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Research Article

EFFECTIVENESS OF LIQCORIS ORGANIC PESTICIDE AS GROWTH INHIBITOR OF PLANT PATHOGENIC MICROORGANISMS

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ARTICLE HIGLIGHTS

- First study on Liqcoris, an organic pesticide made from coconut waste.
- 15% Liqcoris concentration effectively inhibits pests and plant pathogens.
- Active compounds include phenol and carbamic acid.
- Pest and fungal pathogens were isolated directly from diseased plants in a school garden.
- Coconut waste-based pesticide offers practical, eco-friendly pest control solutions.

Article Information

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ABSTRACT

Sustainable control of plant diseases is essential to maintain biodiversity. Learning using prototypes is an effort to support students in understanding and learning science and technology in order to answer problems related to biodiversity loss. The research aimed to: 1) produce a prototype of Liqcoris organic pesticide (BLM 06) from coconut shell and shell waste, 2) measure the ability of the Liqcoris organic pesticide in inhibiting the growth of plant pathogenic microorganisms, and 3) study the content of Liqcoris organic pesticide. The data obtained were statistically analyzed using the SAS Version 9.0 program. The analysis carried out was the analysis of variance (ANOVA). Duncan's advanced test was used at the 5% level to determine the differences among the treatments, i.e., 00 = control or without PDA + pesticide; 10 = PDA + pesticide concentration 10%; 15 = PDA + pesticide concentration 15%, 20 = PDA + pesticide concentration 20%, and 25 = PDA + pesticide concentration 25%. Based on in vitro test, the study showed that Liqcoris concentration of 15% proved effective in killing phytopathogen fungi ($P < 0.05$), including Fusarium equiseti, Fusarium graminearum, Nigrospora sphaerica and Colletotrichum gloeosporioides. Treatments with various concentrations of 15%, 20%, and 25% effectively produced inhibitory values in the diameter of pathogenic fungal colonies, respectively 0.118 cm, 0.000 cm, and 0.000 cm that were significantly different from concentrations of 10% (2.7140 cm) and control 0% (5.2180 cm). Considering the economic value of production, the best concentration of organic pesticides chosen was 15%. On the other hand, in vivo test results indicated that in controlling mosaic viruses on diseased curly chili variety TM 999, without Liqcoris treatment (control), the intensity of disease attack increased by 20.22% after 6 weeks after treatment (wat). Meanwhile, after applying Liqcoris organic pesticide with a concentration of 15%, the percentage of mosaic virus attacks decreased to 2.72% after 6 wat ($P < 0.05$). This study showed that the content of 15% Liqcoris organic pesticide was dominated by active compound phenol (35.16%) and carbamic acid, phenyl ester (23.61%).

Keywords: biodiversity, carbamic acid, in vitro test, in vivo test, phenol, plant diseases

INTRODUCTION

The impacts of climate change are increasing the current loss of biodiversity (Linders *et al*. 2019; Ceballos *et al*. 2015; National Research Council 2013). The decline and loss of biodiversity can have serious impacts not only on the environment, but also on human welfare in many aspects, such as health, economy, culture, food security, air and others. Therefore, it is important to investigate and understand the status of biodiversity and the driving factors causing biodiversity loss. Several factors causing the decline or extinction of a species include loss of habitat, air, soil and air pollution, climate change, warming seawater, ocean acidification, the emergence of disease, exploitation of flora and fauna, introduced species and industrialization of agriculture and forests.

Biodiversity plays an important role in the framework of climate change mitigation strategy development. It refers to the living variability and ecological complex of an organism, including: a) diversity of ecosystems or communities, habitats and landscapes, b) biodiversity, and c) genetic diversity within species different. Biodiversity is a prerequisite for the healthy and natural development of all living individuals and ecosystems, therefore, it is important to study how global change affects ecosystem function (Allan *et al*. 2015), as well as the importance of biodiversity conservation activities in providing structural diversity as well as sustainable development of human civilization in the future.

Moreover, the use of prototypes is one effort to facilitate understanding on the role of science and technology in solving problems related to biodiversity loss. A prototype is a basic working model for developing a learning method, in the form of a module, product model, or tool model. A prototype is a visual form of media that is using the sense of sight (eyes) to facilitate the learning process. Apparently, there is a significant influence between knowledge and visual representation (Arum & Abdurrahman 2014). In this context, the existence of superior prototypes can help encourage the younger generation to save flora and fauna biodiversity from eventual decline.

Mitigating biodiversity decline through plant disease control using organic pesticides has increasingly gained public recognition and acceptance. On the other hand, mitigating biodiversity decline can increase the awareness and desire for a healthier life and prevent further environmental damage. This paradigm shift considering that organic pesticides leave no residue on plants after its use. Other benefits of using organic pesticides include: 1) faster decomposition process so the pesticide do not leave residues in the soil or on plants (Arif 2015; Dharmadewi & Suryatini 2022), 2) organic pesticides are readily available at a more affordable price (Wulandari *et al*. 2019) compared to other chemical pesticides, 3) apart from being safe for health, organic pesticides from fruits and vegetables are also environmentally friendly (Wulandari *et al*. 2019; Yunus, 2022), and 4) the raw materials for making organic pesticides are easy to find (Daryanti 2024; Firyanto 2023).

MATERIALS AND METHODS

Study Location

The study was conducted in the Biosystems Landscape Management (BLM) Laboratory, Phytopathology Laboratory, and greenhouse of SEAMEO BIOTROP, Bogor, West Java Province, Indonesia.

Sampling Plant Commodities Attacked by Phytopathogen Fungi

Sampling of diseased plants was carried out in two school gardens located at State Vocational School 1 Pacet, Cianjur, and Agricultural Development State Vocational High School Cianjur, West Java Province, Indonesia. The sampling was carried out using exploration method on leaves and fruits to obtain phytopatogen microorganism. The parts of plant that were infected by the disease were cut and put into an envelope, and labeled accordingly. Subsequently, the sample was taken to the laboratory for analysis.

Isolation and Identification of Phytopathogen Fungi

The phytopathogen *fungi* were isolated from the leaves and fruits of the diseased plants. The leaves and fruits were taken to the laboratory and incubated for ± 2 weeks at 22 ^{o}C in a closed container and lined with damp tissue. Isolation of the phytopathogen *fungi* was carried out by cutting the part of the leaf that has spores growing by cutting half the diseased part and half the healthy part, then growing those parts on PDA (Potato Dextrose Agar) media, followed by incubation until the pathogen growth occurred on the media. The fungus was isolated using the spread method at a dilution of 10^{-1} to 10^{-6} grown on

Figure 1 (a) Mosaic symptoms due to virus attack on chili plants,
(b) rotten symptoms on chili fruits suspected of being attacked by *Colletroticum*

PDA media in duplicate. Incubation was carried out at 37 °C for 24 - 48 hours. Purification of the isolate was carried out using the quadrant streak technique. The phytopathogen isolates that were successfully isolated were then rejuvenated and identified molecularly. Molecular identification of phytopathogen fungi had been done at the ICBB (Indonesian Center for Biodiversity and Biotechnology) Laboratory, PT Biodiversitas Bioteknologi, Bogor, Indonesia.

Preparation of Pesticide Organic Liqcoris

Raw materials in the form of coconut shell waste and coconut fiber were cleaned and dried before the pyrolysis process was carried out. The liquid smoke that had been obtained was then packaged in the 1,000 mL plastic bottle and labeled Liqcoris. Furthermore, the liquid smoke was analyzed and tested to obtain data on its content before being tested for its effectiveness.

Analysis of Liqcoris Organic Pesticide Content

The analyzed data were: a) pH measurements (APHA 23rd (2017): 4500-H+B); b) Phenol Content (GC-MS), c) Total Nitrogen (IK.LP-04.16-LT-1.0); d) Total Phosphorus as P_2O_5 (AOAC (2012) 942.05)); e) Total Potassium as K_2O (IK.LP-04.10-LT-1.0); f) Protein Content (IK.LP-04.5-LT-1.0); g) C-organic (Trimetry); and h) total Sugar (SNI 01-2892-1992 (Luff Schrool). The content of the organic pesticide was analyzed at the Testing and Certification Services Laboratory Unit, Bogor Agricultural Institute, Bogor, Indonesia.

In Vitro Test on the Effectiveness of Liqcoris Organic Pesticide

Isolates of phytopathogen fungi that had been identified molecularly were used as material for Liqcoris organic pesticide effectiveness tests, i.e., *Fusarium equiseti, Fusarium graminearum, Nigrospora sphaerica, Colletotrichum gloeosporioides*, and *Fusarium equiseti*. The effectiveness test was analyzed using a Completely Randomized Design (CRD) with 2 factors. The first factor was 5 concentrations of Liqcoris organic pesticides (0%, 10%, 15%, 20%, and 25%), while the second factor was 5 isolates of phytopathogen fungi that had been identified molecularly. The total experimental units were 125. Phytopathogen fungi isolates that had been rejuvenated and incubated for 7 days at room temperature were planted on PDA media, which were mixed with Liqcoris organic pesticide concentrations (0%, 10%, 15%, 20%, and 25%), then the inhibitory effect of organic pesticides in controlling the growth of phytopathogen fungi was observed.

The growth of phytopathogen fungi was observed by calculating the increase in growth diameter of the five phytopathogen fungi isolates from the time they were planted until the period of treatment application. Measurement of the growth diameter of phytopathogen fungi isolates was stopped after the growth of the isolates was hampered/stopped due to the influence of Liqcoris organic pesticide treatment.

In Vivo Test on the Effectiveness of Liqcoris Organic Pesticide

The in vivo test on the effectiveness of Liqcoris organic pesticide was carried out using a diseased chili plant variety, red arrow curly TM 999 variety chili , which was 3 months old. Mosaic symptoms had attacked the diseased chili plants used in the study due to viruses. The concentration of Liqcoris organic pesticide used at this stage was the best concentration resulting from in vivo effectiveness testing using the pour method. The 15% Liqcoris concentration was sprayed on the diseased chili plants using a knapsack sprayer according to the level of attack by Plant Pest Organisms (PPO). Spraying was twice per week. Observations were carried out every week on 40 sample plants. The observed parameters were plant growth and attack intensity. Measurement of attack intensity was conducted following Aâ *et al.* (2013). The assessment of mosaic symptom attacks in the study is presented in Figure 2 and Table 1.

On the other hand, the attack intensity of the diseased TM 999 variety chili plants was calculated using the following formula:

$$P = \frac{\sum(n \; x \; v \;) x \; 100\%}{N \; x \; Z}$$

where:

P = Attack intensity
n = Number of leaves from each attack category
v = Scale value of each category
N = Number of sample plants observed
Z = The highest attack category value

Statistical Analysis

The data obtained were statistically analyzed using the SAS Version 9.0 program. The analysis was carried out using analysis of variance (ANOVA). In determining the differences among treatments, Duncan's advanced test was used at the 5% level.

RESULTS AND DISCUSSION

Molecular Identification of Pathogenic Fungi

In the in vitro test of the effectiveness of Liqcoris organic pesticide, the fungi that cause plant disease were isolated and identified molecularly to prove and make sure whether all the isolates that would be used in the effectiveness test were actually the plant phytopathogen fungi or not. The results of this research showed that five isolates (*F. equiseti*; *F. graminearum*; *N. sphaerica*; *C. gloeosporioides*; *F. equiseti*) that had been isolated successfully were phytopathogen fungi.

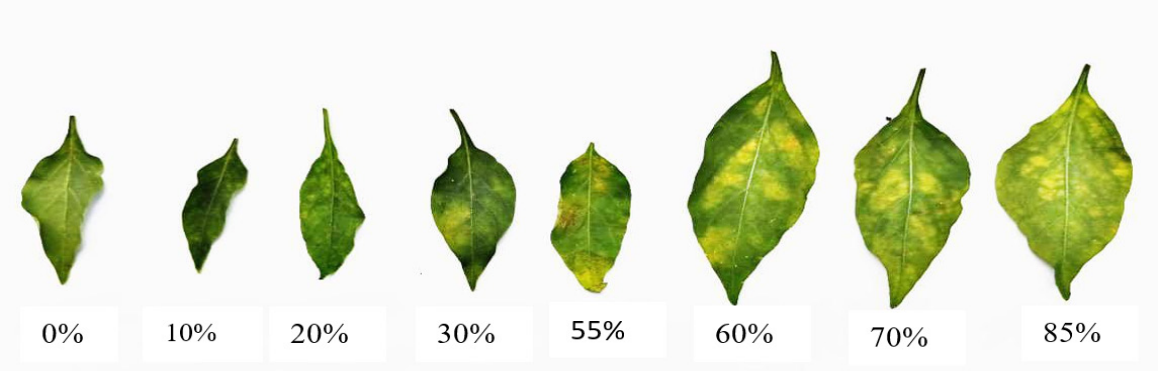

Figure 2 Chart of mosaic symptom scores due to virus attack on TM 999 variety chili plants

Table 1 Categories of mosaic symptoms due to virus attack on TM 999 variety chili plants

No.	Range (%)	Symptoms
1.	0	Plants did not show virus symptoms (healthy)
2.	1 -10	Plants showed very mild mosaic symptoms
3.	11- 29	Plants showed moderate mosaic symptoms
4.	30 - 59	Plants showed symptoms of heavy mosaicism or heavy striping without leaf shrinkage or deformity
5.	60 - 100	Plants showed very heavy mosaic or mottled symptoms with severe leaf shrinkage or deformation, stunting or death

The identification results obtained 5 species of phytopathogen fungi (Fig. 3). The BLAST results of the five isolates in the NCBI database are presented in Table 2.

Table 2 BLAST results on the NCBI database for five isolates of phytopathogen fungi

Isolate	Sample number	Name of comparison species	Accession code	Homology (%)
1	2211.07586	*Fusarium equiseti* isolate QN0826.1 large subunit ribosomal RNA gene, partial sequence	MN368509.1	100
2	2211.07587	*Fusarium graminearum* culture CBS:131572 strain CBS 131572 large subunit ribosomal RNA gene, partial sequence	MH877356.1	100
3	2211.07588	*Nigrospora sphaerica* isolate Velamen roots of Rhyncostylis retusa large subunit ribosomal RNA gene, partial sequence	MH259869.1	100
4	2211.07589	*Colletotrichum gloeosporioides* strain APVR 29 28S ribosomal RNA gene, partial sequence	MF100150.1	100
5	2211.07590	*Fusarium equiseti* isolate QN0826.1 large subunit ribosomal RNA gene, partial sequence	MN368509.1	100

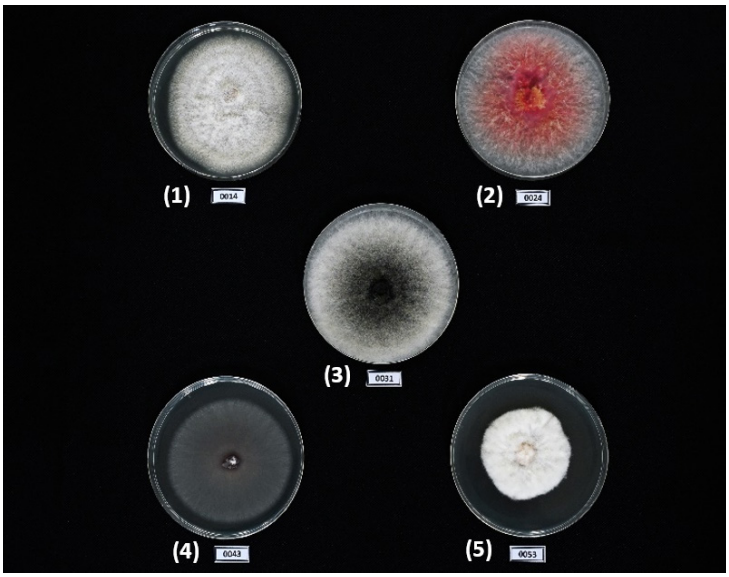

Figure 3 Morphology of five isolates phytopathogen fungi

Notes: (1) = *Fusarium equiseti;* (2) = *Fusarium graminearum;* (3) = *Nigrospora sphaerica;* (4) = *Colletotrichum gloeosporioides;* (5) = *Fusarium equiseti.*

The results of the molecular test were in accordance with the morphological characteristics of the five isolates. This is supported by the statement of Hami *et al.* (2021) and Rizki *et al.* (2021) who stated that *F. equiseti* is a fungal pathogen that attacks chili and lettuce plants (Tziros *et al.* 2022). Symptoms of an attack caused by *F. equiseti* include changes in leaf color from green to yellow then wilting and eventually the plant dies. According to Hazzat *et al.* (2023), on PSA media, isolate N3 developed colonies with abundant, fluffy, creamy white aerial mycelium. On the other hand, the color of the *F. graminearum* colony (isolate ES15) is reddish with a circular shape, lunar-shaped macroconidia, 2-3 septates. Oval microconidia are not septate (Hakim & Kasiamdari 2023).

Meanwhile, the phytopathogen fungi *N. sphaerica* is a phytopathogen fungus on blueberries (Wright *et al.* 2008). The results of macroscopic identification of *N. sphaerica* colonies were white to light gray to dark on the seventh day. Colony *N. sphaerica* were initially white and gradually become light gray to dark gray with the onset of sporulation (Zheng *et al.* 2021; Maulidiyah *et al.*, 2023). According to Amrullah *et al.* (2023), *C. gloeosporioides* colonies appear to grow concentrically with a creamy white upper color at the age of 5 days. At the age of 11 days, the colony produces orange acervulus which contain a collection of conidia masses.

In Vitro Test on Effectiveness of Liqcoris Organic Pesticide

The application was tested in vitro by measuring the inhibitory effect of the organic pesticide at various concentrations (0%, 10%, 15%, 20%, and 25%) on the growth of the five isolates of phytopathogen fungi. In the test, the parameters measured were the diameter of the fungal growth colony during the application of Liqcoris organic pesticides. Results of the observations of pesticide inhibitory effect are presented in Table 3. Treatment with various concentrations (0%, 10%, 15%, 20%, and 25%) had a very significant effect in inhibiting the increased colony diameter of phytopathogen fungi ($P < 0.05$) for 6 days after incubation.

Table 3 The ability of Liqcoris organic pesticide to inhibit the growth of phytopathogen fungi in vitro for 5 days after incubation

Conc. (%)	Treatment	Increase in diameter (cm)
00	Control or without PDA+ pesticide	5.2180[a]
10	PDA+ pesticide concentration 10%	2.7140[b]
15	PDA+ pesticide concentration 15%	0.1180[c]
20	PDA+ pesticide concentration 20%	0.0000[c]
25	PDA+ pesticide concentration 25%	0.0000[c]

Notes: Numbers followed by the same letter in the same column indicate that they are not significantly different based on the DMRT test at the $\alpha \leq 5\%$ level; PDA = Potato Dextrose Agar.

Based on Table 3, Duncan's further test results stated that the best inhibitory ability of organic pesticides in inhibiting/controlling the growth of phytopathogen fungi was shown at a concentration of 15% with an average increase in colony diameter of 0.118 cm. ($P < 0.05$). The treatment with various concentrations differed significantly from the control (0%). Treatments with various concentrations of 15%, 20%, and 25% effectively produced increased colony diameter of phytopathogen fungi that were not significantly different, respectively 0.118 cm; 0.000 cm; 0.000 cm ($P < 0.05$). By considering the economic value of production, the best concentration chosen was 15% which would be used in the in vivo test.

In Vivo Test on the Effectiveness of Liqcoris Organic Pesticide

Before applying the 15% concentration of Liqcoris organic pesticide to the diseased plant, the test plants chosen were chili plants, which were attacked by mosaic symptoms due to viruses. The Intensity of Disease Symptoms (IDS) of plants was measured before and after Liqcoris organic pesticides were applied. The intensity of mosaic virus disease and aphid attacks were observed over a period ranging from 1 week to 6 weeks. After applying the Liqcoris organic pesticide at a 15% concentration, the results showed a significantly different effect compared to the control ($P < 0.05$) at 6 wat (weeks after treatment) (Table 4).

Based on the Table 4, control or plants that were not given a application had a higher IDS value than those plants that were given the 15% Liqcoris organic pesticide. The results showed that compared to the control, which IDS value was 33.04% at 0 wat and increased to 53.26% at 6 wat, the application of 15% Liqcoris organic pesticide could control the development of mosaic virus disease. The application of 15% Liqcoris organic pesticide showed that the IDS value at 0 wat was 21.13% and decreased to 18.41% at 6 wat. The lowest symptoms of mosaic virus disease were observed when applying the organic pesticide Liqcoris compared to the control. Based on the result, the active compound content of Liqcoris may have been able to control plant mosaic viruses.

Test Results on the Content of Liqcoris Organic Pesticide

After obtaining the best concentration that would be used, the content of the Liqcoris organic pesticide was tested. The test results showed that the active compound contained in the 15% Liqcoris organic pesticide were phenol (35.16%); carbamic acid; phenyl ester (23.61%); butyric acid hydrazide (2.87%); cyclotene (1.27%); o-cresol (3.33%); m-cresol (4.25%); phenol 2-methoxy (14.28%); p-creosol (3.20%); phenol 4-ethyl-2-methoxy (1.31%); syringol (8.16%); phenol 2,6-dimethoxy-4-methyl (1.56%); and trans-13-octadecenoic acid methyl ester (0.98%).

According to Aminu and Abdullahi (2021), phenol and cresol are a group of disinfectants. *Disinfectants* are materials used to eradicate pathogenic bacteria and microorganisms. Phenol and its derivatives function to prevent pest attacks, plant diseases and virus (Aminu & Abdullahi 2021; Hagner 2013; Zhang 2014). Phenol is a class of secondary metabolites with hydroxyl groups (Tijjani *et al.* 2018). These compounds are synthesized in plants as part of the host's defense mechanism against pathogens.

Table 4 Intensity of symptoms mosaic virus and aphids during treatment

Sample plant	Type of disease symptom	Treatment	IDS		Result
			0 wat (%)	6 wat (%)	
Diseased TM 999 variety chili plant	Mosaic symptoms due to virus attack	Control	33.04[a]	53.26[a]	IDS increase +20.22%
		15% Liqcoris Organic Pesticide	21.13[b]	18.41[b]	IDS decrease -2.72%

Notes: IDS = Intensity of Disease Symptoms; wat = weeks after treatment; numbers followed by the same letter in the same column indicate that they are not significantly different based on the DMRT test at the α ≤ 5% level.

Table 5 Liqcoris organic pesticide nutrient content

Parameter*	Result	Regulatory Limit**	Unit	Method
C-Organic (wet base)	0.50	Min. 15	% w/w	Titrimetry
C/N	1.39	< 25	% w/w	Calculation
Macro nutrients (Total N+P_2O_5+K_2O)	20.86	Min. 2	% w/w	Calculation
Total Nitrogen	0.36	-	% w/w	IK.LP-04.16-LT-1.0
Phosporous as P_2O_5	20.50	-	% w/w	AOAC(2012)942.05
Potassium as K_2O	0.001	-	% w/w	IK.LP-04.10-LT-1.0
pH	4.57	4 - 9	-	APHA23rd(2017):4500+B
Protein content	2.16	-	% w/w	IK.LP-04.5-LT-1.0
Total sugar	38	-	% w/w	SNI 01-2892-1992(Luff Schrool)

Notes: * = Outside the scope of accreditation; ** = Regulation of the Minister of Agriculture of the Republic of Indonesia No. 261/Permentan/KPTS/SR.310/M/4/2019 regarding Technical Requirements for Solid Organic Fertilizer.

Phenolics have large and complex chemical structures (Walton 2003). Flavonoids are the largest class of phenolics. Phytoalexins are isoflavonoids that present antibiotic and antifungal properties in response to invading pathogens. They are toxic molecules that disrupt pathogenic biochemical processes or cellular structures (Sharma *et al*. 2022).

Meanwhile, carbamates are organic pesticides derived from carbamic acid, including carbaryl, carbofuran, and ami nocarb (Zacharia 2011). The principle behind the use of carbamate pesticides is to influence the transmission of nerve signals, resulting in the death of the pest through poisoning (Yadav *et al*. 2015). This pesticide can be easily degraded in the natural environment with minimum environmental pollution. Hagner (2013) reported that acetic acid and furfural contained in liquid smoke have the ability to repel snail pests. The effectiveness of disinfectant compounds is greatly influenced by their concentration and exposure time. The higher the concentration and the longer the exposure, the more influential the disinfectant compound will be. The macronutrient content in Liqcoris organic pesticide is presented in Table 5.

In this study, the C/N, macronutrients, total nitrogen, phosphate, potassium, pH, protein, and total sugar are in accordance with the regulation of the Minister of Agriculture of the Republic of Indonesia no. 261/Permentan/KPTS/SR.310/M/4/2019 regarding Technical Requirements for Solid Organic Fertilizer (Table 5). Our study showed that Liqcoris contained low concentration of C-organic and should be increased to optimal levels as an organic fertilizer. Organic pesticides can be mixed with other natural ingredients with high organic C content to increase organic C.

Based on the results of content tests, the Liqcoris Organic Pesticide that has been successfully produced can be used to improve soil quality and help plants grow better, stronger, and more resistant to pests and diseases. This result is supported by Yatagai (2002), who reported that liquid smoke contains chemical components, such as acetic acid that accelerate plant growth and prevent disease in plants. Liquid smoke can be applied to plants by spraying it on all the leaves, twigs, and branches. The concentration of liquid smoke used is 1 - 2% (with water solvent) to maintain healthy plants,

and if pests attack the plants, the concentration can be increased to 3 - 5% depending on the attack level. To increase diameter and height for forestry plants and increase production for agricultural plants, the application of liquid smoke can be combined with compost charcoal. Utilization of organic waste into friendly pesticides has promising prospects, because organic waste is available with various nutrient contents and the raw materials are abundant in nature. Organic pesticides function as a plant pest control. Besides, organic pesticide is also environmentally friendly because the materials used are active and easily decomposed in nature.

CONCLUSION

Liqcoris, an organic pesticide made with coconut shell waste at 5% concentration, effectively killed phytopathogen fungi compared to the control ($P < 0.05$). In vitro tests revealed that treatments with concentrations of 15%, 20%, and 25% effectively inhibited the diameter of pathogenic fungal colonies by 0.118 cm, 0.000 cm, and 0.000 cm, respectively, which was significantly different from concentrations of 10% (2.7140 cm) and control 0% (5.2180 cm). Given the economic value of output, the optimal concentration of organic pesticides is 15%. In vivo test results showed that in controlling mosaic viruses on diseased TM 999 variety curly chili, without Liqcoris treatment (control), the intensity of disease attack increased by 20.22% at 6 weeks after treatment (wat). Meanwhile, after applying 15% Liqcoris organic pesticide, the percentage of mosaic virus attacks decreased -2.72% at 6 wat. The Liqcoris organic pesticide at 15% concentration is dominated by active compound phenol (35.16%) and carbamic acid, phenyl ester (23.61%).

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