

## Bioassay Techniques of Insecticidal Plant-extracts on Tropical Cattle Ticks (*Boophilus microplus*)

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### ABSTRACT

The engorge female of tropical cattle ticks survived *in vitro* more than 72 h and began laying at 70-75 h after taking from cattles. The larvae were hatches 14-23 days after laying. The unfed larvae survived more than 2 months in humid bottle. The leg contact method was used for larvical bioassay. The larvae were left to contact with the dry film of plant-extract. The mortality of larvae in empty, evaporated ethanol- and ethyl acetate-coated bottles as control were considerably low and no significant difference were found among them. In insecticidal bioassay of adult ticks, both body dipping and leg contact methods could be applied. The dipping method was rapid and easy procedure for screening bioassay. The leg contact method was used for bioassay in the fractionation of compounds dissolved in non polar organic solvents. The mortality of engorge female ticks was very low at 48 h after dipping with water, methanol, ethanol, acetone and 2% polyoxyethylene sorbitan mono-oleate. After dipping with ethyl acetate and toluene the mortality were high.

The survival check of larvae was observed on its leg movement after exposing to fresh air. The treated engorge female tick was observed in its leg and body movements after exposing to warm-air, 100w lamp's light, sunshine and direct press to the body. The non survival ticks were confirmed by its non laying.

This study demonstrated that the cattle ticks can be bioassayed for larvical and insecticidal activities *in vitro* before further application *in vivo*.

### INTRODUCTION

It is well known that cattle ticks make a great economic loss to raise livestock. The heavy infestation of ticks causes anemia, skin disease and direct losses of weight gain as well as milk lactation. It also play a vector to transmit viruses, rickettsiae, bacteria and protozoa. In Thailand, common cattle tick is *Boophilus microplus*, which is a one host tick. This tick has been reported to be resistant to the chlorinated hydrocarbon insecticides (Hitchcock, 1953). The recent major problem in tick control is the development of resistant strains to insecticides. The effective insecticides from plants such as pyrethrin, are known to be low toxic to mammals (Martin, 1965). Recently, there are many development of the synthetic pyrethroides which are more effective and less liable to induce resistant strains than the chlorinated hydrocarbons, organophosphates or carbamates (Elliot et al., 1978). Therefore, the tick bioassay techniques is necessary to study for screening as another new effective insecticidal plant-extracts.

### MATERIALS AND METHODS

The engorge female of *Boophilus microplus* (6X9 -8X11 mm. in size) were collected from cattle. The 20 ticks were left in each petri dish which opened the lid in the daytime and closed at night or closed whole day long at room temperature (27-33°C). The survival period, initial laying time and hatch period after collection were observed. The survival period of unfed larvae in humid bottle (3-4 drops of water once a week) were also observed. The unfed larvae were used for screening the larvical activity of plant-extracts by leg contact method. Therefore, the mortality of unfed larvae was observed after contacting with dry film of plant-extract dissolving solvents such as ethanal and ethyl acetate. The 1 cc of solvents was put into 60 cc bottle. Then the bottle was rolled horizontally at room temperature to evaporate solvent. The larvae (~150-300) were left to contact inside the bottle for 15-30 min, and transferred into dry and humid resting bottle (240 cc). The humid resting bottle had water-wet filter paper inside the lid, but the dry resting bottle had no wet paper. The mortality of larvae was

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observed in 10 replicates at 24 h after transferring to the resting bottles. The empty 60 cc bottle was used as control bottle.

In screening the insecticidal activity of plant-extracts, the engorge female ticks were collected and tested by dipping method or leg contact method. In dipping method, the mortality of engorge female ticks were observed after dipping (ca. 2 sec.) with dissolving solvents for plant-extracts such as methanol, ethanol, acetone, ethyl acetate, toluene, polyoxyethylene sorbitan mono-oleate (Tween 80®, emulsifier) and mixture of Tween 80® and ethanol. In the leg contact method, the solvent was dropped scattering on filter paper in petri dish and allowed to dry in vacuum desicator for 2 h. Then 1.5 cc distilled water were dropped every 24 h on the filter paper for wetting and left the ticks walking around. The mortality of ticks was observed in 4 replicates (20 ticks/rep.) after dipping and leg contact at 24 and 48 h. The mortality of engorge female ticks was checked by observation of their legs and body movements after exposing to warm-air from hair dryer, 100W lamp's light, sunshine and direct press to the body, and were confirmed by their non laying.

## RESULTS

The survival period of unfed engorge female

ticks after collecting were more than 72 h *in vitro*. After collecting they began laying at 60-70 h in continuously closed petri dish, but at 70-75 h in alternately opened and closed the lid (Table 1). The larvae were hatched at 14-23 days after laying. The egg color changed from brown to white on 3-4 days before hatching. The unfed larvae survived more than 2 months in humid bottle.

Mortality rate of the unfed larvae contacting with evaporated solvent bottle such as ethanol - and ethyl acetate were 1.99% and 2.44% respectively (Table 2). There was no significant difference in mortality of larvae from the control (empty) bottle, when the larvae were transferred into humid resting bottle. However, if they were in dry resting bottles, the mortality of larvae became 66.46% after contacting with evaporated ethanol bottle. The survival check of tick larvae was observed in its leg movement after exposing to the fresh air.

There were no significant difference in the mortality of engorge female ticks among dipping in solvents; methanol, ethanol, acetone, 1-2% Tween 80®, and mixture of 1% Tween 80® 9 parts and 100% ethanol 1 part, and water (Table 3). The mortality of ticks after dipping 48 h with ethyl acetate and toluene were 53.75% and 100% respectively, while the mortality were 0.00% and 0.00% after leg contacted with evaporated filter paper of ethyl acetate and toluene

**Table 1** Survival period of cattle tick (*Boophilus microplus*) *in vitro*

Stage	Condition	No./ rep.	No. of rep.	Survival period
Engorge female	Closed petri dish	20	35	66-70 h (begin laying)
Engorge female	Opened & closed petri dish	20	52	70-75 h (begin laying)
Unfed larvae	Closed moisture bottle	~5,000	70	more than 2 months

**Table 2** Mortality of unfed larvae of *Boophilus microplus* *in vitro* after contacted with evaporated solvent bottle\*

Larvae contacted bottles	Mortality rate at 24 h (Meen, %)**
Ethanol evaporated bottle (60 cc) & transfer into humid bottle (240 cc)	1.99 a
Ethyl acetate evaporated bottle (60 cc) & transfer into humid bottle (240 cc)	2.44 a
Empty bottle (60 cc) & transfer into humid bottle (240 cc)	1.95 a

\* Contacting time 15-30 min.

\*\* LSD .05 = 1.1661, LSD .01 = 1.5979, C.V. = 58.26%, 10 replicates (~150-300 larvae/rep.)

**Table 3 Mortality of engorge female cattle ticks *in vitro* after dipping with solvents.**

Solvents	Mortality rate (Mean, %)*	
	24 h	48 h
Water (control)	0.00 c	0.00 c
Methanol	1.25 c	1.25 c
Ethanol	2.50 c	2.50 c
Acetone	5.00 c	5.00 c
1% Tween 80® aq. solution	0.00 c	0.00 c
2% Tween 80® aq. solution	0.00 c	3.75 c
1% Tween 80® 9 parts + 100% Ethanol 1 part	0.00 c	0.00 c
Ethyl acetate	48.75 b	53.75 b
Toluene	95.00 a	100.00 a

\* LSD .05 = 5.752, LSD .01 = 7.642, C.V. = 22.95%, 4 replicates (20 ticks/rep.)

evaporated filter paper for 48 h.

The survival adult ticks moved their legs and body after exposing (2-3 min.) to warm-air from hair dryer, 100W lamp's light, sunshine and direct press to the body. The survival tick flexed its legs or retained the body when they were pressed. Finally, the dead ticks could not lay at 5 day after dipping.

## DISCUSSION

The engorge female ticks survived more than 72 h after collecting from cattle. Their survival period were long enough for insecticidal bioassay since the bioassay for screening was done by observation of mortality rate after treatment at 24 and 48 h. The engorge female ticks also survived after dipping with polar solvents; water, methanol, ethanol, acetone, 2% Tween 80® and mixture of 1% Tween 80® 9 parts + 1 part of 100% ethanol. Therefore, any of these solvents and emulsifier mixture can be used in dipping method for insecticide bioassay. The dipping procedure was easy and rapid method for screening insecticidal activities of plant crude-extracts.

The non polar solvents were toxic to the engorge female ticks after dipping (Table 3), but non toxicity after leg contact method. Therefore, the leg contact method was useful for screening the insecticidal substances in fractionation by non polar solvents.

The production of tick larvae for *in vitro* larvicidal bioassay was easy and they do not need food for their long survival. The unfed larvae survived more than two months in moisture bottle. Unfortunately, in this study survival check was not observed to their

dead. It has been reported that the non-parasitic stage of *Boophilus microplus* larvae survive up to 5 months (Harley, 1966). The larvae hatch period *in vitro* (14-23 days) was not so different from the natural hatch (14-146 days) (Soulsby, 1982).

The mortality of larvae after contacting with solvent (ethanol and ethyl acetate) evaporated bottles were very low and had no significant difference from the control of empty bottle (Table 2). It showed that the solvent evaporated bottles had no toxic residues to larvae. The mortality of larvae might be caused by mechanical trauma during transfer. So that leg contact method is useful for screening larvicidal activity of plant-extracts. This bioassay method can also be applicable for testing larvicidal activity of fractionation and purification compounds which dissolved in non polar organic solvents.

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