Natural plant growth regulator effect on the vegetative growth of Long Pepper (*Piper retrofractum* Vahl.)

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Abstract Long pepper (*Piper retrofractum* Vahl.) is an important medicinal plant for industry. Long pepper is grown by cuttings, but the obstacles encountered in planting through cuttings are easy to wilt and slow growth. The use of natural growth regulators (PGR) is one method to accelerate the growth of plant cuttings. The study showed an interaction between PGR of banana weevil extract and cow urine on the time of shoot emergence, percentage of shoot emergence, shoot length, shoot number, leaves number, and fresh root weight. The fastest shoot emergence resulted from a combination treatment of 0% banana weevil extract and 50% cow urine, namely at two weeks after treatment (WAT), and the longest shoot emergence was in the treatment of 75% banana weevil extract and 50% cow urine, namely at 6WAT. The combination of 0% banana weevil extract and 50% cow urine resulted in the highest shoot length, shoots number, leaves number, root length, roots number, root fresh weight, and root dry weight of 18.98 cm; 2.47; 13.00; 12.61 cm; 3.93; 0.56 g; and 0.11 respectively. The highest shoot diameter resulted from the treatment of 75% banana weevil extract and 25% cow urine of 1.31 cm.

Keywords: Banana weevil extract, Cow urine, Cuttings, Long pepper, Medicinal plants

Introduction

Long pepper (*Piper retrofractum* Vahl.) is known as chili jamu "*Cabya*" that is an essential medicinal plant in the pharmaceutical industry in Indonesia. Long pepper is a family group of Piperaceae (Vasavirama and Upender, 2014). Generally, this plant is used as raw material for health drinks, cooking ingredients (Kartasapoetra, 2004), natural insecticides (Umami and Purwani, 2015), oral inflammation medicine (Evizal, 2013), bronchitis (Syarif, *et al.*, 2011), cramps. Stomach, colds, asthma (Jamal *et al.*, 2013), cholesterol-lowering (Kim *et al.*, 2011), childbirth (Vinay *et al.*, 2012), influenza, asthma (Chaveerach *et al.*, 2006), as well as antioxidants and anticancer (Mulia, 2015).

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Long pepper production in Indonesia is still deficient, and this is due to the absence of appropriate exploration and cultivation activities for long pepper commodities (Nurhuda *et al.*, 2017). Generally, the propagation of long pepper plants is done vegetatively through cuttings. The cutting method is used because it has the advantage of maintaining the original nature and character of the parent and has a high success rate. In addition, propagation by cuttings is the best way to propagate long pepper plants. However, the obstacles faced in planting through cuttings are easy to wilt and slow growth. The use of natural growth regulators (PGR) is one method to accelerate the development of plant cuttings (Kurniati *et al.*, 2017). Natural plant growth regulators are directly available in nature and come from organic materials, for example, cow urine or extraction from certain plant parts (Nurlaeni, 2015).

Banana weevil extract contains cytokinins and gibberellins, which are beneficial for plants (Lindung, 2014). Karimah *et al.* (2013) stated that cow urine contains Indole Acetic Acid (IAA), which is included in the auxin group and consists of the gibberellin hormone. The combination of natural PGR in banana weevil extract and cow urine has been widely used. According to Asmara's research (2019), soaking banana weevil extract at a concentration of 40% gave the best effect on plant height, leaves, wet stover weight, dry stover weight, dry stover weight, dry tuber weight, and banana weevil weight. Diameter of bulbs of shallot plants at 24 DAP. Also added by Muvidah (2017) showed that soaking banana weevil extract at a concentration of 75% could provide optimum results for plant height, number of leaves, wet weight, and dry weight of mung bean plants.

Meanwhile, Gaol *et al.* (2016) suggested that the best soaking cow urine on Arachis pintoi plants was at a concentration of 50% on the number of leaves, plant length, and root weight. However, no research has been conducted on the optimum concentration of growth of long pepper cuttings in the seedling phase. Therefore, it is necessary to study the effect of several natural PGR concentrations of banana weevil extract and cow urine on the growth of long pepper cuttings to determine the optimum concentration to increase the growth of long pepper cuttings in the seedling (vegetative) phase.

Materials and methods

Time and location of research

The research was carried out at the Experimental Garden, Faculty of Agriculture, Bengkulu University. This research was conduct from Mei to August 2020.

Research materials

Soil cuttings used as plant material. Polybag is used as a container for planting media. Organic and inorganic fertilizers and natural plant growth regulators (PGR) are used as a source of nutrition.

Research design

The study used a completely randomized block design (RCBD) with two factors. The first factor was the concentration of banana weevil extract consisting of 4 levels, namely 0%, 5%, 50%, and 75%, and the second factor was the concentration of cow urine, consisting of 3 levels, namely 0%, 25 %, and 50%.

The observational data were analyzed using the F test. If the treatment showed a significant effect at the 5% level, the analysis was continued with DMRT (Duncan Multiple Range Test) at the level $\alpha = 5\%$.

Population and sample

After combining two factors. There were 12 treatments and replicated three times, the total number of experimental units were 36. The long pepper cuttings were planted at the polybags. The arrangement of polybags were arranged as follows: the distance between polybags was 20 cm x 20 cm, and the distance between replications was 50 cm.

Research stage

Stages the research was carried out by preparing a nursery and planting media by mixing soil, sand, and cow dung in a ratio of 1:1:1 (v/v). Growing media was put into polybags with a diameter of 15 cm and a height of 20, a length of 3.85 meters, and 4 meters. The shade poles are made of bamboo with a height of 1.5 meters in front and 1 meter behind, then the top of the shade is closed using 75% paranet.

Long pepper cuttings were taken along two segments. The cuttings used for propagation are soil vines. Making a combination of a solution of natural growth regulators between banana weevil extract and cow urine according to each treatment, weighing using an analytical balance for each concentration, each concentration dissolved in 1 liter of water measured using a 1-liter measuring degree, then stirred until evenly. After that, the cuttings cut into 10 cm were immersed in the PGR treatment solution for 20 minutes. Planting was carried out with Javanese chili cuttings soaked again in a solution of Menkozeb's active ingredient fungicide at a dose of 3 grams/L and mixed with water for 15 minutes, then planted in the morning in polybags filled with planting media and watered first so that the conditions of the growing media were moist. Maintenance is done by watering around the plant.

Observation variable

The observed variables were the time of shoot emergence, percentage of shoot emergence, shoot length, shoot number, leaves number, shoot diameter, root length, roots number, root fresh weight, and root dry weight.

Results

The results of the analysis of variance on all observed variables (Table 1) showed that the concentration of banana weevil extract had a significant effect on the variables of shoot length, a number of shoots, and the number of leaves starting from 6 weeks after treatment (WAT) to 12 weeks after treatment (WAT). The concentration of cow urine also significantly affected the shoot length and the number of leaves from 8 WAT to 12 WAT, and the number of shoots at 6 WAT and 8 WAT. The interaction between the combination of banana weevil extract concentration and cow urine had a significant effect on the variables of shoot length, the number of shoots, and the number of leaves starting from 4 weeks after treatment to 12 weeks after treatment as root wet weight.

Natural Plant Growth Regulator of Banana Weevil Extract and Cow Urine Effects on Vegetative Growth of Long Pepper (Piper retrofractum Vahl.)

The results showed a significant effect between banana weevil extract concentration and cow urine on shoot length variables starting from 4 weeks after treatment to 12 weeks after treatment (WAT). At 12 WAT observations, the highest shoot length variable was indicated by a combination of 0% banana weevil extract and 50% cow urine of 18.98 cm. In contrast, the lowest shoot length was indicated by a combination of 75% banana weevil extract and 50% cow urine of 75% banana weevil extract and 50% cow urine was not significantly different from 75% banana weevil extract and 50% cow urine, which was 5.08 cm (Table 2).

retrofractum Vahl.)	C C		
Variable	В	С	B x C
Shoot length			
2 WAT	2.10ns	0.33ns	2.04ns
4 WAT	7.48**	1.02ns	5.60**
6 WAT	5.17**	1.59ns	5.46**
8 WAT	4.68**	3.56*	7.39**
10 WAT	5.75**	3.92*	7.00**
12 WAT	6.11**	3.80*	8.46**
Shoot number			
2 WAT	4.81**	0.16ns	2.05 ns
4 WAT	1.91**	1.50ns	3.84**
6 WAT	6.09**	6.60**	4.23**
8 WAT	7.58**	8.74**	8.09**
10 WAT	3.62**	2.51ns	5.23**
12 WAT	151**	0.62ns	3.66**
Leaves number			
2 WAT	4.44**	0.41ns	4.24**
4 WAT	4.94**	0,30ns	3.81**
6 WAT	2.34**	1.87ns	4.16**
8 WAT	10.32**	4.63*	10.98**
10 WAT	11.21**	6.34**	11.08**
12 WAT	6.68**	5.17**	9.91**
Root length	1.08ns	5.53**	2.30ns
Root numbers	4.11**	0.11ns	1.39ns
Shoot diameter	0.67ns	1.14ns	2.31ns
Root fresh weight	2.02ns	2.10ns	2.53*
Root dry weight	1.00ns	1.77ns	1.95ns

Table 1. Recapitulation of Analysis Variance of Natural Plant GroethRegulator Effects on The Vegetative Growth of Long Pepper (*Piper retrofractum* Vahl.)

Notes: * = Significant effect based on F Table 0.05, ** = very significant effect based on F Table 0.01, ns = Not significant effect, B = banana weevil extract, C = cow urine, B x C = Interaction between extracts banana hump and cow urine, WAT = week after treatment.

Combination of Natural PGRs (%)	Shoots length (cm)				
	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
B1C1	1.28cd	1.94b	2.29c	3.39b	5.08c
B1C2	2.04bcd	2.97b	4.13bc	5.83b	7.61bc
B1C3	3.50a	6.09a	9.16a	13.12a	18.98a
B2C1	2.12bc	2.47b	3.39bc	4.50b	6.62bc
B2C2	1.57cd	3.47b	5.19b	6.61b	10.33b
B2C3	1.40cd	2.13b	3.56bc	4.55b	6.42bc
B3C1	2.56b	2.86b	4.67bc	6.35b	9.15bc
B3C2	1.90bcd	2.52b	3.66bc	4.92b	6.80bc
B3C3	1.77bcd	2.30b	4.13bc	5.95b	8.06bc
B4C1	1.44cd	2.23b	3.38bc	4.39b	6.18bc
B4C2	1.04d	1.90b	3.05bc	4.07b	5.64bc
B4C3	1.07d	1.70b	2.44c	3.27b	4.62c

Table 2. Natural Plant Growth Regulator Effect on shoots length of *Piper* retrofractum Vahl

Noted : Values followed by the same letter in the same column are not significantly different based on DMRT at level = 5%. B1C1 = 0% banana weevil extract 0% cow urine; B1C2 = 0% banana weevil extract 25% cow urine; B1C3 = 0% banana weevil extract 50% cow urine; B2C1 = 25% banana weevil extract 50% cow urine; B2C2 = 25% banana weevil extract 25% cow urine; B2C2 = 25% banana weevil extract 25% cow urine; B3C2 = 50% banana weevil extract 0% cow urine; B3C2 = 50% banana weevil extract 5% cow urine; B3C3 = 50% banana weevil extract 5% cow urine; B4C1 = 75% banana weevil extract 25% cow urine; B4C2 = 75% banana weevil extract 25% cow urine; and B4C3 = 75% banana weevil extract 5% cow urine;

The results showed a significant effect between banana weevil extract concentration and cow urine on the variable number of shoots starting from 4 weeks after treatment to 12 weeks after treatment (WAT). At 12 WAT observations, the highest number of shoots was shown by combining 0% banana weevil extract and 50% cow urine of 2.47 pieces. In contrast, the lowest number of shoots was indicated by a combination of 0% banana hump extract and 0% cow urine of 1.22 pieces. Meanwhile, the combination of 25% banana weevil extract and 25% cow urine was not significantly different from the combination of 0% banana hump extract and 50% cow urine of 1.73 pieces was not significantly different from the nine combinations of banana weevil extract and other cow urine (Table 3).

Combination of Natural PGRs (%)	Shoots number (piece)				
	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
B1C1	1.00b	1.11b	1.07b	1.13b	1.22b
B1C2	1.13b	1.37b	1.40b	1.47b	1.60b
B1C3	1.87a	2.13a	2.27a	2.27a	2.47a
B2C1	1.33b	1.15b	1.20b	1.20b	1.30b
B2C2	1.30b	1.27b	1.27b	1.67b	1.92ab
B2C3	1.00b	1.28b	1.28b	1.27b	1.38b
B3C1	1.11b	1.15b	1.22b	1.53b	1.73b
B3C2	1.28b	1.25b	1.40b	1.47b	1.47b
B3C3	1.11b	1.08b	1.12b	1.13b	1.33b
B4C1	1.00b	1.00b	1.08b	1.13b	1.53b
B4C2	1.00b	123b	1.27b	1.27b	1.33b
B4C3	1.12b	1.22b	1.20b	1.20b	1.27b

Table 3. Natural Plant Growth Regulator Effect on shoots number of *Piper* retrofractum Vahl

Noted : Values followed by the same letter in the same column are not significantly different based on DMRT at level = 5%. B1C1 = 0% banana weevil extract 0% cow urine; B1C2 = 0% banana weevil extract 25% cow urine; B1C3 = 0% banana weevil extract 50% cow urine; B2C1 = 25% banana weevil extract 50% cow urine; B2C2 = 25% banana weevil extract 25% cow urine; B2C2 = 25% banana weevil extract 25% cow urine; B3C2 = 50% banana weevil extract 0% cow urine; B3C2 = 50% banana weevil extract 5% cow urine; B3C3 = 50% banana weevil extract 5% cow urine; B4C1 = 75% banana weevil extract 25% cow urine; B4C2 = 75% banana weevil extract 25% cow urine; and B4C3 = 75% banana weevil extract 5% cow urine;

The results showed a significant effect between banana weevil extract concentration and cow urine on the variable number of leaves starting from 4 weeks after treatment to 12 weeks after treatment (WAT). At 12 WAT observations, the highest number of leaves was indicated by a combination of 0% banana weevil extract and 50% cow urine of 13.60 pieces. In contrast, the lowest number of leaves was indicated by a combination of 0% banana weevil extract and 0% cow urine of 3.32 pieces. Meanwhile, the combination of 0% banana weevil extract and 0% cow urine was not significantly different from the combination of 75% banana hump extract and 25% cow urine and a combination of 75% banana weevil extract and 50% cow urine which were 3.57 pieces and 3.60 pieces respectively. Successive, but significantly different from the combination of 25% banana weevil extract and 25% cow urine of 7.23 pieces. The combination of 50% banana hump extract and 0% cow urine was not significantly different in the other six combinations (Table 4).

Combination of	Leaves number (piece)				
Natural PGRs (%)	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
B1C1	0.33bc	1.50b	2.08b	2.47b	3.32c
B1C2	1.33bc	1.29b	2.42b	3.47b	4.93bc
B1C3	3.13a	4.27a	7.27a	9.93a	13.60a
B2C1	1.89ab	2.22b	2.69b	2.60b	4.17bc
B2C2	1.44bc	2.09b	3.13b	4.00b	7.23b
B2C3	1.00bc	1.50b	2.03b	2.63b	4.20bc
B3C1	1.83ab	2,25b	3.07b	4.15b	5.80bc
B3C2	1.33bc	1.69b	2.91b	3.27b	5.00bc
B3C3	0.67bc	1.39b	2.27b	2.63b	4.73bc
B4C1	0.44bc	1.15b	1.63b	2.42b	3.91bc
B4C2	0.00c	1.22b	1.70b	2.07b	3.57c
B4C3	0.33bc	1.67b	1.83b	2.28b	3.60c

Table 4. Natural Plant Growth Regulator Effect on leaves number of *Piper* retrofractum Vahl

Noted : Values followed by the same letter in the same column are not significantly different based on DMRT at level = 5%. B1C1 = 0% banana weevil extract 0% cow urine; B1C2 = 0% banana weevil extract 25% cow urine; B1C3 = 0% banana weevil extract 50% cow urine; B2C1 = 25% banana weevil extract 0% cow urine; B2C2 = 25% banana weevil extract 25% cow urine; B2C3 = 25% banana weevil extract 50% cow urine; B3C1 = 50% banana weevil extract 0% cow urine; B3C2 = 50% banana weevil extract 25% cow urine; B3C3 = 50% banana weevil extract 5% cow urine; B4C1 = 75% banana weevil extract 0% cow urine; B4C2 = 75% banana weevil extract 25% cow urine; B4C2 = 75% banana weevil extract 25% cow urine; B4C2 = 75% banana weevil extract 25% cow urine; B4C2 = 75% banana weevil extract 5% cow urine; B4C3 = 75% banana weevil extract 5% cow urine.

The results showed a significant effect between the concentration of banana weevil extract and cow urine on the variables of tuna diameter, root length, number of roots, root wet weight, and root dry bob. In the shoot diameter variable, the combination of 75% banana weevil extract and 25% cow urine had the highest shoot diameter of 1.31 cm. However, it would not be significantly different from 25% banana weevil extract and 0% cow urine of 1.28 cm. Meanwhile, the combination of 0% banana weevil extract and 0% cow urine had the lowest shoot diameter of 1.03 cm. However, it would not be significantly different from 75% banana weevil extract and 50% cow urine of 1.05 cm (Table 5).

Combin ation of	Variable					
Natural PGRs (%)	Shoot diameter (cm)	Root length (cm)	Root number (piece)	Fresh root weight (g)	Dry root weight (g)	
B1C1	1.03b	6.02b	2.27c	0.26b	0.057bc	
B1C2	1.20ab	6.83b	3.33abc	0.37ab	0.07abc	
B1C3	1.22ab	12.61a	3.93a	0.56a	0.11a	
B2C1	1.28a	6.11b	3.07abc	0.30b	0.06bc	
B2C2	1.13ab	8.82b	2.87abc	0.47ab	0.10ab	
B2C3	1.20ab	8.27b	2.93abc	0.30b	0.06abc	
B3C1	1.10ab	5.49b	3.62ab	0.33b	0.05c	
B3C2	1.18ab	7.09b	3.25abc	0.45ab	0.08abc	
B3C3	1.11ab	8.50b	2.87abc	0.35b	0.07abc	
B4C1	1.15ab	7.83b	2.97abc	0.32b	0.07abc	
B4C2	1.31a	5.76b	2.74bc	0.26b	0.056bc	
B4C3	1.05b	7.14b	2.62bc	0.26b	0.057bc	

Table 5. Natural Plant Growth Regulator Effect on shoot diameter, root length, root number, fresh root weight, and dry root weight of *Piper retrofractum* Vahl

Noted : Values followed by the same letter in the same column are not significantly different based on DMRT at level = 5%. B1C1 = 0% banana weevil extract 0% cow urine; B1C2 = 0% banana weevil extract 25% cow urine; B1C3 = 0% banana weevil extract 50% cow urine; B2C1 = 25% banana weevil extract 0% cow urine; B2C2 = 25% banana weevil extract 25% cow urine; B2C3 = 25% banana weevil extract 50% cow urine; B3C1 = 50% banana weevil extract 0% cow urine; B3C2 = 50% banana weevil extract 25% cow urine; B3C2 = 50% banana weevil extract 25% cow urine; B3C2 = 50% banana weevil extract 5% cow urine; B4C1 = 75% banana weevil extract 0% cow urine; B4C2 = 75% banana weevil extract 25% cow urine; and B4C3 = 75% banana weevil extract 5% cow urine.

The highest root length in the combination of 0% banana weevil extract and 50% cow urine was 12.61 cm. In comparison, the lowest root length in the combination of 75% banana hump extract and 0% cow urine was 5.49 cm but not significant with the other ten combinations. Meanwhile, for the variable, the highest number of roots was also in the combination of 0% banana weevil extract and 50% cow urine of 3.93 pieces, while the lowest number of roots was in the combination of 0% banana hump extract and 0% cow urine of 2.27 pieces.

Root wet weight and root dry weight showed no significant difference between banana weevil extract and cow urine. The highest root wet weight was in the combination of 0% banana weevil extract and 50% cow urine of 0.56 grams, while the lowest root wet weight was in the combination of 0% banana weevil extract and 0% cow urine, 75% banana weevil extract and 25% cow urine, and 75% of banana weevil extract and 25% of cow urine is 0.26 grams. Meanwhile, root dry weight was also not significantly different between the combinations of natural growth regulators, where the highest root dry weight was also in a combination of 0% banana weevil extract and 50% cow urine of 0.11 grams, and the lowest root dry weight was in a combination of 50% banana weevil extract. And 0% cow urine of 0.05 grams.

Discussion

The results showed that the combination treatment of natural growth regulators between banana weevil extract and cow urine significantly affected long pepper vegetative growth, such as shoot length, the number of shoots, and the number of leaves. The combination of 0% banana weevil extract and 50% cow urine had the best performance for the variables of shoot length, the number of shoots, and the number of leaves starting from 2 weeks after treatment to 12 weeks after treatment (WAT). This is different from Hariani et al. (2018), which states that the best natural effect on shoot length growth is the administration of ZPT banana weevil extract because P or phosphorus helps add plant nutrients. Also added by Maspary (2012), banana weevil extract contains PGR gibberellins and cytokinins. It also contains seven beneficial microorganisms such as Azospirillium, Azotobacter, Bacillus, Aeromonas, Aspergillus, phosphate solubilizing microbes, and cellulose microbes to stimulate cell division quickly. Gibberellins and cytokinins can help regulate cell division in the meristem area so that the growing point grows typically. Its role includes regulating the growth rate of each network and integrating its parts.

Shoot length in the combination of 0% banana weevil extract and 50% cow urine showed the best results compared to the combination of banana weevil extract and other concentrations of cow urine. Giving cow urine as a growth regulator can stimulate faster shoot growth, and the number of shoots is more to a certain extent. It is suspected that the auxin and cytokinin hormones contained in cow urine can work optimally to stimulate the formation of meristem tissue so that it continues to divide and cause shoot growth. better than other treatments (Yunanda *et al.*, 2015).

The combination of 0% banana weevil extract and 50% cow urine produced the highest number of leaves, namely 13.60 leaves. This follows the research of Gaol *et al.* (2016) that giving cow urine with a concentration of 50% gave the best effect on increasing growth, especially the number of leaves. However, in this study, giving cow urine up to 50% combined with banana weevil extract up to 75% can reduce leaves. It is suspected that the banana weevil extract containing gibberellins is sufficient to grow the number of leaves. Giving cow urine that contains gibberellins can reduce the number of leaves because it has exceeded the optimum concentration. According to Bidadi *et al.* (2010), the application of gibberellins can increase plant stems and leaves, but if in excessive doses, it will reduce the growth of the number of leaves.

Root length variables showed no significant difference for the combination of banana weevil extract and cow urine. Combining 0% banana weevil extract and 50% cow urine produced the longest root length of 12.61 cm. Increasing the concentration of cow urine up to 50% with a combination of banana weevil extract from 0% to 75% resulted in the best root length. The hormone auxin in cow urine can increase cell elongation, in this case, root cells. Sofwan *et al.* (2018), growth regulators that have an essential role in the root growth process come from the auxin group. Meanwhile, the combination of banana weevil extract and cow urine did not affect the number of roots. It is suspected that the concentration given is not appropriate in stimulating root formation. The number of roots is closely related to the length of the resulting root to determine the root volume. Yunanda *et al.* (2015) said that if the number of roots formed is large, the ability of the roots to absorb nutrients is also higher, and the photosynthesis process runs well so that the photosynthate is produced and allocated to all parts of the plant for root growth also increases.

In summary, the fastest shoot emergence resulted from a combination treatment of 0% banana weevil extract and 50% cow urine, namely at two weeks after treatment (WAT), and the longest shoot emergence was in the treatment of 75% banana weevil extract and 50% cow urine, namely at 6WAT. The combination of 0% banana weevil extract and 50% cow urine resulted in the highest shoot length, shoots number, leaves number, root length, roots number, root fresh weight, and root dry weight. The highest shoot diameter resulted from the treatment of 75% banana weevil extract and 25% cow urine

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