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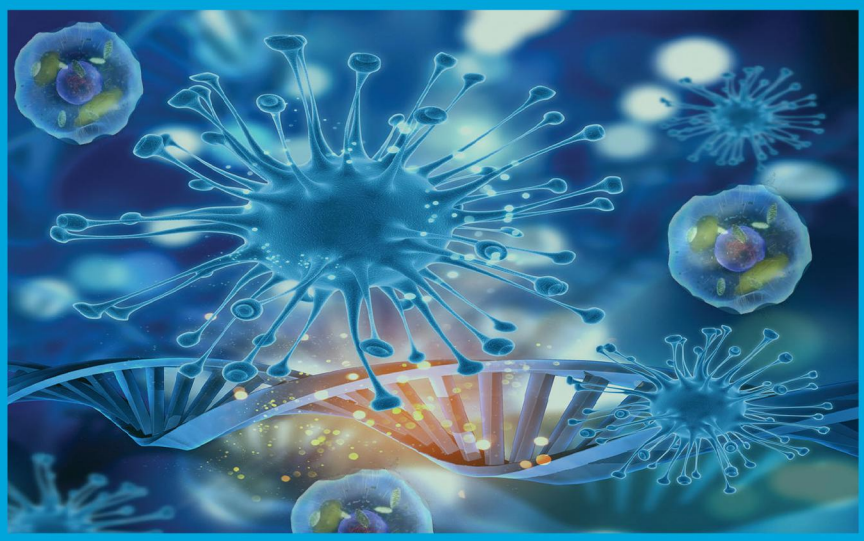

Malang City, Indonesia
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Editors
Dian Siswanto, Retno Mastuti, Fahrul Zaman Bin Huyop and Chairat Treesubsuntorn

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Preface: The 9th International Conference on Global Resource Conservation (ICGRC) and AJI from Ritsumeikan University

The 9th International Conference on Global Resource Conservation-AJI from Ritsumeikan University is a consolidation effort to provide a scientific forum for the scientist from Indonesia and abroad to share their research interest related to environmental issues. This conference took place at Ijen Suites Hotel Resort & Convention, Malang – East Java, in 7-8th March 2018.

The awareness to eradicate all forms and dimensions of poverty, including extreme poverty, is the greatest global challenge and the absolute requirement for sustainable development. In the context of Sustainable Development Goals, the Green Campus Program in Indonesia is an implementation of the effectively and efficiently utilization of existing resources in the campus environment.

Some universities have a focus on the green campus activities such as save energy, paperless, and zero-waste program. Sustainability of this program is a major concern to maintain the environment. The program may encourage any researchers from multidiscipline area to have a collaboration. Merging basic and applied sciences to solve the complex environmental issue is one of the important agenda for accelerating the contribution to society. Scientific meeting for facilitating the expert from botany, zoology, ecology, environmental health and conservation, and also conservation education and policy is one of strategy to overcome the recent issue in environments.

Hopefully, the information in this proceeding will support green campus program, its sustainability, and related technology development in Indonesia. We also would like to express our gratitude to PPIKID (Peningkatan Publikasi Karya Ilmiah Dosen) and Green Campus Program of Brawijaya University for financially support the conference and its proceeding.

Malang, August 2018

Dian Siswanto, M.Si, M.Sc., Ph.D
Chairman of ICGRC 2018

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Bio-larvicidal effervescent preparation development based on locally isolated Bacillus sphaericus from Lombok Island (West Nusa Tenggara, Indonesia) against Anopheles larvae

Bambang Fajar Suryadi, Baiq Wiwin Maruni Diarti, Yunan Jiwantarum, Baiq Laily Zainiati, and Santi Pristianingrum

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Bio-Larvicidal Effervescent Preparation Development Based on Locally Isolated *Bacillus sphaericus* from Lombok Island (West Nusa Tenggara, Indonesia) Against *Anopheles* Larvae

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Abstract. The aims of this study were to design an effervescent preparation using *Bacillus sphaericus* locally-isolated from lombok island previously grown on fish flour medium and to evaluate the toxicity of the preparation against field-captured 3rd instar *Anopheles* larvae. *B. sphaericus* was grown on fish flour medium at 30°c for 72 hours. The culture was dried and mixed with effervescent components (consisting of citric acid, tartaric acid, and calcium bicarbonate) in five combinations. From the five combinations, two combinations, namely combination 4 (10% citric acid : 10% tartaric acid : 55% calcium bicarbonate : 25% *B. sphaericus*) and combination 5 (5% citric acid : 5% tartaric acid : 65% calcium bicarbonate : 25% *B. sphaericus*), were effective in killing *Anopheles* larvae in the first 24 hours of observation. Besides effective larval killing, they both showed the fastest starting bubbling time and bubbling duration when put in water. In conclusion, fish flour medium combined with effervescent materials could be used to develop *B. sphaericus*-based bio-larvicidal preparations to control 3rd instar *Anopheles* larvae in a laboratory experiment.

Keywords: Effervescent, observation, toxicity.

INTRODUCTION

Malaria is still a health problem in Lombok Island, West Nusa Tenggara, Indonesia. In 2015 alone, it was predicted that roughly 58,500 people suffered from malaria on this island. Malaria cases were widely found in people inhabiting villages close to beach areas in Lombok Island.[1,2] Malaria parasites are spread by *Anopheles* mosquitoes, which consist of 26 species found on this island.[2]

There are some approaches that can be used to control *Anopheles*. However, most of them involve chemical pesticide agents that cause unwanted environmental effects with long term application. One approach that is considered effective and safe is by using biological control agents (bio-larvicidals), such as bacteria. The best known bacterium able to control *Anopheles* larvae is *Bacillus sphaericus*.[3]

One problem in battling mosquito-borne diseases is dependency on imported bio-larvicidal products. This includes *B. sphaericus*-based bio-larvicidal products. However, entomopathogenic *B. sphaericus* were reported to exist in almost every part of the world.[4] Therefore, locally-isolated entomopathonic *B. sphaericus* could be used for developing bacterial-based bio-larvicides to reduce the dependency. Suryadi et al.[5] were successful in isolating entomopathogenic *B. sphaericus* from Lombok Island. In 2017 [6], it was reported that some natural ingredient media can be used to grow the bacteria. From those cultures, *B. sphaericus* tested still showed high toxicity against naturally-captured 3rd instar *Anopheles* larvae.

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B. sphaericus-based bio-larvicidal preparations are commercially available in various forms. These preparations, albeit effective to control mosquito larvae, all have similar limitations. All commercial products were made as hydrophobic preparations, which allow the bacteria to be slowly liberated into the environment.[7] Therefore, fast-release formulations that can readily liberate an adequate number of bacteria for suppressing the targeted larvae should be made. In this study, we managed to make effervescent bio-larvicidal formulations using locally-isolated *B. sphaericus* and tested them to observe their effectiveness against *Anopheles* larvae.

EXPERIMENTAL DETAILS

B. sphaericus (Isolate MNT) stock was refreshed in nutrient agar solid medium supplemented with streptomycin (30 µg/mL). The bacterial culture was incubated at 30°C for 24 hours. The culture was then subcultured in liquid medium, containing fish flour and water (concentration was 30% w/v), then incubated at 30°C for 72 hours in a shaking incubator. Bacterial culture was then standardised to the concentration of a 10 McFarland standard (approximately 3 x 10^9 cells/mL). The bacterial culture was dried and mixed evenly with effervescent components (citric acid, tartaric acid, and sodium bicarbonate) in a volume-based mixture. Effervesce was made in five combinations. All combinations are listed in Table 1 below.

TABLE 1. Combination of *B. sphaericus* suspension and effervescent material to make effervescent bio-pesticide preparation

Combination #	*B. sphaericus* Concentration	Citric Acid	Tartaric Acid	Sodium Bicarbonate	*B. sphaericus* Suspension
Combination 1	10 McFarland Unit	25%	25%	25%	25%
Combination 2		20%	20%	35%	25%
Combination 3		15%	15%	45%	25%
Combination 4		10%	10%	55%	25%
Combination 5		5%	5%	65%	25%

Percentage is in volume-based manner

These five combinations were then tested for starting bubbling time and bubbling duration. Only combinations that showed the fastest starting bubbling time and the fastest bubbling duration were tested for LC (Lethal Concentration) value calculation. The LC value was calculated based on cell concentration.

The bioassay was performed on naturally-captured 3rd instar *Anopheles* larvae. This assay started when 20 grams of effervescence combination was mixed with 20 larvae in 200 mL water. The amount of the effervescence was gradually lowered in 10 time intervals and the procedure was replicated three times. Larval death and observation time were recorded and calculated to obtain LC values using Minitab V16 statistical application for Windows.

RESULTS AND DISCUSSION

An effervescent is a formulation that when mixed with water will release CO_2 rapidly. These formulations are made by mixing active ingredients including mixtures of sodium bicarbonate and organic acids such as citric and tartaric acid. The main advantages of effervescence are quick production of target ingredients in water. The most frequently used acid is citric acid. Other acids such as tartaric, fumaric, adipic, and malic acid can be used as well. Potassium and sodium carbonate, sodium and potassium bicarbonate, and arginine carbonate are examples of alkali sources. Sodium bicarbonate is the most used carbonate because of its high solubility, fast reaction, and low cost [8].

In this study, the basic effervescent formulation for bio-larvicidal was made based on Lombok locally-isolated *B. sphaericus*. Field-captured *Anopheles* larvae were chosen as the target. This approach aimed to determine the capability of the formulations to kill targeted larvae in a certain amount of time. Observations of the five effervescent combinations are presented in Table 2.

TABLE 2. Bubbling time and bubbling duration on 5 effervescence combination

Combination #	Start Bubbling (hh:mm:ss)	Stop Bubbling (hh:mm:ss)	Bubbling Duration (hh:mm:ss)
Combination 1	0:07:52	1:40:25	1:32:33
Combination 2	0:05:37	1:54:54	1:49:17
Combination 3	0:00:00	1:50:23	1:50:23
Combination 4	0:00:00	1:02:40	1:02:40
Combination 5	0:00:00	0:56:49	0:56:49

Combinations 4 and 5 showed the best combination of starting bubbling time and bubbling duration. The fastest bubbling time and bubbling duration found in those two combinations ensured the bacterial content of the preparation would be dispersed more rapidly in smaller sizes that could be easily swallowed by larvae. These combinations showed 100% larval death after 24 and 48 hours of observation, indicating they were very effective in killing *Anopheles* larvae. The formulations of the effervescent preparations are presented in Fig. 1.

FIGURE 1. Effervescent preparation consists of bacterial powder, citric acid and tartaric acid.

Bioassay results for combination 4 and combination 5 are presented on Table 3.

TABLE 3. Bioassay result on Combination 4 and 5 against *Anopheles* larvae

Combination #	24 hours			48 hours		
	Larval death on 10% conc.	LC_{50} (cell/mL)	LC_{90} (cell/mL)	Larval death on 10% conc.	LC_{50} (cell/mL)	LC_{90} (cell/mL)
Combination 1	100%	8.96×10^4	3.19×10^5	100%	5.54×10^3	2.83×10^4
Combination 2	100%	5.64×10^4	3.87×10^5	100%	2.07×10^3	2.06×10^4
Combination 3	100%	3.74×10^4	2.74×10^5	100%	3.54×10^3	2.70×10^4
Combination 4	100%	1.20×10^4	2.71×10^5	100%	4.84×10^2	2.10×10^3
Combination 5	100%	4.89×10^3	2.66×10^5	100%	3.55×10^3	2.67×10^4

From Table 3 it can be seen that all combinations, despite showing varied bubbling durations, could cause 100% larval death in a 10% culture concentration. The LC values (LC50 and LC90 on 24 and 48 hours observations) varied, but the differences were not great. It was clear that various effervescent combinations had little effect on larval death and LC values, as the concentration and proportion of the culture remained the same. The toxin produced by *B. sphaericus* grown in fish flour medium was very potent, as it maintained its toxicity until 10^{-7} dilution in 24 and 48 hours observations. *B. sphaericus* cannot use carbohydrates as a carbon source, it relies on protein and/or fat. These components were well provided by the fish flour used as growth medium. Besides fish flour, almost any ingredient (preferably from natural sources) can be used as a good growth medium for *B.*

sphaericus as long as it is rich in protein and/or fat. These media can be varied, from animal or plant sources.[3,9] Therefore, natural growth medium combined with effervescent ingredients could be proposed as a good candidate for developing simple bio-larvicidal effervescent formulations.

SUMMARY

In this study, we succeeded in developing a basic effervescent bio-larvicidal preparation using Lombok Island locally-isolated *B. sphaericus* cultivated in fish flour-based medium. The preparation showed 100% effectivity against 3rd instar *Anopheles* larvae in 24 and 48 hours testing. Thus, these are potentially good candidates for developing a bio-larvicidal preparation based on an indigenous biological agent to reduce dependency on imported products.

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CERTIFICATE

This is to certify that

BAMBANG FAJAR SURYADI

has participated at

A JOINT CONFERENCE OF BRAWIJAYA UNIVERSITY
& RITSUMEIKAN UNIVERSITY:
THE 9th INTERNATIONAL CONFERENCE ON GLOBAL RESOURCE CONSERVATION (ICGRC)
& AJI FROM RITSUMEIKAN UNIVERSITY

"Toward Sustainable Development Goals:
Green Campus, Sustainability and Technology"

as ***ORAL PRESENTER***

Biolarvicidal Effervescent Formulation Development Based on Local Isolate Bacillus sphaericus from Lombok Island, Indonesia

Ijen Suites Hotel Resort & Convention, Malang
7 - 8th March 2018

Prof. GyongSu Mun	**Prof. Dr. Ir. Mohammad Bisri, MS**	**Dian Siswanto, Ph.D**
Vice-Director Asia-Japan Research Institute	Rector of Universitas Brawijaya	Conference Chairperson

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"Toward Sustainable Development Goals:
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