m^{-1}. The experiment was laid out using a split-plot design where N levels were considered for the main plots and bio-stimulants to the sub -plots. The main plot comprised 3 nitrogen treatments: N_1: 0 kg ha^{-1}, N_2: 60 kg ha^{-1} and N_3: 120 kg ha^{-1}. The sub-plots included four bio-stimulant concentrations, namely, B_1: 0 mL L^{-1}, B_2: 5 mL L^{-1}, B_3: 10 mL L^{-1} and B_4: 15 mL L^{-1}. Each treatment was replicated thrice and this arrangement resulted in a total of 12 treatment combinations. The variety considered for the study was RNR-15048, sown in the nursery bed. The transplanting of rice was done on 31st of July and the seedlings were transplanted to the main field with a spacing of 20 cm between rows and 15 cm between plants. For nutrient management, nitrogen was applied in three splits: 50 % as a basal dose, 25 % during the active tillering stage and the remaining 25 % at panicle initiation. Phosphorus and potassium were both applied fully as basal doses at the time of transplanting. Bio-stimulant was applied at 0 mL, 5 mL, 10 mL and 15 mL L^{-1} at 15, 30 and 45 days after transplanting (DAT), respectively. For weed management pre-emergence herbicide, Pretilachlor 50 % EC, was applied at the rate of 2.5 mL L^{-1} of water, followed by manual weeding and a post-emergence foliar spray of Bispyribac-Sodium 10 % EC at 0.4 mL L^{-1} at 40 DAT. For pest control, Chlorpyriphos 50 % EC was used to manage leaf folder infestation, while Chlorantraniliprole 0.4 % GR was applied at 45 days after sowing (DAS) to control stem borer infestation. The crop was harvest on 16th November and the growth parameters (plant height, number of tillers m^{-2}, LAI, dry matter accumulation) were noted at 30, 60 DAT and harvest; however, yield parameters and yields were recorded at harvest. Moreover, the nitrogen content and uptake were calculated by considering the following formulae:

$$\text{Total N in plant sample (\%)} = \frac{(V_S - V_B) \times S \times 0.014 \times 100}{W}$$

Where V_S and V_B represent the volumes (in mL) of H_2SO_4 used for titrating the sample and the blank, respectively; S denotes the strength of the sulfuric acid solution (0.02 N) and W is the weight of the plant sample analysed.

$$\text{Plant N uptake (kg ha}^{-1}) = \frac{\text{Nitrogen content (\%)} \times \text{Yield (kg ha}^{-1})}{100}$$

The data collected during the study were organised and analysed statistically to draw reliable conclusions. Analysis of variance (ANOVA) was conducted following the standard methods outlined by Gomez and Gomez (15). Treatment effects were assessed using the 'F' test. The standard error of the mean was calculated for all measurements. Differences between treatment means were compared using the critical difference (CD) at a 5 % significance level.

Results and Discussion

Growth attributes

The application of nitrogen at a rate of 120 kg ha^{-1} resulted in the highest plant heights of 68 cm, 98 cm and 123 cm at 30 DAT, 60 DAT and the harvest stage, respectively. Additionally, the same treatment recorded the highest dry matter accumulation with the values of 305 g m^{-2}, 651 g m^{-2} and 946 g m^{-2} at 30 DAT, 60 DAT and at harvest, respectively. The application of 120 kg N ha^{-1} recorded significantly higher height and dry matter accumulation during all the growth stages of rice than the remaining nitrogen management treatments (Table 1). In the case of the highest LAI at 60 DAT (4.3) and number of tillers m^{-2} at harvest (249), the treatment 120 kg N ha^{-1} registered its significant superiority over all other nutrient levels. However, the lowest values of the above-mentioned growth attributes at different growth stages were observed in the treatment with 0 kg $ha^{-1}$of nitrogen application. The higher values recorded with 120 kg ha^{-1} for all the growth attributes could be due to optimum availability of nitrogen, which might result in an enhanced chlorophyll synthesis, improved leaf area and increased photosynthetic efficiency, which in turn, would

Table 1. Effect of nitrogen levels and *Kappaphycus alvarezii* sap on growth parameters of kharif rice

Treatments	Plant height (cm)			Dry matter accumulation (g m^{-2})			Leaf area index	Number of tillers m^{-2}
	30 DAT	60 DAT	Harvest	30 DAT	60 DAT	Harvest	At 60 DAT	At harvest
Nitrogen (kg ha^{-1})								
0	53	78	101	191	467	745	3.1	172
60	61	85	110	263	569	844	3.9	212
120	68	98	123	305	651	946	4.3	249
S. Em. ±	1.50	2.27	1.57	5.77	9.09	25.13	0.05	4.76
CD at 5 %	5.87	8.90	6.17	22.6	35.7	98.6	0.2	18.67
Bio-stimulant *Kappaphycus alvarezii* sap (mL L^{-1})								
0	55	79	103	231	491	748	3.4	193
5	58	83	108	246	533	829	3.7	205
10	62	90	113	265	589	854	4	215
15	69	95	122	289	635	949	4.3	232
S. Em. ±	1.06	1.61	1.76	6.59	10.98	15.71	0.11	2.66
CD at 5 %	3.14	4.77	5.23	19.57	32.63	46.66	0.34	7.91
Nitrogen × Bio-stimulant								
S. Em. ±	1.83	2.78	3.05	11.41	19.02	27.20	2.78	4.61
CD at 5 %	NS	8.26	NS	NS	56.52	80.81	8.26	13.71

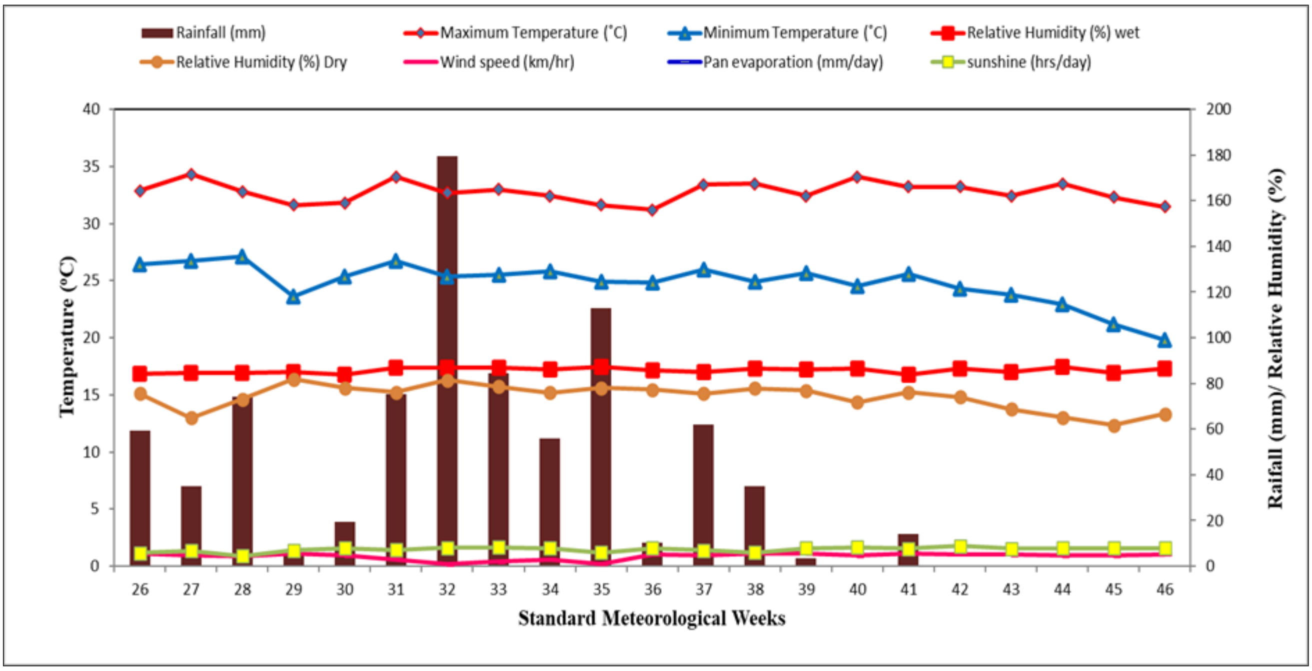

Fig. 1. Meteorological observation prevailed during the crop period (June 2024 to November 2024). Data source: Agro-Meteorological Observatory, Centurion University of Technology and Management, Paralakhemundi, Odisha, India.

promote taller plants, greater dry matter assimilation and higher tillering (16, 17). Moreover, the foliar spray of *K. alvarezii* sap at 15 mL L^{-1} resulted in the highest plant height of 69 cm, 95 cm and 122 cm at 30 DAT, 60 DAT and at harvest, respectively. The same treatment also recorded the highest dry matter production of 289 g m^{-1}, 635 g m^{-1} and 949 g m^{-1} at 30 DAT, 60 DAT and harvest, respectively. The highest LAI of 4.3 at 60 DAT was noted with the 15 mL L^{-1} bio-stimulant treatment. Similarly, the highest number of tillers m^{-2} at harvest (232) was recorded in the same treatment of the study. However, 15 mL L^{-1} bio-stimulant spray remained significantly superior to all other bio-stimulant treatments of the study in the expression of plant height, dry matter production, LAI and number of tillers of rice in the above-mentioned growth stages. The lowest values of growth attributes were registered with the foliar application of *K. alvarezii* sap at 0 ml L^{-1}. The improvements could be attributed to increased nutrient uptake and enhanced physiological activity promoted by bioactive compounds in the seaweed extract, including hormones like auxins and cytokinins. Such compounds might support cell elongation, cell division and chlorophyll production, contributing to overall vegetative growth (2, 18, 19). Further, there were significant interactions between N levels and bio-stimulants in the expression of plant height at 60 DAT, dry matter accumulation at 60 DAT and harvest, LAI at 60 DAT and number of tillers at harvest.

Yield attributes

Different nitrogen levels significantly influenced the yield attributes of rice (Table 2). Among the nutrient levels, N at 120 kg ha^{-1} resulted in the highest number of panicle-bearing tillers m^{-2} (238), number of filled spikelets $panicle^{-1}$ (118), panicle length (24 cm) and 1000-grain weight (16 g) and it remained significantly superior to the other N doses. In contrast, the lowest values of yield attributes were observed in the control (0 kg N ha^{-1}), with the values of 157 panicle-bearing tillers m^{-2}, 102 filled spikelet's panicle $^{-1}$, 19 cm panicle length and a 1000-grain weight of 13 g. The application of 60 kg N ha^{-1} did not show any significant influence on the yield attributes of rice and remained significantly inferior to 120 kg ha^{-1} N application. The enhanced yield-attributing characters under the 120 kg N ha^{-1} treatment might be attributed to improved nutrient availability, which supported a higher photosynthate assimilation, more productive tillers and enhanced grain filling (20–22).

Furthermore, foliar application of *K. alvarezii* sap at 15 mL L^{-1} registered the highest yield attributes in rice, with values of 217 panicle-bearing tillers m^{-2}, 119 filled spikelets $panicle^{-1}$, a panicle length of 24 cm and a 1000-grain weight of 16 g. This treatment was

Table 2. Effect of nitrogen levels and *Kappaphycus alvarezii* sap on yield parameters and yield of kharif rice

Treatments	Panicle bearing tillers m^{-2}	Filled spikelets $panicle^{-1}$	Length of panicle (cm)	1000 grain weight (g)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
Nitrogen (kg ha^{-1})						
0	157	102	19	13	2.3	3.4
60	199	107	22	14	3.7	4.3
120	238	118	24	16	4.1	5.1
S. Em. ±	5.40	1.69	0.49	0.41	0.07	0.16
CD at 5 %	21.18	6.62	1.93	1.61	0.29	0.62
Bio-stimulant *Kappaphycus alvarezii* sap (mL L^{-1})						
0	179	100	20	13	2.9	3.7
5	191	105	22	14	3.3	4.2
10	204	113	22	15	3.5	4.5
15	217	119	24	16	3.8	4.8
S. Em. ±	3.17	1.25	0.47	0.32	0.10	0.18
CD at 5 %	9.41	3.71	1.39	0.96	0.29	0.52
Nitrogen × Bio-stimulant						
S. Em. ±	5.49	2.16	0.81	0.56	0.17	0.30
CD at 5 %	16.31	6.43	NS	NS	0.50	0.90

significantly superior to other bio-stimulant treatments studied. The control treatment recorded the lowest values for all yield attributing parameters, namely, panicle-bearing tillers m^{-2} (179), filled spikelets per panicle^{-1} (100), panicle length (20 cm) and a 1000-grain weight of 13 g. The improved performance in 15 mL L^{-1} *Kappaphycus* sap could be due to the presence of phytohormones and bioactive compounds in the seaweed extract, which might facilitate the development of reproductive parts and nutrient translocation during the reproductive period of rice. These findings agree with the observations recorded in previous studies (23–26). The interaction effect between N levels and bio-stimulant was significant for panicle bearing tillers, filled spikelets, grain yield and straw yield.

Yield

Applying nitrogen significantly boosted both grain and straw yields in rice (Table 2). Grain yield was consistently enhanced with higher nitrogen doses, from 2.3 t ha^{-1} in the control (0 kg N ha^{-1}) to 4.1 t ha^{-1} at 120 kg N ha^{-1}, reflecting an almost 78 % improvement. Straw yield followed a similar trend to grain yield, with yield increment from 3.4 t ha^{-1} in the untreated control to 5.1 t ha^{-1} with the application of 120 kg N ha^{-1}. Additionally, the 120 kg N ha^{-1} treatment remained significantly superior in both grain and stover yields of rice. These notable results can be attributed to the sufficient nitrogen supply, which ensured optimal availability during the critical growth stages. This likely enhanced nitrogen uptake, leading to improved photosynthate assimilation and greater biomass accumulation, ultimately resulting in increased yield. These results align with previous studies that observed yield gains with higher nitrogen application (27, 28). The foliar spray of *K. alvarezii* sap notably improved rice yields. The highest grain yield (3.8 t ha^{-1}) was noted with application of *K. alvarezii* sap at 15 mL L^{-1}, while the lowest was recorded in the control (2.9 t ha^{-1}). There was a yield enhancement of 31 % with the application of *K. alvarezii* sap at 15 mL L^{-1} over control. Similarly, straw yield was increased from 3.7 t ha^{-1} in the control to 4.8 t ha^{-1} with the highest dose of bio-stimulant application. However, the foliar spray of *K. alvarezii* at 15 mL L^{-1} remained significantly superior to all other bio -stimulant levels studied. The improved yields in treated plots could be attributed to bioactive compounds present in *K. alvarezii* sap, such as phytohormones (auxins and cytokinins), essential amino acids, micronutrients. and polysaccharides, which might promote cell division, nutrient uptake and overall physiological performance of the crop resulting in higher grain and straw yields of rice (12, 13, 29). The interaction effect between N levels and bio-stimulant was significant for grain yield and straw yield of rice.

Nitrogen content (%) and uptake (kg ha^{-1})

The nitrogen content in both grain and straw was increased with higher nitrogen application (Table 3). The highest nitrogen content in grain (0.69 %) and straw (0.44 %) was observed with nitrogen application of 120 kg ha^{-1} and it remained statistically superior over 60 kg ha^{-1} of N and N omission treatment (control). Similarly, the N uptake by rice grain and straw was the highest with 120 kg ha^{-1} N, with the respective values of 29 and 23 kg ha^{-1} in grain and straw and a total N uptake of 52 kg ha^{-1}. In contrast, the control (0 kg N ha^{-1}) recorded the lowest N content and uptake in rice grain and straw. The increases in nitrogen content and uptake with higher N dose could be attributed to enhanced nitrogen availability to plants, improved chlorophyll synthesis, increased enzyme activity and better synchronisation of nitrogen supply as per the crop needs. Such physiological benefits might support the higher nitrogen assimilation and translocation to the grain and straw. These findings are in line with earlier reports by previous researchers (30–32).

The foliar application of *K. alvarezii* sap influenced N content and uptake of rice (Table 3). Among the bio-stimulant treatments, the highest N content in rice grain (0.67 %) and rice straw (0.42 %) was registered with the foliar application of 15 mL L^{-1} of *K. alvarezii*. This treatment remained significantly superior to all other bio-stimulant treatments in enhancing the higher N concentrations of both grain and straw. Further, the aforesaid treatment resulted in the highest nitrogen uptake with the values of 26 kg ha^{-1} in grain and 20 kg ha^{-1} in straw with a total nitrogen uptake of 46 kg ha^{-1} by rice. The improvement in nitrogen content and uptake at higher concentrations of the seaweed extract can be due to the presence of phytohormones, which enhanced root development, nutrient mobilisation, enzymatic activity and stomatal conductance resulting in better nutrient uptake by the rice plant. Similar results were recorded by (14, 33–35).

Conclusion

Nitrogen and bio-stimulant (*K. alvarezii*) application significantly influenced the growth, yield attributes, productivity and nutrient uptake of rice during the kharif season. Among the nitrogen levels, the application of 120 kg N ha^{-1} significantly enhanced the productivity and nitrogen uptake of rice. The foliar application of

Table 3. Effect of nitrogen levels and *Kappaphycus alvarezii* sap on nitrogen content and uptake of kharif rice

Treatments	Nitrogen content (%)		Nitrogen Uptake (kg ha^{-1})		
	Grain	Straw	Grain	Straw	Total
Nitrogen (kg ha^{-1})					
0	0.51	0.28	11.74	9.57	21.31
60	0.62	0.38	22.64	16.53	39.17
120	0.69	0.44	28.75	22.54	51.29
S. Em. ±	0.01	0.01	0.56	0.62	0.89
CD at 5 %	0.02	0.02	2.20	2.41	3.51
Bio-stimulant *Kappaphycus alvarezii* sap (mL L^{-1})					
0	0.55	0.32	16.08	12.17	28.25
5	0.58	0.35	19.97	15.31	35.28
10	0.61	0.37	22.16	17.20	39.36
15	0.67	0.41	25.97	20.18	46.15
S. Em. ±	0.01	0.00	0.65	0.72	0.95
CD at 5 %	0.02	0.01	1.93	2.13	2.83
Nitrogen × Bio-stimulant					
S. Em. ±	0.01	0.01	1.13	1.24	1.65
CD at 5 %	0.03	0.02	3.35	3.68	4.91

K. alvarezii sap at 15 mL L^{-1} showed a significant impact on the overall performance of rice. Based on the above findings, the study may conclude that applying 120 kg ha^{-1} of nitrogen combined with foliar application of *K. alvarezii* sap at 15 mL L^{-1} during critical growth stages can be an effective agronomic practice for enhancing rice growth, productivity, nitrogen content and uptake of kharif rice. Future research may focus on validating these findings across diverse environments, exploring the physiological mechanisms behind nitrogen–bio-stimulant interactions, assessing long-term effects on soil health and integrating these practices with precision nitrogen management tools for improved efficiency.

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Authors' contributions

Conceptualisation was carried out by SM, PC, BD and PVN. Methodology was developed by SM, PC, MS and PVN. Validation was performed by SM, PC and MS. Formal analysis was conducted by PVN, SM, MS, BD and PC. Data curation was undertaken by PVN, PC, SM, MS and SR. Draft preparation was done by MS, SM, PVN and SR. Writing review and editing were carried out by PVN, SM, MS, PC and SR. Supervision was provided by PVN, PC, SM, MS, BD and SR. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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