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Lemongrass Powder in Bio-Bs Effervescent Formulation of Lombok Island Local Isolate on Viability and Amount of Bacillus Sphaericus Toxin Crystal Protein for Control of Anopheles sp. Larvae

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ABSTRACT

Bio Formula – BS effervescent (Bio- Bacillus sphaericus) is an Effervescent powder-shaped formula that is easy to use by the public. Lombok's local effervescent bio-BS isolated formula has a weakness in terms of smell. The results from the literature search that fragrant lemongrass has a distinctive smell and can kill Aedes aeygpti, but scientific data has not been obtained about the ability of fragrant lemongrass to kill Anopheles Sp in the form of larvae and mosquitoes. The study aimed to find out the effect of the combination formulation of Bio-BS Effervescent local isolate Lombok Island with the addition of lemongrass powder for viability and the amount of toxin crystal protein Bacillus sphaericus. This research method is exploratory and experimental in the laboratory with the design of Post Test Only Control Group Design. The study used six treatment formulations. Samples of Anopheles sp larvae research in Batu Layar lagoon, West Lombok regency and pelur lagoon in Peringgesela, East Lombok regency and from the results of colonization of larvae. The independent Variable is a combination formulation of Bio-BS Effervescent isolated locally from Lombok Island with the addition of fragrant lemongrass powder. The dependent variable is the mortality of Larvae Anophels Sp, Viability of B. Sphaericus, and Amount of Toxin Protein Production of B. Sphaericus. The larvae death rate, the concentration of cells/endospores, and the number of repeats in each container are then tabulated and analyzed using Probit Analysis with the help of MINITAB 16 software. B. sphaericus viability data and the amount of endospore toxin protein crystal production were descriptively analyzed.

INTRODUCTION

Development of a model for a biopesticide formula with the basic ingredients of B. sphaericus bacteria can be developed using simple media and simple formulas that can be used effectively and efficiently by the community for eradicating mosquitoes and controlling mosquitoes when they are in their larval stages. The use of biopesticides has been proven to be effective and safe to apply. FormulasBio – BSeffervescent (Bio-*Bacillus sphaericus)* is a formula in the form of an Effervescent powder so that it is easy to use by the public and gives an interesting impression because the effect of this powder when put in water gives

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foam due to the release of carbon dioxide gas in the formula and is expected to kill the larvae more quickly. This form of the Bio-BS effervescent (Bio-B. sphaericus) formula makes it attractive to the public for larval control because of how it works. By using effervescent preparations, bacteria can be forced to spread (vertically and horizontally) by the foam produced by these preparations. This resulted in the possibility of contact between the larvae and the B. sphaericus bacteria to be greater. Increased contact between the larvae and B. sphaericus bacteria will theoretically lead to a higher mortality rate of the larvae.

Research resultFikri & Jiwintarum, (2016)also succeeded in making 5 Bio-BS effervescent formulas (Bio- B. sphaericus). The formula consists of a composition of citric acid, tartaric acid, sodium carbonate, local B. sphaericus bacteria on the island of Lombok and fish meal. The addition of a natural media formula in Bio-BS effervescent (Bio-B. sphaericus) uses fish meal, because it contains high protein. Protein is a source of carbon and nitrogen for the growth of B. sphaericus bacteria. Formula Bio-BS effervescent (Bio-B. sphaericus) 4 consisting of 10% citric acid, 10% tartaric acid, 55% sodium carbonate, 25% fish meal and 10 units of B. sphaericus bacteria. Farland (3.0 x 109 cells/ml) with a duration of 01:02:40 froth. Formula Bio-BS effervescent (Bio-B. sphaericus) 5 consisting of 5% citric acid, 5% tartaric acid, 65% sodium carbonate, 25% fish meal and B bacteria. sphaericus 10 units Mc. Farland (3.0 x 109 cells/ml) with a long time for foam to appear 00:56:49. Bioaasay Test Larvicidal Formula Bio-BS effervescent 4 gave B. sphaericus 100% entomopathogenic ability from 24 hours of observation to 10-5 dilution, and 72 hours of observation 100% entomopathogenic ability to 10-6 dilution. The entomopathogenic ability of the Bio-BS effervescent 4 formula on Anopheles Sp larvae was still visible until 10-8 dilution, namely at 24 hours (35%), 48 hours (35%) and 72 hours (55%). Meanwhile, the Larvicidal Formula Bio-BS effervescent 5 Bioasasay Test showed 100% entomopathogenic ability of B. sphaericus bacteria from 24 hours of observation to 10-3 dilution, 48 hours 10-4 observation time of 72 hours 100% entomopathogenic ability to 10-6 dilution.(Fikri & Jiwintarum, 2016).

Research Fikri & Jiwintarum, (2017) also proves that the Bio-BS effervescent formula (Bio-B. sphaericus) is good for controlling Anopheles Sp larvae in the aquatic environment. Because B. sphaericus contained in the formula releases toxins contained in endospores which are capable of killing Anopheles Sp. mosquito larvae. Various analytical techniques cannot predict the killing power of B. sphaericus against larvae of certain mosquito species. The most effective detection method is to test B. sphaericus directly on mosquito larvae(Charles et al., 1988). The results of laboratory-scale trials of Bio-BS effervescent (Bio-B. sphaericus) which are useful for eradicating Anopheles sp mosquito larvae based on the area of the test area show no difference in control effectiveness Anopheles Sp larvae between formula 4 and formula 5 based on the area of the test areaFikri & Jiwintarum, (2017). Formulas of Bio-BS effervescent (Bio-B. sphaericus) 4 and Bio-BS effervescent (Bio-B. sphaericus) 5 when spread in the water environment, it is expected that B. sphaericus bacteria can spread in environmental water and can survive by producing a protein toxin for kills Anopheles Sp. larvae. Research Fikri & Jiwintarum, (2017). proves that the viability and protein crystal toxin produced by B. sphaericus is best when the formula is spread on water media originating from pond water, paddy field water and river water, followed by well water, and the worst is seawater media. and tap water.

Formulas Lombok's local isolate Bio-BS effervescent (Bio-B. sphaericus) has a weakness in terms of smell, so when it is used in society it is less attractive. To be able to use a model of a biopesticide formula with the basic ingredients of the B. sphaericus bacteria,

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a local isolate from Lombok Island, which can be developed using simple media and simple formulas that can be effectively and efficiently used by the community for eradicating mosquitoes and controlling mosquitoes when they are in the larval stage, and so easy to apply in society, we need a mixture of natural ingredients that have aromatherapy which scientifically can also function as a larvicide, such as citronella. The results of a literature search proved that citronella has a unique therapeutic aroma and can kill*Aedes aegypti*in the form of larvae and mosquitoes, but scientific data has not been obtained regarding the ability of citronella to kill Anopheles Sp in the form of larvae and mosquitoes and how it affects the viability of potential bacteria as biolarvicides.

METHODS

This research is exploratory and experimental in the laboratory with the research design Post Test Only Control Group Design. This study used 6 treatment formulations, negative control and positive control. Types of treatment are distinguished by variations in formulation combination Bio-BS Effervescent local isolate from Lombok Island with the addition of citronella powder. The number and form of treatment as shown in table 1.

Table 1 Combination formulation of Bio-BS effervescent (Bio-*Bacillus sphaericus)*4 and 5 with citronella powder

No	formulation	Bacterial Colonies of Bacillus sphaericus	Sour Citric	Tartric Acid	Natrium carbonate	Sea fish meal	Formula Dosage	Fragrant Lemongrass Powder
1.	Bio-BS effervescent 4	10 units *Mc. farland*	15%	15%	45%	25%	10 gram/L	10 gram/L
2.	Bio-BS effervescent 5	10 units *Mc. farland*	10%	10%	55%	25%	10 gram/L	10 gram/L
3.	Bio-BS effervescent 4	10 units *Mc. farland*	15%	15%	45%	25%	10 gram/L	5 gram/L
4.	Bio-BS effervescent 5	10 units *Mc. farland*	10%	10%	55%	25%	10 gram/L	5 gram/L
5.	Bio-BS effervescent 4	10 units *Mc. farland*	15%	15%	45%	25%	5 gram/L	10 gram/L
6.	Bio-BS effervescent 5	10 units *Mc. farland*	10%	10%	55%	25%	5 gram/L	10 gram/L
7.	Negative Control (Aquadest)							
8.	Positive Control (Methanol)							

The samples in this study were Anopheles Sp larvae in the Batu Layar lagoons, West Lombok Regency and the lagoons of Pelur Village, Peringgesela District, East Lombok Regency and from the results of larval colonization. The sample size needed for research on the Bio-BS effervescent formula (Bio-B. sphaericus) local isolate of Lombok Island on Anopheles Sp larvae because each experiment requires 20 Anopheles Sp instar III larvae, the number of larvae needed is 20 x 8 x 3 larvae = 480 Larvae + 25% correction factor (480 larvae + 120 = 600 Larvae). So the total number of larvae needed is 600 Anopheles Sp Instar III larvae.

The sampling technique uses the Non-Random Purpusive Sampling technique, namely sampling based on criteria made by the researchers themselves. The criteria for the larvae used in this study were the larvae of the Anopheles Sp Instar III mosquito. The independent variable (independent variable) formulation combination Bio-B Effervescent local isolate

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from Lombok Island with the addition of citronella powder. The dependent variable (dependent variable) was the death of Anophels Sp. larvae, the viability of B. Sphaericus and the total production of B. Sphaericus Toxin Protein.

RESULTS AND DISCUSSION

1. Death of Anophels Sp. Larvae

The results of testing the death of Anophels Sp larvae can be seen in table 2.

Table 2. The results of the description of the death of the larvae of Anopheles Sp instar III formulationcombinationBio-B Effervescent local isolate from Lombok Island with the addition of citronella powder

Formulation	Time	Average	Standard deviation	N
Bio-BS effervescent 4 + Citronella Powder (10 10) g/L (A)	0 Hours	25,0000	.00000	3
	24 hours	25,0000	.00000	3
	48 Hours	25,0000	.00000	3
	72 Hours	25,0000	.00000	3
	Total	25,0000	.00000	12
Bio-BS effervescent 5 + Citronella Powder (10 10) g/L (B)	0 Hours	25,0000	.00000	3
	24 hours	25,0000	.00000	3
	48 Hours	25,0000	.00000	3
	72 Hours	25,0000	.00000	3
	Total	25,0000	.00000	12
Bio-BS effervescent 4 + Citronella Powder (10 : 5) g/L (C)	0 Hours	25,0000	.00000	3
	24 hours	22.3333	.57735	3
	48 Hours	23.6667	.57735	3
	72 Hours	25,0000	.00000	3
	Total	24,0000	1.20605	12
Bio-BS effervescent 5 + Citronella Powder (10 5) g/L (D)	0 Hours	25,0000	.00000	3
	24 hours	22.3333	.57735	3
	48 Hours	23.6667	.57735	3
	72 Hours	25,0000	.00000	3
	Total	24,0000	1.20605	12
Bio-BS effervescent 4 + Citronella Powder (5 : 10) g/L (E)	0 Hours	25,0000	.00000	3
	24 hours	21.0000	1.00000	3
	48 Hours	22.3333	.57735	3
	72 Hours	25,0000	.00000	3
	Total	23.3333	1.87487	12
Bio-BS effervescent 5 + Citronella Powder (5 10) g/L (F)	0 Hours	25,0000	.00000	3
	24 hours	21.0000	1.00000	3
	48 Hours	22.3333	.57735	3
	72 Hours	25,0000	.00000	3
	Total	23.3333	1.87487	12
Positive Control (Methanol)	0 Hours	25,0000	.00000	3
	24 hours	25,0000	.00000	3
	48 Hours	25,0000	.00000	3
	72 Hours	25,0000	.00000	3
	Total	25,0000	.00000	12
Negative Control (Aquadest)	0 Hours	25,0000	.00000	3
	24 hours	.0000	.00000	3
	48 Hours	.0000	.00000	3
	72 Hours	.0000	.00000	3
	Total	6.2500	11.30668	12

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Formulation	Time	Average	Standard deviation	N
Total	0 Hours	25,0000	.00000	24
	24 hours	20.2083	7.98901	24
	48 Hours	20.8750	8.13641	24
	72 Hours	21.8750	8.44580	24
	Total	21.9896	7.22203	96

Table 3. Results of statistical analysis of the effect of the combination formulation of Bio-B Effervescent local isolate Lombok Island with the addition of citronella powder on mortality

Source	Type III Sum of Squares	df	MeanSquare	F	Sig.
Corrected Model	4946.990a	31	159,580	1276642	.000
Intercepts	46420010	1	46420010	37136008 3	.000
formulation	3439,406	7	491,344	3930750	.000
Time	323,781	3	107,927	863,417	.000
Formulation * Time	1183,802	21	56,372	450,972	.000
Error	8,000	64	.125		
Total	51375,000	96			
Corrected Total	4954990	95			
a. R Squared = .998 (Adjusted R Squared = .998)					

2. Viability of B. Sphaericus

Effect research resultsformulationcombinationBio-B Effervescent local isolate of Lombok Island with the addition of citronella powder on the viability of B. Sphaericus can be seen in tables 4 and 5.

Table 4. Viability of B. Sphaericus using Total Plate Count (TPC) method for 30 days incubation

Formulation	Replication	Number of colonies/cfu
Bio-BS effervescent 4 + Citronella Powder (10 : 10) g/L (A)	Plate 1	225
	Plate 2	220
	Plate 3	225
	Plate 4	225
	Total	895
	Average	223.75

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Formulation	Replication	Number of colonies/cfu
Bio-BS effervescent 5 + Citronella Powder (10 : 10) g/L (B)	Plate 1	224
	Plate 2	225
	Plate 3	222
	Plate 4	225
	Total	896
	Average	224
Bio-BS effervescent 4 + Citronella Powder (10 : 5) g/L (C)	Plate 1	215
	Plate 2	198
	Plate 3	200
	Plate 4	210
	Total	820
	Average	205.75
Bio-BS effervescent 5 + Citronella Powder (10 : 5) g/L (D)	Plate 1	200
	Plate 2	210
	Plate 3	210
	Plate 4	196
	Total	816
	Average	204
Bio-BS effervescent 4 + Citronella Powder (5 : 10) g/L (E)	Plate 1	200
	Plate 2	198
	Plate 3	215
	Plate 4	289
	Total	902
	Average	225.5
Bio-BS effervescent 5 + Citronella Powder (5 : 10) g/L (F)	Plate 1	200
	Plate 2	210
	Plate 3	189
	Plate 4	186
	Total	785
	Average	196.25
Positive Control (NYSM Media)	Plate 1	215
	Plate 2	210
	Plate 3	190
	Plate 4	189
	Total	804
	Average	201

Table 5. Viability of B. Sphaericus using Total Plate Count (TPC) method for 60 days incubation

Formulation	Replication	Number of colonies/CFU
Bio-BS effervescent 4 + Citronella Powder (10 : 10) g/L (A)	Plate 1	110
	Plate 2	160
	Plate 3	167
	Plate 4	178
	Total	615
	Average	198.25
	Plate 1	121

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Bio-BS effervescent 5 + Citronella Powder (10 : 10) g/L (B)	Plate 2	126
	Plate 3	130
	Plate 4	165
	Total	542
	Average	135.5
Bio-BS effervescent 4 + Citronella Powder (10 : 5) g/L (C)	Plate 1	153
	Plate 2	130
	Plate 3	178
	Plate 4	180
	Total	641
	Average	160.25
Bio-BS effervescent 5 + Citronella Powder (10 : 5) g/L (D)	Plate 1	153
	Plate 2	135
	Plate 3	178
	Plate 4	180
	Total	646
	Average	161.5
Bio-BS effervescent 4 + Citronella Powder (5 : 10) g/L (E)	Plate 1	132
	Plate 2	129
	Plate 3	158
	Plate 4	154
	Total	573
	Average	143.25
Bio-BS effervescent 5 + Citronella Powder (5 : 10) g/L (F)	Plate 1	120
	Plate 2	150
	Plate 3	167
	Plate 4	178
	Total	615
	Average	153.75
Positive Control (NYSM Media)	Plate 1	110
	Plate 2	150
	Plate 3	160
	Plate 4	170
	Total	590
	Average	190

3. B. Sphaericus Toxin Protein Amount

Table 6. Data analysisThe amount of Bacillus sphaericus toxin protein was calculated using the direct method (slide) with a calculated area of 1 cm2 exposed to 30 days

Formulation	Replication	Bacillus sphaericus Toxin Protein Amount
Bio-BS effervescent 4 + Citronella Powder (10 : 10) g/L (A)	Slide 1	83
	Slide 2	87
	Slide 3	87
	Total	257
	Average	85.7
Bio-BS effervescent 5 + Citronella Powder (10 : 10) g/L (B)	Slide 1	85
	Slide 2	80
	Slide 3	87
	Total	252
	Average	84

Bio-BS effervescent 4 + Citronella Powder (10 : 5) g/L (C)	Slide 1	70
	Slide 2	85
	Slide 3	90
	Total	245
	Average	81.7
Bio-BS effervescent 5 + Citronella Powder (10 : 5) g/L (D)	Slide 1	70
	Slide 2	90
	Slide 3	86
	Total	246
	Average	82
Bio-BS effervescent 4 + Citronella Powder (5 : 10) g/L (E)	Slide 1	66
	Slide 2	56
	Slide 3	69
	Total	191
	Average	63,7
Bio-BS effervescent 5 + Citronella Powder (5 : 10) g/L (F)	Slide 1	60
	Slide 2	54
	Slide 3	66
	Total	180
	Average	60
Positive Control (NYSM Media)	Slide 1	90
	Slide 2	92
	Slide 3	87
	Total	269
	Average	89.7

Table 7. Data analysisThe amount of Bacillus sphaericus toxin protein was calculated using the direct method (slide) with a calculated area of 1 cm2 exposed to 60 days

Formulation	**Replication**	**Bacillus sphaericus Toxin Protein Amount**
Bio-BS effervescent 4 + Citronella Powder (10 : 10) g/L (A)	Slide 1	100
	Slide 2	100
	Slide 3	99
	Total	299
	Average	99.7
Bio-BS effervescent 5 + Citronella Powder (10 : 10) g/L (B)	Slide 1	98
	Slide 2	97
	Slide 3	99
	Total	294
	Average	98
Bio-BS effervescent 4 + Citronella Powder (10 : 5) g/L (C)	Slide 1	96
	Slide 2	95
	Slide 3	99
	Total	290
	Average	96.7

Bio-BS effervescent 5 + Citronella Powder (10 : 5) g/L (D)	Slide 1	91
	Slide 2	89
	Slide 3	90
	Total	270
	Average	90
Bio-BS effervescent 4 + Citronella Powder (5 : 10) g/L (E)	Slide 1	80
	Slide 2	86
	Slide 3	90
	Total	256
	Average	85,3
Bio-BS effervescent 5 + Citronella Powder (5 : 10) g/L (F)	Slide 1	68
	Slide 2	85
	Slide 3	89
	Total	242
	Average	80.7
Positive Control (NYSM Media)	Slide 1	100
	Slide 2	100
	Slide 3	99
	Total	299
	Average	99.7

Biopesticides formulas based on local natural resources for the growth and reproduction of B. sphaericus which can erase people's thoughts and fears using biopesticides with bacterial ingredients called Bio - BSeffervescent (Bio-*Bacillus sphaericus)* Lombok island local isolatesfor controlling Anopheles Sp. larvae. This form of the Bio-BS effervescent (Bio-B. sphaericus) formula makes it attractive to the public for larval control because of how it works. This is in accordance with theoretical studies which state that Effervescent is a dosage form that will provide efficient delivery for effective absorption, this preparation will completely dissolve in water. By using effervescent preparations, bacteria can be forced to spread (vertically and horizontally) by the foam produced by these preparations. This resulted in the possibility of contact between the larvae and the B. sphaericus bacteria to be greater. Increased contact between the larvae and B. sphaericus bacteria will theoretically lead to a higher mortality rate of the larvae.

FormulasLombok's local isolate Bio-BS effervescent (Bio-B. sphaericus) has a weakness in terms of smell, so when it is used in society it is less attractive. To be able to use a model of a biopesticide formula with the basic ingredients of the B. sphaericus bacteria, a local isolate from Lombok Island, which can be developed using simple media and simple formulas that can be effectively and efficiently used by the community for eradicating mosquitoes and controlling mosquitoes when they are in the larval stage, and so easy to apply in society, we need a mixture of natural ingredients that have aromatherapy which scientifically can also function as a larvicide, such as citronella. The results of this study provecombination formulationBio-BS Effervescent local isolate of Lombok Island with the addition of lemongrass powder can be used as a biopesticide, especially for test larvae of Anopheles sp instar III and proves that the formulationcombinationBio-BS Effervescent local isolate of Lombok Island with the addition of lemongrass powder did not affect the viability of Bacillus Sphaericus and the longer the incubation period in the formula exposure, the greater the amount of toxin crystal protein.

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The results of this study indicate that the combination formulation of Bio-BS effervescent 4 + Citronella Powder (10 : 10) g/L and the combination formulation of Bio-BS effervescent 5 + Citronella Powder (10 : 10) g/L exposure 24 hours, 48 hours and 72 hours showed the death of 25 larvae (100%). The combination formulation of Bio-BS effervescent 5 + Citronella Powder (10: 5) g/L showed the average mortality of larvae at 24-hour exposure of 22 individuals, 48-hour exposure of 23 individuals and 72-hour exposure of 25 individuals. Formulations of Bio-BS effervescent 4 + Citronella Powder (5 : 10) g/L and Bio-BS effervescent 5 + Citronella Powder (5 10) g/L showed the average mortality of larvae at 24-hour exposure of 21, 48-hour exposure of 22 individuals and 72 hours of exposure totaling 25 individuals. For the positive control using methanol the number of larvae deaths from 24 hour exposure, 48 hours and 72 hours as many as 25 birds. The results of statistical analysis test the effect of the formulationcombinationBio-B Effervescent local isolate of Lombok Island with the addition of citronella powder on the death of third instar Anopheles Sp larvae. The results of the multiple comparisons statistical test showed that there was no significant effect between formulations A, B and the control group, while between the other formulations there was a significant effect.

Research Fikri & Jiwintarum, (2016) also succeeded in making 5 Bio-BS effervescent formulas (Bio- B. sphaericus). The formula consists of a composition of citric acid, tartaric acid, sodium carbonate, local B. sphaericus bacteria on the island of Lombok and fish meal. The addition of a natural media formula in Bio-BS effervescent (Bio-B. sphaericus) uses fish meal, because it contains high protein. Protein is a source of carbon and nitrogen for the growth of B. sphaericus bacteria. Result of identification of Bio-BS effervescent formula (Bio- B. sphaericus) from researchFikri & Jiwintarum, (2016)namely formulas 4 and 5 are the best formulas from the results of the foam test performed. This is because the two formula combinations provide the fastest effervescent effect and a longer effervescent effect or longer time. This allows the B. sphaericus bacteria in the preparation to spread well in the water environment being tested. Bioaasay Test Larvicidal Formula Bio-BS effervescent 4 gave B. sphaericus 100% entomopathogenic ability from 24 hours of observation to 10-5 dilution, and 72 hours of observation 100% entomopathogenic ability to 10-6 dilution. The entomopathogenic ability of the Bio-BS effervescent 4 formula on Anopheles Sp larvae was still visible until 10-8 dilution, namely at 24 hours (35%), 48 hours (35%) and 72 hours (55%). Meanwhile, the Larvicidal Formula Bio-BS effervescent 5 Bioasasay Test showed 100% entomopathogenic ability of B. sphaericus bacteria from 24 hours of observation to 10-3 dilution, 48 hours 10-4 observation time of 72 hours 100% entomopathogenic ability to 10-6 dilution. The entomopathogenic ability of the Bio-BS effervescent 5 formula against Anopheles Sp larvae was still visible until 10-8 dilution, namely at 24 hours (30%), 48 hours (45%) and 72 hours (60%)(Fikri & Jiwintarum, 2016).

Research Fikri & Jiwintarum, (2017) also proves that the Bio-BS effervescent formula (Bio-B. sphaericus) is good for controlling Anopheles Sp larvae in the aquatic environment. Because B. sphaericus contained in the formula releases toxins contained in endospores which are capable of killing Anopheles Sp. mosquito larvae. This is supported by theoretical studies which state that B. sphaericus is generally capable of killing mosquito larvae of the genus Culex and Anopheles Sp, but is less capable of killing larvae of the genus Aedes.(Berry et al., 1993). The ability to kill the larvae of various types of mosquitoes varies greatly, depending on the mosquito species and the B. sphaericus strain. It was also reported that the same B. sphaericus strain had different abilities in killing the same species of

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mosquito larvae (Thiery & de Barjac, 1989).

Research (Aji, 2017) proved that of the 62.5% of residents who planted citronella grass in their yard, 77.5% of them did not find Aedes aegypti larvae in the water reservoir environment.StudyArifin, (2014)which aims to determine the effect of citronella extract at various concentrations on the period of sucking blood from Aedes aegypti mosquitoes using citronella extract n-Hexane in the form of spray concentrations of 3.12%, 6.25%, 12.5% and 25% which are sprayed onto The results showed that citronella extract (Cymbopogon nardus (L.) Randle) which was effective at repelling mosquitoes during the observation period was a concentration of 25%. Research resultSatriawan, (2014)showed that citronella extract (Cyambopogon citrus) was effective as a larvicide against Aedes Sp where 0% mortality was obtained at a concentration of 0 ppm and at a negative control, 0%. 4% death at a concentration of 312.5 ppm, 42% death at a concentration of 652 ppm, 50% death at a concentration of 1250 ppm and 90% death at a concentration of 2500 ppm. The concentration that can kill 50% (LC50) of larvae is in the interval between 599.9 ppm and 1798.5 ppm, with an estimated 973.7 ppm or 0.097%.Research resultMirawati et al., (2018)proved that by using the steam distillation process of essential oils from zodia leaves and lemongrass stems made in lotion formulations, the effectiveness of repelling mosquitoes from lotion preparations was highest at 0 hours with 100% repellent power. The best combination of lotion preparations, a combination of essential oils of zodia leaves and essential oils of lemongrass stems as a repellent for Aedes aegypti mosquitoes, is 7:3. Research resultRiris et al., (2019) showed that citronella leaf essential oil combined with eucalyptus leaf essential oil and VCO had a repellent effect on Aedes aegypti mosquitoes. 1) and C (1:1:3) with a total protection against mosquitoes for 4 hours of 83%. The most effective total mosquito repellent was composition B (1:3:1) and C (1:1:3). with a total protection against mosquitoes for 4 hours of 83%.

The results of the post-hoc statistical test showed that the comparison of the compositions B and C had only a slight difference and there was a significant effect of applying the repellent on the frequency of Aedes aegypti mosquitoes perching as expressed by a P value <0.05.The results of a literature search proved that citronella has a unique therapeutic aroma and can kill*Aedes aegypti*in the form of larvae and mosquitoes, but scientific data has not been obtained regarding the ability of citronella to kill Anopheles Sp in the form of larvae and mosquitoes and how it affects the viability of potential biolarvicidal bacteria.Resultsviability of B. Sphaericus exposed formulation combination Bio-B Effervescent local isolate of Lombok Island with the addition of citronella powder with an incubation period of 30 days and 60 days of colony growth, this shows that B. Sphaericus survives(viable) to exposureformulationcombinationBio-B Effervescent local isolate from Lombok Island with the addition of citronella powder. The number of colonies that grew with exposure for 30 days from formulation A averaged the number of colonies 223.75/CFU, formulation B averaged the number of colonies 224/CFU, formulation C averaged the number of colonies 205.75/CFU, formulation D averaged the number of colonies 204/CFU, formulation E the average number of colonies was 225.5/CFU and formulation F the average number of colonies was 215/CFU and on NYSM control media the average number of colonies was 201/CFU. The number of colonies that grew with exposure for 60 days from formulation A average number of colonies was 198.25/CFU, formulation B average number of colonies was 135.5/CFU, formulation C average number of colonies was 160.25/CFU, formulation D average number of colonies was 161, 5/CFU, the average number of colonies in formulation E was 143.25/CFU and the average number of colonies in formulation F was

153.75/CFU and on NYSM control media the average number of colonies was 190/CFU.

The test results for calculating the number of endospore toxin protein crystals produced in each treatment were carried out using the breed slide method with CBB Spore staining + Acetic Acid. The average number of endospore toxin protein crystalswith exposure for 30 consecutive days from formulation A was 85.7 endospore toxin protein crystals, formulation B was 84, formulation C was 81.7, formulation D was 82, formulation E was 63.7, formulation F was 60 and on control media NYSM amounted to 89.7 crystals of endospore toxin protein. HThe results of the calculation test for the number of endospore toxin protein crystals produced in each treatment were carried out using the breed slide method with CBB Spore staining + Acetic Acid. The average number of endospore toxin protein crystalswith exposure for 60 consecutive days from formulation A was 99.7 endospore toxin protein crystals, formulation B was 98, formulation C was 96.7, formulation D was 90, formulation E was 85.3, formulation F was 80.7 and on NYSM control media amounted to 99.7 crystals of endospore toxin protein.

CONCLUSION

Combination formulation Bio-BS Effervescent local isolate of Lombok Island with the addition of lemongrass powder had no effect on test larvae of Anopheles sp instar III and the viability and amount of Bacillus Sphaericus toxin crystal protein for the control of Anopheles sp. larvae.

REFERENCE

Aji, R. (2017). The Effect of Lemongrass on the Presence of Aedes Aegypti Larvae in Water Reservoirs. Health Vocational Journal. http://ejournal.poltekkes-pontianak.ac.id/index.php/JVK 3 (1)

Arifin, MN (2014). Effect of n-Hexane Extract of Lemongrass (Cymbopogon nardus (L.) Randle) at various concentrations on the blood-sucking period of Aedes aegypti mosquitoes.

Berry, C., Hindley, J., Ehrhardt, AF, Grounds, T., & de Souza, E. (1993). Genetic determinants of host ranges of Bacillus sphaericus larvacidal toxins. J. Bacteriol, 175, 510–518.

Charles, JF, Kalfon, A., Bourgouin, C., & de Barjac, H. (1988). Bacillus sphaericus asporogenous mutants: ultrastructure, mosquito larvacidal activity and protein analysis. Ann. Inst. Pasteur/Microbiol, 139, 243–259.

Dulmage, T., Yousten, AA, Singer, S., & Lacey, LA (1990). Guidelines for Production of Bacillus Thuringiensis H-14 and Bacillus Sphaerirus. WHO Special Program for Research and Training in Tropical Diseases (TDR).

Fikri, Z., & Jiwintarum, Y. (2016). Simple Culture Media For Breeding Bacillus Sphaericus Local Isolate Of Lombok Island For Controlling Anopheles Sp. Mosquito Larvae. Research Development Report for Beginner Lecturer Scheme at the Mataram Health Polytechnic, Ministry of Health, Republic of Indonesia.

Mirawati, P., Simaremare, ES, & Pratiwi, RD (2018). Repellent Effectiveness Test of Lotion Preparations Combination of Essential Oil of Zodia Leaves (Evodia suaveolens Scheff) and Essential Oil of Lemongrass (Cymbopogon citratus) Against Aedes aegypti L. Mosquitoes. PHARMACY: Indonesian Pharmaceutical Journal (Pharmaceutical Journal of Indonesia), 15(1), 1. https ://doi.org/10.30595/pharmacy.v15i1.2286

Riris, M., Umar, M., & Fitriani, J. (2019). Repellent Effectiveness Test of Citronella Leaf

Essential Oil Cymbopogon nardus (L) Randle Combined with Eucalyptus Leaf Essential Oil (Melaleuca leucadendron Linn.) and VCO (Virgin Coconut Oil) Against Aedes aegypti Mosquitoes. Medika Tadulako, Scientific Journal of Medicine, 6 (1 January 2019).

Satriawan, A. (2014). Effectiveness of Lemongrass Kitchen (Cyambopogon citrus) as a larvicide on Aedes Sp Instar III/IV mosquito larvae. Faculty of Medicine Syariff Hidayatullah Jakarta.

Support., M. 17. (2015). Probit Analysis. http://support.minitab.com/en-us/minitab/17/topic-library/modeling-statistics/reliability/types-of-reliability-analyses/probit-analysis/

Susilawati, L., & Susiawati Purnomo, E. (2016). Viability of Bacterial Cells with Different Cryoprotectant Agents (As a Reference in Culture Collection Preservation in the Microbiology Laboratory). Biogenesis: Scientific Journal of Biology, 4(1), 34–40. https://doi.org/10.24252/bio.v4i1.1118

Thiery, I., & de Barjac, H. (1989). Selection of the most potent Bacillus sphaericus strains, based on activity ratios determined on three mosquito species. app. Microbiol. Biotechnol, 31, 577–581.