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Research article

Effects of pellet fertilizer prepared from Azolla and zinc oxide nanoparticles on rice growth and yield

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Abstract

Importance of the work: The combination of zinc oxide nanoparticles (nZnO) and Azolla as fertilizer could reduce chemical fertilizer use and alleviate zinc deficiency.
Objectives: To evaluate the impact of pellet fertilizers containing nZnO and dried Azolla on Pathum Thani 1 rice growth and yield.
Materials and Methods: A one-factor completely randomized design was used. The weight contents of Azolla and nZnO in pellet fertilizer were 60.96% and 0.04%, 60.92% and 0.08% and 60.88% and 0.12%, respectively. The performance was evaluated of the different pellet fertilizers on rice growth, yield and yield components and on the concentration of zinc in plant organs.
Results: The pellet fertilizer significantly improved the SPAD index of Pathum Thani 1 rice, compared to the control. This improvement was not significantly different from the use of urea fertilizer. Similar to urea fertilizer, the pellet fertilizer significantly enhanced plant height, the number of tillers per hill, the number of panicles and the number of grains per panicle. However, the treatments did not significantly affect the weight of paddy and brown rice. The inclusion of nZnO led to a significant increase in zinc accumulation in the rice roots, leaves and grains. There was greater zinc accumulation in the treatments with increasing nZnO dosages.
Main finding: The Azolla-nZnO pellet fertilizer effectively improved rice growth and increased the zinc content in grains. This eco-friendly pellet fertilizer could be used to improve zinc nutrition in rice-consuming regions. Further study is required to improve its performance in promoting grain weight.

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Introduction

Climate change poses risks to agricultural production and food security and is an obstacle to achieving the Sustainable Development Goals of the Thai government (Office of the National Economic and Social Development Council, 2025). Rice, a key cereal crop that feeds billions of people, is susceptible to extreme weather events (Wassmann et al., 2009; Li et al., 2024). Rice farmers in developing tropical nations are particularly at risk of being affected by climate change (Firdaus et al., 2020). In Thailand, projections have indicated that the alterations in temperature and precipitation could reduce mean rice yield by up to 33% within 10 yr (Sinnarong et al., 2019). Furthermore, alterations in weather will reduce soil fertility, disrupt nutrient uptake and exacerbate mineral deficiency, including zinc, in rice-consuming regions (St. Clair and Lynch, 2010; Myes et al., 2015). Therefore, strategies to mitigate the impact of climate change and enhance rice productivity are required. However, relying more heavily on traditional chemical fertilizers presents several limitations, including high costs, the long-term deterioration of soil fertility, water eutrophication and a considerable contribution to greenhouse gas emissions (Kumar et al., 2019; Ashitha et al., 2021). Thus, more sustainable methods for promoting rice cultivation should be explored such as the use of organic fertilizers (Verma et al., 2020) and highly efficient nanomaterials (Verma et al., 2022).

A promising source of organic materials for agricultural use is Azolla, an aquatic freshwater fern in the Salviniaceae family, commonly found in Asia and Africa (Roy et al., 2016). Azolla can grow and reproduce rapidly in various environments and is resilient to elevated temperatures (Lestari et al., 2024). As such, Azolla is regarded as a renewable and inexpensive source of biomass, suitable for applications in biofuels (Miranda et al., 2016) and livestock feed (Katole et al., 2017). In addition, Azolla forms a symbiosis with *Anabaena azollae*, a phototrophic cyanobacterium that resides in its leaves, which enables nitrogen fixation (Setiawati et al., 2018). Consequently, Azolla is rich in nitrogen and has been used as a biofertilizer in agriculture, including rice cultivation (Yao et al., 2018). Azolla is utilized in fresh, composted or dried forms (Setiawati et al., 2018) to promote rice growth and yield (Razavipour et al., 2018). In addition, Azolla can improve long-term soil fertility by reducing ammonia volatilization, increasing soil microbial activity and providing organic matter (Subedi and Shrestha, 2015). However, the limitations of Azolla use in rice production include high labor requirements for its maintenance and incorporation into fertilizer regimes, as well as its deficiency in micronutrients (Li et al., 2020; Marzouk et al., 2023).

Enhancing Azolla-based fertilizers with materials that efficiently deliver micronutrients might address these limitations. Nanoparticles have been increasingly used to improve the productivity and yield quality of various crops (Singh et al., 2021). Zinc oxide (ZnO) nanoparticles are cost-effective materials widely used in medical, electronic and cosmetics applications (Dey et al., 2025). In agriculture, ZnO nanoparticles (nZnO) provide a crucial nutrient for plants, where, due to their small size and chemical stability, they can be readily transported into plant tissues (Reddy Pullagurala et al., 2018). As a result, the use of ZnO nanoparticles at low dosages can be highly effective in promoting plant growth. For example, the application of ZnO nanoparticles promotes photosynthesis and metabolism in rice (Sabir and Singh, 2013). For example, Zhang et al. (2021) found that the addition of nZnO to soil improved the photosynthetic potential of Nanjing 9108 rice and increased plant weight, panicle number, grain weight and grain quality. Mazhar et al. (2023) used nZnO suspensions to improve the growth and yield of IR-6 rice more effectively than the use of bulk zinc sources. In addition, ZnO nanoparticles have been shown to alleviate stress induced by heavy metal contamination (Prakash et al., 2022) and drought conditions (Rameshraddy et al., 2017). Nevertheless, these studies did not examine the potential of nZnO to reduce reliance on conventional chemical fertilizers.

Since Azolla is rich in nitrogen and nZnO efficiently supplies a key micronutrient, their combined use may exert a synergistic effect on rice growth and reduce reliance on conventional chemical fertilizers. A few studies have examined this combined application. For example, the effects were investigated of nZnO foliar spray, combined with rice straw compost and gypsum amendments, on wheat yield and soil quality (El-Sharkawy et al., 2024). This integrated approach improved the grain yield and nutrient content relative to the use of individual fertilizer components. In rice, the combined use of nZnO and biochar was more effective in decreasing cadmium accumulation in roots; however, the addition of biochar had no impact on rice growth (Ali et al., 2019). Notably, these studies did not compare the performance of nZnO-organic material combinations to that of conventional chemical fertilizers. In particular, no reported studies have compared Azolla-nZnO pellet fertilizer directly with urea fertilizer in rice cultivation.

The absence of an assessment of combined organic-nZnO fertilizers compared to conventional treatments may discourage the agricultural sector from adopting these fertilizers on a larger scale.

The current study examined the combined use of nZnO and Azolla in rice production to address the existing research gap concerning nZnO-organic fertilizer approaches. By varying the content of nZnO, the combined fertilizer may serve as a viable alternative to urea fertilizer for enhancing rice growth and yield. The findings could provide insights into the relative efficacy of the combined fertilizer and serve as a guideline for the use of nZnO and Azolla as sustainable alternatives to conventional fertilizer practices.

Materials and Methods

Synthesis of ZnO nanoparticles, Azolla cultivation and preparation of pellet fertilizer

ZnO nanoparticles (nZnO) were synthesized by the co-precipitation method modified from Mazhar et al. (2023). A 0.1 M aqueous solution of zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) and a 0.2M aqueous solution of sodium hydroxide (NaOH) were prepared under constant stirring until the solutions became clear and equilibrated to room temperature (22ºC). The NaOH solution was slowly added dropwise to the zinc nitrate solution during 30 min. The reaction was allowed to proceed for 12 hr under continuous stirring. The ZnO nanoparticles were washed four times with deionized water and subsequently dried at 80ºC in an open-air oven for 12 hr. The dried precipitates were ground into a fine powder and annealed at 550ºC for 4 hr. The nZnO were characterized using transmission electron microscopy (TEM) and X-ray diffraction (XRD) with Cu K-alpha radiation.

Initially, the *Azolla microphylla* was provided by the Department of Agriculture, Thailand. To increase the biomass of Azolla, additional cultivation was conducted in a greenhouse using a rectangular well measuring 30 cm in height, 50 cm in width and 100 cm in length. An amount (437.5 g) of cow manure and 125 L of water were added to the well. After 3 d, the Azolla was added so that it covered 10% of the water surface. The Azolla was harvested after 20 d of cultivation and air-dried for 2 d.

The pellet fertilizer raw materials were thoroughly mixed according to three weight formulas. In the first formulation (Azolla-nZnO 1), the weight proportions of Azolla and nZnO were 60.96% and 0.04%, respectively. In the second (Azolla-nZnO 2) and third (Azolla-nZnO 3) formulations, the proportions were 60.92% and 0.08%, respectively, and 60.88% and 0.12%, respectively. In all three formulas, the mixture included cow manure (35%), hydraulic lime (3%) and molasses (1%). Then, the mixture was ground and cut into pellets (1 cm long) using a pellet mill. A pellet fertilizer with 100% dried Azolla was also prepared. The fertilizer pellets were dried in the open air for 2 d before use.

Rice cultivation experiment

A completely randomized design experiment was conducted to evaluate the effects of the different pellet fertilizers on Pathum Thani 1 rice compared to Azolla, the nZnO control and urea fertilizer. Each treatment consisted of 10 pots, each containing a single rice plant, with three replicates per treatment. The Pathum Thani 1 rice seeds were provided by the Pathum Thani Rice Research Center, Thailand. The seeds were soaked in distilled water for 24 hr and germinated in seedling trays. After 7 d, the seedlings were transplanted into pots filled with local topsoil for the experiment. A soil sample was sent to the Science Center at Valaya Alongkorn Rajabhat University, Pathum Thani, Thailand for analysis. The properties of the soil and the characterization methods are presented in Table 1. Throughout the experiment, the rice plants were watered daily and subjected to seven distinct fertilizer treatments, as detailed below. In the control group, no fertilizer was applied. The six treatment groups received the following applications: 1.5 g of 46% urea fertilizer; 6 g of Azolla pellet fertilizer without nZnO; 0.75 g of nZnO powder; 3 g of Azolla-nZnO 1 pellet fertilizer; 3 g of Azolla-nZnO 2 pellet fertilizer; and 3 g of Azolla-nZnO 3 pellet fertilizer. The fertilizer application rates were selected to ensure that treatments containing urea or Azolla received equivalent amounts of total nitrogen.

Table 1 Properties of experimental soil characterization methods.

Soil property	Value	Method
pH	7.90	pH meter
Electrical conductivity (mS/cm)	1.02	Soil conductivity meter
Carbon-to-nitrogen ratio	1.80	Walkley and Black method and Kjeldahl method
Total N (%)	1.35	Titration
Total P_2O_5 (%)	1.30	Colorimetry
Total K_2O (%)	1.04	Flame photometry
Moisture (%)	30.80	Drying and gravimetric method
Zinc content (ppm)	7.30	Inductively coupled plasma mass spectrometry
Soil texture	Clay	Hydrometer

All treatments were applied on the first day after transplanting (DAT). Typically, Pathum Thani 1 rice reaches the reproduction period and the ripening period on 60 and 90 DAT, respectively, and is usually harvested during 100–120 DAT. The experiment proceeded until 120 DAT.

Growth parameters were recorded throughout the cultivation period. The number of tillers per hill was measured at 60 DAT. Plant height was measured with a ruler on 60 and 90 DAT. Leaf SPAD indices at 40, 50, 60, 70, 80 and 90 DAT were measured during 0900–1000 hours using a SPAD 502 Plus chlorophyll meter (Spectrum Technologies Inc.). Measurements were taken at three positions along the leaf blade at one-quarter, one-half and three-quarters of the distance from the leaf base. The values were averaged before being recorded. On the day of full harvest, the harvest date and yield components were recorded, consisting of the number of panicles per plant, the number of grains per panicle, percentage of good grains (defined as those free from chips or cracks) and the weight per 1,000 grains for both paddy and brown rice.

The rice root, leaf and grain samples were collected and sent to the National Nanotechnology Center (Thailand) for zinc quantification using inductively coupled plasma mass spectrometry (Shimadzu ICPMS-2030). Data were analyzed using one-way analysis of variance followed by Fisher's least significant difference tests at the 0.05 significance level, with the SPSS software (version 13; SPSS Inc.). Normality and homogeneity of variance of the data used for the analysis were confirmed based on the Shapiro-Wilk test and Levene's test, respectively, in the SPSS software.

Results and Discussion

The TEM images (Fig. 1) revealed that the synthesized nZnO had an average rod-like size of approximately 320 nm in length and 100 nm in width. There was a notable proportion of nZnO measuring below 50 nm in size. This morphology is typical of nZnO synthesized via the co-precipitation method (Wang, 2004). The XRD peaks (Fig. 2) confirmed the hexagonal wurtzite crystal structure of ZnO. Although the average particle size exceeded that of nZnO typically reported in other studies, the rod-shape morphology, with a width of 100 nm, may enhance cellular penetration compared to spherical counterparts (Qi et al., 2017; Castiglione et al., 2023; Li et al., 2023). This narrow width, along with the smaller particles below 50 nm, likely facilitates uptake and translocation in rice plants. Furthermore, studies have reported that nanoparticles larger than 100 nm can still be absorbed by plants, despite the smaller size exclusion limit of plant cell walls (Zhao et al., 2016; Ma and Yan, 2018; Ali et al., 2021). Therefore, the nZnO treatments were expected to impact rice growth and zinc accumulation.

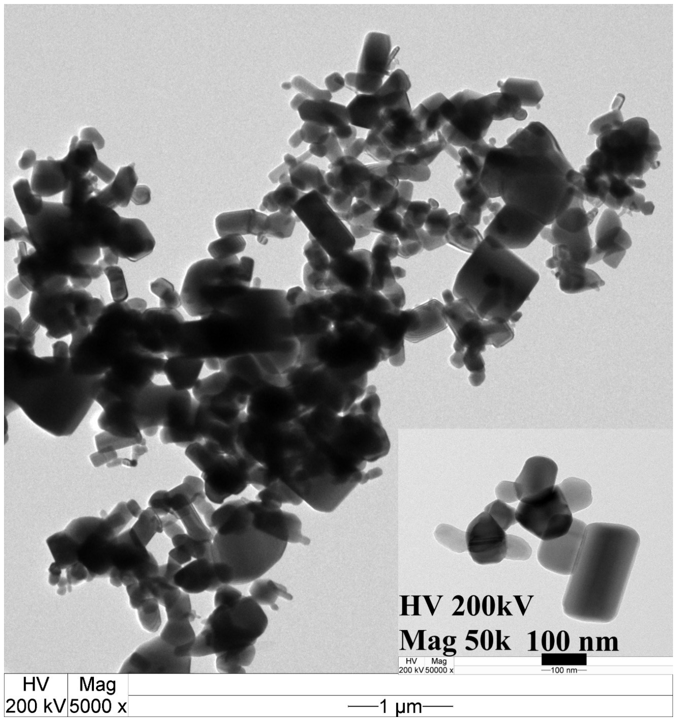

Fig. 1 Transmission electron microscopy images of synthesized ZnO nanoparticles, showing rod-shaped ZnO particles with average width of ~100 nm and an average size of ~320 nm.

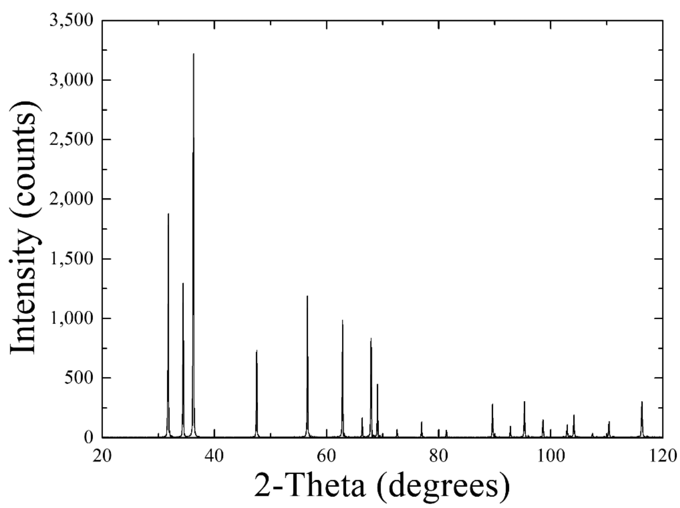

Fig. 2 X-ray diffraction spectrum of synthesized ZnO nanoparticles, with peak pattern consistent with the wurtzite crystal structure.

The effects of the treatments on SPAD indices (Fig. 3) were significant across all six measurement days. Between 40 and 80 DAT, the urea treatment had the highest SPAD index, but it was not significantly different from Azolla-nZnO 3 (Fig. 3). At 90 DAT, both the Azolla-nZnO 3 and Azolla-nZnO 2 treatments tended to have higher SPAD indices than the urea treatment (Fig. 3). In contrast, treatments with only dried Azolla or nZnO resulted in significantly lower SPAD indices than both the urea and the Azolla-nZnO treatments. Based on these results, the Azolla-nZnO pellet fertilizer enhanced the chlorophyll content as effectively as the urea fertilizer and with the optimal nZnO content, it might outperform the individual components.

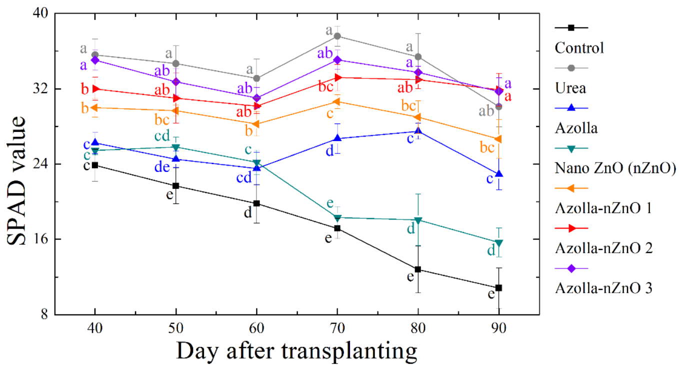

Fig. 3 Leaf SPAD values of rice at 40, 50, 60, 70, 80 and 90 days after transplanting under different treatments. Different lowercase letters denote significant ($p < 0.05$) difference among means at each time point, based on Fisher's least significant difference test. Error bars = ± SD.

The enhanced effectiveness of the Azolla-nZnO pellet fertilizer stems from the synergistic effects of its components. Azolla is rich in nitrogen, an essential element of chlorophyll in rice (Prabakaran et al., 2022). In its dried powder form, Azolla increases the availability of phosphorus (Setiawati et al., 2018; Al-Bdairi and Kamal, 2021), a crucial constituent of adenosine triphosphate and nicotinamide adenine dinucleotide phosphate, both of which are required for the Calvin cycle (Kayoumu et al., 2023). Concurrently, nZnO supplies zinc, which promotes the activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO), an enzyme used in carbon dioxide fixation, by promoting the expression of RuBisCO subunit-coding genes (Rehman et al., 2012; Yoshihara et al., 2019; Guo et al., 2024). By integrating Azolla and nZnO into the pellet fertilizer, both nutrient-delivery mechanisms are likely sustained until the fertilizer is fully depleted. Notably, although the Azolla-based fertilizer contained the same total nitrogen as urea fertilizer, its lower available nitrogen content led to fewer photosynthetic benefits. Similarly, nZnO alone was less effective than urea fertilizer, as zinc could not replace nitrogen for photosynthetic function. Nonetheless, the synergistic mechanisms within the nanomaterial-organic framework (Tang et al., 2024) enhanced the performance of Azolla-nZnO pellet fertilizer, making it comparable to that of urea fertilizer.

The rice treated with the Azolla-nZnO 3 produced the highest number of tillers per hill at 60 DAT (Table 2), significantly exceeding the values observed in the urea, Azolla-only (g and nZnO-only treatments, but not significantly different from the Azolla-nZnO 1 and 2 groups. At 60 DAT, plant height was the same in the Azolla-nZnO 2, 3 treatments and the urea fertilizer treatment (Table 2). The superior performance of the pellet fertilizer treatment relative to the Azolla-only and nZnO-only treatments was consistent with the research by El-Sharkawy et al. (2024), showing that combinations of nZnO and organic matter improved crop growth. The similarity in growth performance between the Azolla-nZnO 2 and Azolla-nZnO 3 treatments and the urea fertilizer treatment also aligned with the observed effects on SPAD indices. Based on these results, the enhancement in rice growth could be attributed to the increased chlorophyll content in the combined treatment groups.

Table 2 Number of tillers per hill at 60 d after transplanting (DAT) and plant height at 60 and 90 DAT of rice under different treatments.

Treatment	Number of tillers per hill	Plant height (cm)	
		60 DAT	90 DAT
Control	5.33±1.53^{c}	55.2±4.0^{c}	77.8±1.4^{f}
Urea	14.67±1.55^{b}	75.0±1.0^{a}	129.3±7.9^{a}
Azolla	7.52±2.31^{c}	61.0±0.9^{bc}	100.6±0.8^{de}
Nano ZnO (nZnO)	7.44±0.51^{c}	58.5±0.6^{bc}	98.4±0.7^{e}
Azolla-nZnO 1	15.70±0.61^{ab}	65.4±4.5^{b}	109.3±0.6^{cd}
Azolla-nZnO 2	17.68±1.54^{ab}	73.0±2.0^{a}	115.0±2.3^{bc}
Azolla-nZnO 3	18.85±1.23^{a}	74.4±1.3^{a}	120.2±0.7^{b}
p value, CV (%)	< 0.001, 44.4	< 0.001, 11.9	< 0.001, 14.2

Weight proportions of Azolla and nZnO in Azolla-nZnO 1 = 60.96% and 0.04%, respectively; in Azolla-nZnO 2 = 60.92% and 0.08%, respectively, and in Azolla-nZnO 3 = 60.88% and 0.12%, respectively; CV = coefficient of variation.
Values (mean ± SD) in each column with different lowercase superscripts are significantly ($p < 0.05$) different, based on Fisher's least significant difference test.

However, by 90 DAT, plant height in the urea treatment group was significantly greater than in all other treatment groups (Table 2), including those treated with Azolla-nZnO. This difference between 60 DAT and 90 DAT was likely due to the changing nutritional requirements of rice at different growth stages. Pathum Thani 1 rice transitions from the vegetative stage to the reproductive stage at around 60 DAT, with the ripening stage beginning around 90 DAT. Notably, the demand for nitrogen is typically highest during the reproductive stage (Sims and Place, 1968). Therefore, the high availability of nitrogen in urea fertilizer more effectively meets this requirement than the Azolla-nZnO treatments during the reproductive stage, resulting in greater plant height by the end of this period.

The yield parameters of rice under the different treatments are presented in Tables 3 and 4. The treatments significantly influenced the number of panicles, number of grains per panicle, percentage of good grains and days of full harvest. Specifically, the Azolla-nZnO 2 and Azolla-nZnO 3 treatments performed best in terms of the number of panicles and grains per panicle (Table 3). Their values were significantly greater than those of the Azolla-only and nZnO-only treatments (Table 3) but not significantly different from the urea treatment. For the percentage of good grains and days to full harvest, all non-control treatments yielded statistically similar results (Tables 3 and 4). However, none of the treatments had a significant effect on the weight of paddy or brown rice.

The lack of significant effects on paddy and brown rice weight may have been due to applying the treatments only once at the beginning of the experiment. It was likely that the nutrients supplied by the treatments were depleted during later growth stages, particularly during grain filling. This contrasts with a study by El-Sharkawy et al. (2024), where a twice-applied combination of nZnO and corn compost significantly increased grain weight. This increased application frequency likely explained the discrepancy in effects. Therefore, additional applications, especially at the onset of the reproductive stage, should be explored to enhance the effectiveness of the pellet fertilizer. Furthermore, the dissolution rate of the pellets might influence fertilizer performance. As the pellets released nutrients, their size decreased, which in turn reduced their surface area and slowed dissolution (Lakshani et al., 2023), thereby limiting nutrient release. Alternatively, the treatments may have simply been ineffective at improving grain weight. Grain filling is a complex process, involving starch biosynthesis and gene expression (Ma et al., 2023), which require multiple nutrients that may not have been adequately supplied by the treatments. Therefore, to better assess the potential of the pellet fertilizer, future studies should examine its application at later growth stages and evaluate its combination with other types of fertilizers.

Table 3 Number of panicles and grains per panicle and percentage of good grains of rice under different treatments, on day of full harvest

Treatment	Number of panicles	Number of grains per panicle	Good grains (%)
Control	2.82±1.05^{c}	107.4±3.8^{c}	72.11±3.2^{b}
Urea	13.00±1.03^{a}	157.9±3.4^{a}	97.52±2.7^{a}
Azolla	8.15±0.79^{b}	132.4±4.0^{b}	96.48±2.3^{a}
Nano ZnO (nZnO)	5.17±0.76^{c}	129.3±1.2^{b}	98.20±1.7^{a}
Azolla-nZnO 1	14.22±1.34^{a}	135.2±1.0^{b}	98.44±1.2^{a}
Azolla-nZnO 2	15.15±1.03^{a}	157.6±3.1^{a}	97.52±4.0^{a}
Azolla-nZnO 3	16.15±1.04^{a}	158.9±1.7^{a}	98.41±2.1^{a}
p value, CV (%)	< 0.001, 45.4	< 0.001, 12.1	< 0.001, 16.9

Weight proportions of Azolla and nZnO in Azolla-nZnO 1 = 60.96% and 0.04%, respectively; in Azolla-nZnO 2 = 60.92% and 0.08%, respectively, and in Azolla-nZnO 3 = 60.88% and 0.12%, respectively; CV = coefficient of variation.

Values (mean ± SD) in each column with different lowercase superscripts are significantly ($p < 0.05$) different, based on Fisher's least significant difference test.

Table 4 Weight of paddy and brown rice and day of full harvest of rice under different treatments

Treatment	Weight of paddy rice (g/1,000 grains)	Weight of brown rice (g/1,000 grains)	Day of full harvest (d)
Control	27.67±0.57	23.76±0.46	108.3±1.5^{b}
Urea	27.71±0.64	24.01±0.30	115.2±0.6^{a}
Azolla	27.74±0.61	24.04±0.40	113.0±1.7^{a}
Nano ZnO (nZnO)	27.58±0.58	24.01±0.14	109.7±0.8^{b}
Azolla-nZnO 1	27.75±0.70	23.87±0.28	114.4±1.3^{a}
Azolla-nZnO 2	27.82±0.83	24.03±0.31	114.7±0.9^{a}
Azolla-nZnO 3	27.92±1.01	24.02±0.32	115.2±1.1^{a}
p value, CV (%)	0.99, 0.36	0.57, 0.40	< 0.001, 2.28

Weight proportions of Azolla and nZnO in Azolla-nZnO 1 = 60.96% and 0.04%, respectively; in Azolla-nZnO 2 = 60.92% and 0.08%, respectively, and in Azolla-nZnO 3 = 60.88% and 0.12%, respectively; CV = coefficient of variation.

Values (mean ± SD) in each column with different lowercase superscripts are significantly ($p < 0.05$) different, based on Fisher's least significant difference test.

The treatments had significant effects on zinc accumulation in the rice roots, leaves and grains, as shown in Table 5. The nZnO-only treatment resulted in the highest level of Zn accumulation (Table 5), followed by the Azolla-nZnO pellet treatments in decreasing order of nZnO concentration. A similar trend was observed in previous studies (Bala et al., 2019; Yan et al., 2021). These findings indicate that zinc content in rice parts can be modulated by adjusting the nZnO content. Even without nZnO, zinc accumulation occurred due to native zinc content in soil, highlighting the importance of considering baseline soil zinc levels in future cost-effectiveness studies. Crucially, all nZnO-containing treatments led to significantly greater zinc accumulation in rice grains compared to the control, urea and Azolla-only treatments. Given widespread zinc deficiency in developing countries (Gibson et al., 2007; Rerksuppaphol and Rerksuppaphol, 2019), nZnO use in rice cultivation could help to address micronutrient malnutrition, with its nutritional benefits likely outweighing potential toxicity risks (Plum et al., 2010). The Azolla-nZnO pellet fertilizer showed promise for sustainable rice production due to Azolla's facile cultivation and the simple, cost-effective synthesis of nZnO. The pelletization process is scalable and could biofortify rice grains with zinc. Unlike conventional methods, such as foliar spraying (Sánchez-Palacios et al., 2023; Bodeerath et al., 2024), the pellet fertilizer enabled gradual zinc release with less input, reducing toxicity risks. However, controlling the nZnO particle size during synthesis remains a challenge and future research should explore industrial-scale nZnO sources for broader adoption.

Table 5 Concentration of Zinc accumulated in roots, leaves and grains of rice under different treatments.

Treatment	Concentration of zinc (ppm) accumulated in		
	Roots	Leaves	Grains
Control	20.0±0.2[g]	10.4±0.4[e]	15.0±0.7[g]
Urea	23.2±0.5[e]	11.3±0.4[e]	17.5±0.3[e]
Azolla	21.6±0.4[f]	10.8±0.3[e]	16.2±0.2[f]
Nano ZnO (nZnO)	35.3±0.8[a]	19.5±0.3[a]	27.2±0.4[a]
Azolla-nZnO 1	26.3±0.4[d]	13.5±0.4[d]	20.4±0.5[d]
Azolla-nZnO 2	29.4±0.4[c]	15.2±0.2[c]	22.4±0.3[c]
Azolla-nZnO 3	31.9±0.2[b]	16.6±0.5[b]	24.6±0.4[b]
p value, CV (%)	< 0.001, 21.0	< 0.001, 24.3	< 0.001, 22.1

ppm = parts per million; Weight proportions of Azolla and nZnO in Azolla-nZnO 1 = 60.96% and 0.04%, respectively; in Azolla-nZnO 2 = 60.92% and 0.08%, respectively, and in Azolla-nZnO 3 = 60.88% and 0.12%, respectively; CV = coefficient of variation.

Values (mean ± SD) in each column with different lowercase superscripts are significantly ($p < 0.05$) different, based on Fisher's least significant difference test.

In conclusion, the current study presented the first comparative evaluation of a pellet fertilizer composed of dried Azolla and nZnO against conventional urea fertilizer for improving rice production. The Azolla-nZnO pellet fertilizer effectively enhanced the chlorophyll content and key growth parameters in rice, performing comparably to urea, while significantly increasing zinc accumulation in the rice grains. This was caused by a synergistic interaction between the nitrogen from the Azolla and nZnO's stimulation of carbon fixation. Due to its single application and incomplete nutrient profile, the pellet fertilizer had no significant effect on grain weight. However, the enhanced zinc content in the grain suggested its strong potential for sustainable rice production, particularly through zinc biofortification and the use of inexpensive, locally accessible materials. Further research should evaluate the effectiveness of multiple applications or co-application with traditional chemical fertilizers to improve yield. Monitoring key nutrient and zinc uptake could elucidate the synergy between zinc and Azolla, leading to optimized fertilizer use. Field-scale trials, encompassing large-scale Azolla cultivation and nZnO synthesis, are required to evaluate economic feasibility. Additionally, investigating nZnO combinations with other locally available organic materials may broaden this approach's applicability where Azolla is unavailable.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Al-Bdairi, S.H.J., Kamal, J.A. 2021. The effect of biofertilizer of Azolla, phosphate and nitrogen fertilizers on some growth traits of rice. IOP Conf. Ser. Earth Environ. Sci. 735: 012064. doi.org/10.1088/1755-1315/735/1/012064

Ali, S., Mehmood, A., Khan, N. 2021. Uptake, translocation and consequences of nanomaterials on plant growth and stress adaptation. J. Nanomater. 2021: 6677616. doi.org/10.1155/2021/6677616

Ali, S., Rizwan, M., Noureen, S., et al. 2019. Combined use of biochar and zinc oxide nanoparticle foliar spray improved the plant growth and decreased the cadmium accumulation in rice (*Oryza sativa* L.) plant. Environ. Sci. Pollut. Res. 26: 11288–11299. doi.org/10.1007/s11356-019-04554-y

Ashitha, A., Rakhimol, K.R., Jyothis, M. 2021. Chapter 2 - Fate of the conventional fertilizers in environment. In: Lewu, F.B., Volova, T., Thomas, S., Rakhimol, K.R. (Eds.), Control. Release Fertil. Sustain. Agric. Academic Press, Cambridge, MA, USA. pp. 25–39.

Bala, R., Kalia, A., Dhaliwal, S.S. 2019. Evaluation of efficacy of ZnO nanoparticles as remedial zinc nanofertilizer for rice. J. Soil Sci. Plant Nutr. 19: 379–389. doi.org/10.1007/s42729-019-00040-z

Bodeerath, S., Jumrus, S., Veeradittakit, J., Utasee, S. 2024. Variation of grain zinc, phytate concentration and phytate:Zn molar ratio in unpolished and polished rice affected by foliar zinc application among Thai rice varieties. Plant Prod. Sci. 27: 304–319. doi.org/10.1080/1343943X.2024.2412867

Castiglione, M.R., Bottega, S., Sorce, C., Spanò, C. 2023. Effects of zinc oxide particles with different sizes on root development in *Oryza sativa*. Rice Sci. 30: 449–458. doi.org/10.1016/j.rsci.2023.03.016

Dey, S., Mohanty, D.L., Divya, N., et al. 2025. A critical review on zinc oxide nanoparticles: Synthesis, properties and biomedical applications. Intell. Pharm. 3: 53–70. doi.org/10.1016/j.ipha.2024.08.004

El-Sharkawy, M., Alotaibi, M.O., Li, J., Mahmoud, E., Ghoneim, A.M., Ramadan, M.S., Shabana, M. 2024. Effect of nano-zinc oxide, rice straw compost and gypsum on wheat (*Triticum aestivum* L.) yield and soil quality in saline-sodic soil. Nanomaterials (Basel) 14: 1450. doi.org/10.3390/nano14171450

Firdaus, R.B.R., Tan, M.L., Rahmat, S.R., Gunaratne, M.S. 2020. Paddy, rice and food security in Malaysia: A review of climate change impacts. Cogent Soc. Sci. 6: 1818373. doi.org/10.1080/23311886.2020.1818373

Gibson, R.S., Manger, M.S., Krittaphol, W., Pongcharoen, T., Gowachirapant, S., Bailey, K.B., Winichagoon, P. 2007. Does zinc deficiency play a role in stunting among primary school children in NE Thailand? Br. J. Nutr. 97: 167–175. doi.org/10.1017/S0007114507250445

Guo, S., Hu, X., Wang, Z., Xing, B. 2024. Zinc oxide nanoparticles cooperate with the phyllosphere to promote grain yield and nutritional quality of rice under heatwave stress. Proc. Natl. Acad. Sci. U.S.A. 121: e2414822121. doi.org/10.1073/pnas.2414822121

Katole, S.B., Lende, S.R., Patil, S.S. 2017. A review on potential livestock feed: Azolla. Livest. Res. Int. 5: 1–9.

Kayoumu, M., Iqbal, A., Muhammad, N., et al. 2023. Phosphorus availability affects the photosynthesis and antioxidant system of contrasting low-P-tolerant cotton genotypes. Antioxidants (Basel) 12: 466. doi.org/10.3390/antiox12020466

Kumar, R., Kumar, R., Prakash, O. 2019. The impact of chemical fertilizers on our environment and ecosystem. In: Res. Trends Environ. Sci. AkiNik Publications, New Delhi, India, pp. 69–86.

Lakshani, N., Wijerathne, H.S., Sandaruwan, C., Kottegoda, N., Karunarathne, V. 2023. Release kinetic models and release mechanisms of controlled-release and slow-release fertilizers. ACS Agric. Sci. Technol. 3: 939–956. doi.org/10.1021/acsagscitech.3c00152

Lestari, S.U., Roeswitawati, D., Syafrani, S., Maftuchah, M., Purnama, I. 2024. Addressing nitrogen-rich biomass production challenges in *Azolla microphylla* cultivation from varying shading and water depth dynamics. Pertanika J. Trop. Agric. Sci. 47: 889–904. doi.org/10.47836/pjtas.47.3.18

Li, J., Lens, P.N.L., Otero-Gonzalez, L., Laing, G.D. 2020. Production of selenium- and zinc-enriched Lemna and Azolla as potential micronutrient-enriched bioproducts. Water Res. 172: 115522. doi.org/10.1016/j.watres.2020.115522

Li, N., Zhao, Y., Han, J., Yang, Q., Liang, J., Liu, X., Wang, Y., Huang, Z. 2024. Impacts of future climate change on rice yield based on crop model simulation—A meta-analysis. Sci. Total Environ. 949: 175038. doi.org/10.1016/j.scitotenv.2024.175038

Li, Z., Yan, W., Li, Y., et al. 2023. Particle size determines the phytotoxicity of ZnO nanoparticles in rice (*Oryza sativa* L.) revealed by spatial imaging techniques. Environ. Sci. Technol. 57: 13356–13365. doi.org/10.1021/acs.est.3c03821

Ma, B., Zhang, L., He, Z. 2023. Understanding the regulation of cereal grain filling: The way forward. J. Integr. Plant Biol. 65: 526–547. doi.org/10.1111/jipb.13456

Ma, X., Yan, J. 2018. Plant uptake and accumulation of engineered metallic nanoparticles from lab to field conditions. Curr. Opin. Environ. Sci. Health 6: 16–20. doi.org/10.1016/j.coesh.2018.07.008

Marzouk, S.H., Tindwa, H.J., Amuri, N.A., Semoka, J.M. 2023. An overview of underutilized benefits derived from Azolla as a promising biofertilizer in lowland rice production. Heliyon 9: e13040. doi.org/10.1016/j.heliyon.2023.e13040

Mazhar, Z., Akhtar, J., Alhodaib, A., et al. 2023. Efficacy of ZnO nanoparticles in Zn fortification and partitioning of wheat and rice grains under salt stress. Sci. Rep. 13: 2022. doi.org/10.1038/s41598-022-26039-8

Miranda, A.F., Biswas, B., Ramkumar, N., et al. 2016. Aquatic plant Azolla as the universal feedstock for biofuel production. Biotechnol. Biofuels 9: 221. doi.org/10.1186/s13068-016-0628-5

Myers, S.S., Wessells, K.R., Kloog, I., Zanobetti, A., Schwartz, J. 2015. Effect of increased concentrations of atmospheric carbon dioxide on the global threat of zinc deficiency: A modelling study. Lancet Glob. Health 3: e639–e645. doi.org/10.1016/S2214-109X(15)00093-5

Office of the National Economic and Social Development Council. 2025. About SDGs–Goal 2: end hunger, achieve food security and improved nutrition, and promote sustainable agriculture. https://sdgs.nesdc.go.th/goal-2/, 10 February 2025. [in Thai]

Plum, L.M., Rink, L., Haase, H. 2010. The essential toxin: Impact of zinc on human health. Int. J. Environ. Res. Public Health. 7: 1342–1365. doi.org/10.3390/ijerph7041342

Prabakaran, S., Mohanraj, T., Arumugam, A., et al. 2022. A state-of-the-art review on the environmental benefits and prospects of Azolla in biofuel, bioremediation and biofertilizer applications. Ind. Crop. Prod. 183: 114942. doi.org/10.1016/j.indcrop.2022.114942

Prakash, V., Rai, P., Sharma, N.C., et al. 2022. Application of zinc oxide nanoparticles as fertilizer boosts growth in rice plant and alleviates chromium stress by regulating genes involved in oxidative stress. Chemosphere. 303: 134554. doi.org/10.1016/j.chemosphere.2022.134554

Qi, K., Cheng, B., Yu, J., et al. 2017. Review on the improvement of the photocatalytic and antibacterial activities of ZnO. J. Alloys Compd. 727: 792–820. doi.org/10.1016/j.jallcom.2017.08.142

Rameshraddy, Pavithra, G.J., Rajashekar Reddy, B.H., et al. 2017. Zinc oxide nano particles increases Zn uptake, translocation in rice with positive effect on growth, yield and moisture stress tolerance. Ind. J. Plant Physiol. 22: 287–294. doi.org/10.1007/s40502-017-0303-2

Razavipour, T., Moghaddam, S.S., Doaei, S., et al. 2018. Azolla (*Azolla filiculoides*) compost improves grain yield of rice (*Oryza sativa* L.) under different irrigation regimes. Agric. Water Manag. 209: 1–10. doi.org/10.1016/j.agwat.2018.05.020

Reddy Pullagurala, V.L., Adisa, I.O., Rawat, S., et al. 2018. Finding the conditions for the beneficial use of ZnO nanoparticles towards plants—A review. Environ. Pollut. 241: 1175–1181. doi.org/10.1016/j.envpol.2018.06.036

Rehman, H., Aziz, T., Farooq, M., et al. 2012. Zinc nutrition in rice production systems: A review. Plant Soil. 361: 203–226. doi.org/10.1007/s11104-012-1346-9

Rerksuppaphol, L., Rerksuppaphol, S. 2019. Zinc deficiency in children with Dengue viral infection. Pediatr. Rep. 11: 7386. doi.org/10.4081/pr.2019.7386

Roy, D.C., Pakhira, M.C., Bera, S. 2016. A review on biology, cultivation and utilization of Azolla. Adv. Life Sci. 5: 11–15.

Sabir, N., Singh, B. 2013. Protected cultivation of vegetables in global arena: A review. Indian J. Agric. Sci. 83: 123–135.

Sánchez-Palacios, J., Henry, D., Penrose, B., et al. 2023. Formulation of zinc foliar sprays for wheat grain biofortification: A review of current applications and future perspectives. Front. Plant Sci. 14. doi.org/10.3389/fpls.2023.1247600

Setiawati, M.R., Damayani, M., Herdiyantoro, D., et al. 2018. The application dosage of *Azolla pinnata* in fresh and powder form as organic fertilizer on soil chemical properties, growth and yield of rice plant. AIP Conf. Proc. 1927: 030017. doi.org/10.1063/1.5021210

Sims, J.L., Place, G.A. 1968. Growth and nutrient uptake of rice at different growth stages and nitrogen levels. Agron. J. 60: 692–696. doi.org/10.2134/agronj1968.00021962006000060033x

Singh, R.P., Handa, R., Manchanda, G. 2021. Nanoparticles in sustainable agriculture: An emerging opportunity. J. Control. Release. 329: 1234–1248. doi.org/10.1016/j.jconrel.2020.10.051

Sinnarong, N., Chen, C.-C., McCarl, B., et al. 2019. Estimating the potential effects of climate change on rice production in Thailand. Paddy Water Environ. 17: 761–769. doi.org/10.1007/s10333-019-00755-w

St. Clair, S.B., Lynch, J.P. 2010. The opening of Pandora's box: Climate change impacts on soil fertility and crop nutrition in developing countries. Plant Soil. 335: 101–115. doi.org/10.1007/s11104-010-0328-z

Subedi, P., Shrestha, J. 2015. Improving soil fertility through Azolla application in low land rice: A review. Azarian J. Agric. 2: 35–39.

Tang, Y., Zhao, W., Gao, L., et al. 2024. Harnessing synergy: Integrating agricultural waste and nanomaterials for enhanced sustainability. Environ. Pollut. 341: 123023. doi.org/10.1016/j.envpol.2023.123023

Verma, B.C., Pramanik, P., Bhaduri, D. 2020. Organic fertilizers for sustainable soil and environmental management. In: Meena, R.S. (Ed.). Nutrient Dynamics for Sustainable Crop Production. Springer. Singapore. pp. 289–313. doi.org/10.1007/978-981-13-8660-2_10

Verma, K.K., Song, X.-P., Joshi, A., et al. 2022. Recent trends in nano-fertilizers for sustainable agriculture under climate change for global food security. Nanomater. 12: 173. doi.org/10.3390/nano12010173

Wang, Z.L. 2004. Nanostructures of zinc oxide. Mater. Today. 7: 26–33. doi.org/10.1016/S1369-7021(04)00286-X

Wassmann, R., Jagadish, S.V.K., Sumfleth, K., et al. 2009. Chapter 3: Regional vulnerability of climate change impacts on Asian rice production and scope for adaptation. In: Advances in Agronomy. Academic Press. pp. 91–133. doi.org/10.1016/S0065-2113(09)01003-7

Yan, S., Wu, F., Zhou, S., et al. 2021. Zinc oxide nanoparticles alleviate the arsenic toxicity and decrease the accumulation of arsenic in rice (*Oryza sativa* L.). BMC Plant Biol. 21: 150. doi.org/10.1186/s12870-021-02929-3

Yao, Y., Zhang, M., Tian, Y., et al. 2018. Azolla biofertilizer for improving low nitrogen use efficiency in an intensive rice cropping system. Field Crops Res. 216: 158–164. doi.org/10.1016/j.fcr.2017.11.020

Yoshihara, S., Yamamoto, K., Nakajima, Y., et al. 2019. Absorption of zinc ions dissolved from zinc oxide nanoparticles in the tobacco callus improves plant productivity. Plant Cell Tiss. Organ Cult. 138: 377–385. doi.org/10.1007/s11240-019-01636-0

Zhang, H., Wang, R., Chen, Z., et al. 2021. The effect of zinc oxide nanoparticles for enhancing rice (*Oryza sativa* L.) yield and quality. Agric. 11: 1247. doi.org/10.3390/agriculture11121247

Zhao, L., Ortiz, C., Adeleye, A.S., et al. 2016. Metabolomics to detect response of lettuce (*Lactuca sativa*) to $Cu(OH)_2$ nanopesticides: Oxidative stress response and detoxification mechanisms. Environ. Sci. Technol. 50: 9697–9707. doi.org/10.1021/acs.est.6b02763