The Induction of Biotic Stress-Resistance and Increasing Growth and Yield of Chili Pepper (*Capsicum Annuum* L.) Using Cow Bio-Urine and Tricho-Compost

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ABSTRACT

This study was aimed at obtaining chili peppers (Jambi local variety) that were resistant to biotic stress through induced-resistance mechanisms, and obtaining optimal combination of cow bio-urine and tricho-compost composition that support plant growth and yield. This research was conducted from April through to September 2019 at the Plant Diseases and Biological Agents Laboratory, followed by field trial at the Experimental Farm, Faculty of Agriculture University of Jambi. A Split Plot design was employed in this study with the concentration of cow bio-urine as main plot and composition of tricho-compost as sub plot. The results showed that the application of 60% cow bio-urine on various tricho-compost compositions within growing media was able to induce plant resistance against biotic stress. The number of plants showing disease symptoms ranged from 13.3% to 16.0%, and was classified as mild biotic stress (< 25%). Likewise, the symptom of fruit disease was classified as mild, indicating that plants were resistant to biotic stress. The application of cow bio-urine at various tricho-compost compositions showed a negative linear correlation against total chlorophyll and sugar content. The application of 60% cow bio-urine at different tricho-compost compositions within the growing media produced better growth and yield of chili pepper rather than other combinations. This was indicated by higher number of productive branches, number of flowers and fruit sets, and total weight of fruit per plant. In general, it can be concluded that the use of 60% cow bio-urine in along with 25% tricho-compost within growing media was an optimal combination in inducing biotic stress resistant, as well as promoting growth and yield of chili peppers.

***Keywords**: Induced-Resistance, Biotic Stress, Vegetable Crops, Organic Farming, Sustainable Agriculture.*

1. INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is one of economically important horticultural crops in Indonesia. Domestic demand for this commodity increased by 1.67 – 3.0 kg per capita per year. On the other hand, foreign market (Malaysia, Singapore, Saudi Arabia and Japan) can be met by only around 30% [1]. Unfortunately, the increasing demand for chilies has not been matched by increasing yields. Data taken from Jambi Central Bureau of Statistics (2018), showed that the average yield of chili peppers in Jambi Province in 2017 was 5.13 tons$\cdot$ha^{-1}, and this was lower than the previous year (7.27 tons$\cdot$ha^{-1}). Nationally, the production of chili peppers in Indonesia currently is 8.47 tons$\cdot$ha^{-1}, while the potential production is more than 20 tons$\cdot$ha^{-1}.

The low productivity of chilies, either in Jambi Province or nationally, is among other caused by the attack of pests and diseases in the chili cultivation area. The high incidence of pest and disease attacks had negative impact on the growth and yield of chili plants in the field. The attack of chilli fruit borer (*Helicoverpa armigera*) had resulted in loss of yields of up to 60%, and the infection of *Colletotrichum* sp. (anthracnose) resulted in production loss of almost 80% [2]. Since early 2003 there are still frequent epidemics of Gemini virus, CMV and other complex infections on chili plants in various parts of Indonesia, which have resulted in crop failure [3].

Based on field conditions and high rate of pest attack on chili plants, current chili cultivation still relies on the high use of synthetic pesticides and fertilizers. In fact, excessive use of synthetic these inorganic chemicals will make the physical condition of soil getting worse. Without being balanced by organic fertilizers (such as compost), the nutrient absorption by plants will not efficient and effective. Therefore, it is necessary to develop alternative cultivation technology to produce plant products are safe for consumption and environmentally friendly. This could be achieved through the development of organic farming technology for chili plants [4].

One of organic methods in chili growing is by the use of antagonistic agents as well as their interactions with biocontrol agents, in the form of the application of cow bio-urine along with tricho-compost (*Trichoderma* sp. in compost). *Trichoderma* sp. has an antagonistic ability to suppress the development of anthracnose (chili fruit rot) by up to 90% [5]. This fungus also accelerates the breakdown of organic waste into tricho-compost (a biofertilizer) which contains macro- and micro-nutrients needed by plants [4]. In addition, cow bio-urine contains solubilizing bacteria of P (*Bacillus* sp.) and *Azotobacter* sp. Cow bio-urine also contains other microorganisms that play an important role as biofertilizer, biostimulant and biopesticide [6].

To our knowledge, the simultaneous application of tricho-compost and cow bio-urine in an integrated package of organic and environmentally friendly cultivation of chili has never been done before. Therefore, and based on results of previous studies we developed a study entitled "The effect of cow bio-urine concentration in inducing the resistance against pest attack of chili (*Capsicum annuum* L.) grown on tricho-compost planting media".

2. MATERIALS AND METHODS

The experiment was conducted at the Plant Disease and Bioagent Laboratory and glasshouse at the Teaching and Research Farm Faculty of Agriculture University of Jambi, Indonesia. The trial was completed within 6 months, from April through to October 2019.

Materials used were seeds of local variety chili, media for identification and multiplication of *Trichoderma* sp. (Potato Dextrose Agar), cow urine, lemon grass, coconut milk, sugar and Effective Microorganism-4, cow dung, and rice husk charcoal. In addition, polybags, para net (50%), ropes, sprayer, scissor, digital balance, oven and digital camera were used in the trial.

The experiment was arranged in a factorial Split Plot Randomized Block Design with 3 replications. Factors investigated were: 1) composition of tricho-compost and soil (sub plot), i.e. 25% tricho-compost + 75% soil (T1), 50% tricho-compost + 50% soil (T2), and 75% tricho-compost + 25% soil (T3), and 2) cow bio-urine concentrations (v/v), i.e. 20% (B1), 40% (B2), 60% (B3), and 80% (B4). Therefore, there were 12 treatment combinations with 3 replications, resulted in 36 experimental units. Each unit consisted of 10 individuals, resulting in 360 plants. Five plants were taken as samples from each experimental unit.

Observation was made on weekly basis (every 7 days) on the following variables: percentage of disease attack, plant height, first flower appearance, number of productive branches, and total flower per plant. Percentage of disease attack was identified based on the level of plant damage, and calculated according to Yoon (2003)[7] method:

$$\text{Percentage of Disease} = \frac{\text{n}}{\text{N}} \times 100\%$$

where: n = number of plants with disease symptoms.

N = total number of plants observed.

Data were analyzed statistically using Analysis of Variance model to see the effect of tricho-compost and bio-urine treatments, and followed by a Duncan's Multiple Range Test (DMRT) to see the difference among treatment means.

3. RESULTS AND DISCUSSION

3.1 Percentage of disease

The first symptoms of pest and disease attacks began to appear at 7 weeks after transplanting, when the plants start to bear fruits. The symptoms observed in the field are as shown in Figure 1 and the number of diseased plants is presented in Table 1.

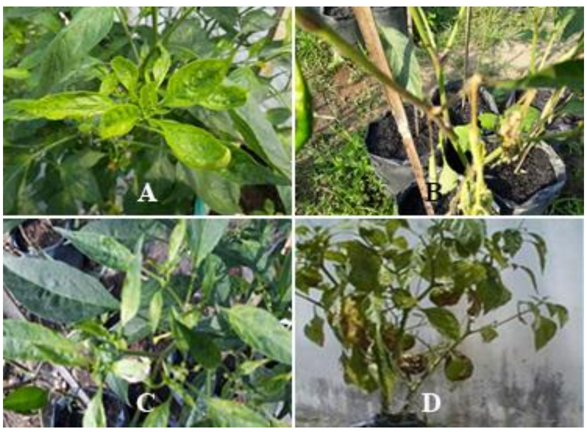

Figure 1 Pest and disease attacks on chili grown in medium with tricho-compost and treated with cow bio-urine (A = *Thrips*; B = *Phytophthora*; C = Mosaic Virus; D = *Fusarium*)

Table 1. The effect by of cow bio-urine and tricho-compost on the percentage of chili plants infected with disease

Cow bio-urine	Tricho-compost composition (%)			
(%)	25	50	75	Means
20	6.67 (L)	13.33 (L)	25.33 (M)	15.11 (L)
40	18.67 (L)	26.67 (M)	50.60 (H)	31.98 (M)
60	13.33 (L)	16.00 (L)	13.33 (L)	14.22 (L)
80	41.33 (M)	28.00 (M)	14.67 (L)	28.00 (M)
Means	20.00 (L)	21.00 (L)	25.98 (M)	-

Notes: The capital letters in brackets indicates the Attack Category which refers to the Technical Guidelines for the Horticultural Protection System - Directorate General of Horticulture in 2018. L = light attack category; M = medium attack category; H = heavy attack category

Table 1 reveals that the application of 60% cow bio-urine in various tricho-compost compositions resulted in 13.33% - 16.00% (average of 14.22%) plants are infected by disease. The range of this number is classified into Light Biotic Stress (< 25%). Meanwhile, the application of 20% cow bio-urine on showed Mild Biotic Stress (6.67% - 13.33%) when combined with 25% and 50% tricho-compost. Figure 1 shows plant with pest and disease attack symptoms. Figure 1 presented visual appearance of various pest and disease attacks on chili grown in medium with tricho-compost and treated with cow bio-urine.

3.2 Plant height

The results of analysis of variance on the effect of cow bio-urine and tricho-compost on main stem height showed that: a) bio-urine concentration as main plot had a significant effect on the height of the main stem, and b) there was no interaction between cow bio-urine concentrations and tricho-compost compositions on main stem height. Furthermore, Duncan's Multiple Range Test was conducted to find out the significances of the difference among treatment means. The results are presented in Table 2.

Table 2. The effect of different concentrations of cow bio-urine on the main stem height of chili paper

Cow bio-urine (%)	Main stem height (cm)	Significance (α = 0.05)
20	35.69	b
40	37.26	ab
60	36.92	ab
80	38.59	a

Notes: Different uppercase in column indicate a significant difference (p < 0.05), and the same uppercase in column indicate a non-significant difference (p > 0.05).

3.3 First flower initiation

The results of analysis of variance on the effect of cow bio-urine and tricho-compost on first flower initiation showed that: a) tricho-compost composition as sub plot had a significant effect on the first flower initiation, and b) there was interaction between cow bio-urine concentrations and tricho-compost compositions on first flower initiation. Further, Duncan's Multiple Range Test was carried out to identify the significances of the difference among treatment means. The results are presented in Table 3 and Table 4.

Table 3. The effect of different compositions of tricho-compost on the first flower initiation of chili pepper.

Tricho-compost (%)	First flower initiation (days after transplanting)	Significance (α = 0.05)
25	25.77	A
50	24.97	AB
75	24.50	B

Notes: Different uppercase in column indicate a significant difference (p < 0.05), and the same uppercase in column indicate a non-significant difference (p > 0.05).

Table 4. The effect of different cow-bio-urine concentrations and tricho-compost compositions on the first flower initiation of chili pepper

Cow bio-urine (%)	Tricho-compost (%)			
	25	50	75	Means
20	29.13 b A	23.73 c B	23.47 c B	25.44 ab
40	24.33 c A	25.27 bc A	26.73 b A	25.44 ab
60	23.67 c A	23.47 c A	23.27 c A	23.47 b
80	25.93 bc AB	27.40 ab A	24.53 c B	25.96 a
Means	25.77	24.97	24.50	-
	A	AB	B	-

Notes: Different uppercase in rows and lower case in columns indicate a significant difference ($p < 0.05$), and the same uppercase in rows and lowercase in column indicate a non-significant difference ($p > 0.05$).

3.4 Productive branches

The results of analysis of variance on the effect of cow bio-urine and tricho-compost on the number of productive branches showed that: a) blocking was significantly effective, b) the concentration of cow bio-urine as main plot significantly affected the number of productive branches, and c) there was interaction between cow bio-urine concentrations and tricho-compost compositions on the number of productive branches. Furthermore, Duncan's Multiple Range Test was carried out to identify the significances of the difference among treatment means. The results are presented in Table 5 and Table 6.

Table 5. The effect of different concentrations of cow bio-urine the number of productive branches of chili pepper

Cow bio-urine (%)	Number of productive branches	Significance ($\alpha = 0.05$)
20	65.33	c
40	74.03	b
60	84.16	a
80	63.76	c

Notes: Different uppercase in column indicate a significant difference ($p < 0.05$), and the same uppercase in column indicate a non-significant difference ($p > 0.05$).

Table 6. The effect of different cow-bio-urine concentrations and tricho-compost compositions on the number of productive branches of chili pepper

Cow bio-urine (%)	Tricho-compost (%)			
	25	50	75	Means
20	51.80 c B	73.00 ab A	71.20 b A	65.33 c
40	68.67 b A	72.56 ab A	80.87 ab A	74.03 b
60	81.93 ab A	81.93 ab A	88.60 a A	84.16 a
80	71.53 b A	70.13 b A	49.60 c B	63.76 c
Means	68.48	74.41	72.57	-
	A	A	A	-

Notes: Different uppercase in rows and lowercase in columns indicate a significant difference ($p < 0.05$), and the same uppercase in rows and lowercase in column indicate a non-significant difference ($p > 0.05$).

3.5 Number of flowers

The results of analysis of variance on the effect of cow bio-urine and tricho-compost on the number of flowers showed that: a) blocking was significantly effective, b) the concentration of cow bio-urine as main plot highly significantly affected the number of flowers, and c) there was interaction between cow bio-urine concentrations and tricho-compost compositions on the number of flowers. Furthermore, Duncan's Multiple Range Test was carried out to identify the significances of the difference among treatment means. The results are presented in the following Table 7 and Table 8.

Table 7. The effect of different concentrations of cow bio-urine the number of flowers of chili pepper.

Cow bio-urine (%)	Number of productive branches	Significance ($\alpha = 0.05$)
20	142,11	c
40	174,51	b
60	235,69	a
80	137,76	c

Notes: Different uppercase in column indicate a significant difference ($p < 0.05$), and the same uppercase in column indicate a non-significant difference ($p > 0.05$).

Table 8. The effect of different cow-bio-urine concentrations and tricho-compost compositions on the number flowers of chili pepper

Cow bio-urine (%)	Tricho-compost (%)			
	25	50	75	Means
20	108.40 e B	186.40 b A	131.53 cde B	142.11 c
40	159.87 bcd A	172.20 bc A	191.47 b A	174.51 b
60	241.67 a A	234.53 a A	230.87 a A	235.69 a
80	170.87 bc A	119.53 de B	122.87 de B	137.76 c
Means	170.20	178.17	169.18	-
	A	A	A	-

Notes: Different uppercase in rows and lowercase in columns indicate a significant difference ($p < 0.05$), and the same uppercase in rows and lowercase in column indicate a non-significant difference ($p > 0.05$).

The application of cow bio-urine at concentration of 60% on chili plants growing on various compositions of tricho-compost was proven to be able to induce plant resistance to biotic stress. Induced resistance is a state of enhanced defensive capacity developed by a plant when appropriately stimulated [8]. This resistance can occur due to non-pathogenic biotic agents such as plant-growth-promoting non-pathogenic rhizobacteria ([9][10][11]), *Pseudomonas*, *Bacillus*, *Trichoderma*, and mycorrhiza species [12]. Vallad and Goodman [13] claimed that this resistance was able to reduce the impact pests attack by 50 - 90%, depending on plant species, physiological conditions, and environmental factors.

According to Harman *et al.*[14], *Trichoderma* sp. produced various compounds that were able to induce local and systemic plant resistance against pathogens attack, and also against unfavorable environmental conditions. Resistance induction mechanism by *Trichoderma* sp. occurs after the spores of the fungus make contact with the root surface of plant. The structure that has been attached to the surface of plant roots will induce the plant to produce at least three chemical substances such as peptides, proteins and low molecular weight compounds that can increase plant defenses. Induction of resistance can occur due to the direct production of pathogenesis-related (PR) protein and phytoalexin compounds by plants as a result of attack by pathogenic microorganisms [15][16].

In our study, the use of tricho-compost in growing media and cow bio-urine spray on plants is proven to increase the number and type of antagonistic microorganisms, so their ability to inhibit and suppress pathogen growth, both in the soil or around the planting area, will increase even more. The ability of compost plus biological agents, especially *Trichoderma* sp. to suppress various soil-borne diseases has been widely studied [17][18]. Besides being able to control various soil-borne diseases, compost plus (tricho-compost) can also suppress air-borne diseases [19][18]. Furthermore, cow bio-urine also contains various insect and pest repellent compounds, and has been used to reduce the spread of pest carrying pathogens (as vectors) ([12] [21]).

The combination of 60% cow bio-urine spray and 25% tricho-compost in growing media was also able to induce growth and yields of chilies better than other combinations. This can be seen in the number of productive branches, the number of flowers and fruits, as well as the fruit weight per plant. It is suspected that various antagonistic microorganisms found in tricho-compost and cow bio-urine are not only able to suppress the population of pathogens around the planting area, but also play an important role in optimizing the availability of macro and micro nutrients. Cow bio-urine is a good source of nitrogen, phosphate, potassium, calcium, magnesium, chlorite and sulphate as well as mineral salts, hormones and enzymes [22]. Urine increased the N concentration of grass and increased the potassium concentration of grass and clover [23]. On *Brassica juncea,* the spray of 50% cow bio-urine was found to increase N, P and K uptake [24].

In addition to bio-urine, the application of tricho-compost into soil was found to suppress pest and disease incidence. With the application of 25% tricho-compost, only 20.00% of plants showed symptoms of pest and disease attacks. Some studies reported the relationship between soil microbial community composition and the suppression of diseases caused by soil-borne pathogens ([25] [26]. Some bacterial (*Pseudomonas*, *Burkholderia*, *Bacillus*, *Serratia*, *Streptomyces*) and fungal (*Trichoderma*, *Penicillium*, *Gliocladium*, *Sporidesmium*, non-pathogenic *Fusarium* spp.) genera have been identified as antagonists of one or more soil-borne plant pathogens ([27][28][29]). Disease suppression also correlated to the production of antibiotics that work against the pathogen, or linked to overlap micro-habitats or similar substrate preferences between antagonists and pathogens ([30][31].

4. CONCLUSION

Based on the results of the investigation and the discussion, it can be concluded that:

1. The application of cow bio-urine at various concentration on chili plants grown on media with different composition of tricho-compost could suppress the intensity of pest attack to moderate level.
2. The spray of 60% cow bio-urine on chili grown on different levels of tricho-compost, might reduce disease infection to less than 25% which is categorized as light biotic stress.
3. Cow bio-urine application showed positive interaction with tricho-compost in accelerating reproductive growth, and increasing number of productive branches as well as number of flowers per plant.

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