Korean J. Plant Res. 19(6) : 721~726 (2006)

Eco-frendly Control of *Culex pipiens* (mosquito) Larvae by *Acorus calamus* (sweet flag) and *Acorus gramineus* (Grassy-leaved sweet flag) Extracts

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Abstract - Mosquitoes are carriers of malaria and encephalitis. This study performed for eco-friendly control of mosquitos by using genus Acorus. Several solvents were used for the extraction of genus Acorus; water, ethanol, and methanol. Grinded leaves and roots were also included. Acorus extracts killed mosquito larvae and the ethanol extract showed the best result. Autoclaved Acorus water needed long time to kill mosquito larvae. LT_{50} of 1% *Acorus calamus* decoction was 13.6 hrs and 1% autoclaved Acorus water was 53.6 hrs. LT_{50} of 0.05% *Acorus calamus* rhizome powder was 28.5 hrs. LT_{50} of 0.5% *Acorus calamus* leaf powder was 10.8 hrs. LT_{50} of 0.1% *Acorus calamus* decoction was 63.4 hrs and 0.1% *Acorus calamus* ethanol extracts was 48.6 hrs and 0.1% *Acorus calamus* methanol extracts was 53.9 hrs. LT_{50} of 0.4% *Acorus gramineus* decoction was 45.5 hrs, 0.4% ethanol extracts was 10.9 hrs, 0.4% methanol extracts was 10.2 hrs. LT_{50} of ethanol extracts was shorter than other extracts. *Acorus calamus* rhizome powder could be used for the eco-friendly control of the mosquito larvae.

Key words - Araceae, Encephalitis, Ethanol extracts, Pest control, Rhizome powder

Introduction

Mosquitoes are well known vectors of malaria and encephalitis (Burfield and Reekie, 2005; Cheng *et al.*, 2003). They are insects of family Culicidae in order Diptera, responsible for spreading human diseases like Japanese encephalitis, dengue, and yellow fever. *Culex pipiens* can take proteins from a septic tank for sewage under apartment without blood feeding in winter (Lee, 2006). Fruitful mosquitos bear eggs throughout the year in a septic tank for sewage or a basement, especially during the summer the population of mosquito increases dramatically. We need to eliminate mosquitos for preventing harmful disease.

Many insecticides are produced and used in a form of mosquito repellent or liquid for mosquito control. First insecticide aimed for keeping toxicity long time. Dichloro-diphenyl-trichloroethane (DDT) was an ideal insecticide. Recently DDT was prohibited by high toxicity of human and long remaining period. Toxicity of chemical insecticide remained long period while eco-friendly insecticide was decomposed relatively easily resulting in low efficiency.

Thomas and Callaghan (1999) made an experiment on the control of mosquito larva with garlic and lemon skin. Cheng *et al.* (2003) performed mortality experiment on mosquito larva with plant oil (Dharmagadda *et al.*, 2005; Kang *et al.*, 2006). Cheng *et al.* (2004) made an experiment on the control of mosquito larva using plant origin natural substances. In this way, the experiments were performed using plant origin elements for the control of mosquito larva. In this study, we made an experiment on the control of mosquito using *Acorus calamus* which was well known for its microcidal and insecticidal property. It has been reported to control mosquito larva using garlic, lemon skin, plant oil and natural substance (Cetin *et al.*, 2004; Cheng *et al.*, 2003; Dharmagadda *et al.*, 2005; Kang *et al.*, 2006; Shaalan *et al.*, 2005). Also, plant extracts have well known for the effects on several pests (Kang *et al.*, 2006; Prakash and Rao, 1997). In this study, we investigated to know the prevention effect of genus Acorus, known as microcidal and insecticidal activity, on mosquito larva (Lee *et al.*, 2004; Prakash and Rao, 1997).

Genus Acorus belongs to family Araceae and composed of *Acorus calamus* and *Acorus gramineus* that are perennial plants. *Acorus calamus* ranges over swamp or shallow pond. Rhizome is bulky and crawls under shallow, stretchs to the next, has many knobs, colours in white or light reddish. Its habitat is known to be Korea, Japan, and

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China. Whole plant of Acorus including leaf, root, and rhizome has genuine aroma (β-asarone). Blooming time is June and July. A flower stalk is slightly shorter than leaf. The length of the stalk is about 5cm of spadix. Leaf of *Acorus calamus* is wider and longer compared to *Acorus gramineus*. *Acorus gramineus* is deciduous and evergreen. Ancient times, *Acorus calamus* was used for washing heads and curing skin disease. *Acorus calamus* contains refined oils which have valuable ingredient such as methyleugenol, asaryl aldehyde and asarone (Lee *et al.*, 2004). Recently, extract of Acorus was reported for their antimicrobial effect on virus and bacterium such as salmonella, dandruff and pimple. The ethanol and methanol extract of *Acorus calamus* are known as insecticide on many harmful insects.

This study was to explore the prevention effect of mosquito larvae using various extracts of genus Acorus.

Materials and Methods

Plant materials

Acorus calamus was purchased from the Ham-pyung agriculture association. *Acorus calamus* was imported from China and was purchased from the Kyung-dong market in Seoul. Domestic *Acorus gramineus* was obtained from the Chonnam oriental medicine agricultural cooperative association.

Plant materials are separated into leaf and rhizome. Leaf samples were prepared into 5cm pieces and root sample were prepared into 2cm pieces. The prepared leaves and rhizomes were grinded in the grinder. Then, the powder was kept for further extraction.

Mosquito Larva

Culex pipens eggs were hatched in water. The larvae were fed and reared until third instar larvae. Each stage of instar larvae takes about three days. When they became third instar larvae, they were used for mortality test.

Extraction of *Acorus calamus* and *Acorus gramineus*

Extraction of *Acorus calamus* used various solvents which are water, ethanol, and methanol. Water extraction of *Acorus calamus* was performed in high pressure cooker into decoction condition. Extraction solvents of *Acorus gramineus* were used by ethanol and methanol. Autoclaved *Acorus calamus* water was produced by putting into 200g *Acorus calamus* in 1L tap water and boiled in pressure cooker for 5 hours. Decoction was prepared using an oriental herb medicine electric brewing pot (DWP-3800T, Daewoong Co. Ltd., Yongin, Korea) using following condition: 200g *Acorus calamus* or 300g *Acorus gramineus* were located into 1.5L tap water and boiled for 5 hours, two cycles of 150 min. Ethanol extracts made by pouring 1L Ethanol to 200g *Acorus calamus* or 300g *Acorus gramineus*. The container was shaken every three days and facilitated the extraction. *Acorus calamus* put into the solution over one month and various elements of *Acorus calamus* was sufficiently extracted from *Acorus calamus*. The methanol extracts were extracted from *Acorus calamus* and *Acorus gramineus* in a same method of ethanol extraction. Ethanol and methanol extracts, leaves and rhizome powder of *Acorus calamus* were used for mortality test of mosquito larva.

Mosquito experminents

Ten mosquito larvae in third stage instar were put into each 200ml capacity bottle. Then, *Acorus calamus* extraction and *Acorus gramineus* extraction were treated in proper concentration. *Acorus calamus* powder was treated 0.5% concentration of leaves and 0.05% concentration of rhizome powder. The treated concentration was 0.1, 0.7, and 1% (v/v) autoclaved *Acorus calamus* water and 1% (v/v) *Acorus calamus* decoction. *Acorus calamus* ethanol or methanol extracts were treated in concentration of 0.1 and 0.7% (v/v). *Acorus gramineus* decoction was treated in concentration of 0.1 and 0.4% (v/v). *Acorus gramineus* ethanol and methanol extracts were treated in concentration of 0.1 and 0.4% (v/v). The treated culture media were mixed by using pipette. Mosquitos were grown as control treatment in distilled water, 1% ethanol, and 1% methanol and compared with treated samples. Then, the number of dead larvae were counted at 1, 3, 5, 24, 48, and 120 hours after treatment. To identify the dead mosquito larva, plastic pipette was used for forming fast-flowing and identified mortality of larva. The larva with motion was counted as live mosquito larva and the larva without motion was counted as dead larva. Three days after treatment, live larvae trapped with mosquito net. Mortality rate of larva was calculated with Excel program (MS Office XP, Microsoft Corporation, USA) and obtained regression formula. The LT_{50} (median lethal time) was calculated using regression formula.

Results

Acorus calamus

Acorus calamus used for mosquito larva control were effective in various forms such as leaf and rhizome powder, decoction and autoclaved water, ethanol and methanol extracts. The strongest prevention effect was observed with ethanol extract, followed by decoction. Autoclaved water showed the weakest effect.

The regression formula and coefficient related to this experiment were shown in table 1. The coefficients were 0.7-0.9. The LT_{50} values of *Acorus calamus* rhizome and leaf powder were 28.5 and 10.8 hours. The LT_{50} value of *Acorus calamus* rhizome powder, a tenth dilution of *Acorus calamus* leaf powder concentration, was about 3 times longer than the leaf powder. As expected, *Acorus calamus* rhizome powder was more effective than *Acorus calamus* leaf powder for the control of the mosquito larva. The LT_{50} values of 0.1% and 0.7% ethanol extract were 48.6 and 5.3 hours, respectively. The LT_{50} values of 0.1% and 0.7% decoction were 63.4 and 28.3 hours, respectively. In treatment of 0.7% extract, the prevention effect of ethanol extract was better than decoction because LT_{50} of decoction was taken about 6 times longer compared to ethanol extracts.

As a result of methanol extract, LT_{50} of 0.1% and 0.7% was taken more time than ethanol extract as 53.9 and 8.4 hours, respectively. In 0.1% and 0.7% treatment, the prevention effect of methanol extract was slightly less than ethanol extract because LT_{50} of methanol was taken more about 1.3 and 1.58 times. Compared with autoclaved water and decoction, LT_{50} value of 1.0% autoclaved water was 53.6 hours, while LT_{50} of decoction was 13.6 hours. LT_{50} was lengthened as the amount of decoction treatment was decreased. LT_{50} was about 28.3 hours in 0.7% decoction and about 63.4 hours in 0.1% decoction.

Acorus gramineus

The extracts of *Acorus gramineus* by ethanol and methanol was also applied to test the prevention effect of mosquito larva. LT_{50} of 0.1% ethanol and methanol extracts and decoction was 34.9, 45.6 and 91.6 hours, respectively (Table 1). LT_{50} of 0.4% ethanol and methanol extracts and decoction were 10.9, 10.2 and 45.5 hours, respectively.

Discussion

Methanol is one of the toxic solvents. Despite of its toxicity, the survival rate of larvae in 1% methanol was similar to the control treatment with water. Consequently, *Acorus calamus* and *Acorus gramineus* were responsible for the prevention effect of methanol extract. The use of methanol has some remoteness with eco-friendly pest control (Ndung'u *et al.*, 2003). In this study, various extracts by different solvents and methods showed the prevention effect which was dosage dependent. For the eco-friendly mosquito control, the use of raw plant material is recommendable than the use of methanol and ethanol as solvents. Thereby, the rhizome powder of *Acorus calamus* and *Acorus gramineus* can be a sustainable way of mosquito control. For the accurate control, the alcohol extract is better to refine and dilute during the preparation. However, the oil compound of Acorus force to apply organic solvent in order to dissolve properly (Lee *et al.*, 2004; Shaalan *et al.*, 2005). So, the *Acorus calamus* extract was made by using ethanol which known as low toxical solvent and directly used without further purification process.

The extractions by ethanol and methanol were compared in order to find out the effect of the solvent. In *Acorus calamus*, ethanol ex-

Table 1. LT_{50}, regression curve, and R^2 value of various forms of *A. calamus* and *A. gramineus*

Plant	Extract	LT_{50}	Regression curve	R^2
A. calamus	0.1% decoction	63.4	y=0.70x+5.62	0.8
	0.7% decoction	28.3	y=1.04x+20.72	0.7
	1.0% decoction	13.6	y=2.61x+14.34	0.7
	1.0% autoclaved water	53.6	y=0.85x+4.69	0.9
	0.5% leaf powder	10.8	y=2.76x+20.20	0.7
	0.05% rhizome powder	28.5	y=1.25x+14.31	0.7
	0.1% ethanol extract	48.6	y=0.99x+2.07	0.8
	0.7% ethanol extract	5.3	y=9.48x+0.08	0.7
	0.1% methanol extract	53.9	y=0.91x+1.13	0.9
	0.7% methanol extract	8.4	y=4.32x+13.87	0.7
A. gramineus	0.1% decoction	91.6	y= 0.47x+6.57	0.7
	0.4% decoction	45.5	y=0.98x+5.62	0.8
	0.1% ethanol extract	34.9	y=0.96x+16.54	0.7
	0.4% ethanol extract	10.9	y=3.90x+7.50	0.9
	0.1% methanol extract	45.6	y=1.08x+9.13	0.8
	0.4% methanol extract	10.2	y=3.98x+9.31	0.9

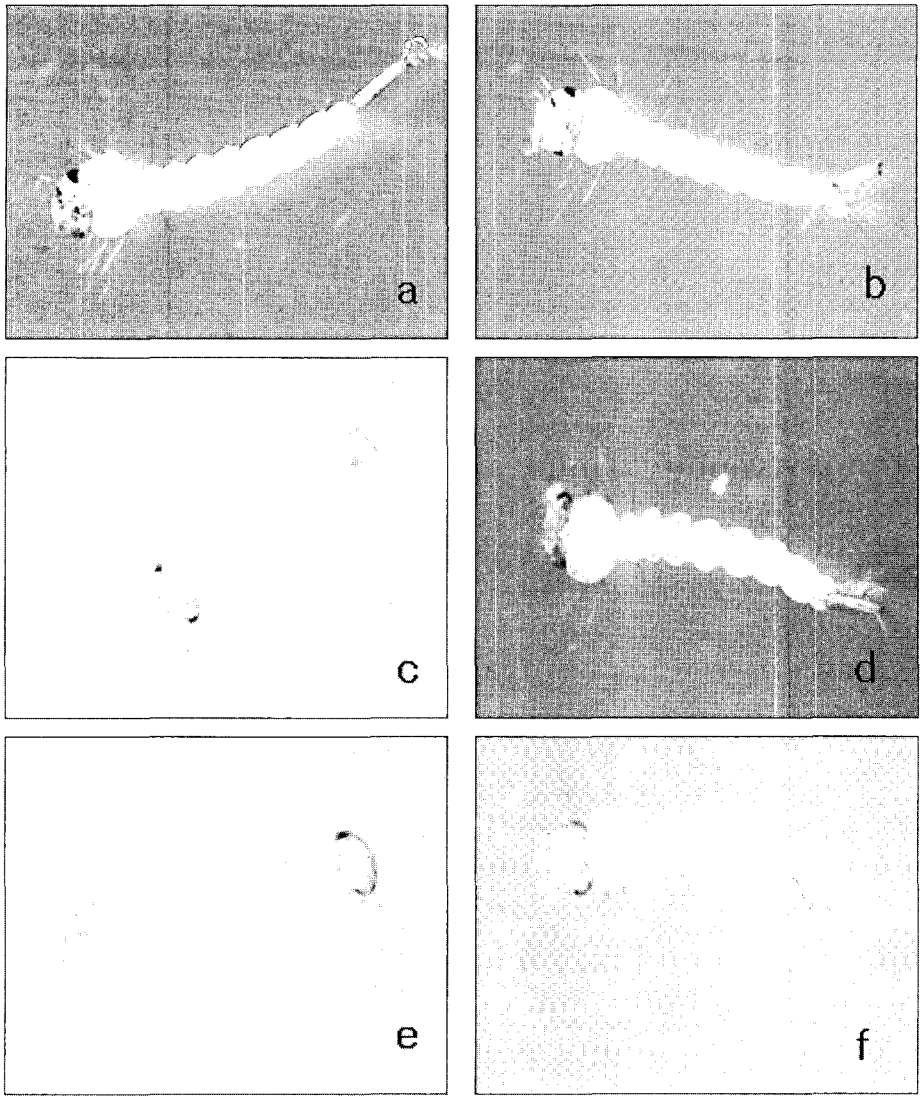

Fig. 1. Mosquito larvae in various extracts and control. (a) and (b) control, (c) methanol extract of *A. gramineus*, (d) ethanol extract of *A. gramineus*, (e) methanol extract of *A. calamus*, (f) ethanol extract of *A. calamus*.

traction method showed better prevention effect than the methanol extraction method. The ethanol extract of *Acorus gramineus* showed good prevention effect even in low amount and additive effect by the increase of the dosage. The ethanol and methanol extracts of *Acorus calamus* gave the internal organs of larva in more yellow color than the control (Fig. 1). A certain chemical of *Acorus calamus* could have attacked the internal organs of mosquito larva. Further studies were warranted with detailed anatomical observation.

The prevention effect is known to be dependent on the species of mosquito (Guillet *et al.*, 1997; Luna *et al.*, 2005; Markouk *et al.*, 2000; Medonca *et al.*, 2005; Nathan *et al.*, 2005; Rahuman *et al.*, 2000; Rajkumar and Jebanesan, 2004). Water extraction method was effective but required relatively higher concentration in the prevention of mosquito larva. Interestingly, concentrated boiled extracts in water were more effective than the autoclaved extracts in terms of mosquito control. As a result, the rhizome of Acorus can be applied for the mosquito control as eco-friendly and sustainable method. Furthermore, the Acorus extracts can be also applied for the other pest control with some modifications.

Acknowledgements

This work was supported by the Regional Research Centers Program (Bio-housing Research Institute), granted by the Korean

Ministry of Education & Human Resources Development.

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(Received 27 October 2006; Accepted 26 December 2006)