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Effect of Biostimulant Formulation of *Centella asiatica* (L.) Urb. Crude Terpenoid Extract with Addition of Micronutrients on the Growth and Yield of Upland Rice (*Oryza sativa* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. Author MY performed the field experiment, statistical analysis and wrote the first draft of the manuscript. Authors Mansyurdin and ZAN reviewed the data analyses and results discussion. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To analyze the effect of biostimulant formulation of *Centella asiatica* terpenoid extract with the addition of micronutrients on the growth and yield of upland rice (*Oryza sativa* L.).
Study Design: Experimental design.
Place and Duration of Study: Laboratory of Plant Physiology, Department of Biology, Andalas University, in agricultural land, Limau Manis, Padang City, Indonesia from January until April 2021
Methodology: The experiment was laid out in a factorial randomized block design having nine treatments and replicated thrice. The first factor was the concentration of the terpenoid extract: a_0 (0 mg/L); a_1 (0.25 mg/L); a_2 (0.5 mg/L). The second factor is the composition of the solution of micronutrients: b_0 (without micronutrients); b_1 ($FeSO_4$ 0,01875%, $MnSO_4$ 0,01562%, $ZnSO_4$ 0,01562%, $CuSO_4$ 0,00312%, H_3BO_3 0,00937%); b_2 ($FeSO_4$ 0,0375%, $MnSO_4$ 0,03125%, $ZnSO_4$ 0,03125%, $CuSO_4$ 0,00625%, H_3BO_3 0,01875%).
Results: The results showed that the biostimulant formulation of terpenoid extract with the addition of micronutrients affected some parameters of growth and yield of upland rice. The highest average number of tillers was 23.00, it was obtained in treatment a_2b_2. Treatment a_1b_2 gave a high average

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dry weight of 100 grains, namely 2.47g. The dry weight of 100 grains was positively correlated (r = 0.702) with the number of tillers, plant height and chlorophyll b with respectively contributions of 9.36%, 7.35% and 3.82%.
Conclusion: The biostimulant formulation of *C. asiatica* crude terpenoid extract with addition of micronutrients was able to increase the number of tillers and dry weight of 100 grains of upland rice.

Keywords: Bio stimulant; Terpenoid extract; Centella asiatica; micronutrients; Oryza sativa.

1. INTRODUCTION

Biostimulants are natural substances or microorganisms that can increase the efficiency of nutrient absorption, tolerance to stress [1], stimulate growth and improve the quality of crop yields [2]. Sources of biostimulants include fulvic acid [3], humic acid [4], protein hydrolysates [5], amino acids [6], and seaweed extract [7]. One of the plant bioactive compounds that have the potential to be developed as a biostimulant is the terpene group. Most of the terpene compounds produced by *Centella asiatica* (L.) Urban (Umbelliferae) are known as medicinal herb [8]. *C. asiatica* has four bioactive components, namely asiatic acid, asiaticoside, madecassic acid, and madecassoside [9].

Application of crude extract of *C. asiatica* by spraying onto leaves at a concentration of 25 mg/l could increase the height and leaf area of soybean plants [10]. Application of crude extract of *C. asiatica* at a concentration of 100 mg/L was able to increase the growth of upland rice [11], and at a concentration of 25 mg/L could increase plant height, number of leaves and fresh weight of corn [12]. According [13], secondary metabolites from plants have potential as biostimulants.

Optimization of biostimulants from natural ingredients that are applied to leaves requires the addition of micronutrients. Several research results show the positive effect of the application of plant extracts with the addition of micronutrients. The application of seaweed extract with the addition of micronutrients (Fe, Zn, mg, B, Mo and Mn) was able to increase the chlorophyll content and the number of flowers of *Dianthus chinensis* and *Ganzania splendern* [14]. Jannah et al. [15] reported that the foliar application of the terpenoid extract of *C. asiatica* with the addition of micronutrients (Zn, Cu, B, Fe and B) was able to increase the growth and yield of maize.

Regarding the potential of *C. asiatica* as a biostimulant, terpenoid extracts and micronutrients have been formulated with the addition of micronutrient to increase the growth and yield of upland rice. It is necessary to develop rice plants which are important food commodities in Indonesia after wheat and corn. The rice harvested area in Indonesia is estimated at 10.66 million hectares or decreased by 20.61 thousand hectares (0.19 percent) compared to 2019 [16]. The decrease in harvested area was caused by the declining interest of farmers in planting upland rice, due to the unavailability of effective fertilizers to increase crop yields

2. MATERIALS AND METHODS

2.1 Experimental Design

The research was carried out on agricultural land at Limau Manis Village, Pauh District, Padang City, Indonesia. The altitude of the study site was ± 246 m above sea level, soil type is latosol. The soil temperature ranges from 27.9-31.9°C (at a depth of 5 cm), pH 6.08, and soil nutrient content are listed in Table 1.

Table 1. The content of soil nutrients in the experimental land

Sample	Content
N-total (%)	0,640%
P-available	25.414 ppm
K	0.297 me/100gr
Na	0.176 me/100gr
Ca	0.707 me/100gr
Mg	0.347 me/100gr
Al	5.933 me/100gr
Co	2.039%
BO	3.516%
C/N	3.186%
Fe	59.279 ppm
Cu	56.073 ppm
Zn	57.077 ppm
Mn	44.540 ppm
B	17.429 ppm

Source: [14]

The experiment was laid out in a factorial randomized block design having nine treatment

and replicated thrice. Each replication has a size of 75 x 75 cm with a planting distance of 25x25 cm. The first factor was the concentration of the terpenoid extract: a_0 (0 mg/L); a_1 (0.25 mg/L); a_2 (0.5 mg/L). The second factor is the composition of the solution of micronutrients: b_0 (without micronutrients); b_1 ($FeSO_4$ 0,01875%, $MnSO_4$ 0,01562%, $ZnSO_4$ 0,01562%, $CuSO_4$ 0,00312%, H_3BO_3 0,00937%); b_2 ($FeSO_4$ 0,0375%, $MnSO_4$ 0,03125%, $ZnSO_4$ 0,03125%, $CuSO_4$ 0,00625%, H_3BO_3 0,01875%).

2.2 Preparation of Terpenoid Extract

Crude extracts from whole organs of *C. asiatica* were provided and macerated with methanol [17]. The phenolic compounds in the crude extract were extracted with activated charcoal to obtain a terpenoid extract [18]. The terpenoid extract was evaporated in a vacuum to obtain a powder. The concentration of the extract was made by dissolving the in water with adding dimethyl sulfoxide to facilitate dissolution [10].

2.3 Planting Upland Rice

Experimental plants using a variety Inpago UNSOED 1 obtained from the Cereal Research Institute, Makassar, Indonesia. Fertilization was adjusted to the recommended dose for upland rice which consisted of 150 kg/ha urea, 135 kg/ha TSP and 60 kg/ha KCL. TSP and KCL were given at the time of sowing. Urea was applied three times at 10, 35 and 55 days after planting, respectively. Treatment of terpenoid extracts with the addition of micronutrients (25 ml per plant) was sprayed onto the leaf surface at 14 days after planting [11].

Parameters observed in this experiment included growth and yield of upland rice. Growth parameters included plant height, number of tillers, wet and dry weight of roots, wet and dry weight of shoot, and chlorophyll content. Yield parameters include number of productive tillers, weight of grain per panicle and dry weight of 100 grains.

2.4 Statistical Analysis

All data on growth parameters and yields were analyzed using analysis of variance (ANOVA) with the SPSS 23.0 program, if there was a significant difference followed by Duncan's significant difference test (DNMRT) at the 5% level. Multiple regression analysis was performed between the independent variables of growth parameters (plant height, number of tillers, chlorophyll a, chlorophyll b, total chlorophyll, wet weight of the shoots, wet weight of roots, dry weight of shoots, and dry weight of roots) with the independent variable being yield parameters (dry weight of 100 grains).

3. RESULTS AND DISCUSSION

The biostimulant formulation of terpenoid extract with the addition of micronutrients affected the number of tillers, wet and dry weight of shoots, and dry weight of roots. The highest average number of tillers was 23.00, it was obtained in treatment a_2b_2, a combination of 0.5 mg/L terpenoid extract with the addition of $FeSO_4$ 0.0375%, $MnSO_4$ 0.03125%, $ZnSO_4$ 0.03125%, $CuSO_4$ 0.00625%, H_3BO_3 0.01875%, while the number of tillers in the control is only 9.00 (Table 2). However, the highest wet weight and dry weight of shoots were obtained in the a1b0 treatment (0.25 mg/L terpenoid extract without micronutrients), which were 80.81g and 19.43g, respectively. As has been reported in previous research (Z Zakiah, Andalas University, Indonesia, Postgraduate Dissertation) that *C. asiatica* terpenoid extract can act as a biostimulant with physiological effects increasing the biosynthesis of gibberellin and auxin. Application of crude extract of C. asiatica at concentration 100 mg/L showed higher plant height with average 79.30 cm than in control 69.60 cm (11). These physiological effects can be enhanced by the addition of micronutrients. According to [19], Zn is required for early growth and development, and Cu acts as an enzyme activator and affects nitrogen metabolism. Meghana et al. [20] reported that the use of micronutrients Fe, Cu, Zn, B and Mn had a significant effect in increasing growth height and number of tillers in rice.

The increase in the number of tillers was in line with the increase in the chlorophyll content of the plant. The highest average chlorophyll content was obtained in the a_2b_2 combination treatment, which was 2.88 mg/l (Table 3). The increase in the number of tillers may be related to the effect of increasing the chlorophyll content. According to [19], Fe plays an important role in the synthesis of chlorophyll and Mn is needed for photosynthesis. Kandoliya et al. [21] reported that the application of micronutrients Fe and Zn was able to increase the content of chlorophyll a, chlorophyll b and total chlorophyll in wheat plants.

Table 2. The effect of terpenoid extract and micronutrients on growth of upland rice

Treatments	Height of plant (cm)	Number of tillers	Wet weight of roots (g)	Wet weight of shoots (g)	Dry weight of roots (g)	Dry weight of shoots (g)
a0b0	54.24[a]	9.00[a]	15.68[a]	28.17[a]	4.08[a]	6.70[a]
a0b1	59.02[a]	22.22[b]	19.23[a]	47.70[bc]	5.58[ab]	11.51[ab]
a0b2	65.42[a]	17.67[b]	21.28[a]	49.99[c]	5.39[ab]	11.52 [ab]
a1b0	54.83[a]	16.11[ab]	29.88[a]	80.81[d]	9.38[c]	19.43[c]
a1b1	57.61[a]	18.00[ab]	20.23[a]	50.98[c]	6.69[abc]	11.48[ab]
a1b2	61.20[a]	15.78[ab]	23.18[a]	58.93[c]	7.39[bc]	12.97[b]
a2b0	54.83[a]	15.33[ab]	14.94[a]	44.68[abc]	3.66[a]	10.39[ab]
a2b1	62.89[a]	15.88[ab]	20.59[a]	31.78[ab]	3.76[a]	7.34[a]
a2b2	67.50[a]	23.00[b]	21.41[a]	51.12[c]	4.85[ab]	15.05[bc]

Table 3. The effect of terpenoid extract and micronutrients on growth of upland rice

Treatments	chlorophyll a (mg/l)	chlorophyll b (mg/l)	Total chlorophyll (mg/l)
a0b0	0.95[ab]	1.21[ab]	2.16[ab]
a0b1	1.08[b]	1.55[b]	2.63[ab]
a0b2	0.91[ab]	1.41[b]	2.20[ab]
a1b0	1.07[b]	1.54[b]	2.61[ab]
a1b1	1.05[b]	0.97[a]	2.09[ab]
a1b2	1.09[b]	1.61[b]	2.70[ab]
a2b0	0.70[a]	0.90[a]	1.56[a]
a2b1	0.91[ab]	1.12[ab]	2.12[ab]
a2b2	0.83[ab]	1.26[ab]	2.88[b]

The application of terpenoid extract with the addition of micronutrients affects the yield of upland rice. Treatment a_1b_2, a combination of terpenoid extract 0.25 mg/L with the addition of $FeSO_4$ 0.01875 %, $MnSO_4$ 0.01562 %, $ZnSO_4$ 0.01562 %, $CuSO_4$ 0.00312%, H_3BO_3 0.00937% gave a high average dry weight of 100 grains, namely 2.47g, while the control was 1.24g (Table 4). Micronutrients play a role in increasing crop productivity and grain yields as a result of improving plant enzymatic systems [22]. Suman and Sheeja [23] reported that the foliar application of Zn micronutrients in rice played a role in increasing growth and yield of rice. According to [24], foliar application of micronutrients for Zn was able to increase rice grain yields. The same finding was also reported by [25] that foliar application of micronutrients (Fe, Zn, B, Cu, and Mn) could increase the number of productive tillers and the weight of 1,000 grains. The combination of *C. asiatica* terpenoid extract and micronutrients affected the yield of corn, where the highest weight of 100 grains and the highest grains weight per cob were obtained at combination of 0.25 mg/L terpenoid extract with addition of 0.0625% $ZnSO_4$; 0.075% $FeSO_4$; 0.0375% H3BO3; 0.0625% $MnSO_4$; 0.0125% $CuSO_4$ (15).

Table 4. Effect of Terpenoid Extract and Micronutrients on Yield of Upland Rice

Treatments	Number of productive tillers	Weight of grain per clump (g)	Dry weight 100 grains (g)
a0b0	8.05^{a}	6.38^{ab}	1.24^{a}
a0b1	15.33^{c}	15.33^{c}	1.93^{b}
a0b2	12.77^{bc}	14.31^{c}	2.43^{b}
a1b0	10.21^{ab}	16.28^{c}	2.33^{b}
a1b1	9.10^{a}	4.52^{a}	2.05^{b}
a1b2	13.11^{bc}	11.15^{bc}	2.47^{b}
a2b0	9.66^{ab}	15.77^{c}	2.08^{b}
a2b1	11.00^{ab}	13.15^{c}	2.29^{b}
a2b2	12.99^{bc}	10.90^{bc}	2.25^{b}

The results of multivariate regression analysis showed that the dry weight of 100 grains (Y) was significantly positively correlated (r = 0.521) with number of tillers with contributions of 11.77% (Table 5). The formulation of the multiple linear regression equation is formulated as follows:

$$Y = 0.806 + 0.314X_1 + 0.178X_2 - 0.395X_3 + 0.244X_4 + 0.185X_5 + 0.443X_6 - 0.080X_7 - 0.485X_8 + 0.303X_9$$

Description:

Y = dray weight of 100 grains
X_1 = plant height
X_2 = total number of tillers
X_3 = chlorophyll a
X_4 = chlorophyll b
X_5 = total chlorophyll
X_6 = wet weight of the shoots
X_7 = wet weight of roots
X_8 = dry weight of shoots
X_9 = dry weight of roots

Table 5. Correlation between Weight of 100 Grains and Growth Parameters of Upland Rice

	Y	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9
Y	1	0.375	0.382*	0.130	0.242	0.235	0.277	0.149	0.265	0.172
X_1		1	0.578**	0.175	0.239	0.406*	0.113	0.011	0.207	-0.179
X_2			1	0.164	0.153	0.258	0.233	0.090	0.223	0.049
X_3				1	0.734**	0.554**	0.138	-0.013	0.010	0.336
X_4					1	0.746**	0.202	-0.050	0.229	0.218
X_5						1	0.107	-0.221	0.333	0.036
X_6							1	0.562**	0.893**	0.728*
X_7								1	0.441*	0.733**
X_8									1	0.566**
X_9										1

*Description: Y = weight of 100 grains; X_1 = plant height; X_2 = number of tillers; X_3 = chlorophyll a; X_4 = chlorophyll b; X_5 = total chlorophyll; X_6 = Wet weight of the shoots; X_7 = Wet weight of roots; X_8= dry weight of shoots; X_9 = dry weight of roots * Significant correlation at 0.1 level; ** Significant correlation at 0.05 level*

4. CONCLUSION

The biostimulant formulation of terpenoid extract with the addition of micronutrients affected growth parameters include the number of tillers, wet and dry weight of shoots, and dry weight of roots. The formulation affects the yield component of upland rice, namely the average dry weight of 100 grains. The dry weight of 100 grains was significantly positively correlated with number of tillers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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