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Growth Responses and Regression Analysis of Zinc Oxide Nanoparticles in *Indica* Rice (*Oryza sativa* L.)

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ABSTRACT

The use of metal nanomaterials to promote plant growth and inhibit plant pathogenic microorganisms is gaining interest. In this study, zinc oxide nanoparticles (ZnONPs) were evaluated for effects on growth and productivity of *indica* rice plants (*Oryza sativa* L.). The rice plant was exposed to ZnONPs with different concentrations i. e. 0, 200, 600 and 800 mg/l. The plant height, weight and the number of panicles per clump were investigated. The result showed that 200 mg/l of ZnONPs induced the highest enhancement of plant height, weight and the number of panicles per clump with a significant difference compared to other groups. The mathematical model of the height, weight and the number of panicles per clump was analyzed by linear regression, and the determination coefficient (R^2) of the linear regression model was 0.86, 0.84 and 0.52, respectively. This linear regression model could be potentially extended to the *indica* rice plant cultivation process in practical applications to predict the rice plant growth and productivity in commercial cultivation.

Key words : Regression analysis, ZnO nanoparticles, growth, productivity, rice

INTRODUCTION

The rice plant (*Oryza sativa* L.) is a major economic crop of the world, especially in Asian countries (Younas *et al.*, 2020). Thailand is one of the most important rice producing countries in the world because rice is the staple food of Thai people, and Thailand has geographical and climatic characteristics suitable for rice cultivation. However, global warming and the reduction of arable land are major problems affecting the decline in the quantity and quality of crops (Attavanich, 2013). To improve crop yields, nanomaterials have become one of the most promising agents in stimulating growth and productivity of crop plants (Shang *et al.*, 2019; Mali *et al.*, 2020). Metal nanomaterials induce stress responses in plants, and thus affect physiological, morphological and biochemical reactions in the plant (Plaksenkova *et al.*, 2020). Previous studies have revealed that metal nanomaterials cause cytotoxicity, genotoxicity and oxidative stress in plants, but depend on the plant size, plant species, the concentration of nanoparticles, and exposure time (Rastogi *et al.*, 2017; Yan and Chen, 2019). Normally, zinc (Zn) is an essential trace element, and plays an important role in many plant physiological processes such as maintenance of membrane integrity, regulation of starch synthesis, root development, pigments formation, activation of the production content of bioactive compounds, induction of protein and hormone synthesis (García-López *et al.*, 2019; Sadax and Bakry, 2020). Zinc oxide nanoparticles (ZnONPs) have been applied in many economic crop studies, but a very few studies have reported their effects on the rice plant. In addition, ZnONPs are used in pharmaceutical medicine, because of their low toxicity and excellent biocompatibility (Vimercati *et al.*, 2020). Therefore, ZnONP is recommended as nano-fertilizer for increasing the growth and productivity of economic plants.

The use of mathematics model could be a promising strategy for estimation of the growth and productivity of economic crop. The

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regression modelling is a generic term for a group of different statistical techniques by using regression analysis. The aim of these techniques is to investigate the relation between observed and predicted variables. Linear regression is the common type of regression, and widely used in various fields (Boldina and Beninger, 2016; Basak *et al.*, 2019). Cultivator could make input decisions and their use to estimate the growth and productivity of plant. The objective of this research was to study the effect of ZnONPs on growth and productivity of rice plants such as plant height, plant weight and the number of panicles per clump, and to generate a mathematical regression model based on the rice plant growth and productivity when it was treated with different concentrations of ZnONPs.

MATERIALS AND METHODS

Seeds of *indica* rice (*Oryza sativa* L.) were germinated in sterile distilled water for two days at room temperature (30°C). The preparation of rice seedling was prepared according to the method of Chutipaijit *et al.* (2018). Sixty-day-old *indica* rice seedlings were treated with ZnONPs suspension (average size 35.80±3.62 nm; Global Chemical Co. Ltd., Thailand) with different concentrations as 0, 200, 400 and 800 mg/l every two weeks. The rice plants were harvested at 2, 4, 6 and 8 weeks after planting to investigate the rice plant heights, weights (fresh weight) and numbers of panicles/clump.

The experiments were designed in randomized complete block design (RCBD), and the number of replicates for each treatment was five (n = 5). Statistical analysis was carried out by the Mstat software (Mstat for Windows, V.1.42, Michigan State University, USA), and analzsed for the significance of changes between different treatments at probability levels of 5 and 1%. Duncan Multiple Range Test (DMRT) was performed for comparison of treatment mean.

The data were analyzed using multiple linear regression analyses to find out the closeness in the relationship between the growth and productivity of rice plant and the concentration of ZnONPs. Microsoft Excel (2013) was used in the data analysis, the equation for a multiple linear regression model was :

$$y = b + a_1x_1 + a_2x_2$$

in which y was the dependent variable predicted values, x_1 and x_2 were the independent variable values, b was the y-intercept when x = 0, and 'a' was the slope (Rizkiana *et al.*, 2019).

RESULTS AND DICUSSION

The *indica* rice plants exposed to the different concentrations of ZnONPs triggered a noticeable enhancement of growth responses compared to the control group (0 mg/l ZnONPs). The results showed that ZnONPs of 200 mg/l enhanced plant heights (Table 1) and plant weights (Table 2) by 105-113 and 122-160%, respectively. The rice plants harvested at week 8 exhibited the greatest growth such as plant heights and plant weights as 124.8±5.11 cm and 28.05±1.52, respectively. Regarding the productivity, the rice plant seedlings treated with ZnONPs of 200 mg/l exhibited the greatest number of panicles per clump when compared with other concentrations, and at 8-week-old rice plant seedling showed the number of panicles per clump at 14.0±1.00 (Table 3). This concentration was greater than those of untreated rice plants. Nanomaterials penetrated into the plant cell through the hydrophilic pathway of the polar aqueous pores, and entered to the plant cells through the vascular or stomatal system. This process

Table 1. The *indica* rice plants height (cm) in various concentrations of ZnONPs

ZnONPs (mg/l)	Time (weeks)			
	2	4	6	8
0	74.4±3.04b	101.1±2.88b	101.2±6.22b	116.4±3.50b
200	82.2±3.05a	105.6±2.07a	114.3±5.51a	124.8±5.11a
400	96.7±2.72b	96.0±5.87b	98.9±3.39b	112.4±3.36b
800	65.1±2.19c	80.2±5.35c	87.7±7.46c	103.4±5.54c

The characters indicate significant differences between the coefficients (P<0.05).

Table 2. The *indica* rice plants weight (g) in various concentrations of ZnONPs

ZnONPs (mg/l)	Time (weeks)			
	2	4	6	8
0	2.75±0.27b	8.13±0.38b	11.5±0.69b	19.51±1.69b
200	3.39±0.15a	12.51±0.67a	18.45±1.31a	28.05±1.52a
400	2.29±0.13b	7.25±0.72c	11.02±0.75b	18.72±1.07b
800	1.70±0.45c	6.72±0.66c	10.21±0.87c	14.61±1.79c

The characters indicate significant differences between the coefficients ($P<0.05$).

Table 3. The number of panicles per clump in various concentrations of ZnONPs

ZnONPs (mg/l)	Time (weeks)			
	2	4	6	8
0	6.20±0.83b	7.00±0.70b	7.60±0.54b	9.40±1.14b
200	8.80±1.78a	11.60±1.14a	12.40±1.34a	14.0±1.00a
400	4.80±0.83b	6.00±0.71b	6.80±0.83b	8.80±1.30b
800	3.20±0.83c	4.60±0.54c	6.20±0.84b	6.80±0.83c

The characters indicate significant differences between the coefficients ($P<0.05$).

depended on the size of nanoparticles (García-López *et al.*, 2019). Many researchers reported that high concentrations of metal nanoparticles led to toxicity responses, while the application of suitable concentrations of nanoparticles showed affectivity in mitigating abiotic and biotic stresses by enhancing plant development and productivity by stimulating the antioxidant enzyme activities (Alabdallaha and Alzahrani, 2020; Song *et al.*, 2021). Kumar *et al.* (2021) reported that photosynthetic pigments produced by the rice plants were highly susceptible to stress. When the rice plants were treated with low toxic nanomaterials such as ZnONPs, pigments necessary for photosynthesis increased, and thus stimulated the rice plant growth. However, the high concentration of ZnONPs resulted in a reduced growth response. Therefore, the appropriate concentration of ZnONPs was crucial for enhancing growth of the rice plants. Madhusudanan *et al.* (2019) investigated the effect of ZnONPs synthesized through sol-gel method on growth and yield of rice (*Oryza sativa* L.), and they reported that the application of ZnONPs at relatively lower doses showed positive effects on rice crop and significantly increased grain zinc concentration. ZnONPs improved seed germination (44.0%), seedling growth (16.9%), root length (26.8%), shoot length (15.2%), chlorophyll content (10.7%) and grain zinc concentration (8.0%). In addition, ZnONP was a stimulant for callus induction and secondary metabolite production in *indica* rice. Chutipaijit and Sutjaritvorakul (2020) demonstrated the effect of ZnO and TiO_2 nanoparticles on callus formation and metabolism of *indica* rice cv. KDML105 in callus induction medium. These two nanoparticles induced callus, and thus led to an increase in biomass and total flavonoid contents.

In regression analysis, the multiple linear regression equation of the relationship between the ZnONPs concentration (Zn : mg/l) and time (t : weeks) on the rice plant height with the ZnONPs application was :

$$y = 15.83 + 0.9232\, t - 0.02217\, Zn,$$

whereas the rice plant weight was :

$$y = - 25.9955 + 0.4087\, t - 0.0051\, Zn$$

Both of the linear regression models were not only obtaining equations but also a coefficient of determination, and determination coefficients of both linear models were 86.28% (R^2= 0.86) and 83.88% (R^2= 0.83) as shown in Figs. 1 and 2. The productivity of rice plants was evaluated and the results were expressed by the number of panicles per clump, and the relationship between the ZnONPs concentration and time on the rice plant productivity was $y = 0.7025 + 0.0925\, t - 0.00494\, Zn$ with $R^2 = 0.52$ (52.03%) (Fig. 3).

The result of equations from three linear regression models was applied to obtain the

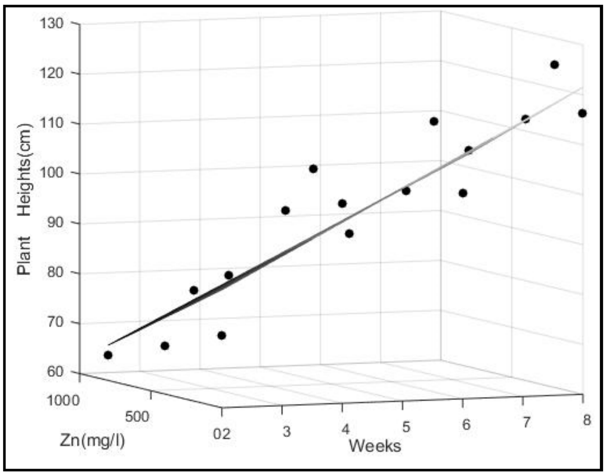

Fig. 1. Regression model of the *indica* rice plants height in various concentrations of ZnONPs, R^2 = 0.86.

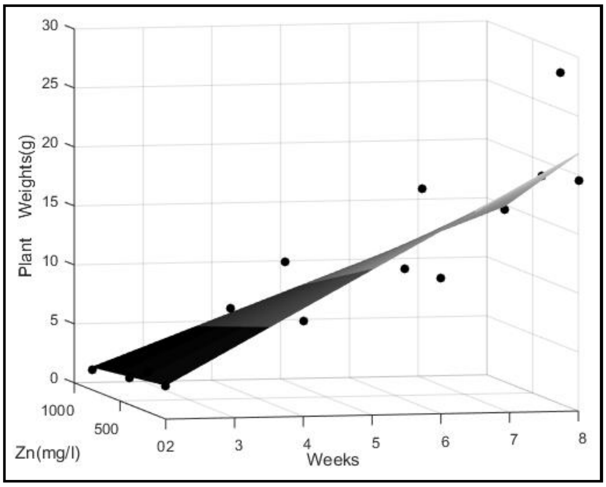

Fig. 2. Regression model of the *indica* rice plants weight in various concentrations of ZnONPs, R^2 = 0.84.

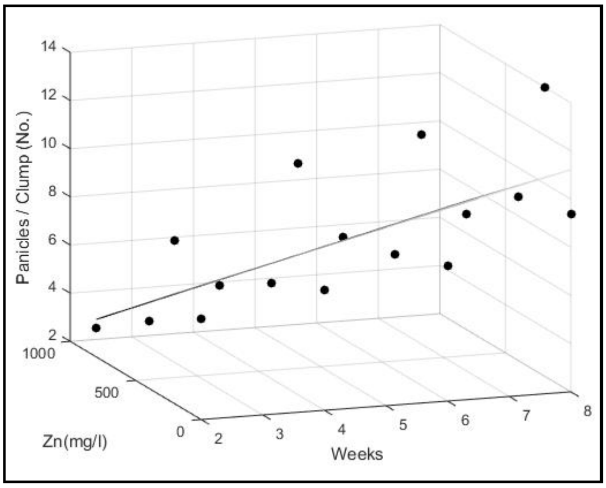

Fig. 3. Regression model of the number of panicles per clump in various concentrations of ZnONPs, R^2 = 0.52.

appropriate prediction for rice treated with ZnONPs cultivation. The actual growth parameters were plugged in the equation and the result estimated growth parameter. Rizkiana *et al.* (2019) studied a mathematical model to predict the vegetative growth of chilli (*Capsicum frutescens*) in an industrial scale. After planting, the height was measured daily for 30 days to obtain the true height (cm). The data were used to develop mathematical models by using linear and polynomial models to obtain the appropriate predictions, and the models were validated and evaluated by using Root Mean Squared Error (RMSE) and Mean Absolute Percentage Error (MAPE). The coefficient (R^2) of the linear model was 0.9667 with error rate of 1.68%. Moreover, the mathematical models predicted growth and also used to predict the accumulation of trace elements in economic crops. Ebrahem *et al.* (2019) developed the regression model for the determination of trace elements including aluminum (Al), copper (Cu), lead (Pb) and zinc (Zn) in faba beans (*Faba sativa* Bernh.) which were safe for human consumption.

CONCLUSION

The rice plant exposed to ZnONPs by treating with 200 mg/l induced the highest enhancement of plant height, weight and the number of panicles per clump with a significant difference. The mathematical model of the height, weight and the number of panicles per clump was analyzed by linear regression, and the determination coefficient (R^2) of the linear regression model was 0.86, 0.84 and 0.52, respectively. This regression model could be potentially extended to the rice plant cultivation process in practical applications to predict the rice plant growth and productivity in commercial cultivation.

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