

## Larvicidal Activity of Essential Oils of Piper betle from the Indonesian Plants against *Aedes Aegypti* L

Dwi Wahyuni

Department of Biology, Faculty of Teacher Training and Education- University of Jember, Indonesia

### ABSTRACT

Among the alternative strategies, the use of plants, insecticidal chemicals appears to be promising. Aromatic plants, and their essential oils, are among the most efficient botanicals. This study was undertaken to assess the larvicidal potential of the essential oil of the *Piper betle* from the Indonesian Plants against *Aedes aegypti* L. *Aedes aegypti* L. is the major vector of dengue fever, an endemic disease in Indonesian. Dengue fever is endemic over large areas of tropics and subtropics. Outbreaks of dengue have repeatedly occurred in Indonesian over the last 10 years. The larvicidal activity of essential oils of *Piper* from the Indonesian Plants was tested using third-instar larvae of *Aedes aegypti*. Methods: Essential oil was hydro distilled in the laboratory from the Indonesian Plants were analyzed by measurement of their LC<sub>50</sub>. The essential oils were extracted by steam distillation. Bioefficacy of the essential oil was evaluated under laboratory conditions using third-instar larvae of *Aedes aegypti*. The results from the *A. aegypti* larvicidal assay using essential oils of *Piper* from the Indonesian Plants. The most active essential oils against third instar larvae of *A. aegypti* were those of the 24hour exposed, LC<sub>50</sub> = 13,1 ppm. For the 48 hour exposed, LC<sub>50</sub> = 11,2 ppm. The Essential Oils of *Piper Betle* from the Indonesian Plants against *Aedes aegypti* L present potential alternatives of new bioinsecticide replacing for insect resistance to insecticidal *Bacillus thuringiensis* toxins expressed in transgenic plant.

**Keywords:** *Aedes aegypti*, Essential Oils, *Piper betle* Plants.

### INTRODUCTION

Insects form the largest class of the animal kingdom and include nearly 80% of known animal species. Among them, tens of thousands of species are considered as high risk species for Man. They have a double impact: (1) medical; insects are pathogenic agents or disease vectors for men and domestic animals and (2) agricultural; they devastate crops. Phytophagous insect's damage rice crops (58% losses), cotton (47%), and cause the loss of more than a third of corn and sugar-cane crops and nearly a fifth of wheat.

*Ae. aegypti* are the major vector most important of insects well-known for their public health importance, since they act as vector for many tropical and subtropical diseases such as dengue fever, yellow fever, Dengue fever is endemic over large areas of tropics and subtropics Outbreaks of dengue have repeatedly occurred in Indonesian over the last 10 years., the major urban vectors of dengue. The approach to combat these diseases largely relied on interruption of the disease transmission cycle by either targeting the *Aedes aegypti* larvae through spraying of stagnant water breeding sites or by killing the adult of *Aedes aegypti*. using insecticides. Larviciding is a successful way of reducing *Aedes aegypti* (Ryan, M.F. and Byrne, O. (1988). While most patients are asymptomatic, reinjection with different serotypes of dengue viruses may lead to hemorrhagic fever with high mortality. During outbreaks, public health authorities in Indonesian have standardized the use of aerolized pyrethroid insecticides that can cause allergies. This measure only partially controls the mosquito population since it eliminates the adult flying insects but does not eliminate the breeding places. In these breeding sites, the larvicide used is usually the organophosphate Temephos, although very slightly toxic may cause headaches, loss of memory, and irritability (Ryan, M.F. And Byrne, O. (1988).

Insect Pest management (IPM) has to face up to the economic and ecological consequences of the use of pest control measures. Fifty years of sustained struggle against harmful insects using synthetic and oil-derivative molecules has produced perverse secondary effects (mammalian toxicity, insect resistance and ecological hazards). The diversification of the approaches inherent in IPM is necessary for better environmental protection. One of the fractions of aromatic plants, most frequently used for industrial applications, and which has shown promise for use in IPM, is the fraction of volatile fragrant compounds commonly called essential oils.

Among current alternative strategies aiming at decreasing the use of classical insecticides, ecochemical control based on plant-insect relationships is one of the most promising methods. For centuries,

\*Corresponding Author: Dwi Wahyuni, Department of Biology, Faculty of Teacher Training and Education- University of Jember, Indonesia

plants and insects have followed a parallel and interdependent evolution. Insects cannot live without plants and vice versa. Chemical mediators are used in interspecies communication, especially allelochemicals. These non-nutritional molecules, produced by an organism, modify the behaviour or the biology of an organism from another species. Consequently, plant allelochemicals exert a wide range of influences on insects: they can be repellent, deterrent or antifeedant; they may inhibit digestion, enhance pollination and capture with their attractive properties; they may increase oviposition or, contrarily, decrease reproduction by ovicidal and larvicidal effects. These molecules generally act at weak doses and have a specific action. Very few are toxic for mammals. Most of them are classed as secondary plant products and therefore have chemical structures that classify them as alkaloids, polyphenolics, terpenes and isoprenoids or cyanogenic glucosides (Ryan, M.F. and Byrne, O. (1988).

The use of plant extracts, including allelochemical compounds such as essential oils, with known effects on insects, could be a useful complementary or alternative method to the heavy use of classical insecticides. This could improve the biodegradability of insecticide treatments and therefore decrease the quantity of toxic insecticide residues, increase insecticide selectivity and develop a better respect for the environment. This alternative strategy based on the identification of plant insecticidal molecules is not recent. (Palsson K, Jaenson GT, 1999)

Activity of essential oils on stored-product insects was investigated. Patchouli, *Pogostemon heyneanus* (Solana- ceae) and *Ocimum basilicum* (Lamiaceae) essential oils showed insecticidal activity against *Sitophilus oryzae* (Coleoptera: Curculionidae), *Stegobium paniceum* (Coleoptera: Anobiidae), *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Bruchus chinensis* (Coleoptera: Bruchidae) (Narasaki, M et al., 1987). The goldenrod *Solidago canadensis* L. (Asteraceae) was strongly toxic to *Sitophilus granarius* (Coleoptera: Curculionidae) and oils from *Eucalyptus* or *Thymus vulgaris* (Lamiaceae) were toxic to *Rhizopertha dominica* (Several essential oils extracted from various spices and pot herbs of the mediterranean area were active against *R. dominica*, *Oryzaephilus surinamensis* (Coleoptera: Cucujidae), *S. oryzae* and *T. castaneum* (Shaaya et al., 1991) and on the bruchid of kidney bean, *Acanthoscelides obtectus* (Coleoptera: Bruchidae) and *Aedes aegypti* (Diptera). The toxic effect of essential oils was not only suitable for granary insects but also for flying insects: *Gaultheria* (Ericaceae) and *Eucalyptus* (Myrtaceae) oils exhibited very high killing power on insects such as the rice weevil *S. oryzae*, the beetles *Callosobruchus chinensis* (Coleoptera: Bruchidae) and *S. paniceum*, and also on *M. domestica*.

(Guenther, E. (1972) Actually, the activities of essential oils on species are manifold. *Mentha*, *Lavandula* (Lamiaceae) or *Pinus* (Pinaceae) essential oils were noted for their toxicity against *Myzus persicae* (Homoptera: Aphididae) and the greenhouse white fly *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) as well as the Colorado beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) and the pear bug *Stephanitis pyri* (Hymenoptera: Stephanidae) (Hummelbrunner LA, Isman MB 2001) Mites (*Dermatophagoides farinae*, Acarina: Pyroglyphidae), termites (*Coptotermes formosanus*, Isoptera: Rhinotermitidae) and cockroaches (*Periplaneta fuliginosa*, Dictyoptera: Blattidae) were exterminated by the hinoki-asunaro (*Thujaopsis dolabrata* hondai, Cupressaceae) leaf oil (Ezeonu FC et al., 2001).

Insects vary enormously in their responses to secondary plants products and it is well known that the sensitivity of different insect species could be quite different for the same substance. Oils from *Cymbopogon nardus* (Graminae) which killed *A. obtectus* in a short time (Regnault-Roger et al., 1993) only knocked down and disabled *Sitotrogace reallela* (Lepidoptera: Gelichiidae) (Krishnarajah et al., 1985). Essential oils are active against both adults and larvae and frequently act to inhibit reproduction. This action could be the result of female sensitivity: essential oils cause adult mortality and also repel carmine spider mite, *Tetranychus cinnabarinus* (Acarina: Tetranychidae) females, and reduce egg laying (Mansour et al., 1986). Reproductive inhibition can also affect the developmental stages of the pest. Leafhopper *Amrasca devastans* (Homoptera: Cicadellidae) oviposition was inhibited by volatiles of *Eucalyptus globulus* (Myrtaceae) and *Coriandrum sativum* (Umbelliferae) (Matos FJA 2000, 1982), and a toxic effect of lavender oils on larvae of *T. vaporariorum* has been shown (Mateeva and Karov, 1983). The LD 50 of some volatiles extracted from the essential oils of *Parthenocissus quinquefolia* (Vitaceae), coniferous trees, *Citrus* and *Mentha* species, was tabulated using larvae of *L. decemlineata* and *Tribolium destructor* (Coleoptera: Tenebrionidae) (Matsumoto, Tet al., 1987). *A. calamus* oil and its active ingredients, asarone and its analogues, significantly reduced fecundity and hatchability of the kelp fly *Coelopa frigida* (Diptera: Coelopidae) and exerted an ovicidal action on *Oncopeltus* Myristicaceae, Graminae, Rutaceae, Myrtaceae families was also observed. This beetle has been shown to be a convenient model to point out with accuracy which reproductive stage is targeted and the speed of the activity of essential oils (Matos FJA 2000).

Some essential oils and their components exhibited both a repellent and a larvicidal action: volatile oils including camphor, cineole, methyl eugenol, limonene, myrcene and thymol, strongly repelled mosquitoes exerted a larvicidal activity. In fact, the mechanisms of the toxic effect on insects are not presently

well known. The neurotoxicity of several monoterpenoids (d-limonene,  $\alpha$ -myrcene,  $\alpha$ -terpineol, linalool and pulegone) which have been identified as important components of essential oils, were tested on the house fly as well as on the German cockroach (Consoli *et al.*, 1988) and linalool was identified as an inhibitor of acetylcholinesterase (Ryan and Byrne, 1988).

## MATERIALS AND METHODS

### *Plant Material*

*Piper betle* from the Indonesian Plants were collected in the Medicinal Plants Garden of the Central Research Health University of Jember, Indonesia. Taxonomic identification of plants was performed by botanists Laboratorium, Department of FKIP Biology, Jember University

### *Essential Oil Analysis*

The essential oils were extracted by steam distillation. The oils were analyzed using a Hewlett-Packard 5971 GC/MS instrument employing the following conditions: column: Dimethylpolysiloxane DB-1 coated fused silica capillary column (30 m x 0.25 mm); carrier gas: He (1 ml/min); injector temperature: 250°C; detector temperature: 200°C; column temperature: 35–180°C at 4°C/min then 180–250°C at 10°C/min; mass spectra: electron impact 70 eV.

### *Larvicidal Bioassay*

Larvae of *A. aegypti* were collected from a mosquito colony maintained strain Jember- Indonesian. For the assay, the essential oil of the plants was placed in a 50 ml beaker and DMSO (0.3 ml) was used to solubilize the oil in water (19.7 ml) that contained 50 larvae (third instar). With each experiment, a set of controls using 1% PP3 and untreated sets of larvae in tap water, were also run for comparison. Mortality was recorded after 24 h of exposure during which no nutritional supplement was added. Each test comprised of three replicates with nine concentrations (1, 1.25, 2.5, 5, 10, 12.5, 25, 50, 100 ppm).

Data were evaluated through regression analysis. From the regression line, the LC50 values were read representing the lethal concentration for 50% larval mortality of *A. aegypti*

## RESULTS AND DISCUSSION

Among the multitude of plant species, some are called aromatic because of the volatile compounds they contain, which give them an odour and a characteristic flavour. Abundant on the Mediterranean periphery, aromatic plants are closely associated with the origins of the western civilization. They have been mentioned from Antiquity for their uses as spices, pot herbs or medicinal plants: A new field could be developed beside these traditional activities: insect pest management (Regnault-Roger, 1995). One of the fractions of aromatic plants, most frequently used for industrial applications, and which has shown promise for use in IPM, is the fraction of volatile fragrant compounds commonly called essential oils. The volatility of essential oils allows them to be easily extracted by water vapours, in contrast to fixed lipid oils and essences (concrete, absolute, oleoresins and resinoids) which are extracted by solvents and alcohol. Distinguished three kinds of water and steam distillation methods for obtaining essential oils. The major chemical compounds were citronella (35.97%), nerol (17.28%), citronellol (10.03%), geranyl acetate (4.44%), elemol (4.38%), limonene (3.98%), and citronellyl acetate (3.51%). The major components of essential oils from *Piper betle* in Indonesian were citronellal (21.8%), geraniol (14.2%), terpineol (19.2%), and cissabinene hydrate. It was reported that the citronellal content varied among the cultivated citronella varieties. Additionally, the composition of essential oils is affected by many factors, including the cultivation conditions of the plants and isolation techniques (Chariandy CM, *et al.* 1999)

The biological significance of essential oils has long been discussed. With regard to the first hypotheses which considered them as wastes of the phytometabolism, it appears today that they present multiple actions. The toxic effect of essential oils was not only suitable for granary insects but also for flying insects. Insects vary enormously in their responses to secondary plant products and it is well known that the sensitivity of different insect species could be quite different for the same substance. Oils from *Cymbopogon nardus* (Graminae) which killed *A. obiectus* in a short time (Carvalho *et al.*, 2003) only knocked down and disabled *Sitotrogacereallela* (Lepidoptera: Gelichiidae) (Akita, K. (1991) Essential oils are active against both adults and larvae and frequently act to inhibit reproduction. This action could be the result of female sensitivity: essential oils cause adult mortality and also repel carmine spider mite, *Tetranychus cinnabarinus* (Acarina: Tetranychidae) females, and reduce egg laying (Mansour *et al.*, 1986).

Larvicidal Activity of Essential Oils of *Piper betle* from the Indonesian Plants against *Aedes aegypti* L of the 48 hour exposed in Table 1.

Table 1.

Concentrations	Mortality was recorded after 48 h of exposure			
	Replika 1	Replika 2	Replika 3	Range
100	100	100	100	100
50	80	80	90	83,33
2.5	50	70	70	63,33
1.2,5	40	50	50	46,66
.10	40	30	30	30,33
5	30	20	20	23,33
.2,5	20	20	10	16,66
.1,25	10	10	10	10
.1	10	0	0	3,33
Kontrol (air)	0	0	0	0,00
Kontrol (PP3)	0	0	0	0,00

LC<sub>50</sub> = 13,1

Larvicidal Activity of Essential Oils of *Piper betle* from the Indonesian Plants against *Aedes aegypti* L of the 24hour exposed

Concentrations (ppm)	Mortality was recorded after 24 h of exposure			
	Replika 1	Replika 2	Replika 3	Range
100	100	100	100	100,00
50	80	80	90	83,33
2.5	60	70	80	70,00
12,5	50	50	60	53,33
10	50	50	40	46,66
5	40	40	30	36,66
2,5	30	30	20	26,66
1.25	10	20	10	13,33
1	10	10	0	6,66
Kontrol	0	0	0	0,00

LC<sub>50</sub> = 11.2

The results from the *A. aegypti* larvicidal assay using the *Piper betle* from the Indonesian Plants. The most active essential oils against third instar larvae of *A. aegypti* were those of the 24hour exposed, LC<sub>50</sub> = 13,1 . For the 48 hour exposed, LC<sub>50</sub> = 11,2 . Causes significant growth inhibition and mortality in later developmental stages of *A. aegypti*. The analysis of the essential oil of this plant from *Piper betle* from the Indonesian Plants were shown to be very active against *A. aegypti* larvae. The essential oils of the *Piper betle* from the Indonesian, although they have insecti-agent is an arbovirus and the major vector is the *Aedes aegypti* mosquito, While most patients are asymptomatic, infection with different serotypes of dengue viruses may lead to hemorrhagic fever with high mortality (Hummelbrunner LA, Isman MB 2001).

During outbreaks, public health authorities in Indonesian have standardized the use of aerolized pyrethroid insecticides that can cause allergies.

Brud, W.S. and Gora, J. (1989) *Matos FJA 2000*.. This measure only partially controls the mosquito population since it eliminates the adult flying insects but does not eliminate the breeding places .. In these breeding sites, the larvicide used is usually the organophosphorate Temephos, although very slightly toxic may cause headaches, loss of memory, and irritability. Secondary metabolites of plants, many of them produced by the plant for its protection against microorganisms and predator insects are natural candidates for the discovery of new products to combat *A. aegypti*. Several studies have on focused natural products for controlling *Aedes* mosquitoes as insecticides and larvicides, but with varied results. The repellency to *A. aegypti* of essential oils from the *Piper betle* from the Indonesian Plants

Was determined in laboratory tests. essential oils suggest that they are promising as larvicides against *A. aegypti*. In the present study essential oils from the *Piper betle* from the Indonesian Plants were tested against third instar *A. aegypti* larvae in a search for effective and affordable natural products to be used in the control of dengue.

Several formulations were proposed to control mosquitoes. The resistance of veneer-faced panels to insects is improved by a deep impregnation of the polymer layer with hiba or kinoki essential oils (Akita, 1991; Tsubochi and Sugimoto, 1992). From all these observations, it could be deduced that essential oils present a widespread range of activities on insects and could be used for environmentally safer pest management. To investigate the mechanisms of toxicity, a comparative study was conducted between larvae of *Aedes aegypti* (Diptera). In fact, the mechanisms of the toxic effect on insects are not presently well known. The neurotoxicity of several monoterpenoids (d-limonene,  $\alpha$ -myrcene,  $\alpha$ -terpineol, linalool and pulegone) which have been identified as important components of essential oils, were tested on the house fly as well as on the German cockroach (Gora, J. (1989) and linalool was identified as

an inhibitor of acetylcholinesterase (Ryan and Byrne, 1988).

However, because of the chemical complexity of essential oils of the paper battle from the Indonesia plant, several mechanisms being contingent on the phytochemical pattern of the oils and the sensitivity of the *Aedes aegypti* could be involved. In these circumstances, the comparison of the toxicity of several essential oils in order to classify them, will be significant only if the evaluation involves experiments conducted with samples of known phytochemical pattern on identical insect populations. Among current alternative strategies aiming at decreasing the use of classical insecticides, ecochemical control based on plant-insect relationships is one of the most promising methods. For centuries, plants and insects have followed a parallel and interdependent evolution. Insects cannot live without plants and vice versa. The observed mortality from the lower concentrations was comparable to the control. The finding of the present experiment revealed that citronella oil possesses oviposition deterrent and ovicidal activities against *H. armigera*. The toxic effect of this oil could be attributed reported that the volatile oils paper battle to major constituents such as citronella, linalool, and geranyl acetate. High toxicity of these compounds was reported against the rice weevil *Sitophilus oryzae* and *Rhyzopertha dominica* (Brud, W.S. and Gora, J. (1989) caused most of the eggs not to develop into adult (abnormality in egg development to adult). The chemical composition and broad spectrum of biological activity of essential oils can vary with plant age, plant tissue, geographical origin of plant, organ used in distillation process, type of distillation, and species and age of targeted pest organism. Essential oils showed that a majority of the inventions focused on household uses. A cleaning solution including clove essential oils and pyrethroid would cleanse and destroy eggs and larvae *Aedes aegypti*.

## CONCLUSION

Diversified use of essential oils from Indonesian plant of their use in the pest management larvacidal activity against *Aedes aegypti*. The most active essential oils against third instar larvae of *A. aegypti* were those of the 24hour exposed,  $LC_{50} = 13,1$  ppm. For the 48 hour exposed,  $LC_{50} = 11,2$  ppm. The Essential Oils of *Piper Betle* from the Indonesian Plants against *Aedes aegypti* L present potential alternatives of new bioinsecticide replacing for insect resistance to insecticidal *Bacillus thuringiensis* toxins expressed in transgenic plant.

## REFERENCES

- Adams RP 2001. *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*, Allured, Illinois, 455 pp.
- Barnard DR 1999. Repellency of essential oils to mosquitoes (Diptera: Culicidae). *J Med Entomol* 36: 625-629.
- Akita, K. (1991) Fukubi Kagaku Kogyo K K, Japan. Insect- repellent and mildewcidal laminate boards. *Patent JP 91-242869 910927*.
- Gora, J. (1989) Biological activity of essential oils and its possible applications. In S. C. Bhattacharyya, N. Sen, K. L. Sethi (eds) *Proceedings 11th International Congress on Essential Oils, Fragrances and Flavours*, vol. 2, pp. 13–23, New Delhi, India: Oxford & IBH.
- Carvalho AFU, Melo VMM, Craveiro AA, Machado MIL, Bantim MB, Rabelo EF 2003. Larvicidal activity of the essential oil from *Lippia sidoides* Cham. against *Aedes aegypti* L. *Mem Inst Oswaldo Cruz* 98: 569-571.
- Chariandy CM, Seaforth CE, Phelps RH, Pollard GV, Khambay BPS 1999. Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. *J Ethnopharmacol* 64: 265-270.
- Ciccio G, Coussio J, Mongelli E 2000. Insecticidal activity against *Aedes aegypti* larvae of some medicinal South American plants. *J Ethnopharmacol* 72: 185-189.
- Consoli RAGB, Mendes NM, Pereira JP, Santos B de S, Lamounier MA 1988. Influência de diversos derivados de vegetais na sobrevivência das larvas *Aedes fluviatilis* (Lutz) (Diptera: Culicidae) em laboratório. *Mem Inst Oswaldo Cruz* 83: 87-93.
- Ezeonu FC, Chidume GI, Udedi SC 2001. Insecticidal properties of volatile extracts of orange peels. *Bioresource Technology* 76: 273-274.
- Guenther, E. (1972) The production of essential oils. In E. Guenther (ed) *The Essential Oils*, 2nd edn., vol.



1, pp. 87–226. Malabar (Florida): Krieger Publishing Company.

Hummelbrunner LA, Isman MB 2001. Acute, sublethal, antifeedant, and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lep., Noctuidae). *J Agric Food Chem* 49: 715-720.

Matos FJA 2000. *Plantas Medicinais*, 2 ed., Imprensa Universitária, Fortaleza, 344 pp. NICC-Comunicação e Saúde. 2003. Pesquisadores criticam o uso de produtos químicos no combate à doença. Available at: [www.cpqam.fiocruz.br/nicc/comunic/jc200101.htm](http://www.cpqam.fiocruz.br/nicc/comunic/jc200101.htm). Accessed on 9 September.

Matsumoto, T., Takaoka, K. and Watanabe, C. (1987) Acaricides, insecticides and insect repellents containing benzaldehyde or perilla aldehyde. *Patent JP-87 176437 870715*.

Narasaki, M., Morita, H. and Fujisaki, T. (1987) Mikasa Chemical Industrial Co, Ltd. Japan. Synergistic insecticides containing insect growth inhibitors. *Patent JP 87-173368 870711*.

Palsson K, Jaenson GT 1999. Plant products used as mosquito repellents in Guinea Bissau, West Africa. *Acta Trop* 72: 39-52

Ryan, M.F. and Byrne, O. (1988) Plant insect coevolution and inhibition of acetylcholinesterase. *Journal of Chemical Ecology* **14**, 1965–75