ISBN 978-602-73585-1-5

PROCEEDING BOOK

THE 4th INTERNATIONAL CONFERENCE ON HEALTH SCIENCE 2017

"The Optimalization of Adolescent Health in The Era of SDGs"

INNA GARUDA HOTEL YOGYAKARTA, INDONESIA
November 5th, 2017

HEALTH POLYTECHNIC OF HEALTH MINISTRY YOGYAKARTA Jl. Tata Bumi No.3, Banyuraden, Gamping, Sleman, Yogyakarta, Indonesia 55293 Phone/Fax.62-274-617601, Email : ichs@poltekkesjogja.ac.id

PROCEEDING BOOK

THE 4th INTERNATIONAL CONFERENCE ON HEALTH SCIENCE 2017

"The Optimalization of Adolescent Health in The Era of SDGs"

INNA GARUDA HOTEL YOGYAKARTA, INDONESIA
November 5th, 2017

ISBN : 978-602-73585-1-5

Published by
Health Polytechnic of Ministry of Health in Yogyakarta 2017

Proceeding of
The 4th International Conference on Health Science 2017
"The Optimalization of Adolescent Health in The Era of SDGs"

Printed in
Yogyakarta
November
2017

Table of Contents

Oral Presentation

Poster Presentation

The 4th International Conference on Health Science 2017 Committee

A. Steering Committee

Advisory Committee :

1. *The Head of Health Practitioners Training and Education Center of The Committee on Development and Empowerment of Health Human Resources of Health Ministry of Indonesia*
2. *The Head of Committee on Development and Empowerment of Health Human Resources of Health Ministry of Indonesia*
3. *Dr. Robert Priharjo, M.Sc, BSN PGCE RN Lecturer Anglia Ruskin University, United Kingdom*

B. Organising Committe

Person in charge	:	Joko Susilo, SKM.,M.Kes
Chairman I	:	Sari Hastuti, S.SiT, MPH
Chairman II	:	Mohamad Mirza Fauzie, S.SiT.,M.Kes
Chairman III	:	Sri Arini Winarti Rinawati, SKM, M.Kep
Secretary	:	Yani Widyastuti, SSiT, M.Keb
Treasurer	:	Suwandi, SE
		Tanto Yuono, SE
		Ns Harmilah, M.Kep, Sp.MB

Members

1. Secretariat :
   1. Dasilah
   2. Evriyani, Amd
   3. Eva Lidya Yunita, AMd.Kg
   4. Astuti Dwi E, Amd
   5. Veronica Anindyati Nugroho Putri, Amd

2. Scientific committee (keynote speaker+materials) :
   1. Hesty Widyasih, SSiT, M.Keb
   2. Achmad Husein, SKM, MPd
   3. Sugeng, Ners.,M.Sc
   4. Almira Sitasari, S.Gz, MPH
   5. Aryani Widayati, SSiT.,MPH
   6. Eni Kurniati, S.SI.,M.Sc

3. Proceeding commitee :
   1. Sabar Santosa, SPd, APP, M.Kes
   2. Dr. drg. Wiworo Haryani, M.Kes.
   3. Siti Nuryani, S.Si.,M.Sc
   4. Dr. Ir. I Made Alit Gunawan, M.Si.
   5. Niken Meilani, SSiT,SPd, M.Kes
   6. Ns.Sutejo, M.Kep.Sp.Kep.J
   7. Dr. drg. Quroti Ayun, M.Kes
   8. Muryoto, SKM.,M.Kes
   9. Ayu Triani, S.T.
   10. Desi Rochmawati, SS.M,Hum
   11. Andika Trisurini, S.Pd
   12. Dina fadhilah, S.Tr
   13. Apriyatni Condro Ekarini, S.SiT.,M.Kes
   14. Sapto Harmoko, SIP

4. Event Committee	:	1. Yanuar Amin, S.ST.,SH 2. Rosa Delima Ekwantini, S.Kp.,M.Kes 3. Dra. Elza Ismail, M.Kes 4. Abdul Majid, S.Kep. Ns.M.Kep. 5. Sarka Ade, SIP, S.Kep. MA 6. Rybob Khomes, S.Kom
5. Promotion, Publication and Bussiness Committee	:	1. Ika Prasetyo Nugroho, SE 2. Uki Wulanggita, SST 3. Nugraheni Tri Lestari, SKM, MPH 4. Mina Yumei Santi, SST., M.Kes 5. Etty Yuniarly, SST.,MPH 6. Haryono, SKM.,M.Kes 7. Trubus Basuki, AMd 8. Bekti Irianto
6. Registration	:	1. Drs. Harya Kunjana 2. Narto, BE., STP.,MP 3. Siti Hani Istiqomah, SKM.,M.Kes 4. Nuriana Kartika Sari, SST 5. Suhardjono, S.Pd.,S.SiT.,M.Kes
7. Logistics		1. Tjarono Sari, SKM.,M.Kes 2 Puti Sudarwati, S.Si 3. Sukarti, SIP
8. Decoration , Place	:	1. Suharyana, SKM 2. Purwanto
9. Documentation	:	1. Heri Purwanto, SE 2. Harsono, AMd. 3. Abdul Hadi Kadarusno, SKM.,MPH
10. Transportation	:	1. Tri Widodo, AMd 2 Agus Pamuji 3. Giyanto

P-09

LARVICIDAL ACTIVITY OF STAR FRUIT EXTRACT (*Averhoa carambola* Linn) AGAINST LARVAE OF *Aedes aegypti*

Siti Zainatun Wasilah
Health Analyst Health Polytechnic of Ministery of Health in Yogyakarta, Indonesia
Email : sitizainatunw@yahoo.co.id

ABSTRACT

Star fruit extract (*Averrhoa carambola* L) is potential as a natural larvicides because it contains chemical compounds of flavonoids, alkaloids, saponins. This study aims to determine the larvacidal activity of star fruitextract to *Aedes aegypti* larvae and LC_{50} value after 24 hours exposure. This study used concentration with 1.5%, 2%, 2.5%, 3%, 3.5% and 1 negative control using tap water and 1 positive control using 0.01% temefos. This study was post test only with control group design, total samples is 525 larvae from third stage instar larvae of *Aedes aegypti*. The mortality larvae will be calculated after 24 hours. The results of the study showed the percent mortality at concentrations of 1.5%, 2%, 2.5%, 3%, 3.5% *Aedes aegypti* larvae were respectively 0; 10.68; 21,36,30,68; 54,68; 61,36. The result of Annova test obtained P <0,05 meaning that there is significant difference between death rate of *Aedes aegypti* larvae with various concentration of star fruit extract (*Averrhoa carambola* L) given. The LC_{50} value of the probit test for *Aedes aegypti* larvae is 3.035%. It can be concluded that the star fruit extract (*Averrhoa carambola* L) can kill *Aedes aegypti* larvae.

Keyword *:Star Fruit Extract (Averrhoa carambola L)* ***,*** *Aedes aegypti larvae, Larvicidal Activities*

INTRODUCTION

Indonesia is one of the tropical countries in the world that has the optimum temperature and humidity for the survival of insects. The mosquitoes is the one of the species of insects that gained great attention in human health, as a potential vector on the transmission of diseases. Some types of mosquito-borne diseases, such as Dengue Hemorrhagic Fever, Dengue Fever, Cikungunya (Break Bone Fever), are transmitted by *Aedes aegypti* mosquitoes. The existence of mosquitoes adjacent to human and animal life is that cause serious problems because mosquitoes act as a vectors.

Eradication of mosquito-borne diseases is to break the chain of life cycle of mosquitoes that consists of four kinds of eliminating the cause of the disease, isolation of the patient, preventing mosquito bites, and vector control[1]. Vector control efforts have been carried out in various ways that is mechanics, biology, and chemistry. These various ways the most popular is the eradication of chemicals by insecticides. However, the use of these chemical insecticides has enormous negative impacts such as environmental pollution, predatory mortality, targeted insect resistance, killing of pets and causing various dangerous diseases in humans [2].

Based on the research of concerning larvae effect of *Averrhoa carambola* Linn of instar larvae of *Aedes aegypti* mosquito, saponin and flavonoid contained in *Averrhoa carambola* Linn have effect as larvicides[3]. Kecombrang (*Etlingera elatior)* stem extract containing flavonoids and saponins is effective as larvicides with concentrations of 0.75% and 1%[4].Based on this fact, an alternative larvicides derived from natural ingredients is needed to reduce the use of chemical insecticides. One of the alternative larvicides used is native Indonesian plants such as starfruit (*Averrhoa carambola* L) which is easy to obtain, cheap and high efficacious. The use of materials derived from plants can be used

as an alternative in mosquito larvae control. The star fruit (*Averrhoa carambola* L) contains compounds such as alkaloids, soponin and flavonoids and other chemical compounds that can affect the nervous system, digestion and breathing in larvae[5].For that, further research on larvicides activity from star fruit extract (*Averrhoa carambola* L) to *Aedes aegypti* mosquito larvae.

MATERIAL AND METHODS

1. Preparation of test materials

   Aedes aegypti mosquito eggs in this study were obtained from the Parasitology Laboratory of Gadjah Mada University Yogyakarta. The mosquitos eggs then placed in a 30x15 cm plastic tray filled with water as for the maintenance of the larvae. Mosquitos eggs will hatch into larvae within 1-2 days. Hatching eggs into larvae are separated by using larval pipettes for colonization and fed by chicken's liver. After the third phase instar larvae, the larvae are removed by using a larval pipette into a plastic cup containing star fruit extract with different concentrations in each cup.

2. Preparation of Test Solution

   This extract made in accordance with the method of maceration, extract used is star fruit washed by tap water and then chopped fine, then dried on room temperature. After dry, star fruit blend dry (without water) then soaked for 24 hours in ethanol 96%. Once soaked, the material is filtered using a gauze cloth. The maserate, concentrated at 40-50^{0}C by a rotary evaporator. Ethanol extract of star fruit dillute by aquadest to 1,5% ; 2%; 2,5% ; 3% ; 3,5%. As for positive control is abate containing 0.01% temefos, and tap water as negative control.

3. Parameters Larvicides Activity of Star fruit

   The larvicidal activity was assesed by the procedur of WHO and Pesticide Commission. According to WHO procedure[6], concentration is considered to have an effect when causing death test larvae of 10-95% which will be used to find the value of lethal concentration. Meanwhile, according to the Pesticide Commission, the use of larvicides is said to be effective if it can kill 90-100% test larvae7.

4. Determination the LC50 Value

   For the bioassay test, larvae were taken into five batch, 25 larvae *Aedes aegypti* of each batch, in 100 ml desired concentration of star fruit extract. The negative control was tap water and 0,01% temefos as positive control. The number of dead larvae were counted after 24 hours of exposure, and the percentage of larvae mortality was reported from the average of three replicate. The mean of death of each treatment group in each unit of observation time was tested by using Probit analysis until LC_{50} value was obtained.

RESULTS AND DISCUSSION

This research was conducted in entomology laboratory of Faculty of Public Health of Ahmad Dahlan University Yogyakarta for 5 days. The making of star fruit extract (*Averrhoa carambola* L) was done in LPPT UGM, it took about 3 weeks. This research started with *Aedes aegypti* egg rearing obtained from Parasitology Laboratory of FK UGM Yogyakarta. The observations were conducted at Ahmad Dahlan Yogyakarta Public Health Laboratory with 3 repetitions with concentration of 1.5%, 2%, 2.5%, 3%, 3.5% with positive control of 0.01% temefos and the tap water as negative control.Before the actual test, firstly done preliminary test to determine the actual concentration for testing third

stage instar larvae of *Aedes aegypti.* The results of the study are presented in the following table:

Table 1. Number of Deaths of *Aedes aegypti* larvae after 24 hours Exposure star fruit extract *(Averrhoa carambola L)*

Group	Number of larvae	Repeat			averagemortality	% mortality
		I	II	III		
0%	25	0	0	0	0	0%
1,5%	25	2	2	4	2,67	10,68%
2,0%	25	5	6	5	5,34	21,36%
2,5%	25	8	8	7	7,67	30,68%
3,0%	25	12	13	16	13,67	54,68%
3,5%	25	18	14	14	15,34	61,36%
Temefos 0,01%	25	25	25	25	25	100%

Source: Primary Data 2017

The highest mortality was found at the highest concentration of 3.5%. The number of larval deaths increases with the increased concentration of star fruit extract (*Averrhoacarambola* L) given. Based on it can be seen that the higher concentration of star fruit (*Averrhoa carambola* L) given the higher the death rate of *Aedes aegypti* larva can be seen in the picture below.

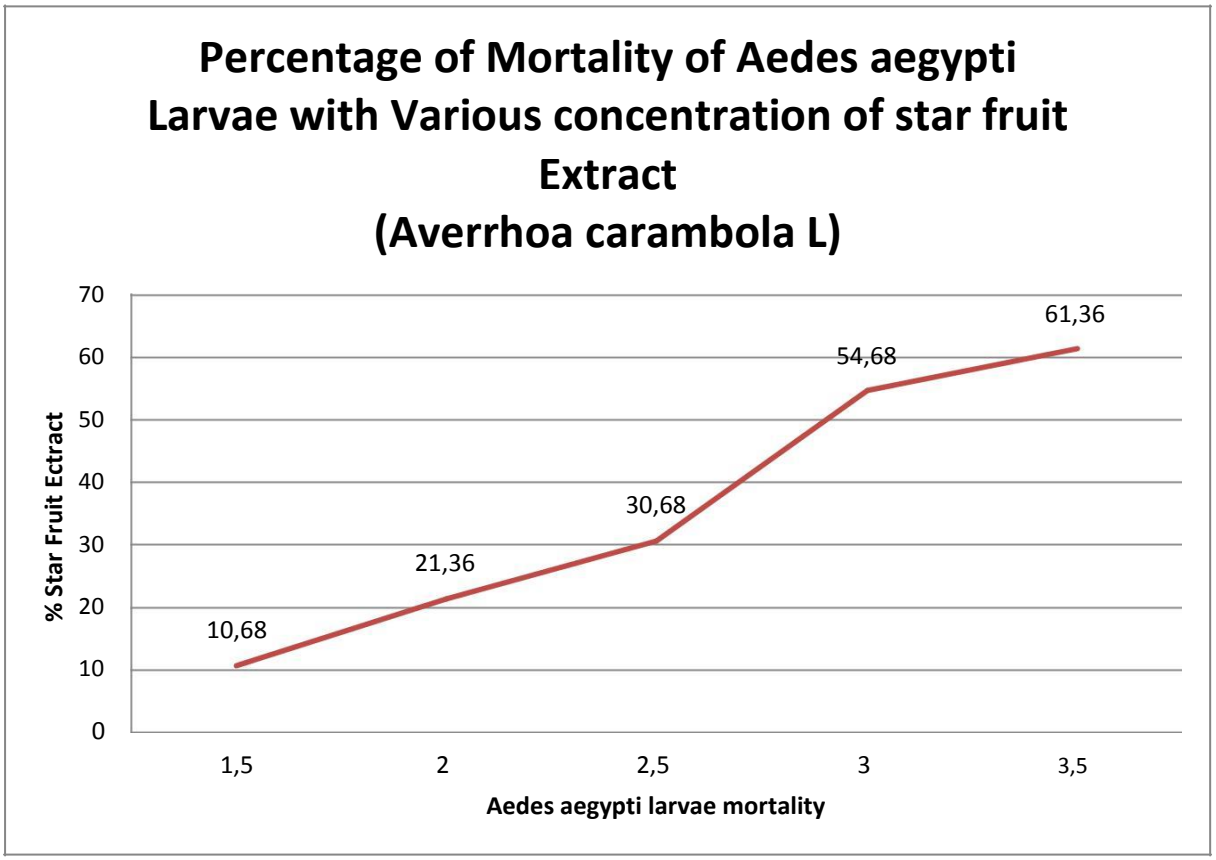

Figure 1. Graph The Percentage of *Aedes aegypti* larvae Mortality With Various Concentrations Of Star Fruit Extract (*Averrhoa carambola* L)

To determine whether there is any difference between treatment groups, statistic test with normality data test is done. Based on the results of statistics conducted by using Kolmogorov smirnov test results obtained as the following table:

Table 2. Results of Probit Analysis Star Fruit Extract (*Averrhoa carambola* L) with Percent Mortality of *Aedes aegypti* larvae

The concentration of star fruit extract (*Averrhoa carambola* L) (%)	Percentage of larval deaths (%)	LC_{50} (%) (IK 99%)	LC_{99} (%) (IK 99%)
1,5	10,68%		
2	21,36%		
2,5	30,68%	3,03581	5,969
3	54,68%	(2,851-3,279)	(5,263-7,155)
3,5	61,36%		

Source: Primary Data 2017

The concentration of 1.5% with 2% and 2.5% with 3% showed no significant difference because (p> 0,05) Furthermore, to find the value of Lethal Concentration 50% (LC_{50}) and 99% (LC_{99}) .The results of probit analysis can be seen in table 2. To determine LC_{50} and LC_{99} a probit test or unit probability is performed. The data of the research were analyzed by using Minitab 14 program with 95% confidence level. From the results of probit analysis, we found a large estimate of concentration resulting in death of *Aedes aegypti* larvae, LC_{50} at 3.03581% with intervals between 2.851% and 3.279%, while for LC_{99} at 5.969% with intervals between 5.263% and 7.155%.

Furthermore, in the figure above can be seen LC_{50} value of 3.035% and LC_{99} of 5.96% means that the effect caused by the extract of star fruit (*Averrhoa carambola* L) can cause death of 50% larvae Ae.aegypti at concentration 3.035%. starfruit is also able to cause death 99% at concentration 5,96% this means concentration 3,035% and 5,96% give effect of mortality in *Aedes aegypti* larvae.

Mosquito is one of the animals that breeding process takes place in two realms. At the egg stage until the pupa lives on the water medium and the adult stage lives on land. At this stage of course the quality of water affects the survival of mosquitoes. Here are the results of measuring the quality of star fruit extract (*Averrhoa carambola* L).

Table 3. Mean Temperature Measurement of Star Fruit Extract *(Averrhoa carambola L)* to Aedes aegypti mortality

Parameter	Concentration (%)	Time 24 hours			Average
		I	II	III	
Temp (^oC)	1,5	27	27,2	27,1	27,1
	2	27	27	27	27
	2,5	27	27	27	27
	3	27	27	27,2	27,06
	3,5	27,1	27	27	27,03

Source: Primary Data 2017

From table 3 it can be seen that the average of temperature measurement on star fruit extract (*Averrhoa carambola* L) in each concentration for 24 hours is 24.03-27,1^{0}C. The temperature range in this medium can still be said to be normal. This is in accordance with the opinion that the *Aedes aegypti* mosquito breeding place is at a temperature of 25-32°C. Thus, larval mortality is not affected by temperature[8].

Table 4. Average Measurement of Star Fruit Extract (*Averrhoa carambola L*) Moisture to Aedes aegypti Mortality

Parameter	Concentration (%)	Time 24 hours			Average
		I	II	III	
moisture (%)	1,5	76,1	76	76	76,03
	2	76	76	76,2	76,06
	2,5	76,2	76	76	76,06
	3	76	76	75,8	75,93
	3,5	76	76	76	76

Source: Primary Data 2017

From table 4 it can be seen that the average of humidity measurements on star fruit extract (*Averrhoa carambola* L) in each concentration during 24 hours is 75,93 - 76%. The normal moisture range in this medium can still be said to be normal. This is consistent with the opinion that the *Aedes aegypti* mosquito breeding place is in moisture> 60%. Thus, larval mortality is not affected by moisture[9].

Table 5. The Average pH Measurement of Star Fruit Extract (*Averrhoa carambola* L) to *Aedes aegypti* Mortality

Parameter	Concentration (%)	Time 24 hours			Average
		I	II	III	
pH	1,5	7,2	7,2	7,2	7,2
	2	7,2	7,2	7,1	7,1
	2,5	7,3	7,2	7,2	7,2
	3	7,2	7,2	7,3	7,2
	3,5	7,2	7,1	7,2	7,1

Source: Primary Data 2017

From table 5it can be seen that the average of pH measurement on star fruit extract (*Averrhoa carambola* L) in each concentration during 24 hours time is 7,1-7,2. The normal pH range in this medium is still normal. Mosquito larvae require a breeding place with pH conditions ranging from 5 to 8. This indicates that the pH of star fruit extract (*Averrhoa carambola* L) did not affect larvae mortality[8].

Thus it can be concluded that the mortality of *Aedes aegypti* mosquito larvae caused only by toxic compounds contained in star fruit extract (*Averrhoa carambola* L)

DISCUSSION

Based on the results of this study, it can be seen that the of star fruit extract(*Averrhoa carambola* L) can be used as larvasida. This occurs because the star fruit extract (*Averrhoa carambola* L) there are active compounds in the form of compounds such as alkaloids, soponin and flavonoids and compounds other chemicals that can affect the nervous system, digestion and breathing in larvae [5].

Mortality of mosquito larvae increases with increasing concentration of star fruit extract (*Averrhoa carambola* L), it indicates that the extract is toxic. In this study the temperature, pH and humidity are still at normal limits, so the possibility of mosquito larvae in this study die caused by external influences such as temperature, pH and humidity. Variation of mosquito larvae mortality caused by the varity of sensitivity and resistance of each larva to the material active in the extract. The death of the larvae is caused by the inability of the larvae to detoxify the toxic compounds that enter the body.

The difference in the percentage of larval mortality is due to the diffusion speed of extracts entering into different cells so that at low concentrations the larvae can still

tolerate these toxic compounds, whereas at high concentrations the larvae can not tolerate the entry of these toxic compounds. This is in accordance with the opinion of [10] who said that the speed of diffusion depends on the difference in the concentration of substances dissolved during the process. This means that if the concentration decreases, the speed of diffusion also decreases. At each concentration showed an increase in the percentage of mortality every 24 hours, this indicates the longer time, the percentage of larval mortality is also increased. The interaction of toxic substances a biological system is determined by the concentration and length of time.Toxic substances that play a role in lethal larvae are alkaloids, saponins, and flavonoids. Alkaloids that enter the body of the larvae through absorption and degrade the skin cell membrane, besides alkaloids can also interfere with the larval nervous system work.

Based on the results of the observations during the test larvae exhibited anxiety symptoms characterized by upward motion movements on the test medium, while the larval control showed a resting state on the surface forming angles. The starfruit extract (*Averrhoa carambola* L) could be as Aedes sp.larvicide this is seen from $LC_{50}$from star fruit extract (*Averrhoa carambola* L) to Ae.aegypti is 3.035%.

Alkaloid compounds act as larvicides by inhibiting the feeding power of the larvae (antifeedant), so the larvae will experience nutritional deficiencies and eventually die. This can also be seen from the results of research about the content of active substances tembelekan (*Lantana* camara L) leaffor *Aedes aegypti* larvae mortality[11]. Based on the results of these studies the alkaloids contained in the leaves of elasticity serves as a poison or poisonning stomach. The content of alkaloids roots and amethyst five time greater than alkaloid content. The alkaloid can also be used as an insecticide[12]. Alkaloids in leaves or fresh fruit taste bitter on the tongue, alkaloid in the form of salt so that it can degrade the cell wall into and damage cells.The alkaloid compound inhibits the work of acetylcholinesterase enzyme that serves in continuing stimulation to the nervous system, so transmission of excitement does not occur[1].

Another active compound contained in the star fruit extract is saponins. Saponins result in decreased activity of digestive enzymes and the absorption of food in insects. In addition, saponins also damage the larvae of the larvae causing the death of larvae. The saponins isolated from Achyranthes aspera plants have larvicidal effects on *Aedes aegypti* and C.quinquefasciatus.

The flavonoid compounds contained in the sweet star fruit extract are also insecticidal because they are respiratory toxins, causing the larvae to not breathe due to respiratory system damage and ultimately causing the death of the larvae. In addition flavonoids also as inhibitors CYP6Z2, family of cytochrome P450 which plays an important role of insecticide resistance in mosquitoes. The flavonoids enter the body of the larvae through siphon located on the surface of the water and cause wilting on the nerves, as well as damage to siphon as a result the larvae can not breathe and eventually die[13]. This study used *Aedes aegypti* mosquito third stage instar larva, has complete organs and body structure of the body has not been hardened so it is suitable for treatment with alkaloid compounds, saponins and flavonoids.

The secondary plant metabolite compounds can disrupt the respiratory system, affect skin immunity and digestive system which eventually leads to mortality[14].The function of active compound content such as alkaloid, saponin, flavonoid as larvicide can also be seen from Tinospora rumphii and Citrus grandis plant can cause *Aedes aegypti* larvae death because it has active ingredient of alkaloids, saponin, flavonoids , steroids and tannins.

Based on the results of this study it can be seen that star fruit extract (*Averrhoa carambola* L) can be used as larvicide to *Aedes aegypti* larvae because there are difference of larvae mortality that signifikan between treatment group and control group.

CONCLUSION

Star fruit extract (*Averrhoa carambola* L) can be used as larvicides against *Aedes aegypti* larvae. LC_{50} value of star fruit extract (*Averrhoa carambola* L.) as larvicides to *Aedes aegypti* mosquito larvae of 3.04%.

SUGGESTION

1. Can be done research of star fruit extract (*Averrhoa carambola* L) against larvae of other species mosquitoes
2. For further researchers can use other techniques in the manufacture of star fruit extract(*Averrhoa carambola* L), to be more effective.

REFERENCES

1. Dinata A. 2009. *Basmi Lalat dengan Jeruk Manis*. Tersedia dari http://litbang.depkes.go.id/lokaciamis/artikel/lalat-arda.htm.
2. Susanna, D., A. Rahman dan E.T. Pawenang. 2003. *Potensi Daun Pandan Wangi Untuk Membunuh Larva Nyamuk Aedes aegypti* L. Jurnal Ekologi Kesehatan 2 (2) : 228-231
3. Setia,W.2010.*EfekLarvasidaAirPerasanBelimbingWuluh Averrhoa bilimbiterhadapLarvaInstar III nyamuk Aedes aegypti*. Skripsi. fakultasKedokteran Universitas lampung. Lampung.
4. Adityani, N. 2012. Uji Efektivitas Ekstrak Batang Kecombrang (Etlingera elatior) sebagai Larvasida terhadap Larva instar III Aedes aegypti. Skripsi. Fakultas Kedokteran Unila. Bandar Lampung
5. Setiawati, D. L. 2000. Mortalitas Larva Culex dengan Ekatrak Umbi Gadung (Dioscorea hispida Dennst) di Laboratorium. Skripsi. Fakultas Biologi. UGM. Tidak Diterbitkan.
6. WHO. 2005. *Guidelines for Laboratory and Field Testing of Mosquito Larvicides*. Geneva.
7. Komisi Pestisida. 1995. *Metode Pengujian Residu Pestisida dalam Hasil Pertanian*. Jakarta: Departemen Pertanian.
8. Hidayat. 1997. *Pengaruh pH Air Perindukan Terhadap Pertumbuhan dan Perkembangan Aedes aegypti Pra Dewasa.* www.kalbefarma.com. Diakses tanggal 16 Juni 2011
9. Martens WJM. 1997. Malaria and climate change. Environt Hlth Perpective, 97:103-116.
10. Poedjiadi. A. 1994. *Dasar-dasar Biokimia.* Universitas Indonesia Press. Jakarta
11. Wardani RS, Mifbakhuddin, Yokorinanti K. Pengaruh Konsentrasi Ekstrak Daun Tembelekan (*Lantana Camara*) Terhadap Kematian Larva *Aedes aegypti*. J Kesehat Masy Indones, 2010; 6(2): 30-38.
12. Mawuntyas,&Tjandra. 2006.Manajemen Administrasi Rumah Sakit. Edisi Kedua. UI-Pres.Jakarta
13. Dinata, A. 2009. Mengatasi DBD dengan Kulit Jengkol. www.miqraindonesia.blogspot.com. Diakses tanggal 1 November 2012
14. Nopianti, S., D. Astuti., S., Darnoto. 2008. *Efektivitas Ekstrak Buah Belimbung Wuluh (Averrhoa bilimbi* L.*) terhadap Kematian Larva Nyamuk Anopheles aconitus Instar III*. Jurnal Kesehatan 1 (2) : 103-114