OPTIMUM CONDITIONS OF USING BIO-EXTRACT FROM URINE WITH VINASSE AND MANURE AFFECTING YIELDS OF MARIGOLD

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Abstract

Factors affecting yields of marigold using fermented bio-extract from urine with vinasse and animal manure were analyzed by response surface methodology with a central composite design. The various concentrations of each bio-extract concentration and volume of bio-extract were investigated. The results revealed that the optimum conditions were bio-extract of 16.38 %v/v and volume of bio-extract of 40.13 mL/plant. Under the optimal conditions, the maximum flower number, flower size and flower fresh weight were found at 7.84 flowers/plant, 5.90 cm and 7.34 g/flower, respectively. The confirmation experiment under the optimal condition showed the flower number of 8.00 flowers/plant, flower size of 5.98 cm and flower fresh weight of 7.38 g/flower. The result was close to the predicted values by linear regression.

Keywords: Optimization, Bio-extract, Marigold

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Introduction

Marigold is an important commercially grown flower crop in Thailand (Kanacharoenpong et al., 2003). Nowadays, marigold has been used for many benefits; a cut flower, dyeing, animal feeding (Department of Agriculture Extension, 2002) and health products (Schalch et al., 2007). Most of the cultivation of marigolds used fertilizer and caused problems with chemical residues and the environment (Yodprech, 2009). The use of marigolds as health product requires chemical-free produced marigolds. As the price of chemical-free marigolds is quite high, farmers have changed the production using chemical fertilizer to organic fertilizer and bio-extract. The production of organic fertilizers and bio-extract contains many sources such as animal manure (Detpiratmongkol & Liphan, 2015), food waste (Thadinjan, 2014), crop weeds (Paekum et al., 2014) and urine (Sreela-or, 2017). The use of organic fertilizer is safe for farmers and consumers health as well as the environment. However, the efficacy of organic fertilizer and bio-extract on the growth and yield of marigold is inferior to those of chemical fertilizer. Therefore, research studies were conducted to develop the production of organic fertilizer and bio-extract that has similar in efficiency to chemical fertilizer. Urine is currently gaining more attention as plant nutrition due to its macronutrient content, easy collection method and large amounts. However, the application of urine alone cannot provide the proper amount of plant nutrients. In order to improve the efficiency of urine, researchers applied fermentation of urine with other raw materials. Karno et al. (2017) applied lactic fermentation and aeration to ferment urine. It was found that after the fermentation, nitrogen and potassium increased. Sreela-or & Tharungsree (2020) have determined the optimum conditions of using fermented urine that affect yields of marigold. Urine was fermented with vinasse and bat feces. After the fermentation of urine, nitrogen, phosphorus, and potassium were increased. Urine can be used relatively safe with plants since bacterial and viral infections are less likely to be transmitted through the urinary tract. The only parasite that can be found in urine is *Schistosoma hematobium*. However, shellfish intermediate hosts for this parasite are

uncommon in Thailand, and no case has been reported in Thailand (Bureau of Environmental Health, 2021). This is also compliant with the World Health Organization (WHO) report that urine poses a minimal risk of infection, especially when it has been stored for an extended period. However, urine should be carefully handled and should not be applied to crops less than one month before they are harvested. This waiting period is significant for crops that are consumed raw. Vinasse is the main final byproduct of ethanol production (Phothilangka et al., 2018) which contains high organic matter content (Nualsri & Sreela-or, 2019). Vinasse also has high potassium, nitrogen, and phosphorous (Tharangsri et al., 2020). The fermented mixtures of urine, vinasse and bat feces provided the highest phosphorus content (Sothearen et al., 2014). The research results found the number of flowers of 6.20 flowers/plant, flower size of 5.70 cm and flower fresh weight of 7.00 g/flower. While the yields of chemical fertilizer produced the of flowers of 7.53 flowers/plant, flower size of 5.92 cm and flower fresh weight of 7.36 g/flower. This research study was thus conducted to develop the production of bio-extract from fermented urine that has similar efficiency to chemical fertilizer, which further developed from the research of Sreela-or & Tharungsree (2020). The oxygen was supplied to the fermentation process to increase the efficiency of microorganisms which is essential for the bio-fermentation process. Cricket manure was added to the production of bio-extract. The cricket manure contained large essential amounts of macronutrients for plant growth, especially nitrogen, phosphorus and potassium (Bukari et al., 2021). The researchers applied knowledge to develop and promote farmers who grow marigolds in organic farming systems.

Materials and Methods

The process of producing the optimization of fermented bio-extract from urine with vinasse and animal manure on yields of marigold in this experiment was shown in the following steps.

1. Preparation of fermented bio-extract from urine with vinasse and animal manure

The urine used in this experiment was obtained from a sample group of 1st to 4th year male students (ages 18-24 years) in the Faculty of Food and Agricultural Technology, Pibulsongkram Rajabhat University. The experiment was tested on 87 students collecting 45 liters of urine at 10 °C for 30 days. Bat feces and cricket manure were contaminated to remove rubble, gravel, silt particles and decayed plant and animal matter. Then contaminant bat feces and cricket manure were dried to the final moisture content of 30% to produce bio-extract from urine with vinasse and animal manure. Then the collected urine was fermented in a 20-liter fermenting bucket according to Sreela-or (2017) method adding 10 liters of urine, 2 liters of vinasse, 0.5 liters of bat feces, and 0.5 liters of cricket manure. The fermentation process was supplied by an air pump at a flow rate of 150 L/min and fermented for 20 days. The raw materials of bio-extract including urine with vinasse, bat feces and cricket manure, were analyzed to find the elements of plant nutrients. The bio-extract solutions were analyzed for nitrogen content by the Kjeldahl method, phosphorus by wet digestion and Atomic Absorption spectroscopy and potassium by Wet digestion and spectrophotometer methods. (Land Development Department, 2010). The chemical composition of raw materials used in the production of fermented bio-extract and bio-extract used in the experiment is shown in Table 1.

Table 1 Chemical composition of urine, vinasse, bat feces, cricket manure and bio-extract

Parameters	Raw materials				Bio-extract
	Urine	Vinasse	Bat feces	Cricket manure	
Nitrogen (mg/L)	2,256.91	649.74	29,441.65	43,587.75	5,098.87
Phosphorus (mg/L)	179.78	179.05	83,005.47	2,4761.19	4,033.71
Potassium (mg/L)	114.33	1,146.54	5,839.01	31,077.26	1,594.19

2. Preparing experimental plants

Plant marigolds into 16-inch diameter clay pots, one seedling in each pot, a totally of 66 pots. Water once a day and add fermented bio-extract according to the experimental design. Apply bio-extract four times on days 14, 21, 28 and 35 respectively, with no use of chemical pesticides and other fertilizers. For the controlled treatment with 15-15-15 fertilizer was applied at 5 grams in each pot four times same as the experimental plants. Root watering was used in the experiment.

3. Experimental Design

Response surface methodology (RSM) was to optimize fermented bio-extracts from urine with vinasse and animal manure on yields of marigold in this experiment. Study the main factors, i.e. bio-extract concentration and volume of bio-extract that can affect the yield of marigolds. Factors of variation corresponds with 5 levels: negative alpha level ($-\alpha$), low level, medium level, high level, and alpha level (α) as shown in Table 2 and a set of experiments to find the optimal ratio for yields of marigold using a central composite design in Table 2. The response was defined as the number of flowers/plant (flower), flower size (cm) and flower fresh weight (g/flower).

Table 2 Experimental variables and levels investigated by central composite design

Code	Variable	Parameter value				
		$-\alpha$	Low	Medium	High	α
X_1	Bio-extract concentration (%v/v)	7.93	10.00	15.00	20.00	22.07
X_2	Volume of bio-extract (mL/plant)	25.86	30.00	40.00	50.00	54.14

4. Recording of experimental results

The data was recorded within 60 days of the experiment. The maximum number of flowers per plant (only the completed flower) with size (cm) and fresh weight (g/flower) was recorded.

5. Data analysis

The central composite design (CCD, expert 7) method was used to determine the recording of the yield of marigolds. A second-order polynomial model is used to

describe the effects of key variables factors on a response based on experimental results from a CCD. In order to predict the optimal condition, the quadratic polynomial equation was fitted to correlate between variables, the response (i.e., flower number, flower size, flower fresh weight and estimated responses as the following equation (Eq. 1):

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i \, X_i + \sum_{i=1}^{k} \beta_{ii} \, X_i^2 + \sum_{i<j}^{k} \beta_{ij} \, X_{ij} \quad (1)$$

where Y is the response, β_0 is the constant and β_i is the linear coefficient, β_{ii} is quadratic coefficient, β_{ij} is the interactive coefficient and X_{ij} is the coded factor level.

6. Confirmation Experiment

The confirmation experiment was found after obtaining the optimal response values for the number of flowers per plant, flower size and flower fresh weight. The experiment was repeated in 6 conditions: optimal level, high level, medium level, low level, chemical fertilized 15-15-15 formula, and unfertilized. Each condition was replicated 3 times.

Results

The effects of key factors, i.e. bio-extract concentration (X_1), and volume of bio-extract (X_2) on the yield of marigold in the number of flowers per plant (Y_1), flower size (Y_2) and fresh weight (Y_3). Regression analysis of the data from Table 3 resulted in the quadratic (Eq. 2-4) as follows:

$$Y_1 = 7.80+0.46X_1+0.26X_2-0.34X_1X_2-1.15X_1^2-1.48X_2^2 \quad (2)$$

$$Y_2 = 5.84+0.33X_1+0.07X_2-0.35X_1X_2-0.47X_1^2-0.60X_2^2 \quad (3)$$

$$Y_3 = 7.30+0.24X_1+0.064X_2-0.29X_1X_2-0.36X_1^2-0.46X_2^2 \quad (4)$$

Statistical analysis of differential equations represented a relationship between the predictor variable and the response variable for the use of bio-extract from urine with vinasse and animal manure on marigold yield in number of flowers per plant, flower size and fresh weight at confidence intervals for 95 % (Table 4). Response surface methodology and central composite design were applied. The R^2

values were 97.78, 99.27 and 99.15 respectively and the design of experiments was appropriate with the model used in this experiment. According to the lack of fit in the model indicated that it was not fit the experimental data, with no statistically significant difference (P=0.5820, 0.0625 and 0.1150, respectively). Consider the concentration of fermented bio-extract (percent) and the amount of fermented bio-extract feeding (mL/plant), which is the main factor affecting the yield of marigold in number of flowers per plant, flower size and fresh weight with a significant level of 95%

Table 3 Central composite experimental design matrix defining bio-extract concentration (X_1) and volume of bio-extract (X_2) on yields of Marigold

Run	X_1 (Code)	X_1 (Observed)	X_2 (Code)	X_2 (Observed)	Flower number (flowers/plant)	Flower size (cm)	Flower fresh weight (g/flower)
1	0.000	15.00	0.000	40.00	8.00	5.81	7.29
2	-1.000	10.00	1.000	50.00	5.33	4.96	6.65
3	0.000	15.00	0.000	40.00	7.67	5.87	7.32
4	1.414	22.07	0.000	40.00	6.33	5.42	6.93
5	-1.000	10.00	-1.000	30.00	4.33	4.03	5.85
6	1.000	20.00	1.000	50.00	5.33	4.85	6.52
7	0.000	15.00	1.414	54.14	5.33	4.66	6.40
8	1.000	20.00	-1.000	30.00	5.67	5.32	6.88
9	0.000	15.00	0.000	40.00	7.33	5.80	7.24
10	-1.414	7.93	0.000	40.00	4.67	4.37	6.21
11	0.000	15.00	-1.414	25.86	4.33	4.59	6.35
12	0.000	15.00	0.000	40.00	8.00	5.84	7.30
13	0.000	15.00	0.000	40.00	8.00	5.90	7.35

Remark -1.414= -α level, -1=Low level, 0=Medium level, 1=High level, 1.414= α level

Table 4 Model coefficients estimated by multiple linear regression (significance of regression coefficients)

Response	Source	Sum of squares	Mean square	P value
Flower number	Model	24.50	4.90	<0.0001*
(flowers/plant)	X_1	1.70	1.70	0.0024*
	X_2	0.54	0.54	0.0353*
	X_1X_2	0.45	0.45	0.0490*
	X_1^2	9.20	9.20	<0.0001*
	X_2^2	15.34	15.34	<0.0001*
	Residual	0.56	0.079	
	Lack of fit	0.20	0.066	0.5820
	Pure error	0.36	0.089	
	R^2	97.78		
Flower size	Model	5.01	1.00	<0.0001*
(cm)	X_1	0.89	0.89	<0.0001*
	X_2	0.039	0.039	0.0293*
	X_1X_2	0.49	0.49	<0.0001*
	X_1^2	1.52	1.52	<0.0001*
	X_2^2	2.52	2.52	<0.0001*
	Residual	0.037	0.005233	
	Lack of fit	0.030	0.009904	0.0625
	Pure error	0.006920	0.001730	
	R^2	99.27		
Flower Fresh	Model	2.97	0.59	<0.0001*
weight	X_1	0.46	0.46	<0.0001*
(g/flower)	X_2	0.033	0.033	0.0200*
	X_1X_2	0.34	0.34	<0.0001*
	X_1^2	0.92	0.92	<0.0001*
	X_2^2	1.48	1.48	<0.0001*
	Residual	0.025	0.003630	
	Lack of fit	0.019	0.006269	0.1150
	Pure error	0.006600	0.001650	
	R^2	99.15		

Remark * ($P<0.05$)

Figure 1 shows the yield of marigolds in the number of flowers per plant, flower size and fresh weight from the experiment at the lower rate of 4.33 flowers/plant, 4.03 cm, and 5.85 g/flower. While the highest marigold yield was 7.84

flowers/plant, 5.90 cm, and 7.34 g/flower, respectively. The Concentration of fermented bio-extract from urine with vinasse and animal manure at 10.00-16.38 %v/v enhanced yield of marigold in three aspects. However, the marigold yield was reduced in all three aspects if the concentration of fermented bio-extracts was more than 16.38 %v/v. The amount of fermented bio-extract from urine with vinasse and animal manure feeding from 30.00-40.13 mL/plant improved the yield of marigold in three aspects. While the amount at over 40.13 mL/plant of fermented bio-extracts feeding lowered marigold yield in three aspects. The experimental results found that the concentration of fermented bio-extracts at 16.38 %v/v and the amounts of fermented bio-extracts feeding at 40.13 mL/plant provided the highest yield of marigold of 7.84 flowers/plant, 5.90 cm, and 7.34 g/flower under the predicted optimum. The yield of marigold in number of flowers per plant, flower size and fresh weight from the experiment was at 8.00 flowers/plant, 5.98 cm, and 7.38 g/flower, respectively (Table 5) were slightly different from the obtained values of the regression coefficients at 2.00 %, 1.34 % and 0.54 %, respectively. Moreover, the use of the experimental model under the optimum condition provided the similar yield of marigold in three aspects to that of chemical fertilizers.

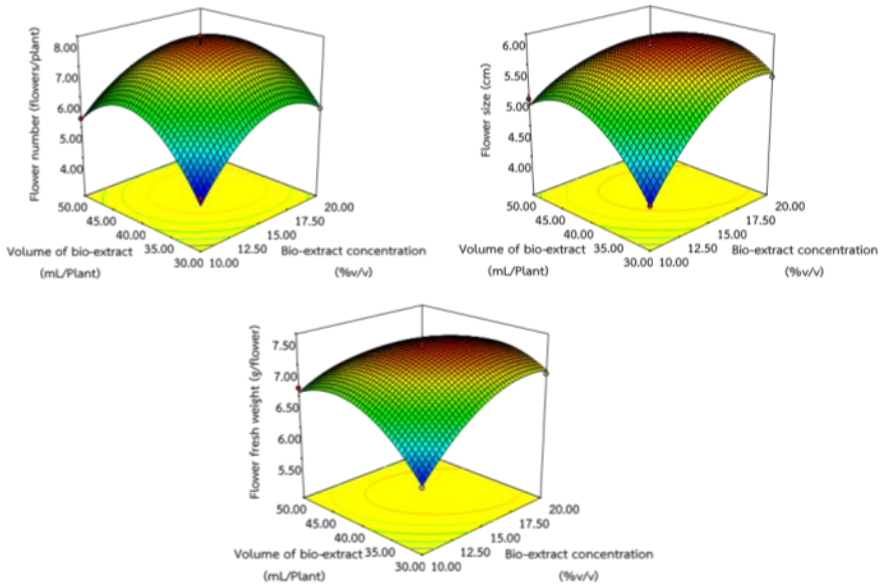

Figure 1 Response surface plots showing the effects of bio-extract concentration (X_1) and volume of bio-extract (X_2) on yields of Marigold.

Table 5 Experimental design and results of confirmation test

Condition	Bio-extract concentration (%v/v)	Volume of bio-extract (mL/plant)	Flower number (flowers/plan)	Flower size (cm)	Flower Fresh weight (g/flower)
Optimum (Predicted)	16.38	40.13	7.84	5.90	7.34
Optimum (Observed)	16.38	40.13	8.00	5.98	7.38
High	20.00	50.00	5.33	4.82	6.49
Medium	15.00	40.00	7.67	5.84	7.31
Low	10.00	30.00	4.33	4.05	5.89
Chemical fertilizer	-	-	8.33	6.02	7.40
Control	-	-	2.67	3.87	4.03

Discussions

Plant nutrients should be applied in optimum concentration and to provide plants to meet the demands of essential nutrients and help them produce maximum yield (Anugoolprasert & Rithichai, 2015). Nutrient deficiency is result in slowing down growth and reducing yield (Treeloket, 2013). However, having too much concentration of nutrients can harm plants, slow plant growth and cause carbon dioxide fixation and reduction in photosynthesis (Osotspa, 2014). Urine contains essential plant nutrients such as nitrogen, phosphorus and potassium (Sreela-or, 2017). There have been studies on application of urine for improving the nutrient composition of various plants; for examples, sweet corn (Rungreung, 2007), kale (Sreela-or, 2017) and marigold (Sreela-or & Tharungsri, 2020). The optimum conditions in this experiment provided more yields of marigold in three aspects (number of flowers of 8.00 flowers/plant, flower size of 5.98 cm and flower fresh weight of 7.38 g/flower) comparing with Sreela-or & Tharungsri (2020) study. As the fermented bio-extract from urine with vinasse and animal manure in this

experiment contained higher essential plant nutrients such as nitrogen, phosphorus and potassium of 5,098.87, 4,033.71 and 1,594.19 mg/L, respectively. While the fermented bio-extract from urine in Sreela-or & Tharungsri (2020) study contained nitrogen, phosphorus and potassium of 2,546.81, 632.90 and 413.25 mg/L respectively. This experiment got higher essential plant nutrients due to cricket manure adding and air pump supplying. Cricket manure contains high amounts of nitrogen and potassium, as well as the air pump helps to increase the efficiency of organic degradation of micro-organisms. Moreover, the bat feces used in this experiment had higher essential nutrients, especially phosphorus than in the experiment of Sreela-or & Tharungsri (2020). This may occur because of different storage periods. When comparing with a chemical fertilizer, the yields of marigold in three aspects were nearly the same as the number of flowers, flower size and flower fresh weight. These results demonstrated the efficiency of bio-extract from urine with vinasse and animal manure in this experiment. As the optimum condition of the bio-extract in this study had all three plant macronutrients in the right amount. Nitrogen promotes the growth of stems and leaves, which enhances cell enlargement and increases number of cells and therefore stimulates elongation and number of leaves (Olaniyi et al., 2008). Phosphorus promotes root growth and propagation of root and at the same time stimulates flowering and controlling flowering time (Kanacharoenpong et al., 2003). Potassium helps in maintaining balance of enzymes, regulating pressures in plant cells, promoting plant respiration and plant growth (Zain & Ismail, 2016). The findings of the study can be used to develop guidelines for reducing the costs of marigold production as it produced nearly the same results as chemical fertilizer use. At the same time, this can promote farmers to plant marigold organic farming system. The study also revealed that most farmers prefer to use organic fertilizer and bio-extract rather than chemical fertilizers if they have similar yields to chemical fertilizer. (Suriyakanont & Roach, 2019).

Conclusions

Fermented bio-extracts from urine with vinasse and animal manure can be applied in cultivation of marigolds in substitution of chemical fertilizers. The production of marigold (flower/plant, flower size and fresh weight) revealed the similar results as the application of chemical fertilizers. The optimum conditions were bio-extract of 16.38 %v/v and volume of bio-extract of 40.13 mL/plant, which caused number of flowers of 8.00 flowers/plant, flower size of 5.98 cm and flower fresh weight of 7.38 g/flower.

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