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EFFECTS OF ARECA CATECHU L. SEED EXTRACT ON MORTALITY ANOPHELES VAGUS LARVAE

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Abstract

Bioinsecticide is an alternative to overcome larva resistance to insecticide and reduce environment contamination. This research is to analyze the effect of areca seed extract to *An. vagus* larva. The research is taken time in 2017. The research design is laboratory experiment with post test only control group design. The areca seed and *An. vagus* larva are collected from Kabupaten Sumba Barat Daya, then the seeds were extracted with ultrasonic method. The effect of the extract is evaluated based on LC50 and LC90 value. The sample from 25 *An. vagus* larva instar III are released into areca seed extract on 500, 1250, 2000, 2750, 3500, 4250 and 5000 ppm and observed within 6, 12, 18 and 24 hours and 6 repetition. The data then analyzed with probit analysis and ANOVA. The areca seed extract eliminates *An. vagus* larva as much as 61.33% under 6 hours exposure with 5000 ppm, 52% larva mortality on 12 hours with 4250 ppm, 56% larva mortality on 18 hours with 3500 ppm, and 55% larva mortality on 24 hours with 2750 ppm. The value of LC50 and LC90 on 6, 12, 18 and 24 hours are 4654,374 and 6320,732; 3717,286 and 5127,489 ppm; 3201,473 and 4775,206 ppm; 2385,297 and 4496,708 ppm. There is variation of larva mortality percentage average on every concentration and exposure time, the higher the concentration and exposure time the higher *An. vagus* larva mortality.

Introduction

Kabupaten Sumba Barat Daya is one of malaria endemic area on Nusa Tenggara Timur Province. Number of malaria cases in 2015 was 4,622 cases below Kabupaten Lembata with 8,887 cases. Puskesmas Kori has highest API on Kabupaten Sumba Barat Daya with API number 24.5 per mile (Dinas Kesehatan Kabupaten Sumba Barat Daya, 2015)

Malaria is affected with environment, climate, vector and the bionomy and community

behavior. The role of Anopheles mosquito as malaria vector is widely reported by ELISA test based researches (sporozoite antigen detection) or microscopically through Anopheles mosquito salivary gland surgery. It is reported that of 420 species of Anopheles, 70 species are malaria vector and 23 species are twins. In Indonesia 80 Anopheles species are found and 22 of them are confirmed as malaria vector and specifically local distributed (Sandy, 2014).

Anopheles species that has been

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confirmed as malaria vector on Nusa Tenggara Timur Province is *An. barbirostris*, *An. subpictus*, *An. sundaicus* and *An. minimus*. The vector suspect is based on ELISA test result with positive result found sporozoite on Anopheles species *An. vagus* and *An. anullaris* while other Anopheles species which does not malaria vector are *An. indefinitus*, *An. aconitus*, *An. flavirostris*, *An. maculatus*, *An. kochi*, *An. tessellatus*, *An. balabacensis*, *An. umbrosus* and *An. hyrcanus* group. These Anopheles species though has not been proven that can transmit malaria disease on Nusa Tenggara Timur Province still must be aware of, since those species has been detected as malaria transmitting vector on other place (Kazwaini, 2013).

One of the approach in effort to control the vector transmitting disease is by preventing direct contact between human and mosquito. The usage of synthetic insecticide (chemical) is widely known as effective, relatively cheap, easy and practical yet has negative effect to environment (Sudrajat, 2010). The bioinsecticide usage to control the vector is one alternative to reduce the effect of synthetic insecticide (Hasanah, 2012).

One of the plant that can be used as bioinsecticide to control bugs and pests is areca plant (*Areca catechu* L.). Areca is palm species vegetating Pacific, Africa and Asia particularly in Indonesia. It contains secondary metabolite such as alkaloid, flavonoid, saponin and tannin. Part of the plant that mostly used for nabati insecticide is the seed since the active ingredient like arekolin kind of flavonoid, able to paralyze and stop breathing on insects is highly found in young areca seed (Eri, 2014).

Gassa (2008) research on Makassar showed that result of areca (*A. catechu* L.) seed extract effect to *Culex* sp mortality is highly effective to eliminate *Culex* sp mosquito larva 9 to 96 hours after the test done. A research conducted on Chennai, Tamil Nadu, India to test effectivity of larvacide to *Ae. Aegypti* using areca (*A. catechu* L.) leaf metanol extract, tobacco (*Nicotiana tabacum*) leaf, and betel (*Piper bettle*). Leaf. The test was conducted on 62,5, 125, 250, 500 dan 1000 ppm concentrate. The mortality is observed after 24 and 48 hours exposure. *A. catechu* L. indicate highest larva

activity followed by *N. tabacum* and *Piper betle* with LC₅₀ value from 124,28 and 95,75; 236,73 and 98,45; 313,58 and 122,99 ppm after 24 and 48 hours (Tennyson, 2012).

On Nusa Tenggara Timur, the banana plant (*A. catechu* L.) is widely found around local's houses. Beside being growth as local house vegetation, it is also used by the people as betel chewing complement, yet has not been utilized as bioinsecticide that can be used to control insect population that relatively environment friendly and un toxic. This research objective is to study the effect of young Areca (*A. catechu* L.) seed as Anopheles sp biolarvacide on Kabupaten Sumba Barat Daya, Nusa Tenggara Timur Province.

Method

The design is laboratory experimental, using control group with Post test only control group design. The research was conducted for 5 months (March – July 2017). The young areca (*A. catechu* L.) seed and Anopheles sp. collection was taken place on Kori Village, Kabupaten Sumba Barat Daya, Nusa Tenggara Timur Province. The areca seed extract was processed on SATREPS Institute of Tropical Disease (ITD) Airlangga University laboratory. The test of areca (*A. catechu* L.) seed extract on Anopheles sp. larva was taken place on Waikabubak R&D Workshop for Animal Sourced Disease Control, Nusa Tenggara Timur Province.

The areca seed taken from Kabupaten Sumba Barat Daya oftenly used by the local for beteling, aged ± 2 bulan since flower stage until areca fruit, the rind is green colored and has tender seed structure. It then being cutted and air dried then blended into powder form. 250 gr of areca seed powder was added by 1500 ml 96% etanol (1:6 proportion) and to accelerate the extraction process, the ultrasonic device is operated. The ultrasonic treatment was repeated 3 times 2 minutes each. The result on every treatment was filtered and contained in the bottle to be evaporated. The filtrat obtained then being vaporized with vaccum rotary evaporator on 40°C, speed 70 rpm and pressure 0.7 bar until a condensed extract was obtained. It then being ovened on 40°C temperature for 24 hours. Final product is scaled with analytic weight scale.

The larva used in this research is *An.*

vagus instar III or IV from forced egg of mature *An. vagus* mosquito collected from Kabupaten Sumba Barat Daya with consideration that larva instar III has had firm, complete and stabil body organ. The sample size is aligned with WHO standard for toxicity study which is 25 mosquitos per group (Misnarni, 2013; Tennyson, 2013). The capturing or *An. vagus* mosquito was conducted at 6pm to 10pm with aspirator around the cowshed belong to the local resident, mosquito captured is then took to the laboratory at Waikabubak for forced egg and tested.

Toxicity test using the solvent with 7 group of various areca (*A.catechu* L.) seed extract concentrate, inside separate treatment and control container. 25 *An. vagus* instar III or IV larvas was taken using pipette and inserted into each container filled with well water and prepared extract concentrate. The larva was remained in contact with test for 24 hour. Treatment was repeated 6 times. Larva mortality on treatment group then compared with *An. vagus* larva on control group. Observation was conducted on 6, 12, 18 and 24 hours.

Data analysis was conduct was conducted with *Statistical Package for the Social Sciences (SPSS)* with probit analysis method. The differences between biolarvacide from various areca (*A. catechu* L.) seed extract concentrate then tested for normality and homogeneity continued with variant analysis statistic test (ANOVA). The variant analysis is included in parametric statistic cathegory. Data normality tes by saphiro-will test. Data that normally distributed can be proceed with post-hoc test

to find out the difference of each group.

Result and Discussion

Global malaria vector control strategy uses synthetic insecticide to control Anopheles mosquito which is *Indoor Residual Spraying (IRS)* and *Insecticide Treated Nets (ITNs)*. The achievement of IRS is depended on resting behavior of the mosquito inside the house, house wall surface and community will to accept IRS. Insecticide usage in vector control has been done for long time. Malathion as one of insecticide widely used nowadays start to be reported resisted by the mosquito (Susanti, 2012). Mature mosquito also can fly and easily detect and avoid syntethic insecticide spray. So the control action applied to pre mature Anopheles mosquito. On pre mature phase, the larva habitat is relatively restricted and unable to avoid the control act. The Anopheles larva in India is controlled with larvacide chemical such as temephos, fention, malathion, cloropyriphos and methopren (Dhiman, 2016). The prevention of Anopheles resistance, unwanted effect on non target organism, environmental and human safety can be achived with alternative insecticide from vegetation based (bioinsecticide) (Tomass, 2011). One of the vegetation that can be used as bioinsecticide is areca.

Toxicity test of area seed extract conducted on *An. vagus* larva with 500 ppm, 1750 ppm, 2000 ppm, 2750 ppm, 3500 ppm, 4250 ppm and 5000 ppm concentration. Table 1 presents that *An. vagus* larva after exposed by areca (*A. catechu* L.) seed extract for 6 hours causes lowest average mortality on

Table 1. Average Mortality Percentage of *Anopheles vagus* Larva Based on Areca (*A. Catechu* L.) Extract Concentration Level and Exposure Time (Hour)

Concentration (ppm)	Larva mortality percentage (%) based on exposure time			
	6 hour	12 hour	18 hour	24 hour
500	0	0	0	0
1250	0	1.33	6	14
2000	2	6.67	18.67	34.16
2750	10.67	29.33	42.67	54
3500	17.67	32	56	75.33
4250	35.33	52.67	68	86
5000	61.33	100	100	100

Source : Primary Data

2000 ppm concentration as much as 2% and highest on 5000 ppm concentration as much as 61.33%. Exposure time 12, 18 and 24 hours causes lowest average mortality on 1750 ppm as much as 1.33%, 6% dan 14% while highest concentration 5000 pp causing 100% mortality of *An. vagus* larva.

The Larva mortality is caused by larva inability to detoxificate the toxic composition come into it's organ (Yunita, 2009). The toxic contained in the areca seed which has insecticide nature are alcaloid, flavonoid, saponin and tanin (Amudhan, 2012). Gassa (2011) said that areca contains arecoline an alcaloid substance that is similar with nicotine. It is an ester *metal-tetrahidrometil-nikotinat* in form of toxic high bases oil and able to cause paralysis and suspended respiratory.

The alcaloid and saponin have a nature as gastrototoxic and obstruct cholinesterase enzim of the larva (Cania, 2013). Alcaloid can cause injury on digestive system cell membran and distrust neuro system by obstruct asetilcholinesterase enzim work system (Cahyati, 2017). The alcaloid make this enzim fail to transmit the stimulation to larva digestive system (midgut), causing uncontrolled intestine motion. The failure of central neuro system obstructs cholinesterase and acetilcholin enzim activity will accumulate so all impuls from central neuro system to muscle and other body organ is blocked leading to mortality (Gassa, 2011 and Salim, 2015).

Flavonoid attacks several neuro organ on several insect vital organ so that sort of paralysis occure, distrust respiratory system and causing mortality. Flavonoid work as respiratory inhibitor. Inhibitor is a substance that obstruct or decrease the chemical speed reaction, distrust energy mechanism in the mitochondria by hamper electron transporting system (Muta'ali, 2015). Tanin can decrease the ability to digest food by decreasing digestive enzim activity (protease and amilase) (Ahdiyah, 2015).

The toxicity test was conducting by placing mosquito larva in an extract suspension with certain concentration, so that whole body of mosquito larva is exposed to toxic substance of areca (*A. catechu L.*) seed extract. The toxic substance contained in areca seed can come

into the body through body wall and mouth since larva usually take food from the habitat (Yunita, 2009)

Larvacide work mechanism to eliminate the larva is through skin contact, then penetrate the insect's integument (cuticula), trachea or sensoric organ. Chemical contained in the insecticide will dissolve the lipid or wax layer of the cuticula so that the active substance can penetrate into insect body (Pradani, 2011).

This larvacide enter the body through mouth (larva food). Then the larva will be deceased by toxic come in along with the food into the body. Then cell metabolism will be obstructed since electron transport is hampered in mitochondria therefore energy generation from food as energy source of the cell does not occure and the cell can't do the activity resulting mortality of the larva (Ahdiyah, 2015).

The observation of tested larva showed that larva movement was slow when it was touched and rolled the body, emerged and submerged in the medium, compare to larva control that show static condition level to water surface. The condition is the symptom caused by bioinsecticide exposure. Anxiousness is one of poisoned symptom caused by alcaloid substance. The substance causes larva slow movement when it was touched and always bend (Cania, 2013). Other symptoms are emerge and submerge in the medium (Yunita, 2009). Larva exposed to areca seed extract indicated poisoned symptoms such as less active, roll, bend and straight movement.

Microscopic observation was done to compare *Anopheles* sp. larva morphology before and after areca seed extract treatment. Body color become dark brown and finally deceased, this is aligned with research on *Argulus* sp. larva, parasite on fish that become dark brown after exposed to areca seed extract (Kinang, 2017). Bioactive materials contained by areca seed is the cause of larva mortality since compared to the larva which was not given the extract, none of them turn to dark brown nor obstructed the movemen. Not even deceased. The larva become longer. From previously intrar II *An. vagus* larva length was 4 mm (0.4 cm), after it was exposed to areca seed extract with 2750 ppm until 5000 ppm concentrat,

Table 2 LC_{50} and LC_{90} Value of Areca (*A. catechu* L.) Seed Extract to *Anopheles vagus* Larva Based on Exposure Time

Extract	Exposure time (hours)	LC_{50}		LC_{90}	
		Value	Interval	Value	Interval
<i>Areca catechu</i> L.	6	4654.374	4489.688-4851.208	6320.732	5987.262-6758.266
	12	3717.286	3569.297-3875.757	5127.489	4876.324-5449.682
	18	3201.473	3083.861-3321.740	4775.206	4581.051-5005.572
	24	2650.236	2535.252-2764.507	4162.762	3990.033-4365.027

Source : Primary Data

become 6 mm (0.6 cm).

Table 2 showed that areca (*A. catechu* L.) seed extract LC_{50} value after 6 hours exposure is 4654.374 ppm and after 24 hours exposure is 2650.236 ppm, this means on 4654.374 ppm concentration with 6 hours exposure can eliminate 50% *An. vagus* larva. The LC_{90} value after 6 hours exposure is 6320.732 ppm and after 24 hours is 4262.762 ppm, this means on 6320.732 ppm concentration and 6 hours exposure can eliminate 90% *An. vagus* instar III and early instar IV larva while on 4262,762 ppm concentration result 90% mortality after 24 hours.

The relation of concentration with *An. vagus* instar III and early instar IV mortality based on probit analysis is displayed by the curve on picture 2 until 4 showing the higher concentration of areca (*A. catechu* L.) seed extract then the less time required to eliminate

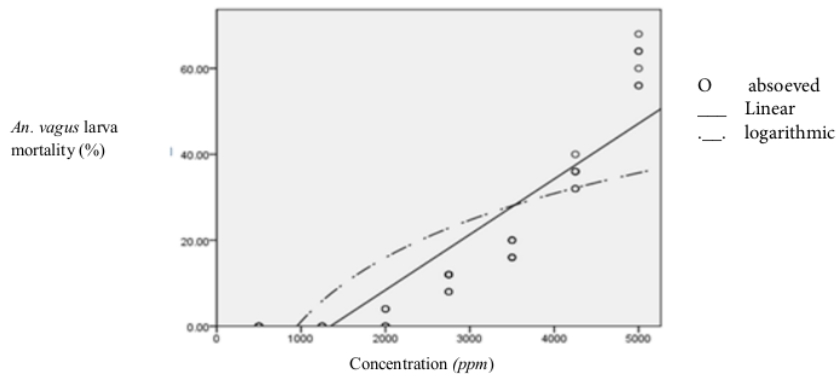
the larva and the lower the concentration then longer time to eliminate the larva. Picture 2 shows that 6 hours exposure time in areca (*A. catechu* L.) seed extract can eliminates 50% of *An. vagus* instar III and early instar IV as many as 4654,371 ppm.

Picture 3 shows that 12 hours exposure time in areca (*A. catechu* L.) seed extract can eliminates 50% of *An. vagus* instar III and early instar IV as many as 3717,286 ppm.

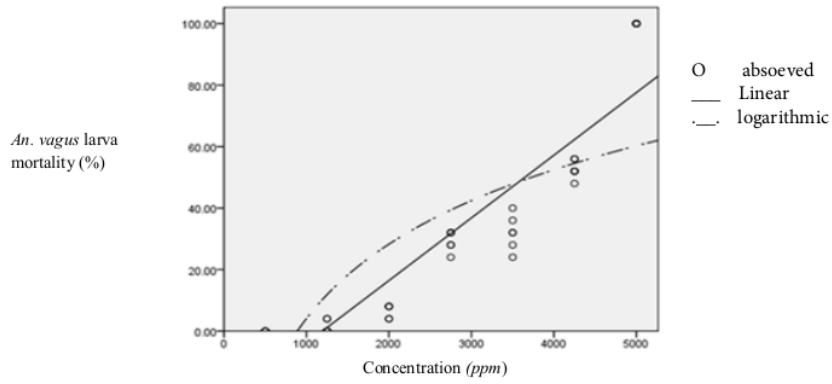
Picture 4 shows that 18 hours exposure time in areca (*A. catechu* L.) seed extract can eliminates 50% of *An. vagus* instar III and early instar IV as many as 3201.474 ppm.

Picture 5 shows that 24 hours exposure time in areca (*A. catechu* L.) seed extract can eliminates 50% of *An. vagus* instar III and early instar IV as many as 2650.236 ppm.

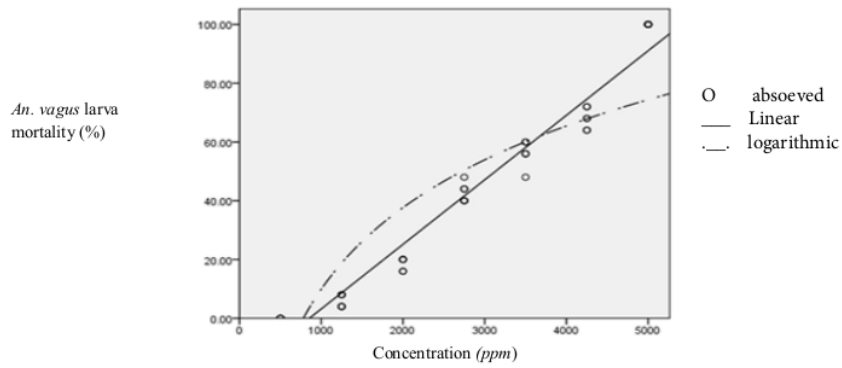
Determining the proper concentration on toxicity test that able to eliminate 50% *An.*



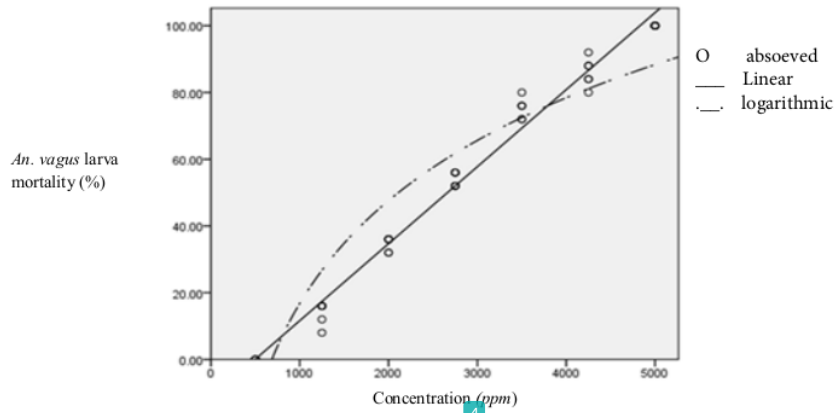
Picture 1. *Anopheles vagus* larva mortality Curve on 6 Hours Exposure Time in Areca (*A. catechu* L.) Seed Extract



Picture 2. *Anopheles vagus* Larva Mortality Curve on 12 Hours Exposure Time in Areca (*A. catechu* L.) Seed Extract



Picture 3. *Anopheles vagus* Larva Mortality Curve on 18 Hours Exposure Time in Areca (*A. catechu* L.) Seed Extract



Picture 4. *Anopheles vagus* Larva Mortality Curve on 24 Hours Exposure Time in Areca (*A. catechu* L.) Seed Extract

vagus mosquito larva by statistic test with probit analysis. The probit analysis resulting that LC_{50} value is obtained on 2458.287 ppm concentraton. This can be intrepreted that areca (*A. catechu* L.) seed extract with 2458.287 ppm concentraton has potency as bio larvacide since it can eliminate 50% population of test larva. WHO stated in Ahdiyah (2015), the larvacide concentration is considered effective when resulting 10-95% mortality rate of test larva that later be used to seek LC value. The insecticide concentration level consider to have sufficient killing ability and environment friendly when it reached LC_{50} . The LC value below LC_{50} is cathegorized as low killing ability. The LC value above LC_{50} should have further test to find out whether it is safe for the environment (Cania, 2013).

Kolmogorov sminov test result show p value (asyp sig) for larva mortality = 0,354 ($p > 0,005$) and p value (concentration) =0,537 with probability (sig.2-tail) $p > 0,05$ means the data is normally distributed. Homogenity test is 0.001 means α value less than 0.05, therefore the decision is used *Post Hoc Test*-Game Howell data.

The ANOVA test result indicates that there is significant variation of *An. vagus* instar III and early instar IV larva mortality caused by areca (*A. catrchu* L.) seed extract with various concentration. The regression test result indicate the effect of *An. vagus* larva mortality percentage is 0,000 ($<0,005$) shows the consentration level have significant effect on *An. vagus* larva mortality percentage. The beta coefficient value is 0.994 indicates the higher areca (*A. catechu* L.) seed extract concentration the higher *An. vagus* larva mortality percentage.

The difference with previous research by Tannyson (2013), using areca extract on *Ae. aegypti* larva with LC_{50} and LC_{90} is 124.28 and 278.73 ppm while on this research obtained LC_{50} and LC_{90} is 2650.236 and 4162.762 ppm. This could be caused by the difference of test larva, kind of solution and environment condition of plant origin so that affect the natures and metabolism compound contained in the plant.

Conclusion

The LC_{50} value of areca seed extract to *An. vagus* larva mortality on 6, 12, 18 and 24 hours are 4654.374 ppm, 3717.286 ppm, 3201.473

ppm and 2650.236 ppm. The LC_{90} value are 6320.732 ppm, 5127.489 ppm, 4775.206 ppm and 4162.762 ppm. There is variation of average larva mortality percentage on every concentration and exposure time, the higher areca (*A. catechu* L.) seed extract concentration then the higher mortality percentage and less time required to eliminate *An. vagus* instar III and early instar IV and the lower the concentration then longer time required to eliminate *An. vagus* instar III and early instar IV.

There should be further research regarding effectivity of areca seed extract with various solution so that it can be applied on field particularly on small breeding site and located around human residential.

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