Influence Of Micronutrients And Terpenoid Extract Of Centella Asiatica Applications On Growth And Yield Of Corn Var. Lamuru

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Abstract : Influence of micronutrients and terpenoid extract of Centella asiatica applications on growth and yield of corn var. Lamuru was expected to be the first step in optimizing yield and field-scale corn production. This study aims to evaluated the effective concentration of terpenoid extracts and micronutrients in increasing growth and yield of corn and to determine the most effective morphological traits in increasing grain yield of corn. This study was carried out in field conditions used Randomized Factorial Block Design with two factors. The first factor was terpenoid extract concentration: (E_0 without extract); (E_1 0.25 mg/L); (E_2 0.5 mg/L). While the second factor was the concentration of micronutrients: (M_0 without micronutrients); (M_1 micronutrients 0.25%); (M_2 micronutrients 0.5%). The results showed that the E_1M_2 treatment was the most effective in increasing total leaf area, plant height, cob diameter, weight of 100 grains and grain weight per cob, 2765.95 cm^2, 212.63 cm, 58.40 mm, 41.17 g and 240.01 g respectively, while control 2459.48 cm^2, 183.60 cm, 46.70 g, 35.97 g, 169.88 g respectively. The weight of 100 grains was significantly positively correlated (r = 0.839) with total leaf area, root dry weight with the contribution of 78.873% and 30.138% respectively. Grain weight per cob was significantly positively correlated (r = 0.594) with stem diameter and root dry weight with contributions of 69.687% and 34.99%, respectively. The results can be concluded that the effect of Centella terpenoids extract on growth and corn yields can be optimized by the addition of micronutrients.

Index Terms : terpenoid extract, Centella asiatica, micronutrients, corn.

1. INTRODUCTION

Biostimulant is natural or microbial material that is applied to plants, seeds or soil in small doses will be able to improve nutrient efficiency (Du Jardin, 2012), growth, development, yield quality and plant tolerance to stress (Du Jardin, 2015). Biostimulant sources includes fulvic acid, humic acid (Canellas et al., 2015), protein hydrolyzate and other compounds containing N (Colla et al., 2015), Trichoderma sp. (benefit fungi) (Lopez-Bucio et al., 2015), rhizobacteria (benefit bacteria) (Nguyen et al., 2018), seaweed extract (Layek et al., 2015) and Moringa oleifera leaf extract (Rana, Tahsina and Anwarul, 2019). Beside that, secondary metabolites also reported as biostimulants including flavonoids (Prabhu et al., 2010), alkaloids (Aniszewski, 2007), triterpenoid saponins (Andresen and Cedergreen, 2010) and Centella asiatica terpenoids (Zakiah et al., 2017). The terpenoid extract of C. asiatica at 0.25 mg / L was able to increase the leaf area, number of leaves, plant height, shoot dry weight, roots and total dry weight, dry weight of grains per plant, number of pods and pod dry weight of soybean. The physiological effects of C. asiatica terpenoid extracts were increasing endogenous of gibberellins and auxins, and chlorophyll b of soybean (Zakiah, 2017). The optimization of biostimulants is generally made in formula form with micronutrients.

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Moringa leaf extract formulation with the addition of micronutrients was able to increased corn yield (Shehu and Okafor, 2017), seaweed extract with the addition of micronutrients was able to increased the growth and yield of potatoes (Al-Juthery et al., 2018). Therefore, to optimizing the effect of terpenoids with addition of micronutrients is requires to be applied to corn, which is included as the second biggest food commodity in Indonesia. National corn production has not fulfilled consumption necessity, so the government imports 732.2 thousand tons in 2018 (BKP, Ministry of Agriculture, 2018). The application of micronutrient and Centella terpenoids extracts is expected to increase corn production, thus it can be the first step in reducing corn imports.

2. MATERIALS AND METHODS

The study was conducted in the Limau Manis farmland, Pauh, Padang from August to November 2018. The altitude of the study site was ± 246 m above sea level. Soil analysis was performed in the 0-10 cm layer, the type of soil was Latosol with the following charactheristic of nutrient content in Table 1.

Table 1. *Soil chemical properties of farmland*

Soil properties	Value
pH	6.08
N-total (%)	0.640
P-available (ppm)	25.414
K (me/100gr)	0.297
Na (me/100gr)	0.176
Ca (me/100gr)	0.707
Mg (me/100gr)	0.347
Al (me/100gr)	5.933
CO (%)	2.039
BO (%)	3.516
C/N (%)	3.186
Fe (ppm)	59.279
Cu (ppm)	56.073
Zn (ppm)	57.077
Mn (ppm)	44.540
B (ppm)	17.429

The study was arranged in a factorial randomized block design with two treatment factors with three groups and 9 plots of each one. Each plot measures 100x75 cm with a distance between plots of 50 cm and a distance between groups of 100 cm. The first factor was the concentration of Centella terpenoid extract: (E_0 : 0 mg/L); (E_1 : 0.25 mg/L); (E_2 : 0.5 mg/L). While the second factor was the concentration of micronutrient: (M_0 : without micronutrients); (M_1 : 0.0625% $ZnSO_4$; 0.075% $FeSO_4$; 0.0375% H_3BO_3; 0.0625% $MnSO_4$; 0.0125% $CuSO_4$); (M_2 : 0.125% $ZnSO_4$; 0.15% $FeSO_4$; 0.075% H_3BO_3; 0.125% $MnSO_4$; 0.025% $CuSO_4$). There were 9 treatment combinations namely E_0M_0 (control); E_0M_1; E_0M_2; E_1M_0; E_1M_1; E_1M_2; E_2M_0; E_2M_1 and E_2M_2.

Methods of Research

Preparation of terpenoid extracts : procedures for preparation crude extracts were provided from all plant organs and macerated with methanol (Singh et al., 2012). Terpenoid extract was purified with activated charcoal (Mora and Fernando, 2012). Then, the purified filtrate was evaporated in vacuo. Extract dilution for concentration treatment was dissolved in distilled water and dimethyl sulfoxide (DMSO) (Zakiah, 2017). Preparation of micronutrients : Zn micronutrient was provided in the form of $ZnSO_4.7\ H_2O$, Fe was provided in the form of $FeSO_4.7\ H_2O$, Mn was provided in the form of $MnSO_4.H_2O$, Cu was provided in the form of $CuSO_4.5H_2O$, and B was provided in the form of H_3BO_3. Each micronutrient was provided as a stock solution. Micronutrient dilution for treatment was dissolved with 1-L distilled water. Planting and application of biostimulant formulas : the corn seed of Lamuru varieties were obtained from the Cereals Research Center, Makassar Indonesia. Corn seeds were dibble 3 seeds per hole with a depth of 3-5 cm, and the distance between seeds/holes 20x75 cm. Fertilizer recommendation viz, Urea (200 kg/ha) and Muriate of Potash (MOP 50 kg/ha) were added to the experiment soil plots. MOP was added to each plot at the 7 DAP, while urea was applied two time at the 7 and 35 DAP (Liptan, 2005). Foliar spray of biostimulant formulas applied at 15 DAP (25 ml per plant) (Zakiah et al., 2017).

Measurement of growth and yield parameters

Growth parameters such as plant height, number and total leaf area measured every week until 49 DAP. Fresh and dry weight of plant were measured at 49 DAP. Yield parameters include the length, weight and diameter of the cob, weight of 100 grains and the weight of the grains per cob measured when the corn at 92 DAP. Statistical analysis : Data (growth parameter and yield) were statistically analyzed using SPSS 23.0 and mean comparison was performed within treatments using Duncan Multiple Range Test at 5% significant level. Multiple regression analysis was performed between the independent variables of growth parameters (total leaf area, height, stem diameter, fresh weight and dry weight of the plant) with the independent variables in the form of yield parameters (weight of grains per cob and weight of 100 grains).

3. RESULT AND DISCUSSION

Application of micronutrients and terpenoid extract of Centella significantly different on plant height, stem diameter, total leaf area, root, and shoot fresh weight, root dry weight, shoot and total dry weight. The highest average plant height and total leaf area were obtained in the E_1M_2 treatment (combination of Centella terpenoid extract 0.25 mg / L and 0.5% micronutrients) 212.63 cm and 2765.68 cm2, while the largest stem diameter (27.73 mm) was obtained in the E_1M_0 treatment (extract terpenoids 0.25 mg / L). The largest average shoot fresh weight (575.42 g) was obtained in the E_1M_1 treatment (combination of Centella terpenoids extract 0.25 mg / L and 0.25% micronutrients), while the average root fresh weight, shoot dry weight, roots, and the largest total dry weight was obtained in the treatment E_1M_0 were 98.43 g, 24.93 g, 115.52 g, and 140.45 g, respectively (Table 2). Plant height and total leaf area were increased with the treatment of Centella terpenoids extract and micronutrient. Terpenoids extract played a role in physiological processes through increasing GA3 and endogenous auxin (Zakiah, 2017). Furthermore, foliar applications with micronutrients (Zn, Mn, and Fe) in corn increased plant height and yield (Ghazvineh and Yousefi, 2012). Micronutrients (Mn, Cu, Fe, Zn and B) played a role in increasing the total chlorophyll content of corn (Dimkpa and Bindraban, 2016). The application of Mn in corn plants increased the shoot fresh weight, photosynthesis rate, and chlorophyll content, thereby it increasing the grain yield (Nozulaidi et al., 2016). Wasaya et al. (2017) reported that the foliar application of combination Zn and B was effective in increasing the fresh weight of plants and assisting in increasing chlorophyll content and total leaf area. Figure 1. shows that the total chlorophyll content tends to increase combination with micronutrients. Root fresh weight, root and shoot dry weight, and total dry weight treated with Centella terpenoid extract alone (E_1M_0) showed significant effect compared to the combination of Centella terpenoid extract and micronutrients (E_1M_2) (Table 2). This indicates that micronutrient elements inhibiting the growth of corn. Gholami et al. (2014) reported that the application of the element Cu can reduce root dry weight and biological yields of corn. Therefore, the element Cu should be considered not to be added to the formulation of Centella terpenoids. The combination of Centella terpenoid extract and micronutrients affected the yield of corn (weight and cob diameter, the weight of 100 grains and weight of grains per cob), except for the length of the cob and the number of rows per cob. The highest average of cob fresh weight was obtained in the E_2M_0 treatment (Centella terpenoid extract 0.5 mg / L) that is 309.73 g, while the highest cob diameter was obtained in the E_1M_2 treatment (combination of Centella terpenoid extract 0.25 mg / L and 0.5% micronutrients) was 58.40 mm. The highest weight of 100 grains and the highest grains weight per cob were obtained at 41.17 g and 240.93 g E_1M_2 treatments (Table 3). Based on the results of multivariate regression analysis, it is known that the weight of 100 grains was significantly positively correlated (r = 0.839) with total leaf area and root dry weight (Table 4) with contributions of 78.873% and 30.138% (formula 1). Grain weight per cob was significantly positively correlated (r = 0.594) with stem diameter and root dry weight (Table 5) with contributions of 69.687% and 34.99%, respectively (formula 2). Souza et al. (2015) reported that plant height and the total leaf area of corn hybrid were positively correlated with the weight of 100 grains in corn. Plant height and total dry weight per plant show positive effects on crop yields (Adesoji et al., 2015). Plant height and total dry weight gave the biggest contribution in

increasing crop yields of corn. Root dry weight and total dry weight were contributed significantly to the increase in corn yield (Ali et al., 2014).

The multiple linear regression equation was formulated as:

$Y_1 = - 8.278 + 0.716 X_1 + 0.028X_2 - 0.309 X_3 + 0.396 X_4$ .....(1)

$Y_2 = 95.947 + 0.058 X_1 - 0.155 X_2 + 0.499 X_3 + 0.292 X_4$.....(2)

Note: Y_1 = Weight of 100 grains, Y_2 = Weight of grains per cob, X_1 = Total leaf area, X_2 = Plant height, X_3 = Stem diameter, X_4 = Root dry weight

Table 2: *Effect of Centella terpenoid extract with addition of micronutrient on corn growth*

Treatment	Plant height (cm)	Stem diameter (mm)	Total leaf area (cm^2)	Leaves number	Root fresh weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)	Total dry weight (g)
E_0M_0	183.60^{c}	20.48^{c}	2459.48^{c}	9.67^{ns}	70.96^{b}	471.73^{c}	14.30^{c}	89.32^{b}	103.62^{b}
E_0M_1	190.77^{b}	25.49^{b}	2530.23^{b}	12.00	67.17^{b}	524.25^{b}	19.62^{b}	88.76^{b}	108.38^{b}
E_0M_2	185.80^{bc}	25.29^{b}	2554.95^{b}	11.33	91.23^{b}	521.13^{b}	18.27^{b}	84.16^{b}	102.42^{b}
E_1M_0	205.13^{ab}	27.73^{a}	2623.05^{a}	13.00	98.43^{a}	548.15^{a}	24.93^{a}	115.52^{a}	140.45^{a}
E_1M_1	200.70^{b}	25.35^{b}	2758.28^{a}	12.33	90.89^{b}	575.42^{a}	20.11^{b}	100.01^{b}	120.12^{ab}
E_1M_2	212.63^{a}	27.10^{a}	2765.68^{a}	13.67	91.73^{b}	522.73^{b}	19.96^{b}	91.45^{b}	111.40^{b}
E_2M_0	201.43^{b}	23.80^{b}	2546.96^{b}	11.00	74.60^{b}	491.13^{c}	19.83^{b}	95.36^{b}	115.19^{b}
E_2M_1	186.90^{b}	25.91^{b}	2467.22^{c}	13.33	80.57^{b}	535.33^{a}	19.78^{b}	96.92^{b}	116.70^{b}
E_2M_2	194.93^{b}	24.97^{b}	2368.71^{c}	12.67	88.40^{b}	511.40^{c}	16.49^{b}	91.39^{b}	107.89^{b}

Values with different superscript letters in the same column indicate significantly different (P<0.05) in DNMRT test.

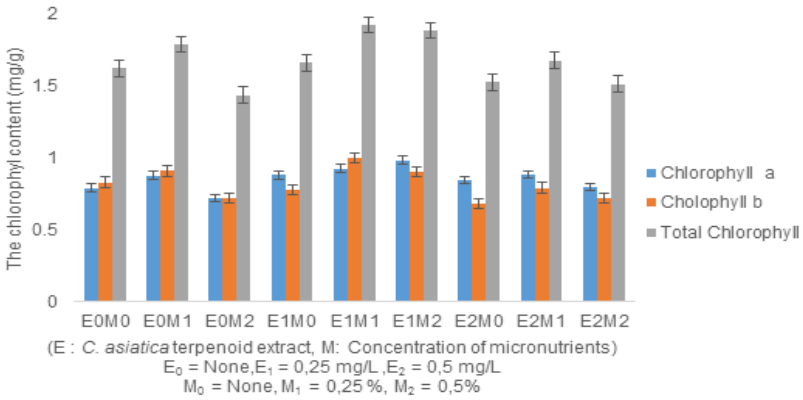

Figure 1. *Chlorophyll a, b and total chlorophyll content of corn (Zea mays) leaves 7 days after application of combination Centella terpenoid extract and micronutrient.*

Table 3: *Effect of Centella terpenoid extracts and micronutrient on corn yield*

Treatment	Lenght of cob (cm)	Fresh weight of cob (g)	Cob diameter (mm)	Weight of 100 grains (g)	Weight of grains per cob (g)	Number of lines per cobs
E_0M_0	18.20^{ns}	228.26^{b}	46.70^{b}	35.97^{b}	169.88^{c}	15.33^{ns}
E_0M_1	22.83	255.43^{b}	58.03^{a}	37.41^{bc}	232.85^{b}	16.67
E_0M_2	22.93	291.10^{a}	56.03^{ab}	35.74^{b}	228.71^{b}	16.67
E_1M_0	21.30	302.11^{a}	57.20^{a}	39.94^{b}	228.46^{b}	16.00
E_1M_1	20.27	299.73^{a}	56.40^{ab}	38.21^{b}	219.58^{b}	16.67
E_1M_2	22.07	306.71^{a}	58.40^{a}	41.17^{a}	240.93^{a}	16.67
E_2M_0	22.13	309.73^{a}	56.77^{ab}	39.31^{b}	239.58^{b}	16.67
E_2M_1	22.43	290.45^{a}	55.90^{b}	34.61^{c}	222.98^{ab}	16.00

Values with different superscript letters in the same column indicate significantly different (P<0.05) in DNMRT test.

Table 4*. Correlation between the weight of 100 grains with corn growth parameters*

	Y_1	X_1	X_3	X_4	X_5	X_6	X_7	X_8	X_9
Y_1	1	0,770**	0,265	0,202	0,090	0,329	0,532**	0,410*	0,169
X_1		1	0,432*	0,440*	0,026	0,236	0,426*	0,306	0,279
X_2			0,501**	0,222	0,160	0,359	0,652**	0,466*	0,398*
X_3			1	0,487*	0,573**	0,291	0,625**	0,403*	0,667**
X_4				1	0,430*	0,327	0,377	0,367	0,465*
X_5					1	0,352	0,571**	0,439*	0,405*
X_6						1	0,586**	0,978**	0,219
X_7							1	0,743**	0,383*
X_8								1	0,281
X_9									1

Table 5*. Correlation between weight of grains per cob with corn growth parameters*

	Y_2	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9
Y_2	1	0,263	0,254	0,572**	0,132	0,306	0,137	0,423*	0,223	0,435
X_1		1	0,594**	0,432*	0,440	0,026	0,236	0,426*	0,306	0,279
X_2			1	0,501**	0,222	0,160	0,359	0,652**	0,466*	0,398*
X_3				1	0,487*	0,573**	0,291	0,625**	0,403*	0,667**
X_4					1	0,430*	0,327	0,377	0,367	0,465*
X_5						1	0,352	0,571**	0,439*	0,405*
X_6							1	0,586**	0,978**	0,219
X_7								1	0,743**	0,383*
X_8									1	0,281
X_9										1

Note: Y_1 = Weight of 100 grains; Y_2 = Weight of grains per cob; X_1 = total leaf area; X_2 = plant height; X_3 = stem diameter; X_4 = shoot fresh weight; X_5 = root fresh
weight; X_6 = shoot dry weight; X_7 = root dry weight; X_8 = total dry weight; X_9 = number of leaves
* indicate significantly different (P<0.05); ** indicate significantly different (P<0.01)

4 CONCLUSION

Application of Centella terpenoid extract and micronutrients was able to optimize growth including plant height, stem diameter, total leaf area, shoot fresh weight, and yields included cob diameter and weight, the weight of 100 grains and weight of grains per cob. The combination of 0.25 mg / L Centella terpenoids and 0.5% micronutrients was most effective in increasing the growth and yield of corn. The weight of 100 grains was significantly positively correlated (r = 0.839) with total leaf area and root dry weight with contributions of 78.873% and 30.138%, respectively. Grain weight per cob was significantly positively correlated (r = 0.594) with stem diameter and root dry weight with contributions of 69.687% and 34.99%, respectively.

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