Thai J. For. 27 (2) : 14-20 (2008) วารสารวนศาสตร์ 27 (2) : 14-20 (2551)

Original article

Effects of Sweet Flag Extracts (*Acorus calamus* L.) on Toxicity and the Levels of Esterase and Glutathione-S-transferase on the Brown Dog Tick (*Rhipicephalus sanguineus* (Latreille))

Chanatda Leenuwongphun[1] **Suraphon Visetson**[1]

Manthana Milne[2]

[1] Faculty of Science, Kasetsart University, Bangkok 10900
Tel. 081-4242701 Email: leenuwongphun@hotmail.com

[2] Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok 10900

Received: September 8, 2008 Accepted: October 2, 2008

ABSTRACT

The tendency of herbal extracts as insecticide alternative is gaining high acceptability to Thai people. Some synthetic pesticides namely pyrethroids and organophosphate have been causing detrimental effects to home pet such as dogs. This research was done to evaluate the efficiency of sweet flag (*Acorus calamus* L.) rhizomes that have been proved to repel and give mortality to some insects. Sweet flag rhizomes was extracted by soaking method at room temperature using 95% ethanol was trialed. The dipping method of herbal extracts was used against brown dog tick (*Rhipicephalus sanguineus*). The completely randomized designs (CRD) with 4 replicates were to analyse their LC50. The toxicity of sweet flag extracts showed Y = 7.28 + 28.65X; LC50 ca. 1.49% w/v. after exposure at 24 hours and after exposure for 36 hours was Y = 10.91 + 34.50X; LC50 ca. 1.13% w/v. on nymph brown dog tick and Y = 18.36 + 29.48X; LC50 ca. 1.07% w/v. after exposure at 24 hours and after exposure for 36 hours was Y =21.28 + 33.34X; LC50 ca. 0.86% w/v. on adult brown dog tick. The detoxification enzyme activities of esterase and glutathione-S-transferase from brown dog tick after 24 hours exposure were also observed. esterase showed stronger activity than other enzymes. The enzyme activities of esterase and glutathione-S-transferase from brown dog tick after 24 hours were induced follow levels of sweet flag extract was increased.

Keywords: Efficiency, Sweet flag extracts, Brown dog tick, Esterase, Glutathione-S-transferase

INTRODUCTION

The relationship between human and dog has been established for thousand of years. Due to this old and close relationship, some ectoparasites of domestic dogs may be seen parasitizing human. This parasitism, though unusal, might be responsible for a simple skin lesion for the transmission of infectious agents. *Rhipicephalus sanguineus* (Latreille), commonly called the brown dog tick or kennel tick, is one of the most widely distributed of all tick.

The heavy infestation of ticks cause vectors of infection, direct damage, immunosuppression, tick paralysis and transmitted diseases. Human always use synthetic chemical insecticides to control the tick. However, most synthetic insecticides are toxic to human as well as animal, and some of them have toxic on other target and remained in environment for a long time. So the insecticides derived from herbal are great demand. The herbal derived insecticides may have low toxicity to mammals and rapid reduction of the toxicity. Essential oil of sweet flag (*Acorus calamus*) has potential for the control of insect pests. The main active ingredient is β-asarone (Mukerjea and Govind, 1960). It affected a wide variety of insects, acting as antifeedant, repellent or chemosterilant (Streloke *et al*., 1989). The crude-extract of sweet flag rhizome showed toxicity to larvae of Boophilus microplus (Chungsamarnyart *et al*., 1988). It has high larvicidal activity on boophilus tick (Chungsamarnyart *et al*., 1988) may be it had toxic on brown dog tick. Esterase and glutathione-S-transferase are groups of detoxification enzymes. These enzymes have a central role in detoxification of xenobiotic and endogenous compounds. In populations with a long history of chemical exposure, high glutathione-S-transferase activity is associated with resistance to insecticides (Wei *et al*., 2001). Resistance to organochlorine and organophosphate insecticides is specifically associated with increased glutathione-S-transferase activity (Vontas *et al*., 2000). The present study of sweet flag extracts can be practically useful as insecticides for the brown dog tick.

MATERIALS AND METHODS

Plant Extracts

Cut sweet flag rhizomes into small pieces and immersed in 95% ethanol for seven days. The ethanol extracted of sweet flag was evaporated at 40 °C by vacuum rotary evaporator and kept at 4 °C.

Mortality of Brown Dog Tick

The mortality of brown dog tick was observed 24 hours and 36 hours after dipping in 1 min. Abbott's formula (Abbott's, 1925) was used to calculate the corrected mortality of ticks. The mean (%) of corrected mortality was the average of 4 replications.

Abbott's formula

$$\% \text{ corrected mortality} = \frac{(\text{alive control} - \text{alive treated}) \times 100}{(\text{alive control})}$$

Insects Treatment for Enzyme Extraction

Live nymphs and adults of dog tick after treated with sweet flag extract was collected at 24 hour after exposure and were used for enzyme extraction using Visetson enzyme assay Visetson *et al*. (2003).

Enzyme Activity Assay

Esterase activity was measured using the PNPA assay which modified from Visetson *et al*. (2003) and Visetson (2004). Glutathione-S-transferase (GST) activity was measued using the CDNB assay which modified from Visetson *et al*. (2002, 2003).

RESULTS AND DISCUSSION

The crude-extract of sweet flag caused 10-90% mortality of ticks at 24 and 36 hours after tested as shown in Table 1-2.

The detoxification enzyme activities of sweet flag extract on brown dog tick after contact at the 24 hours as follows Table 3-4.

The results of LC_{50} of sweet flag extract showed linear respond with Y= 7.28+ 28.65X; LC_{50} ca. 1.49% w/v. after exposure at 24 hours and after exposure for 36 hours was Y= 10.91+34.50X; LC_{50} ca. 1.13% w/v. on brown dog tick nymph and Y= 18.36+29.48X; LC_{50} ca. 1.07% w/v. after exposure at 24 hours and after exposure for 36 hours was Y= 21.28+33.34X; LC50 ca. 0.86% w/v. on brown dog tick adult (Table 5), (Figure 1).

Table 1. The acaricidal activity of sweet flag crude-extract on nymph of the brown dog tick

Concentration of extract	% average mortality of tick after dipping 24hours*	% average mortality of tick after dipping 36hours*
Control (water)	2.50±5.00[a]	2.50±5.77[a]
0.5%	28.06±3.89[b]	43.61±4.75[b]
1.5%	47.22±3.21[c]	52.78± 3.20[b]
2.5%	79.44±1.11[d]	100.00± 0.00[c]

Remark: *Means ± SD followed by the same letter in the same column are not significantly different at 5% level as determined by DMRT, n = 10.

Table 2. The acaricidal activity of sweet flag crude-extract on adult of the brown dog tick

Concentration of extract	% average mortality of tick after dipping 24h*	% average mortality of tick after dipping 36h*
Control (water)	1.67±3.33[a]	1.67±3.33[a]
0.5%	52.50±1.67[b]	62.74±3.31[b]
1.5%	65.48± 1.40[c]	70.23±2.84[c]
2.5%	86.35± 0.55[d]	100.00± 0.00[d]

Remark: * Means ± SD followed by the same letter in the same column are not significantly different at 5% level as determined by DMRT, n = 15.

Table 3. The detoxification enzyme activities of sweet flag extract on nymph of the brown dog tick

Concentration of extract	% average esterase activities (nM paranitrophenol/ mg protein/ ml) *	% average glutathione –S– transferase activities (nM conjugated product /mg protein/ ml) *
Control (water)	5.56±0.44[a]	0.012±0.001[a]
0.5%	19.46±0.43[b]	0.132±0.002[b]
1.5%	20.39±0.37[c]	0.141±0.005[c]
2.5%	23.19±0.17[d]	0.173±0.001[d]

Remark: *Means ± SD followed by the same letter in the same column are not significantly different at 5% level as determined by DMRT, n = 10.

Table 4. The detoxification enzyme activities of sweet flag extract on adult of the brown dog tick

Concentration of extract	% average esterase activities (nM paranitrophenol/ mg protein/ ml) *	% average glutathione –S– transferase activities (nM conjugated product /mg protein/ ml) *
Control (water)	4.08±0.43[a]	0.0025±0.0002[a]
0.5%	4.45±0.32[a]	0.0034±0.0004[b]
1.5%	7.63±0.53[b]	0.0032±0.0009[b]
2.5%	7.73±0.15[b]	0.0041±0.0002[c]

Remark: *Means ± SD followed by the same letter in the same column are not significantly different at 5% level as determined by DMRT, n = 15.

Table 5. Results of Linear respose, LC50 and R square of sweet flag on brown dog tick

	Linear response		LC_{50}	R square
Nymph	Linear respond at 24h.	Y= 7.28+28.65X	1.49%	0.98
	Linear respond at 36h.	Y= 10.91+34.50X	1.13%	0.91
Adult	Linear respond at 24h.	Y= 18.36+29.48X	1.07%	0.82
	Linear respond at 36h.	Y= 21.28+33.34X	0.86%	0.80

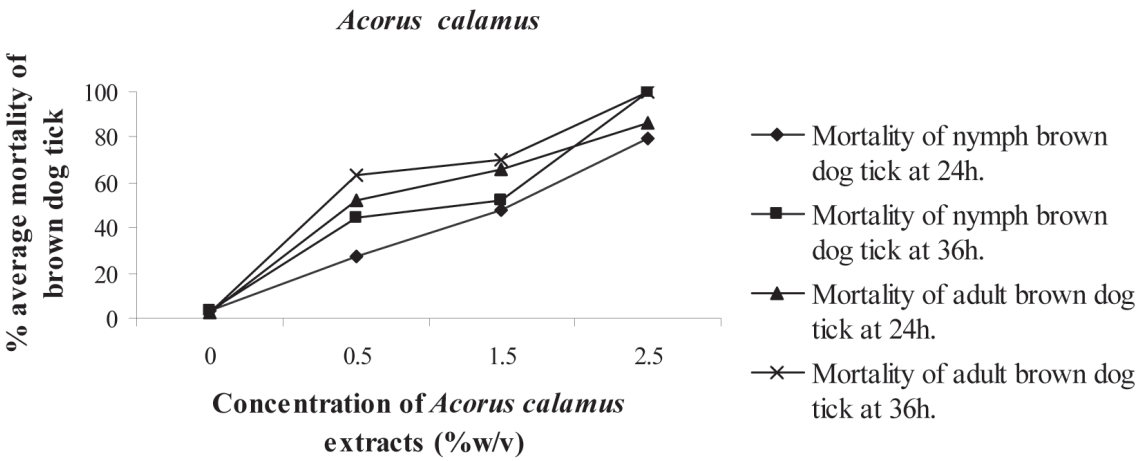

Figure 1. Mortality percentage of brown dog tick at 24 and 36 hours.

The enzyme activities of esterase and glutathione - S - transferase from brown dog tick after 24 hours were induced follow levels of sweet flag rhizomes was increased. Glutathione - S - transferase increased a letter when compare with control.

Sweet flag extract showed acaricidal activity on nymph and adult brown dog tick. Comparing the acaricidal activity results of nymph and adult the brown dog tick. Sweet flag extract showed toxic of acaricidal activity at 24 and 36 hours on adult brown dog tick is higher than nymph brown dog tick. May be enzyme detoxification, esterase and glutathione-S-transferase, on nymph is higher than adult of brown dog tick. Esterase and glutathione-S-transferase are groups of detoxification enzymes. These enzymes have a central role in detoxification of xenobiotic and endogenous compounds. The enzyme activities of esterase and glutathione-S-transferase from brown dog tick after 24 hours were induced follow levels of sweet flag extract was increased. The result of glutathione-S-transferase activity on nymph is higher than adult of brown dog tick. May be these result shown the future on nymph of brown dog tick can resistance to sweet flag extract is faster than adult brown dog tick. Because of the high of glutathione-S-transferase activity is associated with resistance to insecticides (Wei *et al.*, 2001). Resistance to organochlorine and organophosphate insecticides is specifically associated with increased glutathione-S-transferase activity (Vontas *et al.*, 2000) These results showed that sweet flag extracts can be used as alternative insecticide for control the brown dog tick. These results of mortality on brown dog tick shown sweet flag extract at 2.5%w/v was appropriately to use control nymph and adult of brown dog tick. Because of at 36 hours of sweet flag extract at 2.5%w/v killed nymph and adult of brown dog tick as 100%. (Figure 2, 3)

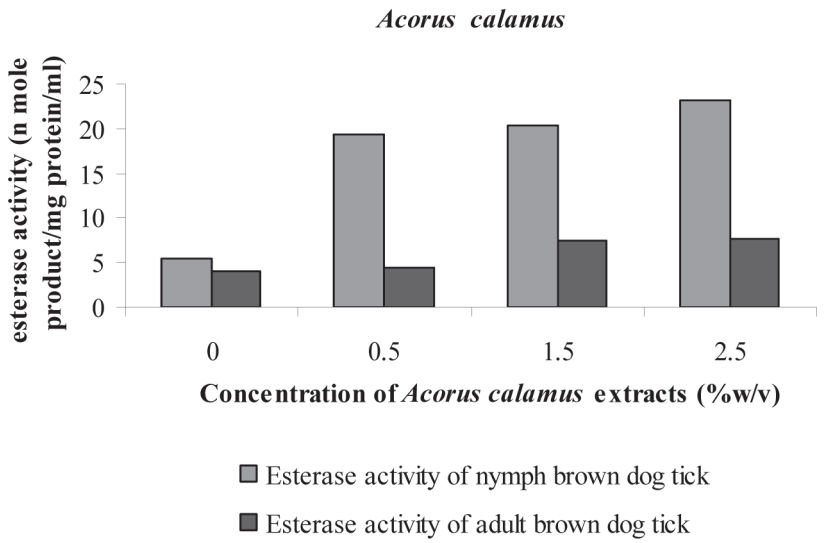

Figure 2. Esterase activities of brown dog tick after tested with various concentrations of sweet flag extracts.

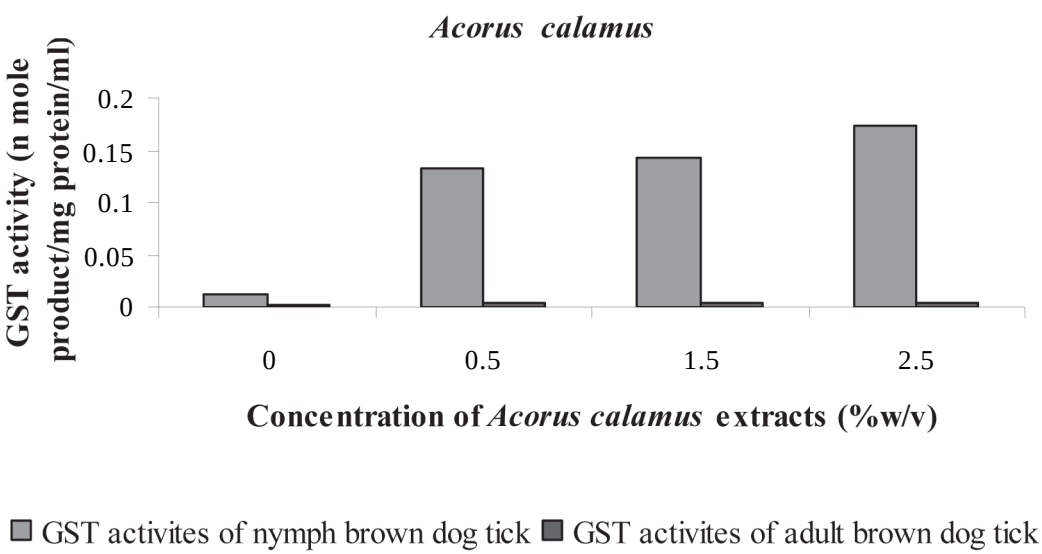

Figure 3. Glutathione-S-transferase activities of brown dog tick after tested with various concentrations of sweet flag extracts.

CONCLUSION AND RECOMMENDATION

Sweet flag extract can be acaricidal activity on nymph and adult brown dog tick and there had many reports on the insecticidal activity of sweet flag on the insects. The crude-extract of Acorus calamus rhizome showed toxicity to *Musca negbulo* and *Culex fatigans* (Dixit *et al*., 1956) and larvae of *Boophilus microplus* (Chungsamarnyart *et al*., 1988). It has high larvicidal activity on boophilus tick (Chungsamarnyart *et al*., 1988). Ethanol extract of sweet flag might be different toxic compounds from the petroleum ether extract which has toxicity to housefly and mosquito (Dixit *et al*., 1956; Mukerjea and Govind, 1959) and high toxicity to *Heteropsylla cubana* (Sharma *et al*., 1992). β -asarone in *A. calamus*

effect on reproduction of *Coelopa frigida* and inhibition to develop of female reproductive (Ramos *et al*, 1986). Mukherjee *et al*. (2007) reported that *A. calamus* rhizomes essential oil is acetylcholinesterase inhibitor. Acetylcholinesterase is found both on the post-synaptic membrane of cholinergic synapses and in other tissues. Acetylcholine binds to acetylcholinesterase and is hydrolysed to acetate and choline. This inactivates the acetylcholine and the nerve inpulse is halted. Effects of acetylcholinesterase inhibitor is actions on the neuromuscular junction will result in prolonged muscle contraction (Wikipedia, 2008).

These experiment can be used to support the growers used for controlling the ticks and the database can be used for active ingredient studies to develop commercial products in the future.

ACKNOWLEDGEMENTS

The researchers wish to thank gratefully acknowledged Department of Zoology, Faculty of Science, Kasetsart University.

REFERENCES

Abbott, W.S. 1925. A Method of Computing the Effectiveness of an Insecticide. **J. Econ. Ent**. 18: 265-267.

Dixit, R.S., S.L. Perti and S.K. Ranganathan. 1956. Evoluation of *Acrorus calaramus* Linn. An insecticidal plant of India. **J. Sci. Indus. Res**. 15: 1622.

Chungsamarnyart, N., S. Jiwajinda, W. Jansawan, U. Kaewsuwan and P. Buranasilpin. 1988. Effective Plant Crude-Extracts on the Tick (*Boophilus microplus*) I. Larvicidal Action. **Kasetsart J.** 22: 37-41.

Mukherjee, P.K., V.Kumar , M. Mal and P.J. Houghton. 2007. In vitro Acetylcholinesterase Inhibitory Activity of the Essential Oil from *Acorus calamus* and its Main Constituents. **Plant A Med**. 73 (3): 283-5.

Mukerjea T.D. and R.Govind. 1959. A plant insecticide-*Acorus calamus* Linn. **Indian J. Ent**. 21: 194-205.

Mukerjea T.D. and R.Govind. 1960. Studies on indigenous insecticidal plant III. *Acorus calamus* L. **Journal of Scientific Indian Research** 19: 112-119.

Ramos-Ocampo V.E., M.T. Hsia and Stephen. 1986. The influence of calamus oil and asarone analogs on the reproduction of *Oncopeltus fasciatus* (Dallas). **Philippine Entomologist**. 6(5): 495-515.

Sharma R.N., P.P. Vrushall and P.H. Vartak. 1992. Toxic effects of some plant oil and their common constituents on the psyllid pest, *Heteropsylla cubana* (Homoptera;Psyllidae) of social forestry tree *Leucaena leucocephala*. **Applied Entomology and Zoology**. 27 (2): 285-7.

Streloke, M., K.R.S. Ascher, G.H. Schmidt and W.P. Neumann. 1989. Vapour pressure and volatility of β -asarone, the main ingredient of an indigenous stored-product insecticide, *Acorus calamus* oil. **Phytoparasitica**. 17: 299-313.

Visetson, S., W. Purivirojkul, P.Kannasutra and H. Rose. 2002. Insecticide Resistance Mechanisms in Various Strains of the Rust-red Flour Beetle (Tribolium castaneum Herbst). **Agr. Sci. J**. 33: 318-324.

Visetson, S., J. Milne, M. Milne and P. Kannasutra. 2003. Synergistic Effects of sesame Oil With Cypermethrin on the Survival and Detoxification Enzyme Activity of *Plutella xylostella* L. Larvae. In The 6th International Conference on Plant Protection in the Tropics **"Globalization and Plant Protection in Developing Economics"** 11-14 August 2003. Pan Pacific Hotel, Kuala Lumpur, Malaysia.

Visetson, S. 2004. **Toxicant Mechanisms in Animals**. Kasetsart University, BKK Thailand.

Vontas, J.G., A.A. Enayati, G.J. Small, and J. Hemingway. 2000. A simple biochemical assay for glutathione-S-transferase activity and its possible field application for screening glutathione-S-transferase-based insecticide resistance. **Pestic. Biochem. Physiol**. 68: 184-192.

Wei, S.H., A.G. Clark and M. Syvanen. 2001. Identification and cloning of a key insecticide-metabolizing glutathione-S-transferase (MdGST-6A) from a hyperinsecticide-resistant strain of the housefly *Musca domestica*. **Insect Biochem. Mol. Biol**. 31: 1145-1153.

Wikipedia. 2008. **Cholinesterase inhibitor**. Available Source: http//en.Wikipedia.Org/wild/ cholinesterase-inhibitor, May 5, 2008.