DISSERTATION

RESISTANCE AND RESISTANT MECHANISMS OF SOME ACARICIDES IN AFRICAN RED MITE, *Eutetranychus africanus* (TUCKER) (ACARI : TETRANYCHIDAE)

TEWIN KULPIYAWAT

GRADUATE SCHOOL, KASETSART UNIVERSITY
2001

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DISSERTATION

RESISTANCE AND RESISTANT MECHANISMS OF SOME ACARICIDES IN AFRICAN RED MITE, EUTETRANYCHUS AFRICANUS (TUCKER) (ACARI : TETRANYCHIDAE)

TEWIN KULPIYAWAT

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The African red mite, *Eutetranychus africanus* (Tucker) is a major pest of tangerine in Thailand. Ten strains of this mite were collected from various tangerine orchards where amitraz and dicofol were widely used. Resistance bioassays were studied in the laboratory condition of 26.4± 1.3°C, 65.3±2.3%RH, and the light from fluorescent lamp for 9 hrs per day. Adult female mites of all field strains including the susceptible one from Thung Khru, Bangkok (TKS) were tested with the acaricides, amitraz and dicofol, by leaf-dip bioassay technique to obtain LC_{50} (ppm) value. The resistance ratios (RR) were obtained by comparing the LC_{50} values of field strains with that of the susceptible strain. The results showed that PSS exhibited the highest resistance to amitraz with the RR of 6.99-fold and HYS exhibited the highest resistance to dicofol with the RR of 31.71-fold. The summarized result showed that all field strains were tolerant to amitraz (RR ranged from 1.57-6.99-fold). FAS, OKS and HYS were resistant (RR ranged from 13.32 - 31.71-fold) while NCS, SPS, BMS, NKS, PSS, NSS and PPS were tolerant to dicofol (RR ranged from 1.16-7.02-fold). The fecundity of fertilized female of susceptible strain (total eggs laid/female=20.33) was significantly less than those of some field strains (total eggs laid/female of FAS, PSS, NSS, SPS, NKS and OKS=35.01-43.20). The relationships between the eggs laid by fertilized female and RR of field strains revealed that there was not a significant positive correlation as well as other biological activities.

The other method was a biochemical technique of two detoxification enzyme extractions, esterase and glutathione S-transferase. The amount of 900 (50mg) adult females of African red mite were ground with polyvinyl polypyrrolidone and potassium phosphate buffer pH 7.5. Supernatant solution was detected after centrifuging at 10,000 x g at 4°C for 5 minutes. The result showed that only glutathione S-transferase had influence upon RR of dicofol with positive relationships. Levels of glutathione S-transferases showed a biochemical mechanism for dicofol resistance in this mite. Laboratory toxicity tests of some candidate acaricides, pyridaben, tebufenpyrad, propargite and fenbutatin oxide were conducted by leaf-dip bioassay technique and the results clearly revealed that all acaricides were highly toxic (LC_{50}<100 ppm) to African red mite of HYS and showed the low levels of RR to this mite. Propargite will be an alternative acaricide for controlling this mite in tangerine and the rests, after registration, will be alternatively used in the near future for resistance management.

Tewin Kulpiyawat — Student's signature

Thesis Advisor's signature — 8 / 10 / 2001

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Tewin Kulpiyawat

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ABBREVIATIONS

a.i.	=	active ingredient
ANA	=	∝-naphthylacetate
AChE	=	acetylcholinesterase
ATCh	=	acetylthiocholine Iodide
βNA	=	β-naphthylacetate
ca	=	approximately
cm	=	centrimeter
conc	=	concentration
^{o}C	=	degree celsius
CDNB	=	1-chloro-2, 4-dinitrobenzene
d	=	day
DT_{50}	=	degradation time 50%(degradation time of pesticide at a 50% decrease)
DDT	=	2, 2-bis (4-chlorophenyl)-1,1,1-trichloroethane
DNA	=	deoxyribonucleic acid
DCNB	=	3,4-dichloronitrobenzene
e.g.	=	*exempli gratia* (for example)
etc.	=	*et cetera* (and the other things; and so on)
EC	=	emulsifier concentration
EM	=	effective microorganisms
EPN	=	O-Ethyl O-4-nitrophenyl phenylphosphorothioate
EDTA	=	ethylene diamine tetraacetic acid
g	=	gravitational force
gm	=	gram
GSH	=	glutathione
GST	=	GSH S-transferase
hrs	=	hours
HCl	=	hydrochloric acid
H_2SO_4	=	sulfuric acid
in	=	inch
kg	=	kilogram

ABBREVIATIONS (Contd.)

KCl	=	potassium chloride
KH_2PO_4	=	potassium dihydrogen phosphate
LC_{50}	=	lethal concentration 50%(concentration causing 50% mortality in the population studied)
LC_{95}	=	lethal concentration 95%(concentration causing 95% mortality in the population studied)
LD_{50}	=	lethal dose 50%(dose causing 50% mortality in the population studied)
mg	=	milligram
ml	=	millimeter
mm	=	milliliter
M	=	molar
MTB	=	methyl thiobutyrate
nM	=	nanomole
ND	=	non-detection
NaOH	=	sodium hydroxide
OD	=	optical density
OP	=	organophosphate
ppm	=	parts per million
P_{450}	=	cytochrome P_{450}
PNPA	=	paranitrophenyl acetate
PVPP	=	polyvinyl polypyrrolidine
R	=	resistant
RH	=	relative humidity
RR	=	resistance ratio
S	=	susceptible
SC	=	soluble concentration
TEPP	=	tetraethyl pyrophosphate
µl	=	microliter
WP	=	wettable powder
W/V	=	weight / volume

RESISTANCE AND RESISTANT MECHANISMS OF SOME ACARICIDES IN AFRICAN RED MITE, *EUTETRANYCHUS AFRICANUS* (TUCKER) (ACARI : TETRANYCHIDAE)

INTRODUCTION

Tangerine (*Citrus reticulata* Blanco) is becoming more and more important as cash crop for fruit growers in Thailand. This is due to the fact that domestic consumption and export of fruits are steadily increasing. Tangerine is commercially grown throughout the country, but mostly at Pathum Thani, Saraburi and Phrae provinces (Planning Division, 1994). The total growing areas of tangerine in Thailand were approximately 264,039 rais with the total production of 585,469 tons. The amount of export tangerine was increasing from 434 tons in 1995 to 574 tons in 1998 with the increase in the total export value from 4,200,000 to 6,800,000 Baht, respectively.

For many years mites have been considered injurious pests of tangerine and pummelo in Thailand. Charanasri *et al.* (1988) reported 10 species of mites as pests of tangerine. Among many species of mites found to be pests of tangerine, the African red mite, *Eutetranychus africanus* (Tucker), is recongnized as the most injurious one. The female of this mite is broad oval and vary in colour from greenish brown to dark brown with the average body size of 396.67 microns long and 344.47 microns wide. The male is smaller and roughly triangular in shape (Charanasri *et al.*, 1988). The spherical and flat-shaped eggs of *E. africanus* are laid on the upper surface of citrus leaves and young fruit. After hatching, the larvae feed on leaves and young fruit and become adults. The infestation by nymph and adult mites results in the appearance of white stippling with empty white cast skin which will gradually spread out all over the entire leaf (Figure 1). The leaves ultimately become yellow and fall off. Heavy infestation may result in premature fruit drops and bared trees. The occurrence of *E. africanus* in citrus orchard is almost all year round but the outbreak can be frequently found during the drought period or water stress (Charanasri *et al.*, 1988).

Study on the seasonal occurrence of this mite in tangerine orchard indicated that their heavy infestations were found during February to May with the highest density in

Figure 1 The appearance of white stippling with empty white cast skin over the entire leaves of tangerine infisted by *E. africanus*

the middle of March. The low incidence of this mite could be observed during June to July and September to January (Kulpiyawat, 1989).

Besides chemicals, many alternative measures have been tried to control this mite both in the laboratory and in the field. Kongchuensin and Kulpiyawat (1994) conducted an efficacy test of EM (Effective Microorganisms) for controlling the African red mite in the laboratory. The result showed that EM was ineffective for controlling the African red mite. The attempts were also made by Kulpiyawat *et al.* (1996) to use neem extract for controlling citrus rust mite. It was found that neem extract at the high dosage of 0.1% azadirachtin could not be applied with the citrus rust mite in pummelo due to its phytotoxicity.

Chemical application was then the only control measure the farmers still use for controlling the African red mite on tangerine, pummelo and other economic crops. Although chemicals have been recognized by citrus growers as the most convenient and effective tools for controlling insect and mite pests. Their inappropriate and indiscriminate use of pesticides cause the arise of various problems such as the increasing cost of production, health hazard to the growers and consumers as well as disruption of the natural balance. Moreover the intensive and uncontrolled spraying programs especially of the organophosphate and carbamate groups of pesticides are initiating pesticide resistant problems.

Today in some planting areas of citrus, the recommend dosage of pesticides cannot be effectively applied for the control of insect and mite pests. Because of the capability of the pests to develop their resistances to the chemicals, the farmers have to increase the application dosage which eventually results in the increasing cost of production and contamination of pesticides in the environments and agricultural products.

In Thailand the information concerning the resistant mechanisms or even the monitoring for the resistance of mites to various groups of pesticides are limited. Most of the works have been done on the resistance of insects to certain groups of insecticides.

Information obtained from the literatures indicated that the first serious and wide-spread failure in chemical control arose from the development of resistances to organophosphates (OPs), including parathion and TEPP, in 1949-50, only 2-3 years after their introductions on greenhouse crops. The fruit orchards and citrus groves also, resistance to parathion and subsequently to many other OPs, soon became apparent between 1950 and 1960 in *Tetranychus urticae* (Koch), *Panonychus ulmi* (Koch) and *P. citri* (McGregor) (Cranham and Helle, 1985).

According to Cranham and Helle (1985), there are two types of OP resistance. The first was discovered by Smissaert (1964) who found that there could be an alteration of the acetylcholinesterase (AChE) in the resistant strain of mites that result in a considerable decrease in sensitivity to AChE inhibitors such as OPs and some carbamates. This type of OP resistance is wide spread in the *T. urticae* complex.

The second mechanism of OP resistance involves a detoxication (Matsumura and Voss, 1964). They studied the metabolism of malathion and parathion and found that there was a substantial difference in carboxyesterase and phosphatase activity between the R and S strains. The OP-R strains of *T. kanzawai* express this type of resistant mechanism.

This study was carried out to determine the acaricide resistance status of the African red mite in tangerine orchard in various parts of Thailand. The data were obtained from this study will certainly provide a very useful information for the management of chemical control for the African red mite in the future. The study was also conducted to examine the resistance of field strains of the African red mite to some acaricides in various parts of Thailand. The study will cover the following experiments : 1. Monitoring for the resistance to some acaricides of the African red mite. 2. Biology and physiology of the susceptible and field strains of the African red mite collected from various tangerine orchards. 3. Toxicity of some candidate of acaricides to the African red mite.

Objectives

1. To determine some acaricide resistance on the African red mite, *E. africanus*, in tangerine orchards of Thialand by leaf-dip bioassay technique

2. To compare the biology between the susceptible and field strains of the African red mite, *E. africanus*

3. To determine the acaricide resistance on the African red mite, *E. africanus*, in tangerine orchards of Thailand by biochemical technique to know resistant mechanisms

4. To investigate the toxicity of some candidate acaricides for controlling the African red mite, *E. africanus*, under laboratory condition

LITERATURE REVIEW

1. Plant-feeding mites

Studying and surveying of plant-feeding mites were conducted by Charanasri (1997). It is found that plant-feeding mite on important economic crops in Thailand belong to the suborder Actinedida. They are divided into four families : 1. Family Tetranychidae; its common name is red mite or spider mite which is the important pest of fruit crops, vegetables, flowers, ornamental plants and field crops. 2. Family Eriophyidae; its common name is four-legged mite which is the important pest of fruit crops and vegetables. 3. Family Tarsonemidae; its common name is thread-footed mite which is the important pest of fruit crops, vegetables, flowers and ornamental plants. 4. Family Tenuipalpidae; commonly known as false spider mite which is the important pest of flower plants such as orchids. The tetranychid mite is the most important of all plant-feeding mites and are the most commonest.

Most plants are destroyed by mites belonging to the family Tetranychidae. They feed on various parts of plants with their colonies. Some mites can spin web like spiders to cover their colonies of eggs, larvas, nymphs and adults. Spin web was secreted from silk gland. The silk gland which contains a big sac, is between palps (appendages on mouthpart). The bodies of tetranychid mites are larger than those in other families of phytophagous mites. They can be seen by naked eyes, they appear to be red spot and move around the leaf surface. When they grow and reach the adult stage, the size is smaller than a needle pin. The bodies of adult females are bigger than the males. The body's colour of the males are more pale than the females. The main characteristic of tetranychid mites is mouthparts by which it uses for sucking up the cellullar contents of plant tissue. Mouthpart possess a pair of needle-like and long recurved whiplike chericerae, set in stylophore which is formed by fusion of chelicerae bases.

Sometimes, host plants are helpful to identify species for tetranychid mites, because many species are host specific. There are many species of these mites that feed on various plants, but not host specific. However, species identification to some tetranychid mites cannot be categorized as host plant in this case. Some characteristics are necessary to use for species identification such as striation, number and location of setae

on female tarsus I and etc. The most important characteristic for identifying mites is male aedeagus, so males should be collected in the collection since most specific identifications depend upon the study of the male aedeagus (Charanasri, 1997).

Mite and tick are classified into phylum Arthropoda the same as prawn, crab, spider and some animals because they have exoskeleton to cover their bodies and joined appendages. Savory (1964) classified animals in the phylum Arthropoda into four subphylums. They are subphylums :

1. Trilobitomorpha
2. Pycnogonida
3. Chericerata
4. Mandibulata

Mites and ticks are animals in the subphylum Chericerata which is the centre of animals without antennae and mandible. The animals in this subphylum have basically a feeding organ which is called chericera instead of mandible. Animals of the Chericerata group are found obiqutusly not only in water, humid area, terrestial but also on plants. They breathe with book lung, book gill or trachea (Chandrapatya, 1992).

Woolley (1988) classified animals in the subphylum Chericerata into three classes : Merostomata, Arachnida and Pycnogonida. Mites and ticks are classified into class Arachnida. Animals in this class have some main characteristics that they share together. Especially, the body consists of about eighteen distinctive or indistinctive segments. Normally, the bodies of arachnids are divided into two parts : joined head and thorax (prosoma or cephalothorax), abdomen (opisthosoma), four pairs of legs, chelicerae for feeding and appendages at mouth termed pedipalp.

Mites and ticks differ from other animals in the same class : two parts of body consist of gnathosoma and idiosoma and the body is not divided distinctly like other arachnid. Krantz (1978) classified animals in the Arachnida into eleven subclasses. Subclass Araneae, the tenth subclass , is the animals of spider groups and subclass Acari, the eleventh subclass, is the animals of mite and tick groups. Animals ot these two subclasses have the same characteristics of undivided or indistinctive divided abdomen. They differ from the other nine subclasses which have distinctly divided abdomens.

Animals in two subclasses, Araneae and Acari have different characteristics. The mouth of Araneae is on the anterior of cephalothorax and abdomen whereas the mouth of Acari is on the anterior of idiosoma which is called gnathosoma without pedicel which is connected between gnathosoma and idiosoma. The legs are connected to the podosoma (Chandrapatya, 1992).

2. Plant destruction of the spider mites

Spider mites destroy the plants with their stylet-or needle-like-chericerae. They feed by penetrating the plant tissue with their chericerae and sucking up the cell contents. The withdrawal of chlorophyll causes local chlorosis with the appearance of small stippled areas which will gradually spread out all over the entire leaf. The leaf ultimately become yellow and fall off. The destruction of various plants were destroyed by the spider mite of the same species may cause different symptoms. Some plants are resistant and some are susceptible to destruction of the spider mites because of the differences in the toxic substance secreted from the salivary gland into plant tissues while they are feeding. It also depends on the kinds of plants which are infested by the mites.

The dispersion of spider mites from one place to another can occur by:1. being taken by human being, 2. crawling from one plant to another , 3. blowing with the wind, 4. ballooning by climbing along the silk web and waiting for the wind blow and 5. attached with the legs of insects or birds (Charanasri, 1997). Wedding *et al.* (1958) mentioned that a heavy infestation of the citrus red mite caused substantial changes in photosynthesis and transpiration rates due to a lesser amount of chlorophyll in the leaves. However, Jeppson *et al.* (1975) found that when a few mites attacked a plant under stress conditions, they would cause the same effect as that of a heavy infestation on a normal plant.

The African red mite , *E. africanus*, which is one of the spider mites in the family Tetranychidae was classified by Baker and Pritchard (1960) into the suborder Prostigmata. Later the suborder Prostigmata was changed to Actinedida by Krantz (1978).

The main taxonomic characteristic history of African red mite

Original name : *Anychus africanus* Tucker (1926)

Later name : *Eutetranychus sambiranensis* Gutierrez and Helle (1971); Meyer (1974)

Current name : *Eutetranychus africanus* (Tucker) Baker and Pritchard (1960); Meyer and Rodrigues (1966); Attiah (1967); Meyer (1974); Meyer (1987); Charanasri *et al.* (1988)

First time, Charanasri *et al.* (1987) identified this mite to be *Eutetranychus orientalis* (Klein) by using some main characteristics in the key of Baker and Pitchard (1960) and including the data from the report of survey of phytophagous mites in Thailand by Baker (1975) and Ehara and Wongsiri (1975). Later, taxonomic characters of the mite in this genus was studied finely. It was found that some characteristics ever used in identifying this mite at that time such as striation pattern between the second and third pairs of dorsocentral setae as well as the shape and the length of dorsal setae are highly variable within populations. Meyer (1987) considered that the two characteristics mentioned above were insufficient for species indentification. She then revised the key to species of *Eutetranychus* collected form South Africa in her publication named "African Tetranychidae (Acari : Prostigmata) with reference to the world genera". Apart from the previous reasons Baker and Pritchard (1960) used the characteristic of strong protuberance at the base of the dorsal setae to separate *E. africanus* from *E. orientalis*. They also indicated *E. africanus* as a synonym of *E. banksi* which is rather confused since the dorsal setae of *E. banksi*, as it was described in the key by Pritchard and Baker (1955), do not set on tubercles. Charanasri *et al.* (1988) then decided to follow the key of Meyer (1987). This key was finely listed the main characteristics for identifying this mite. The key of Meyer (1987) made Charanasri *et al.* (1988) sure that the mite in the genus *Eutetranychus*, formerly identfied into *orientalis* species was probably *africanus*.

Specimens of mites in the genus *Eutetranychus* collected from various host plants in Thailand were then sent to Dr. Meyer, a taxonomist of the Republic of South Africa, who was an expert in identifying mites of the family Tetranychidae and Tenuipalpidae, whereas some specimens were sent to Dr. Krantz, a taxonomist expert, in the United States of America to attest accurately about identifying the mite in this genus.

Both of them attested that the specimens of mites collected from various host plants in Thailand especially on durian and citrus, were *E. africanus* (Figure 2) truely, in spite of the former identification into *E. orientalis*.

3. Biology and chemical control of African red mite

3.1 Life history The life history of African red mite, *E. africanus* was reared on *C. reticulata* leaflets by Kulpiyawat (1989) at 28±2°C and 58±4% RH. The life history were:

3.1.1 Egg (Figure 3) Females usually laid eggs in a row along the mid-rib on the upper leaf surface. The newly laid egg was round, 0.14±0.007 mm, pale and transparent with smooth surface. As time progressed, the egg turned opaque and finally orange in colour. Two red spots were clearly visible through the chorion before hatching. The incubation period was 4.67±0.48 days.

3.1.2 Larva (Figure 4) A newly-hatched larva, 0.14±0.02 mm wide and 0.19±0.02 mm long, possesses only 3 pairs of legs. It was orange in colour with a pair of red eyes clearly discernible on the propodosoma. Larvae usually fed almost immediately after hatching, consequently, the body colour changes into dark-green. The larval stage lasted 1.2±0.31 days then, the resting period called protochrysalis (Figure 5) in which the larva stopped feeding and stayed motionless. This protochrysalis was 0.14±0.001 mm wide and 0.2±0.001 mm long. At this stage the first 2 pairs of legs extended forward whereas the last pairs of legs pointed backward. The old skin became dry and shiny before ecdysis. After 0.55±0.15 days, the old cuticle split transversely along the mid-line of the body. Then, the protonymph would separate itself from the old cuticle.

3.1.3 Protonymph (Figure 6) The protonymph was oval in shape, 0.16 ±0.02 mm wide and 0.2±.02 mm long, dark-green in colour, with 4 pairs of legs. Body hair and leg setae were distinctly longer than those of the larva. Protonymphs feed immediately after molting before entering another resting stage named deutochrysalis (Figure 7) in which the protonymph stoped feeding and stay motionless. The protonymphal period required 0.67±0.27 days. The deutochrysalis, yellow-green in colour

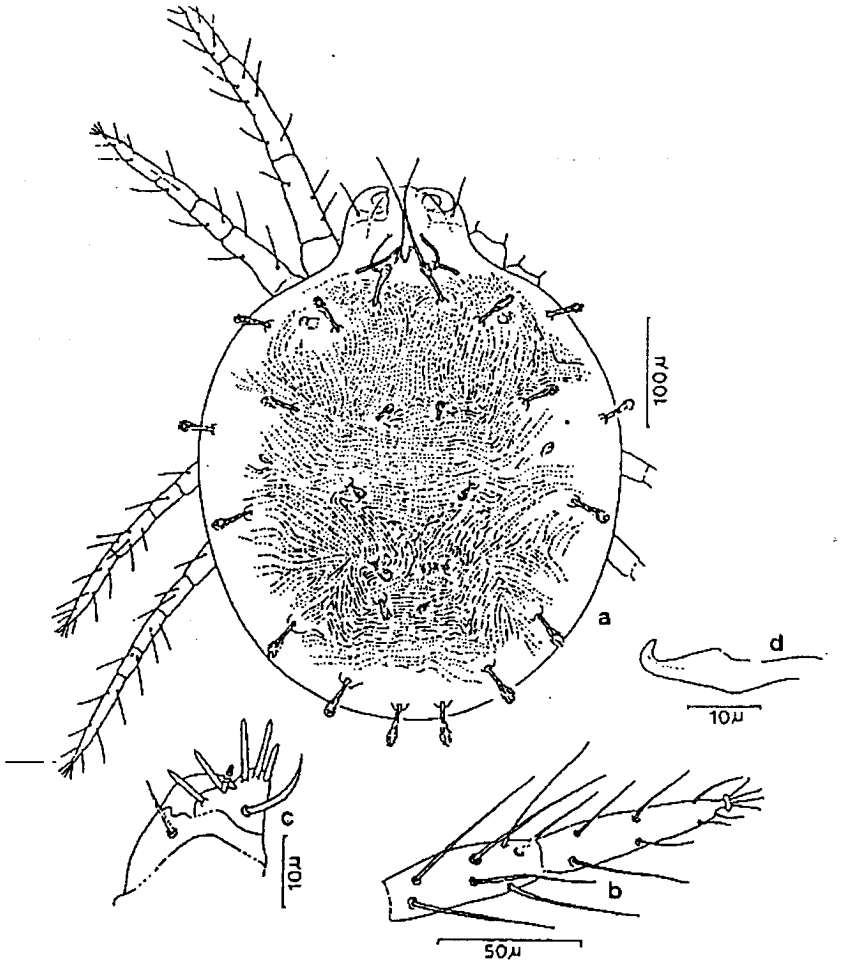


Figure 2 African red mite, *E. africanus* : **a.** dorsum of female; **b.** tibia and tarsus of female; **c.** distal segment of male palpus; **d.** aedeagus

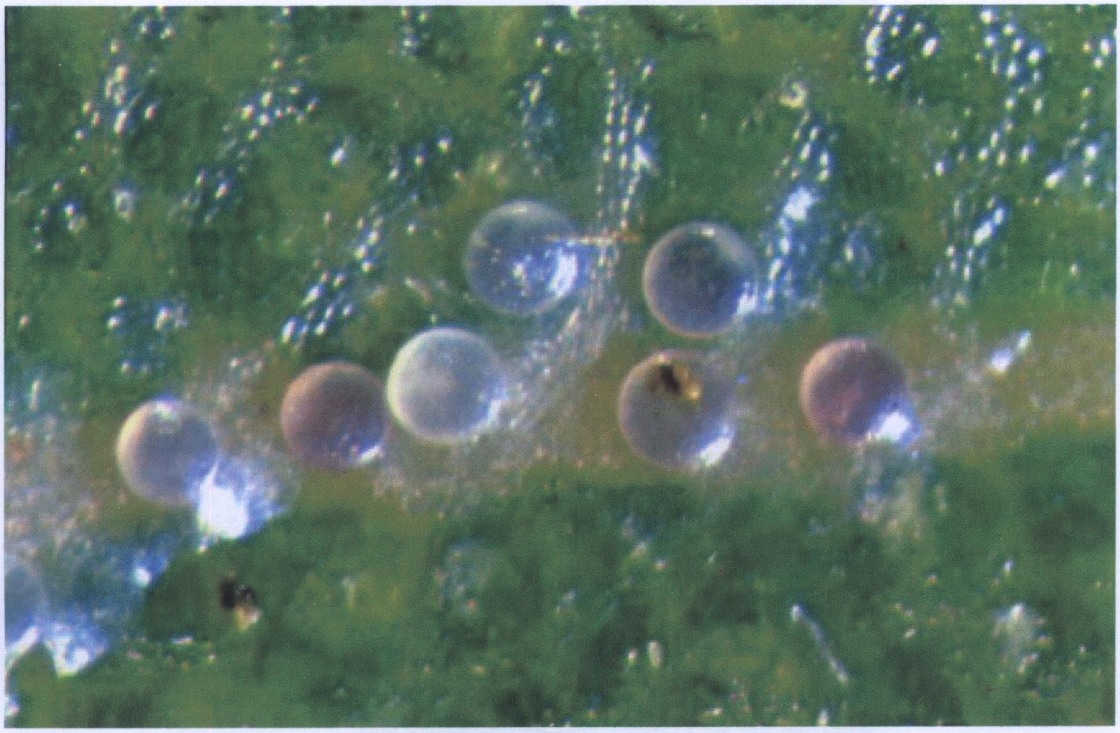


Figure 3 The eggs of African red mite, *E. africanus*

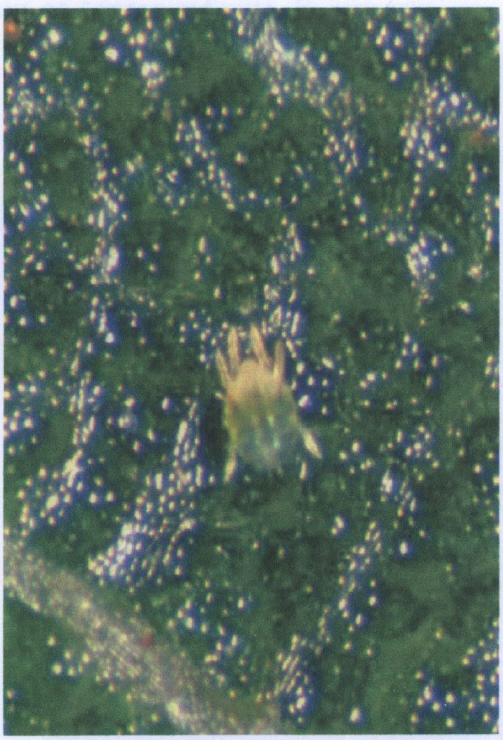


Figure 4 The larva of African red mite, *E. africanus*

Figure 5 The protochrysalis of African red mite, *E. africanus*

Figure 6 The protonymph of African red mite, *E. africanus*

with the body size of 0.17±0.003 mm wide and 0.25±0.01 mm long, required 0.55± 0.15 days.

3.1.4 Deutonymph (Figure 8) The deutonymph was 0.19±0.02 mm wide and 0.25±0.02 long, rather flattened, and dark-green in colour. Sexes could be distinguished in this stage. The female was longer and with a rounded posterior-end as compared to the male which had a slightly pointed posterior-end. After 0.98±0.28 days, both sexes enter into a resting stage known as teliochrysalis (Figure 9) before molting into adult. The teliochrysalis was 0.2±0.001 mm wide and 0.3±0.02 mm long, it had a dry shiny skin. Male teliochrysalis required 0.7±0.26 days before eclosion whereas female teliochrysalis required 0.75±0.26 days.

3.1.5 Female (Figure 10) Female mite was flatly-round in shape, 0.28±0.02 mm wide and 0.34±0.02 mm long, brown or dark-red in colour. All 4 pairs of legs were relatively long and yellowish in colour. While resting, the first 2 pairs of legs are directed forward and the last 2 pairs backward. Female of *E. africanus* was normally sedentary in its feeding habit and confined its feeding on the dorsal surface which caused a large amount of damage by creating many pale spots on the leaf. Female mites completed their life cycles (Figure 11) within 9.40±0.66 days and lived 9.8± 2.04 days.

3.1.6 Male (Figure 12) Male was more triangular in shape, pale brown in colour and 0.17±0.005 mm wide and 0.25±0.009 mm long. The life cycle of male required 9.35±0.78 days and males had a shorter longevity (6.9±1.2 days) than the females. Adult male mite usually enclosed before the female. One to 3 males were observed guarding a female teliochrysalis. Some males climbed on the female dorsum during her resting stage. The male would assist the molting of female by using its mouthparts and 2 pairs of front legs to pull her from the exuvia. Copulation occurred immediately after the female finished molting (Figure 13). In case of many males, there would be competition, and the winning male would have a chance to copulate. The male copulative behavior began when the male was under female and used its front pair of legs to catch the hind legs of female, then it began to copulate. The period of copulation was 10-65 seconds, a female could be inseminated many times and the male would copulate with more than one female.

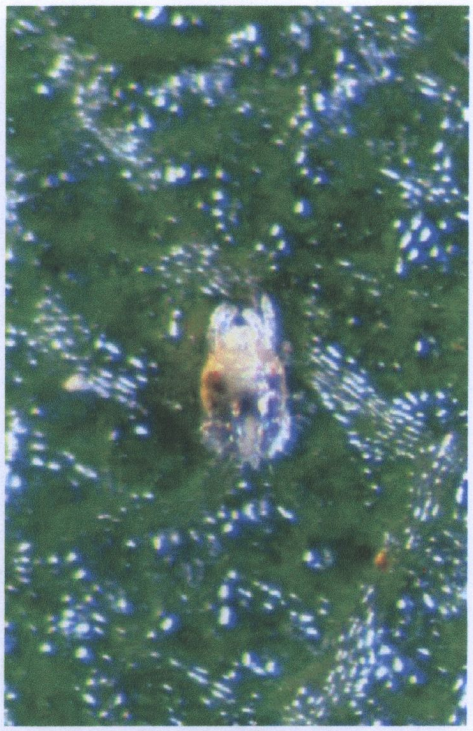


Figure 7 The deutochrysalis of African red mite, *E. africanus*

Figure 8 The deutonymph of African red mite, *E. africanus*

Figure 9 The teliochrysalis of African red mite, *E. africanus*

Figure 10 The adult female of African red mite, *E. africanus*

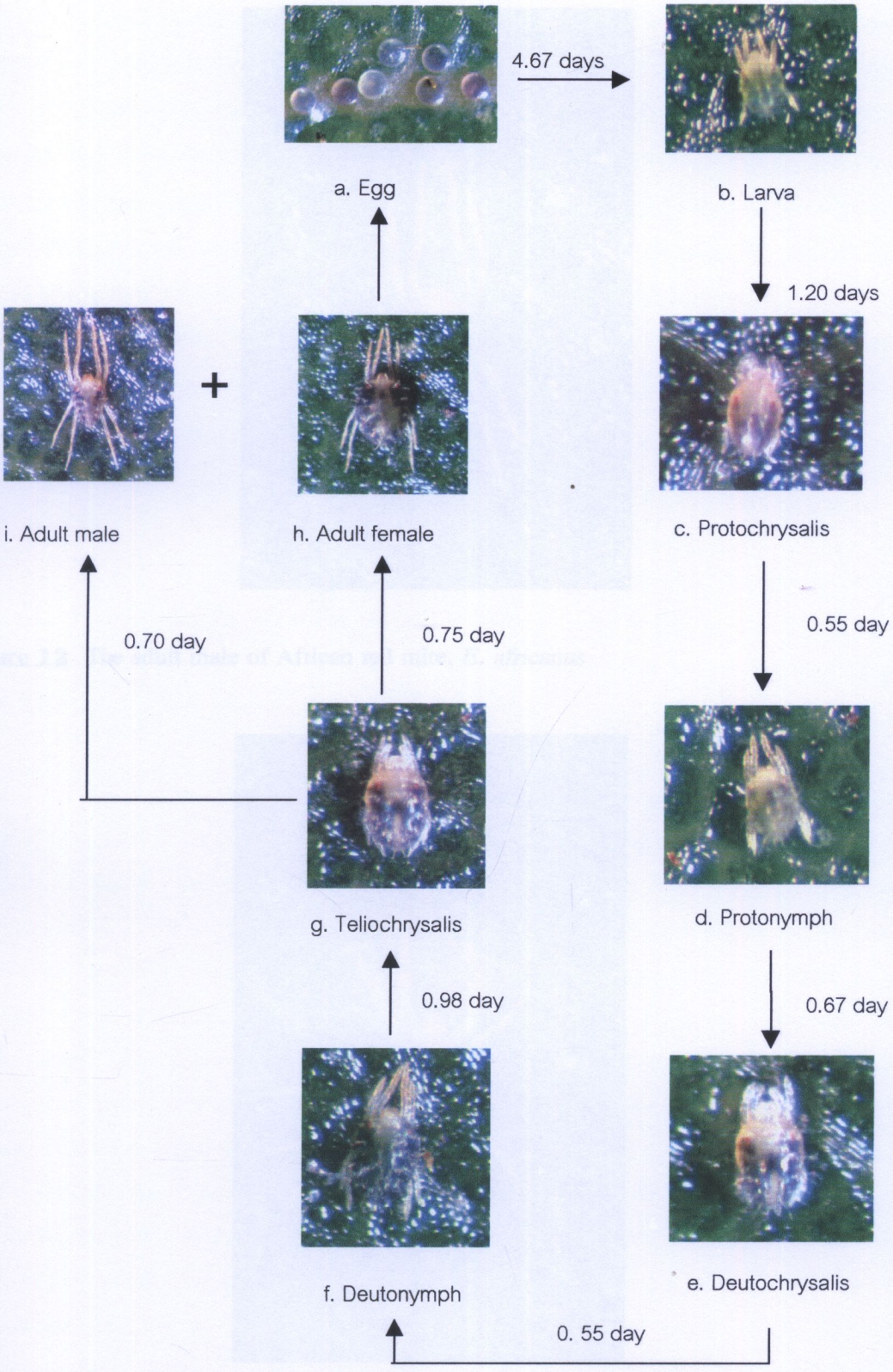


Figure 11 Life cycle of African red mite, *E. africanus*

Figure 12 The adult male of African red mite, *E. africanus*

Figure 13 Guarding of a female teliochrysalis of African red mite, *E. africanus*, by a newly emerged male

3.2 Longevity and fecundity of females Mating of *E. africanus* occurred immediately after a female emerged from teliochrysalis. The pre-oviposition, oviposition and post-oviposition periods of fertilized females were 1-2, 2-6 and 0-2 days, respectively. Females lived up to 6.9±1.66 days. The total number of eggs laid by a fertilized female varied from 12.7±3.13 eggs with a daily production of 3.19±0.93 eggs per female. It was found that only male offspring were produced by unfertilized females and this reproductive type is called arrhenotokous parthenogenesis. The pre-oviposition, oviposition and post-oviposition periods were 1-3, 3-9 and 1-3 days, respectively. Unfertilized females lived slightly longer (9.8±2.04 days) than the fertilized female. Dealing with the number of eggs laid, one female produced 10.3±1.7 eggs during her life span with a daily production of 1.99±0.57 eggs per day.

3.3 Life table From one hundred eggs, they can hatch to become larvae at the rate of 100% and the larvae can develop to become adults at the rate of 100%. Sex ratio of males to females equalled 1:3.25. The net reproductive rate of increased (R_0) was 18.85 and cohort generation time (T_C) was 17.03.

3.4 Host plant In Thailand, the African red mite, *E. africanus* is not only the pest of tangerine (*C. reticulata* Blanco) and pummelo (*C. maxima* Merr.), the mite is also a pest of many other economic crops. The host plants are jack fruit (*Artocarpus heterophyllus* Lamk), jujube (*Zizyphus mauritiana* Lam.), lime (*Citrus aurantifolia* Swingle), lancet (*Lansium domesticum* Corr.), carambola (*Averrhoa carambola* L.), durian (*Durio zibethinus* L.), passion fruit (*Passiflora edulis*), grapevine (*Vitis vinifera* L.), cotton (*Gossypium* sp.), soybean (*Glycine max* (L.)Merr.), cassava (*Manihot esculenta* Crantz), castor bean (*Ricinus communis* L.), bird chilli (*Capsicum* spp.), water melon (*Citrullus vulgaris* Schrad), water convuloulus (*Ipomoea aquatica* Fersk), winged bean (*Psophocarpus tetragonolobus* (L.)DC.), *Alocasia cucullata* Schott, neem tree (*Azadirachta indica* A. Juss), croton (*Codiaeum variegatum* Blume), para rubber (*Hevea brasiliensis* Muell.-Agr.), pagoda plant (*Jatropha podagrica* Hook), plumeria (*Plumeria acuminata* Ait), narra (*Pterocarpus indicus* Wild.), rose (Rosa spp.), peach (*Prunus persica* Batsch), papaya (*Carica papaya* Linn.), bread fruit (*Atrocarpus communis* Forst), leach lime (*Citrus hystrix* DC.), green pea (*Pism sativum* Linn.), peanut (*Arachis hypogaea* Linn.), groundnut (*Arachis hypogaea* L.), Fremont (*Citrus reticulata* var.

Fremont), sweet orange (Ehara and Wongsiri, 1975; Saringkaphaibul *et al.*, 1982; Charanasri *et al.*, 1987; Kulpiyawat, 1989). In Africa *E. africanus* has been recorded from oranges, lemons, frangipani, *Tectona grandis*, *Bauhinia candida*, *Pterospermum semisagitatum* and *Cryptostegia madagascariensis* in South Africa and from peach, loquat and citrus in Mauritius. It has been collected from *Plumeria alba*, *Vitis lobrusca*, *Cordia utilissima*, *R. communis*, *Artocarpus integrifolia*, *A. incisa* and *Citrus* sp. in the Malagasy Republic. In Egypt it was found on eggplant, cotton, fig, prune, apple, orange, *Dalbergia sisso*, *Morus alba*, *Psidium guajava* and *Eucalyptus globulus* (Meyer, 1987).

3.5 Distribution and population fluctuation *E. africanus* was reported as a serious citrus pest in citrus growing areas. It was found in tangerine growing areas at Nan, Chiang Mai, Phrae, Samut Prakan, Petchaburi, Pathum Thani, Saraburi, Nakhon Pathom, Ratchaburi, Bangkok, Nakhon Si Thammarat and Yala provinces (Charanasri *et al.*, 1988; Kongchuensin and Kulpiyawat, 1997) and pummelo growing areas at Chiang Mai, Nakhon Phanom, Petchabul, Pichit, Pathum Thani, Kanchanaburi Nakhon Pathom, Chai Nat, Rayong, Prachin Buri and Trang provinces (Charanasri *et al.*, 1991). Study on the seasonal occurrence of this mite in tangerine orchard indicated that high population of this mite occurred in the drought period during December to March and in the rainy season especially no raining for a long times (Baker and Pritchard, 1960; Kongchuensin and Kulpiyawat, 1997). Boudreaux (1963) stated that most phytophagous mites trended to spread rapidly at high temperature and low relative humidity. High temperature can accelerate the mite development by its unusual loss of water through the body wall and to compensate for this, so mites need to suck up more cellular contents from their host plants. Huffaker *et al.* (1969) had set forth a hypothesis that the mite outbreaks were:1. The result of modern agriculture such as monocropping system for large areas made the outbreak more severe. 2. The modern agriculture affected the development and breeding of the spider mites. 3. The application of some chemicals such as DDT, carbaryl, diazinon, parathion and bordeaux mixture would wipe out all the natural enemies. 4. the chemical composition within the leaf which affected mite oviposition.

3.6 Natural enemies Survey of natural enemies of African red mite on pummelo and tangerine revealed the occurrences of predaceous mites in the family Phytoseiidae (Charanasri *et al.*, 1993), the spider (Wangsilabutr, 1995) and the fungus, *Hirsutella thompsoni* Fisher (Chandrapatya, 1987). The highest density of predaceous

mite, *Amblyseius longispinosus*, was found in pummelo orchard during May to July (Kulpiyawat and Charanasri, 1994). From the laboratory study, it was found that an female of *A. longispinosus* can consume 17 eggs per day, 16 protonymphs per day and 3 female of *E. africanus* per day from larval stage until adult stage (Kongchuensin *et al.*, 1991). The spider, *Hylyphantes graminicola* (Sundevall) in family Linyphiidae was found on pummelo at 11% out of the total spiders. Laboratory study showed that 3 sizes of spiders, 1.0-1.4 mm, 1.5-2.2 mm and 2.3-3 mm, were found consuming the African red mite. Each of them can consume 9.5, 9.5 and 12.9 adult females, respectively (Wangsilabutr, 1995). High population of lady beetles, *Stethorus* sp. were found together with the African red mite in pummelo orchard in 1993 and 1996 (Smith and Papacek, 1993; Morakote and Nanta, 1996).

3.7 Chemical control of African red mite in Thailand The acaricides were applied in pummelo and tangerine orchards when the leaves are observed and found to be pale green with the magnificatino of ten-time handlens, this symptom indicated high population of *E. africanus* on the upper surface of leaves. The acaricides were sprayed alternatively such as 1. propargite 30%WP with the rate of 30 g per 20 liters of water, 2. hexythiazox 2%EC with the rate of 40 ml per 20 liters of water 3. amitraz 20%EC with the rate of 30 ml per 20 liters of water, 4. bromopropylate 25%EC with the rate of 30 ml per 20 liters of water. If the mites are still found, the spray should be repeated at 5 day interval. Some acaricides are recommended to use in durian orchard such as 1. hexythiazox 2%EC with the rate of 40 ml per 20 liters of water, 2. propargite 30%WP with the rate of 30 gm per 20 liters of water, 3. amitraz 20%EC with the rate of 30 ml per 20 liters of water (Kongchuensin and Kulpiyawat, 1997). Some acaricides are recommended to use in papaya orchard such as dicofol 18.5%EC with the rate of 30 ml per 20 liters of water (Entomology and Zoology Division, 2000). Survey of acaricides used in tangarine orchards in Pathum Thani and Saraburi provinces by Statistics Sub-Division (1994) indicated that tangerine grower prefered to use acaricides such as 1. propargite 30%WP, 2. amitraz 20% EC, 3. tetradifon 7.52%EC, 4. hexythiazox 2%EC and 5. dicofol 18.5%EC.

4. Insecticide and acaricidal insecticide problem to grower and the environment

4.1 Harzardous problem Insecticide and acaricidal insecticide in organophosphate and carbamate groups are harzardous to human being and other animals because insecticides in organophosphate and carbamate groups can permeate into blood circulation system of the body and because acute toxicity is rather severe, so the growers or other pets are not allowed through the spraying insecticide area. Normally, growers who obtain high amount of these pesticides will die within minutes or two to three hours and induce chronic toxicity to growers who obtain low amount of these insecticides. Cholinesterase enzyme operation is inhibited and make the insecticides harzardous. These insecticides are not only toxic to growers but also toxic to other non-target animals. Most of these insecticides are toxic to insect pollinators, especially bees. The residue was degraded quite quickly not exceeding twelve weeks. If the high amounts of insecticides are sprayed continuously for a long time, it may accumulate in soil. For example; after spraying parathion for sixteen years, it was found that the rest of the soil mites decreased from 28 to 10 species (Brown, 1978). There was a report that bluegill fish would extremely stretch out fins before death after they obtained malathion (Eaton, 1970). There was a report that azodrin was highly severe toxic to birds. It caused death to a lot of birds when it was used with the rate of 1 pound per acre and the population of birds decreased 75-80% (Brown, 1978).

Insecticide and acaricidal insecticide in organophosphate and carbamate groups are harzardous to environment, especially contamination of these insecticides in environment are less than insecticides in organochlorine group, because organophosphate has low persistence in environment. It can permeate more quickly or is altered in chemical structure or is altered by livingthing more quickly than organochlorine. In addition, the potential of accumulation in fat tissue is less than organochlorine group, so the accumulation is not found in the food chain. However, over spraying of insecticide and acaricidal insecticide causes contamination in agricultural products and water (Nattavatananon, 1978), so the study of biological control is necessary and considered to be an alternative method to replace chemical control or using in combination with chemicals.

4.2 Resistant problem

4.2.1 Insecticide resistance is defined as "the development of an ability in a strain of insects (or any animals) to tolerate doses of toxicants which would prove lethal to the majority of individual in a normal population of the same species" (Anonymous, 1957). Insecticide resistant strains seem to have arisen as a result of natural selection as the insects that have genetic characteristic for resistance to insecticides. They can survive after selection pressure by using insecticide and can transfer this genetic characteristic to descendants from one generation to another generation. Growers prefer using insecticides for controlling insect pests because they are convenient, quick and satisfyingly effective. The growers later found that the effectively used insecticide became less effective, they then try to apply more frequently with higher dosage of insecticides. New expensive insecticide was used instead of the old one and a few insecticides were mixed and sprayed at the same time, so the survival insects from selection pressure of insecticide could develop resistance to insecticide with increasing rate (Ounchaichon, 1989).

Krieger *et al.* (1971) reported that polyphagous insects have oxidase enzyme in detoxifying toxicatns more than non polyphagous insects, so polyphagous insects can tolerate synthetic insecticides more than non-polyphagous insects. Georghion (1983) stated that insect of the same species can develop resistance to insecticides differently when they were selected by selection pressure with different conditions, so the recommendation of spraying insecticides in controlling pests was that care should be taken of insecticide resistance. It is difficult for management when the insecticide resistance occur (Ounchaichon, 1989). It was not new incidence that insects can develop resistance to insecticides. Melander (1914) reported that San Jose scale was resistant to lime sulfur. The California red scale was resistant to cyanide in 1946. DDT was used to control insect pests after successful registration in 1942 and until 1947. There were reports from Italy and Spain that DDT was less toxic to house fly. After that there were reports that insecticide resistance of various insects occurred increasingly. It was found that 400 species of insects were resistant to insecticides (Sombatsiri, 1997).

Ability of insecticide resistant development for insects will occur in population of insect according to theory of selection by using different genotype in that population. The insects that survive from inscticide treatment have to have gene

appearance before. From selection of many generations make insecticide resistant population increase more and more. The insects can resist to insecticide quickly or slowly depend on: 1. Kind of insects; the insects that have high fecundity and many generations in 1 year, will set up insecticide resistance quickly. 2. Environmental conditions; they affect to insecticide resistance. If they are appropriate to growth and fecundity, the insects will resist to insecticides more quickly. 3. Kind of insecticides and their application. Each insecticide contributes to insecticide resistant development of insects. Over applying and repeating the same kind of insecticide for a long time. These cause insecticide resistance more quickly.

4.2.2 Acaricide resistance Insects and mites were classified as the animals in phylum Arthropoda. Different species of mite appear to have sufficient genetic potencies to react with a resistance response to chemical stress. In this respect mites do not differ from insects. Mite resistance to acaricides are the same as insect resistance to insecticides which occur generally. Acaricides resistance have been known since 1937 when Compton and Kearns found ineffective control by Selocide sprays in two-spotted spider mite, *Tetranychus urticae* (Koch) population. Selocide resistance occurred because of unlimited acaricide applications after the second world war following the use of several kinds of synthetic insecticides. The resistance response in tetranychid mites occurred frequently. However, the frequency of the resistance phenomenon in mites is a great problem. This is because of the great economic importance of the spider mites undoubtedly. As a consequence there have more use of chemicals in tetranychid mites than other groups of mite (Helle, 1965).

In 1948 two-spotted spider mite, *T. urticae* resisted to HETP-aerosols. After 1948 parathion was ineffective for controlling this mite on rose in glasshouses at Connecticut and New Jursey because of the transport of infested plants from Pennsylvania resistance, parathion resistance spread to many other glasshouses the same as from New Jursey and Connecticut. In many to these rosehouses new chemicals were applied, but most of these compounds cause new resistance in many spider mite population within a short time. It is real that resistance occurred earlier in glasshouse than outside because of the greater number of generation and more intensive control program in glasshouses. Resistance in *T. urticae* against organophosphate compounds in the field was found in 1954 in peach orchards and in 1955 in apple orchards. Except *T. urticae*, it was found that *Tetranychus cinnabarinus* was resistant to demeton and parathion on cottons. The

history of the resistance developed by the two-spotted spider mite in Europe was about the same as in the United states.

In Europe resistance first appeared in glasshouses in Netherlands and in Norway about 1950-1951. In the southern part of France parathion resistance appeared in *T. cinnabarinus* on rose and carnation grown inside and outside glasshouse in 1951. About this time, the resistance of two-spotted spider mites appeared in cucumber houses and rosehouses in Denmark and in a carnation house in Germany. In different glass areas in Europe, OP resistance was extended with resistance against other acaricides and has become a major problem. Also from other continents information existed about resistance of the two-spotted spider mite. In 1959, Cwilich and Ascher found resistance to organophosphate in *T. cinnabarinus* on sugar beets. In South Africa, *T. cinnabarinus* became a major pest in apple orchards after application of DDT, OP resistance appeared in the Elgin district in 1955-1956. A few years later, resistant *T. cinnabarinus* population on apple and peach also occurred in other districts. In 1960 control by Kelthane became difficult. Parathion control of the two-spotted spider mite in cotton became resistance in Tanganyika in 1958. In carnation fields in Kasazu Town in Japan, Metasystox has been used since 1957 for the control of *T. cinnabarinus*. Within one year resistance against this compound was found.

A report from Australia dealed with the occurrence of resistance in the two-spotted spider mite on the continent as well (Helle, 1965). In South Korea Lee and Kim (1996) reported that acaricide resistance bioassay method to two-spotted spider mite by slide-dip and leaf-dip method were not significantly different. Hackyo, a resistant strain showed 24-fold resistance by slide-dip method and 23-fold resistance by leaf-dip method. Keumchun, a resistant strain showed 71-fold resistance by slide-dip method and 74-fold resistance by leaf-dip method from comparing against susceptible strain of dicofol. In China Shen and Zhong (1994) reported that *Tetranychus viennensis* Zacher was resistant to dicofol and amitraz with medium level, propargite and monocrotophos with high level and zecocarbophos and quinalphos with the highest level in peach orchards. In Japan Funayama and Takahashi (1995) reported that *T. urticae* in apple orchards at Akita, Honshu island was resistant to fenpyroximate in 1992-1994.

In America Bruce-oliver and Grafton-Cardwell (1996) reported that alternate application of acaricides in cotton fields such as dicofol, propargite and abamectin had been used for three years. It was found that *T. turkestani* was susceptible to dicofol, propargite and abamectin and *T. urticae* was resistant to dicofol and propargite with high resistant level. It is a great problem to try to solve the problem of insects and their resistance to insecticides successfully. It is impossible to inhibit insecticide resistance of insects but delaying resistance can be manipulated effectively by application of insecticide when necessary such as changing of chemical groups and using mixed chemicals between synergists and insecticides in order to change the detoxification of xenobiotic in the insect body (Sombatsiri, 1977).

5. Acaricides used in resistance testing

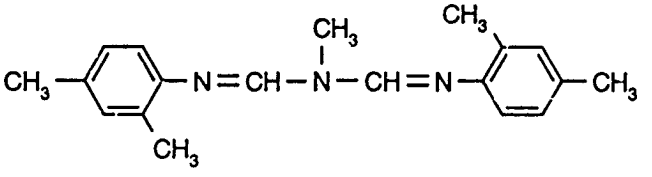
5.1 Amitraz

5.1.1 Common name Amitraz

5.1.2 IUPAC name N-methyl bis (2,4-xylyliminomethyl) amine

5.1.3 Trade name Mitac

5.1.4 Chemical structure



5.1.5 Mode of action Formamidine acaricide with mode of action probably involves an interaction with octapamine receptors in the tick nervous system, causing an increase in nervous activity. Non-systemic, with contact and respiratory action. Expellent action causes ticks to withdraw mouthparts rapidly and fall off the host animal.

5.1.6 Toxicity Oral : Acute oral LD_{50} for rats 800 mg/kg; Acute dermal LD_{50} for rats > 1600 mg/kg

5.1.7 Formulation 20% W/V EC

5.1.8 Uses Control of all stages of tetranychid mite such as *Eutetranychus africanus* in durian, tangerine and pummelo, *Oligonychus mangiferus* in grape, tarsonemid mite such as *Polyphagotarsonemus latus* in chilli and eriophyid mite

such as *Eriophyes litchii* in litchi, *Eriophyes tulipae* in garlic and *Phyllocoptruta oleivora* in tangerine and pummelo (Entomology Division, 2000), pear suckers, scale insects, mealybugs, whitefly, aphids and eggs and first instar larvae of Lepidoptera on pome fruit, citrus fruit, cotton, stone fruit, bush fruit, strawberries, hops, cucurbits, aubergines, capsicums, tomatoes, ornamentals and some other crops. Also used as an animal ectoparasiticide to control ticks, mites and lice on cattle, dogs, goats, pigs and sheep.

5.1.9 Application Recommended at the dose of 20-60 ml per 20 liters of water (approximately 2-6 tablespoonfuls per 20 liters of water). Sprayed all parts of the tree entirely.

5.1.10 Phytotoxicity At high temperatures, young capsicums and pears may be injured.

5.1.11 Compatibility Incompatible with alkaline materials, parathion and others.

5.1.12 Physical chemistry. White/pale yellow crystalline solid. A little solubility in water. Soluble in most organic solvents; in acetone, toluene, xylene. Unstable when pH<7.

5.1.13 Environmental fate

- Animals Rapid breakdown leading to excretion as a conjugate of 4-amino-3 = methylbenzoic acid and to a lesser extent to N-(2,4-dimethylphenyl) - N′-= methylformamidine.

- Plants Rapidly degraded, mainly to N-(2,4-dimethyl-phenyl)-=N′-metylformamidine and to a smaller extent to 2,4-dimethyl-formanilide.

- Soil Rapidly broken down in soil under aerobic conditions. DT_{50} in soil <1 day. Degradation occurs more rapidly in acid than in neutral or alkaline soils. Very strongly adsorbed to soil.

5.1.14 Poisonous decreasing If the skin is contacted with amitraz, wash it with much soap water. If the eye is contacted with it, wash it with clean water for many times. There are not medicines for therapy, so the patient is cured according to the symptom of appearance.

5.1.15 Remark - Toxic to fish
- Don't mix it with alkali chemicals
- Safe to all useful insects
- Good effectiveness when it is drought condition and don't use when it rains

- Harmful to man when he is contacted with it or swallowed into the body
- less toxic to bee
- 2-7 weeks before harvesting

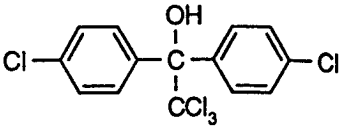
5.2 Dicofol

5.2.1 Common name Dicofol

5.2.2 IUPAC name 2,2,2-Trichloro-1,1-bis(4-chlorophenyl) ethanol

5.2.3 Trade name Kelthane

5.2.4 Chemical structure



5.2.5 Mode of action Non-systemic acaricide with contact action.

5.2.6 Toxicity Oral : Acute oral LD_{50} for rats 690 mg/kg; Dermal: Acute dermal LD_{50} for rats 1,000-1,230 mg/kg

5.2.7 Formulation 18.5%EC

5.2.8 Uses Non-systemic acaricide with little insecticidal activity, recommended at 0.05-2.0 kg a.i./ha for controlling of many species of phytophagous mites (including *Panonychus*, *Phyllocoptruta*, *Tetranychus* and *Brevipalpus* spp.) on various plants such as vegetables, fruit crops, flowers, ornamental and field crops.

5.2.9 Application Recommended at the dose of 20-60 ml per 20 liters of water (approximately 2-6 tablespoonfuls per 20 liters of water)

5.2.10 Phytotoxicity Non-phytotoxic when used as directed, but aubergines and pears may be injured.

5.2.11 Physical chemistry Colourless solid; non-soluble in water but good soluble in various solvent; stable to acids but unstable in alkali media.

5.2.12 Stable $\leq 80^{\circ}C$

5.2.13 Compatibility Incompatible with highly alkaline materials

5.2.14 Environmental fate

- Animals : In rats,following oral administration, 4-4′-dichlorobenzophenone and 2,2′-dichloro-1,1′-bis (chloro-phenyl) ethanol are the

principle metabolites. The same metabolites have been detected in laying hens and dairy goat. Plants : The principal metabolite in plants is 4,4′-dichlorobenzophenone.

- Soil : Photodegradation DT_{50} (silt loam) 30 days. No mobility of parent or metabolites are detected. Dichlorobenzophenone is a major degradation in all processes.

5.2.15 Poisonous decreasing If the skin contacted with dicofol, wash it with much soap water. If the eye is contacted with it, wash it with clean water for many times. If it is swallowed into the body, must send the patient to the doctor. Before sending him to the doctor, let him drink 1-2 glasses of water to make him vomit until the vomitting stops. If he is unconcious, don't make him vomit or give some food to him, maybe give the purgative to him but not oil purgative and don't make him drink milk. For the doctor, if the patient has convulsion or shakiness, inject with barbiturate and calcium solution of 10% into blood-vessel, don't use morphine.

5.2.16 Remark
- 2-7 days before harvesting
- Harmful when in contact with the skin or taken orally
- Irritation with eye and respiratory system
- Toxic to fish
- Mixed with other pesticides that are generally used
- Non toxic to useful insects
- Non toxic to bees (Tomlin, 1997)

6. Techniques for testing of insecticide resistance of insects and mites

6.1 Bioassay technique This technique is used for testing the insecticide resistance of insect and mite in laboratory. A range of bioassay types are available.

6.1.1 Residual test is a test by using poisonous residue of insecticide on materials or leave and exposed to insects or let insects feed on them.

- Adult vial test is a test by enameling insecticide in a glass container or a glass vial. The adults of insects are transferred to be exposed to the insecticide. The dead insects are recorded after 24 hours of testing.

- Leaf-dip bioassay is a test by using the edible leaves for insects. The simplest method to do this is by dipping the leaves in various concentrations of the insecticide, then the leaves are dried. The larvae of insects are released on the

leaves when they are dried. The dead insects are recorded after 24 hours of testing. A residual cell assay has been developed for use on spider mites (White *et al.*, 1994). This method is advised for spider mite not able to use the slide-dip or slide-spray method. Leaves are either dipped or sprayed in a spray tower to produce leaf residues. Disks are cut from the leaves and placed on wet cotton wool. Adult female mites are transferred to each disk. Mortality is recorded after an appropriate period, depending on the speed of action of the acaricide. Clearly, this method can also be used for mites that are not suitable for the slide-dip. Survivors can be isolated to propagate on fresh leaves (Helle and Overmeer, 1985). Leaf-dip can be developed into leaf-spray method. Aliniazee and Cranham (1980) tested pesticides on *Typhlodromus pyri* Scheuten placed on leaves of myrobalan plums laid on wet cotton wool in petri dishes. Thirty *T. pyri* were placed on each leaf infested with *Panonychus ulmi* (Koch) from an insectary culture. Thereafter the leaves were sprayed in a potter spray tower. A disadvantage of dipping is that, depending on the amount of wax present on the surface of the leaf, coverage with the pesticide may not be optimal. Spraying leaf disks is felt to be more accurate. However, if no spraying tower is available dipping is a good alternative. Coverage can be improved by adding a small amount of detergent to the test solution (Helle and Overmeer, 1985).

6.1.2 Topical bioassay is a test by dropping technical insecticide on the second thorax of insect with microapplicator which look like syringe. It has the fixed control of dropping insecticide solution with the amount of one microlitre to drop on the body of insect with various concentrations. The dead insects are recorded after 24 hours of dropping. This method was designed by Harrison (1961) for testing mite. Each adult female mite was treated by expelling the insecticidal contents of a capillary over its ventral or dorsal surface. By this method, the dosage of toxicant per mite is known. It has a high accuracy and repeatability, since the toxicant is not applied to a leaf surface, or to another variable substrate, but directly to the mite itself. Contrary to the slide-dip method.

6.1.3 Dip bioassay is a test by dipping insects into solutions of insecticide. The dead insects are recorded after 24 hours of dipping. This method is used for aquatic insects.

6.1.4 Feeding bioassay is important especially for the testing of microbial insecticides especially to bacterial pathogen (*Bacillus thuringiensis*). This insecticide is mixed into insect food for testing.

6.1.5 Fumigant assay is important especially for the testing of fumigant insecticides to stored product insects to the testing of fumigant insecticide resistance.

6.2 Biochemical technique This technique is an assay of insecticide resistance by using the biochemical reaction.

6.2.1 Esterase activity Esterase is a kind of biochemical technique. The insects will produce carboxylesterase in a lot amount if they are resistant to insecticides. They will produce carboxylesterase in a little amount if they are susceptible to insecticides. These principles are evaluated for insecticide resistance of insects. Spectrophotometer is used for this biochemical detection.

6.2.2 Microtitreplate assay (AChE assay) Principle of AChE (Acetylcholinesterase) is used to biochemical detection. Acetylcholine is detoxified by AChE, to release the thiol and give a yellow colour. AChE from insecticide susceptible insects will be inhibited by the insecticide and no colour will be produced. Reactions can be done by microtitreplate then evaluated by spectrophotometer.

6.2.3 Molecular method It is the molecule detection in body of insect. Insecticide resistance of insects which occur from mutation in gene or DNA. Esterase enzyme synthetic is controlled by gene or DNA in an insect and it makes insecticides non toxic to insects. Insecticide resistance detection of insects can be done by using DNA marker with resistant gene, but not in the susceptible. This method can be done by electrophoresis.

6.3. Neurophysiological technique Neurophysiological assays are available to detect and monitor nerve sensitivity to the insecticide response. Nerve firing rate is used to evaluate insecticide resistance to insect by using the neurophysiological apparatus. The insect which is susceptible to the insecticide will have the peak of nerve firing rate higher than the insect which is resistant to the insecticide.

7. Metabolic Process

Metabolism is a changeable process of various compounds in living organisms in biochemistry. The result from metabolism make the endogenous compounds become the new forms. This process uses tittle heat energy because the enzymes are involved in the reaction as catalytic agent. The metabolism is a process that is very essential to living organisms. The result from metabolism make the organs obtain the energy from the change of the large molecules of substance into the small molecules or synthetic molecules of

substance into the small molecules or synthetic molecules that are different from the original molecules in order to be the precursor of the next reaction.

In toxicology, the importance of metabolism is involved two main cases: Some xenobiotics are involved in metabolism process of the organs and make them more toxic, or made them dissolve in water well appropriate to be eliminated from the organs.

8. Metabolism of xenobiotic compounds

Xenobiotics as well as active metabolites in the body called metabolic precursors or protoxicants. They entered by ingestion, skin and inhalation and followed by absorption, metabolism, temporary storage, distribution or excretion. The most important process in toxicology is metabolism. The metabolism is a process of xenobiotics which is metabolized to be the intermediates or final products. They are more toxic than the parent compounds because they may be intermediary xenobiotic metabolism which has different structure from the parent compounds.

Xenobiotics are changed by enzymatic metabolism in the mammalian body and organisms of other phyla-plants, insects, fish or birds (Hodgson and Levi, 1997) and depend on the structure and the nature of those xenobiotics. The xenobiotics with high polarity property can absorb through organs in the body slowly. When they have been absorped already, they trend to be eliminated from the body. Dichloromethane, a kind of xenobiotic with high volatile property absorped into the body. It becomes non toxic chemical quickly before enzymatic operation.

The xenobiotics that enter the body tissues are non-polar lipophilic, a property that enable them to penetrate lipid membranes well. These xenobiotics are metabolitzed well by a number of relatively non-specific enzymes and accumulated in the body. The xenobiotics that enter the body tissue are metabolized by the various tissues. Metabolism take place first at the portal of entry, the skin or in such organs as the lung epithelium intestine including gastrointestinal tract and especially the liver with the highest metabolism. The xenobiotics can pass into gastrointestinal tract and they are transported to liver to metabolize.

The process of xenobiotics elimination for altering the structure generally consists of two phases.

8.1 Altering process in phase 1 The reactions in phase 1 are adding the functional group to the structure of xenobiobics which contain hydrocarbon chain or ring or modify the parent compounds to other forms. The most important effect is to render the xenobiotic a suitable substrate for phase 2 reactions, the altered compounds in phase 1 can be formed with the other compounds which are synthesized in the body by conjugation process in phase 2, then they are eliminated easily out from the body later. Most phase 1 reactions are oxidations, reductions or hydrolyses, the hydration of epoxides and dehydrohalogenations also occur. The oxidation reactions occur with the xenobiotics which contain C, N, S and P structures, the reduction reactions occur with the xenobiotics which contain alkenyl, carbonyl and nitro groups and the hydrolysis reactions occur with the xenobiotics which contain carboxyl groups.

8.2 Altering process in phase 2 The reactions in phase 2 for xenobiotics that have not been altered, the altered, the structures or Phase 1 metabolites of foreign compounds. Phase 2 reactions include all of the conjugation reactions in which polar groups on them are combined with an endogenons compound such as glucuronic acid, glutathione, sulfate, etc. to form a highly water-soluble conjugate and less toxic. That can be easily eliminated from the body than the parent compound (Visetson, 1991; Hodgson and Levi, 1997).

9. Enzymes system of animals

Animals have enzymes the functions of which are metabolism of xenobiotics (foreign compounds) in the bodies (Manson *et al.*, 1965). In insects and mites, there are three major detoxification enzymes in metabolism of xenobiotics, namely esterase, glutathione S-transferase and monooxygenase in order to detoxify xenobiotics for survive (Dauterman and Hodgson, 1978; Yu 1983,1984; Founier *et al.*, 1985). In this review, the functions of three major enxymes are summarized.

9.1 Esterases Esterases are a group of detoxification enzyme which catalyze the hydrolysis of ester, amide or phosphate linkages of insecticides. Phosphatases attack R-O-P, carboxylesterases attack R.COOR and amidase attack R.CO.NHR linkages. This group of enzymes includes B-esterases (aliesterase, carboxylesterase and cholinesterase), A-esterases (arylesterase, phosphatase, phosphotriesterase, amidase, paraoxonase, malaoxonase) etc. These enzymes can be inhibited by TEPP, EPN, and etc. and these inhibitors can synergize toxicity of insecticidal esters. These cross-substrate specificity may indicate one enzyme with broad (low) substrate specificity or several enzymes with high substrate specificity (Dauterman, 1976). A-esterases that are not inhibited by organophosphate and B-esterases that are inhibited by several OP insecticides due to irreversible phosphorylation of the active serine site (Dauterman, 1985). Esterases are specific enzymes : A-esterases hydrolyze p-nitrophenyl phosphate faster than p-nitrophenyl butyrate, B-esterases hydrolyze p-nitrophenyl phosphate slower than p-nitrophenyl butyrate. Carboxylesterases are important in the metabolism of certain OPs e.g. malathion (Kao *et al.*, 1984).

Esterases are involved in the metabolism of many insecticides in phase 1, so they are grouped into hydrolases. In insects, esterases are found in cytosol, microsomes, mitochondria and nuclei. The highest specific activity was found in the mitochondrial fraction (Zhu and Brindley, 1990). Esterase activity varies enormously among species of insects or even the same species of insects (Yu, 1990). Cohen *et al.* (1977) found that different stages of insects show different patterns of esterase activity. In *Tribolium castaneum* (Herbst) esterase (non-specific) activity was low in egg stage, increased in the larval stage, and declined in the pupa ; in the adult stage, the activity rose sharply. The two-spotted spider mite, *T. urticae* Koch, esterase activity was low in larval stage, increased in the adult stage of 2-3-days age, and declined in the older age. This enzyme with high efficiency was found in midgut, reproductive system and brain (McEnroe, 1965). Esterase activities are involved in the metabolism of xenobiotics that induced insect resistance to organophosphate, pyrethroid (Dauterman, 1985) and chitin inhibitor insecticides (Ishaaya and Degheele, 1988).

The substrates (*in vitro*) such as PNPA, ANA, βNA, phenyl acetate, MTB and ATCh for detecting levels of general esterase activity were appropriate ways to study the involvement of esterase mechanisms in resistant insects (Soderlund and Bloomquist,

1990). The substrates, ANA and PNPA in malathion resistant strains compared to a susceptible strain of *Tribolium castaneum* (Herbst) were not significant in esterase activities (Mackness *et al.*, 1983). The endogenous inhibitors could denature enzymes and reduce their activities. Therefore, to solve this problem, appropriate buffered conditions should be used. Some researcher used distilled water as a homogenizing medium (Ishaaya and Degheele, 1988). Willladen *et al.* (1987) used phosphate buffer with DDT and EDTA. Price (1984) used phosphate buffer without adding any other chemicals. Yu (1990) washed the empty midguts of insects first with KCl then homogenized with Tris/HCl. All the homogenizing buffers were used the pH between 7.2 to 7.6 and centrifuged firstly to sediment mitrochondrial fragments at around 10,000 x g upto 20 minutes. The hydrolysis product of esterase degradation of an artificial substrate (e.g. naphthyl acetate) can be reacted with a dye to give a colour, for example; β-naphthyl acetate is broken down to acetic acid and β-naphthol, the reaction of naphthol with an azo dye will produce a red coloured product at wavelength of 550 nanometer the intensity of the colour can be measured on a spectrophotometer. The more intense the colour, the greater breakdown of the substrate and therefore the more esterase activity (Callaghan *et al.*, 1993).

9.2 Glutathione S-transferases Glutathione S-transferases are a group of soluble detoxification enzyme which catalyze the conjugation of reduced glutathione with various compounds containing an electrophilic center, including insecticides (Chasseaud, 1979)

$$RX + GSH \xrightarrow{GST} GSR + X^- + H^+$$

They are found in mammals, insects, protozoa, algae, fungi and bacteria (Jakoby, 1978) and also in plants (Schroder *et al.*, 1990). The conjugates are further metabolized to form mercapturic acids by several steps or excreted because of their increased water-solubility. In this way compounds may be dealkylated, dehalogenated or cyanide may be released from organic thiocyanates (Visetson, 1991). GSTs are involved in the metabolism of many insecticides. Reactions are catalyzed by nonspecific enzymes include phase 2 conjugation of reactive metabolites formed by microsomal oxidations, as well as phase 1 conjugations with insecticides themselves. These enzymes are found largely in the cytosol in insects and in rat liver microsomes (Jakoby, 1978). They are well

established high levels of GSH S-transferases activity and are important in resistance to organophosphate and organochlorine insecticides (Motoyama and Dauterman, 1980). GSH S-transferase has multiple forms (Motoyama *et.al.*, 1977). These enzymes can be divided into three group: 1. GSH-S-aryl transferase contains active thiol groups and unite with substrates to give off hydrogen halide. They can combine with the leaving group of organophosphate. 2. GSH-S-alkyl transferase can demethylate OP, for example; methyl-parathion, fenitrothion. 3. Epoxy S-transferase, GST can also open epoxides of naphthalene, styrene, etc. Different forms and amounts of GST present in different animal species and strains determine their tolerance of chemicals detoxified by GST. Dauterman (1983) reported that the detoxification rate for diazinon and azinphosmethyl was 6-times higher in resistant strain than susceptible strain and was as high as 120-fold. Cohen and Gerson(1986) stated that the bulb mite, *Rhizoglyphus robini* and the carmine spider mite, *Tetranychus cinnabarinus* had relatively high levels of GSH S-transferase. This suggested a significant physiological role in acarine detoxification processes. Gunderson *et al.* (1986) reported that one species of lepidopterous larvae, *Spodoptera eridania* with CDNB conjugation rate was about 30 times higher than that of DCNB conjugation in guts and fat bodies. Cohen (1986) found that the larval and adult stages of *T. castaneum* are characterized by relatively high levels of GSH S-transferase activity, reduced in pharate pupae and pupae, and no activity was deteced in eggs. Apart from controlling pH and use of low temperature(0-4°C) during homogenization, the adding of compounds such as polymers and reducing agents such as mercaptoethanol or cysteine, etc were useful in the isolation of enzymes. These techniques were used for neutralize vaculolar acids and preventing ionization of these compounds. PVPP was used to inhibit the activity of endogenous inhibitors (Motoyama *et al.*, 1978). GST activity can also be measured spectrophotometrically. This is based on the catalysis by GST of the conjugation of CDNB with reduced glutathione, to produce a yellow product at wavelength of 400 nanometer. This method can also be miniaturized for use in a microtitreplate reader (Callaghan, 1989). Visetson (1991) found that GST activity was very stable for 120 days when stored at -85°C.

9.3 Monooxygenases (Mixed function oxidase of Cytochrome P_{450})

Monooxygenases are involved in the metabolism of many insecticides in phase 1. In insects, there are monooxygenase enzymes in microsome, membrane of fat tissue, intestine and malpighian tubules (Rose, 1985). Monooxygenases, activities are involved in the

metabolism of xenobiotics that induced insect resistance to pyrethroid and organophosphate insecticides (Yu and Nguyen, 1992).

10 Good properties of detoxification enzymes

10.1 In the cells which always obtain toxicants or xenobiotics such as fat tissue and intestine of insects.

10.2 Non - specific to toxicants or xenobiotics, so they can react with many toxicants or xenobiotics.

10.3 Synthesized quickly in each stage of life when the body obtains toxicants or xenobiotics (Schoknechy and Otto, 1989).

11 Insecticide and acaricide resistant mechanism

11.1 Insecticide resistance is developed by an increasing of lipids in the cuticle of insects and mites. A decrease in the absorption of insecticides or acaricides into insects or mites tissue is frequently the basis for a resistant mechanism known as prenetration resistance.

11.2 Insecticide resistance is developed by altering behavior such as ability of the population to avoid the lethal effect of which toxicant by going away.

11.3 Insecticide resistance is developed by biochemical reaction with insect body such as increasing esterase enzyme synthesis. This enzyme will detoxify a part of ester bond of the insecticide and make that insecticide no longer non-toxic.

11.4 Insecticide resistance is developed by accelerating excretion to destroy toxicant or by fat tissue synthesis to absorb more toxicant and make the toxicant of insecticide decrease.

11.5 Altered target sensitivity (Helle, 1965; Carbonaroxi *et al.*, 1986; Chayopas, 1996).

12. Other candidate acaricides used in toxicity testing

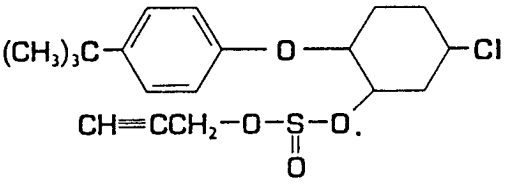
12.1 Propargite

12.1.1 Common name propargite

12.1.2 IUPAC name 2-(4-tert-butylphenoxy) cyclohexyl prop -2-vinyl sulfite

12.1.3 Trade name Omite

12.1.4 Chemical structure



12.1.5 Mode of action Non-systemic acaricide with predominantly contact action and long residual activity

12.1.6 Toxicity Oral : Acute oral LD_{50} for rats 2800 mg/kg

12.1.7 Formulation 20%EC

12.1.8 Uses Control of many species of phytophagous mites (particularly motile stages) on a variety of crops including vines, fruit trees (including top fruit, stone fruit, citrus fruit), hops, nuts, tomatoes, vegetables, ornamentals, cotton, maize, peanuts, and sorghum. Used at rates from 0.75-1.8 kg/ha on row crops and foliar sprays of 0.85-3.0 kg/ha on perennial fruit and nut crops.

12.1.9 Phytotoxicity Phytotoxic to pears, strawberries, roses and cotton under 10 inches in height. Citrus fruit and beans may also show some injury.

12.1.10 Physical chemistry Good solubility in water. Miscible with most organic solvents, such as acetone, benzene, ethanol, hexane, heptane and methanol. Stability : DT_{50} 80 days (pH7); decomposed by strong acid and alkalai (pH>10). No degradation in packing at 20°C for 1 year and 50%RH.

12.1.11 Compatibility Incompatible with alkaline materials, oil sprays and pesticides containing a large amount of petroleum solvents.

12.1.12 Environmental fate Animals : In mammals, propargite is hydrolysed at the sulfite ester linkage to 1-[4-(1,1-dimethylethyl) phenoxy]-2-cyclohexanol and subsequent hydroxylation of the tert-butyl side-chain. Additional metabolites are formed by further oxidation or sulfation of the tert-butyl group and oxidation of the cyclohexyl moiety.

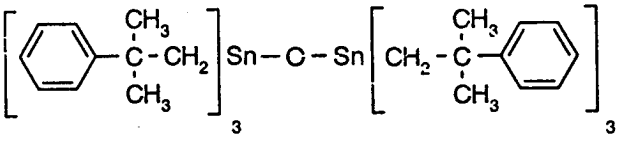
12.2 Fenbutatin oxide

12.2.1 Common name Fenbutatin oxide

12.2.2 IUPAC name bis[tris(2-methyl-2-phenylpropyl)tin] oxide

12.2.3 Trad name Torque

12.2.4 Chemical structure



12.2.5 Mode of action Non-systemic acaricide with contact and stomach action

12.2.6 Toxicity Oral : Acute oral LD_{50} for rats 2631 mg/kg, mice 1450, dogs>1500 mg/kg, irritating to skin and severely irritating to eyes

12.2.7 Formulation 55%SC

12.2.8 Uses Control of all motile stages of a wide range of phytophagous mites on pome fruit, stone fruit, citrus fruit, soft fruit, vines, bananas, cucurbits, ornamentals, and glasshouse crops. Gives long residual control.

12.2.9 Phytotoxicity Phytotoxic to tangerines, tangelos, and some varieties of grapefruit otherwise, non-phytotoxic when used as directed.

12.2.10 Physical chemistry Colourless crystals, non-soluble in water, extremely stable to heat, light and atmospheric oxygen

12.2.11 Environmental fate Soil : In soil, it is metabolised to dihydroxy-bis(2-methyl-2-phenyl=propyl) stannae and 2-methyl-2-phenylpropyl stannonic acid, presumably ultimately forming tinoxide/hydroxide. In tests in commercial use, there was minimal movement of fenbutatin oxide or its metabolites out of the top 30 cm of soil.

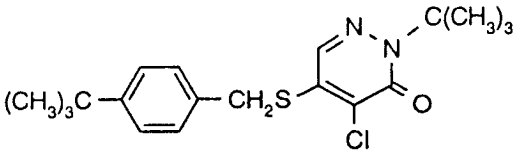
12.3 Pyridaben

12.3.1 Common name Pyridaben

12.3.2 IUPAC name 2-tert-butyl-5-(4-tert-butylbenzyl-thio)-4-chloropyridazin-3(2H)-one

12.3.3 Trade name Sanmite

12.3.4 Chemical structure



12.3.5 Mode of action Non-systemic insecticide and acaricide. Rapid knock-down and long residual activity. Active against all developing stages, espcially against the larval and nymphal stages.

12.3.6 Toxicity Oral : Acute oral LD_{50} for male rats 1350 mg/kg, female rats 820 mg/kg

12.3.7 Formulation 20%WP

12.3.8 Uses Control of Acari, Aleyrodidae, Aphididae, Cicadellidae and Thysanoptera on field crops, fruit trees, ornamentals and vegetables at 100-300 kg/ha

12.3.9 Physical chemistry Colourless crystals, non - soluble in water, stable at 50°C for 90 days, unstable to light

12.3.10 Environmental fate Animals : In the rats, an orally administered dose is excreted mainly in the faces. Elimination is rapid and almost complete within 96 hours. Plants : After application to citrus and apple, pyridaben degrades gradually photochemically and is not translocated into the pulp. Soil : Readily degrades microbiologically in aerobic soil, DT_{50}<21 days in natural water, DT_{50} 10 days (25°C, in dark).

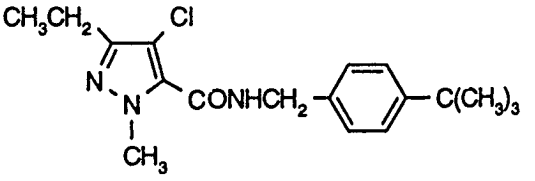
12.4 Tebufenpyrad

12.4.1 Common name Tebufenpyrad

12.4.2 IUPAC name N-(4-tert-butylbenzyl)-4-chloro-3-ethyl-1-methylpyrazole-= 5-carboxamide

12.4.3 Trade name Pyranica

12.4.4 Chemical structure



12.4.5 Mode of action Non-systemic acaricide active by contact and ingestion. Exhibits translaminar movement following application to leaves and thus inhibits the development of mite eggs oviposited on the undersides of leaves.

12.4.6 Toxicity Oral : Acute oral LD_{50} for male rats 595 mg/kg, female rats 997 mg/kg. Non-irritating to skin, slightly irritating to eyes (rabbits). No mutagenic effects were observed in the following tests; Ames mammalian micronucleus, *Drosophila* wing spot, *in vitro* cultured human lymphocytes, *in vivo* bone marrow erythrocytes. Low toxicity to honeybees.

12.4.7 Formulation 5%EC

12.4.8 Uses Control of all stages of *Tetranychus*, *Panonychus*, *Oligonychus* and *Eotetranychus* spp. On top fruit, vines, citrus, vegetables, hops, ornamentals, melons and cotton. Applied at 3.3-10 g a.i./100 l.

12.4.9 Physical chemistry Colourless crystals, non-soluble in water, stable to hydrolysis, DT_{50} > 28 days (pH 5,7 and 9)

12.4.10 Environmental fate Plants : Residues in the unpolished grain of rice plants harvested 113 and 119 days after treatment with pyributicarb (40 kg / ha) were found to be less than the detect able limit (0.005 mg / kg). Soil / Environment Half-life in paddy soil is 13-18 d.

MATERIALS AND METHODS

Materials

1. Ten strains of the African red mite, *E. africanus* were collected from a variety of regions of tangerine orchards in Thailand. The Thung Khru strain (susceptible strain) was introduced from non-pesticide-treated orchard. This susceptible strain was used as the standard check for the value of resistance ratio.

2. The adult females of the African red mite

3. Shelves for rearing the African red mite including fluorescent lamps

4. Other experimental materials
   - Indian coral, *Erythrina orientalis* (Linn.) Merr. leaves
   - plastic trays
   - cotton pads
   - tissues
   - petri-dishes
   - plastic boxes
   - beakers
   - volumetric flasks
   - forceps
   - small fine brushes
   - spreader
   - stereomicroscope

5. Candidate acaricides
   - amitraz 20%EC
   - dicofol 18.5%EC
   - tebufenpyrad 2%EC
   - propargite 20%EC
   - fenbutatin oxide 55%SC
   - pyridaben 20%WP

6. Experimental materials for enzyme preparation
   - mortar and pestle
   - ice box
   - microtubes
   - centrifuge (KUBOTA 6800)
   - micropipettes
   - spectrophotometer (HITACHI U-2,000)
   - balance
   - cuvettes
   - parafilm
   - pipette tips
   - pH meter

7. Chemicals
   - polyvinyl polypyrrolidone (PVPP)
   - potassium dihydrogen phosphate (KH_2PO_4)
   - ethylene diamine tetraacetic acid (EDTA)
   - glutathione (GSH)
   - sulfuric acid (H_2SO_4 conc)
   - sodium hydroxide (NaOH)
   - paranitrophenyl acetate (PNPA)
   - 1-chlorodinitro-2,4-benzene(CDNB)
   - ethanol

Method

Mass rearing of African red mite

The susceptible strain of African red mite, *E. africanus*, was obtained from Indian coral, leaves collected from a long period non-pesticide-treated orchard for a long period in Thung Khru district, Bangkok province. It has been continuously isolated and reared on Indian coral leaves in laboratory for 2 years (approximately 56 generations). The field strains of the African red mite obtained from tangerine were leaves collected from various tangerine orchards in Thailand (Figure 14,15) with spraying amitraz and dicofol. These strains were isolated for mass rearing in laboratory condition of $26.4\pm1.3^{o}C$ and $65.3\pm2.3\%RH$. The adult female mites from each strains were introduced on the upper surface of the leaves with the amount of 50 mites per leaf (ca 1.25 x 3 in^2). The leaves were placed on a moisted-cotton pad on a plastic tray (8.5 x 12 in^2) to maintain the vitality of leaves. The trays were placed on the shelves exposed to the fluorescent lamps for 9 hrs per day (Figure 16). The leaf that showed sign of deterioration was cut into small pieces and placed on a new leaf. The small dried pieces on a new leaf were kept out when the mite moved on a new leaf.

1. Study on acaricide resistance in African red mite by leaf-dip bioassay technique

- Acaricide solution of amitraz and dicofol were prepared by using distilled water with 1.5-2 concentration times of 5-7 dosages in the volumetric flasks (Figure 17). Each concentration comprises 250 ppm of the spreader.

- Ten strains of African red mites from various tangerine orchards were treated with amitraz and dicofol. The Thung Khru susceptible strain was used as a standard susceptible strain in all tests. The adult females were introduced to test the toxicity of acaricides when we obtained a large number of them enough to test.

- The mature leaf with the size of 1.25 x 1.25 in^2 was dipped into the solution in a petri-dish for 5 seconds, rested on a moisted-tissue pad in a petri-dish

Figure 14 Tangerine orchard at Pathum Thani province in the Central region

Figure 15 Tangerine orchard at Chiang Mai province in the North region

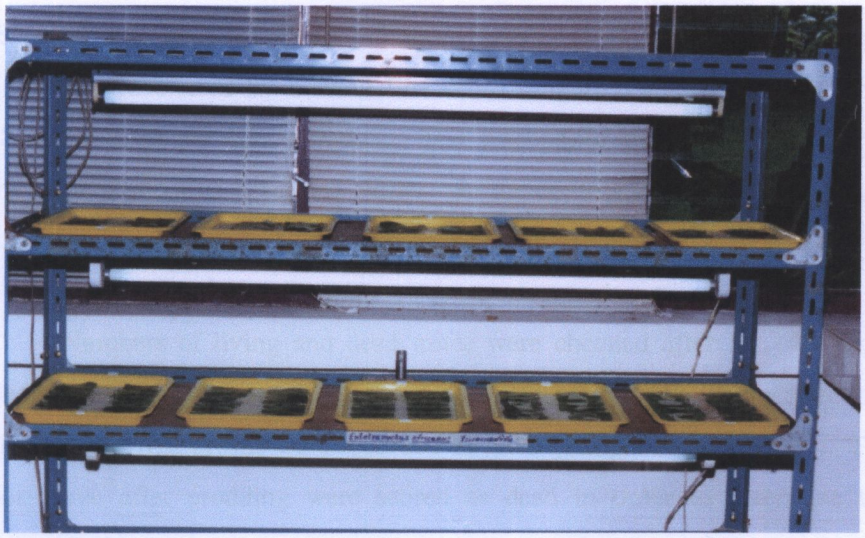


Figure 16 Mass rearing of African red mite, *E. africanus*, on the Indian coral leaves in the trays, placed on the shelves exposed to the fluorescent lamps for 9 hrs per day

Figure 17 Acaricide solution prepared by using distilled water with various dosages in the volumetric flasks

(Figure 18) to dry in the laboratory condition. Twenty adult females with the same age (3-5 d old) were transferred by a small fine brush (Figure 19) and released on a dried leaf with 3 replications per concentration. The susceptible strain and field strains were tested in the same manner as described earlier. In the control treatment of all strains were dipped in distilled water contained 250 ppm of spreader.

- Numbers of living and dead mites were checked after 24, 48 and 72 hrs and recorded. The mites that were able to walk at least one body length after they were touched with a small fine brush, were recorded as alive (Knight *et al.*, 1990). The mites that did not move after prodding were scored as dead in order to determine the LC_{50} (Welty *et al.*, 1988).

- If mortality data in the control treatment appeared, the data would be corrected by Abbott's formula (Abbott, 1925).

$$\% \text{ corrected mortality} = \left[\frac{\% \text{ test mortality} - \% \text{ control mortality} \times 100}{100 - \% \text{ control mortality}} \right]$$

- If the control batch mortality exceeds 20 percent, the test should be repeated to reduce or eliminate the causes of such mortality (Anonymous, 1969). The data were analyzed by probit analysis (Finney, 1971) with microcomputer program to obtain LC_{50} and LC_{95} values.

2. Study on developmental stages, female longevity and fecundity of susceptible and field strains of African red mite

The developmental stages, female longevity and fecundity study of African red mite both susceptible and field strains were conducted in the laboratory on Indian coral leaves. Each leaflet (1 cm in diameter) was placed on a moisted-tissue pad in a plastic box (Figure 20) to maintain the vitality of the leaf. The leaf that showed signs of deterioration was replaced by a new leaf. The plastic boxs were maintained under the laboratory condition. Two female mites were introduced on each individual leaf with a total

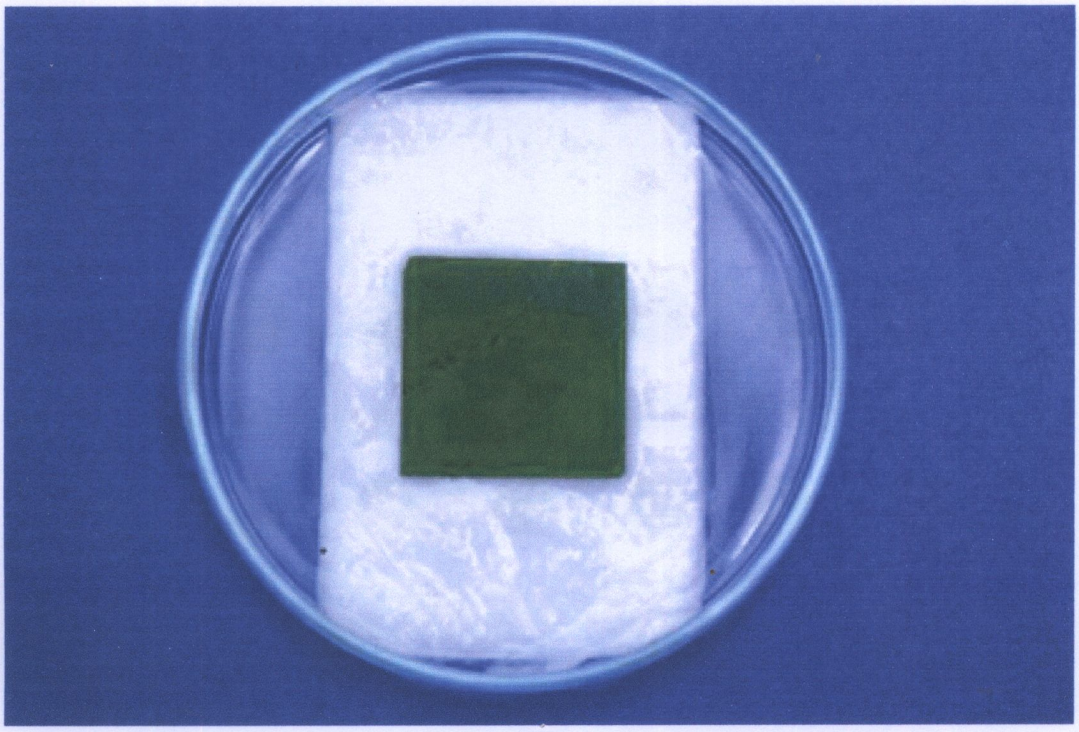


Figure 18 Laboratory testing unit for acaricide toxicity of African red mite, *E. africanus*

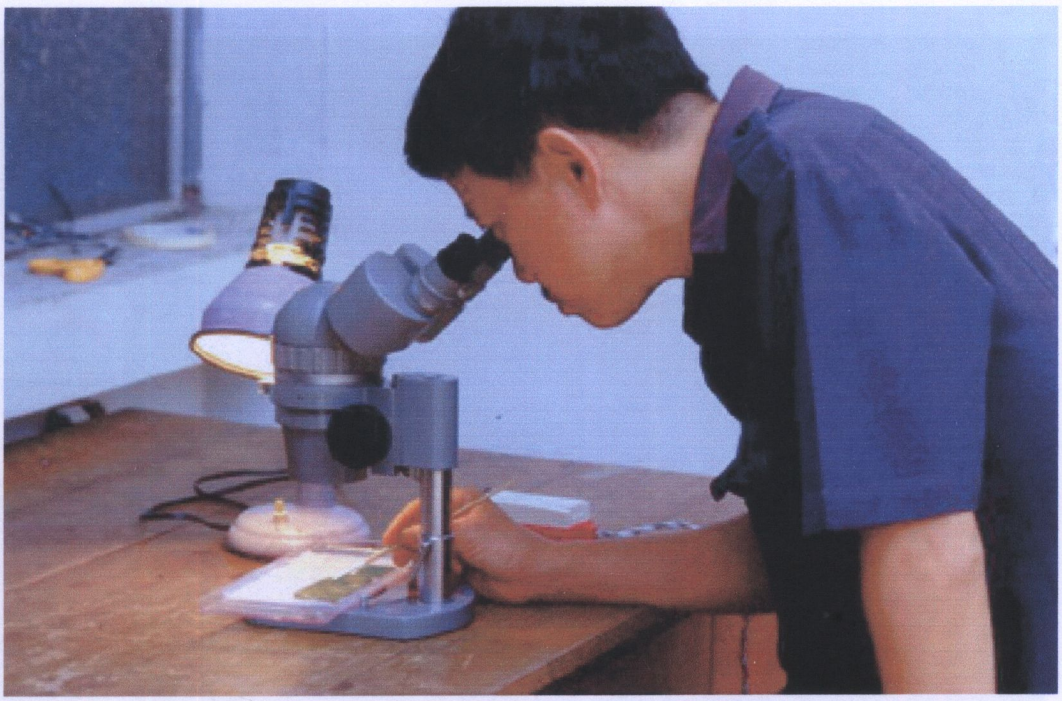


Figure 19 The adult females of African red mite, *E. africanus*, transferred by a small fine brush

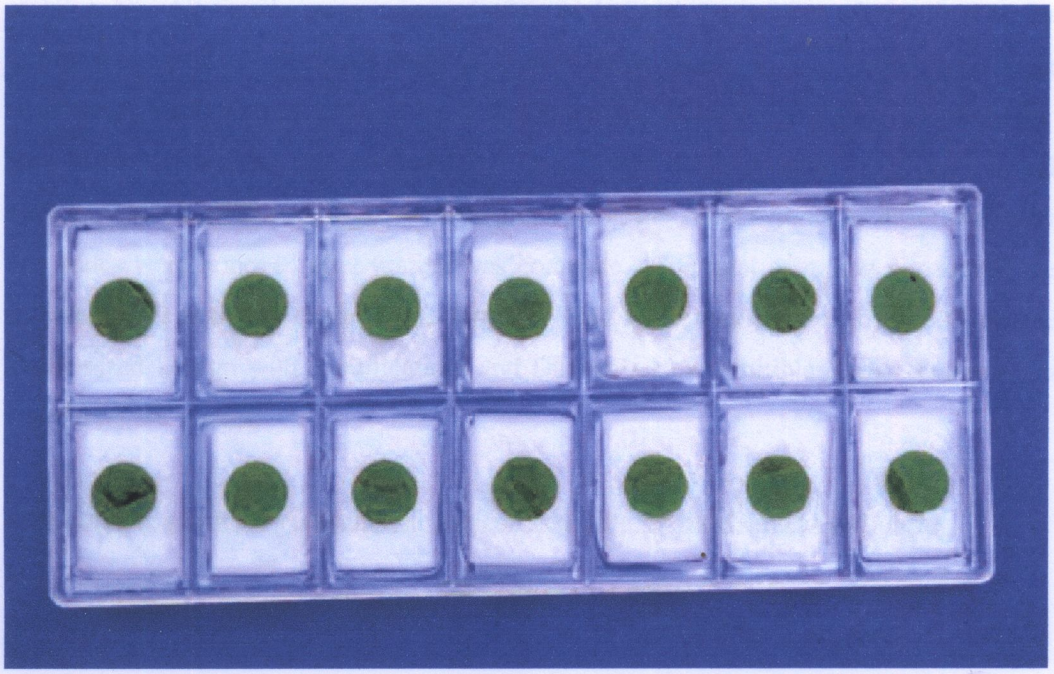


Figure 20 Rearing units for biological studies of African red mite, *E. africanus*

Figure 21 A pestle in a mortar on the icebox for crushing the adult females of African red mite, *E. africanus*

of 18 units. All females were removed after 24 hrs and the number of eggs laid on each leaflet were noted. Eighteen eggs randomly taken from various boxes were reared. individually until adult enclosion. Each leaflet was examined every 12 hrs and duration of each developmental stage was recorded. One female teliochrysalis and 2 active males were introduced on to each of 18 leaflets prepared in the same manner as described earlier. The males were removed after mating was complete. The total eggs laid per female were recorded daily until the death of the female. This study was planned by the Completely Randomized Design with 3 replications and Duncan's New Multiple Range Test was used to separate the means.

3. Study on acaricide resistance in African red mite by biochemical technique

Preparation of the detoxification enzymes from African red mite

Extraction of detoxification enzymes : Two detoxification enzymes, esterase and glutathione S-transferase were detected from the enzyme extraction in this study.

- Adult females with the same age (3-5 d old) were transferred from the stocks by the small fine brush under stereomicroscope. The mites from various strains were placed separately on Indian coral leaves. Each strain was placed on a moisted-tissue pad in a plastic tray. The mites of 900 units (ca. 50 mg) were transferred with the small fine brush and placed in a mortar on the ice box (Figure 21). Polyvinyl polypyrrolidone of half the weight of the mites was added. The mites were crushed finely by the use of a pestle and then 0.1 M potassium phosphate buffer pH 7.5 was added gradually.

- The mixture was rinsed into microtube (Figure 22) after it had been mixed well.

- The microtubes of various strains of the mites were taken to spin by the centrifuge (Figure 23) at 10,000 x g (Fournier *et al.*, 1988; Visetson, 1991; Phiancharoen, 1996) at a temperature of 4^{o}C for 5 minutes.

Figure 22 The microtubes containing the enzyme solution

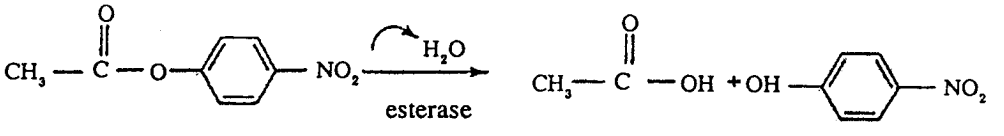
Figure 23 The enzyme solution spinned by the centrifuge

- The supernatant liquid was sucked by a micropipette (Figure 24) into a cuvette (Figure 25) for the detection of esterase.

- Another portion of the supernatant liquid was sucked by a micropipette for the detection of glutathione S-transferase.

Detection of esterase activity

The sample contained 2,900 µl of 0.1 M potassium phosphate buffer pH 7.5 in a cuvette, followed by 50 µl of enzyme solution and then 50 µl PNPA. The reference standard contained 2,900 µl of 0.1 M potassium phosphate buffer pH 7.5 in a cuvette, 50 µl of potassium phosphate + EDTA + GSH pH 7.5 solution and 50 µl of PNPA. Each cuvette was sealed by parafilm, made the solution mix together quickly and took to detect esterase activity. Absorbance examining method of paranitrophenyl acetate (PNPA) was read immediately for 3 minutes at room temperature by a spectrophotometer (Figure 26) at wavelength of 400 nanometer. The yellow colour of paranitrophenol was formed from hydrolysis reaction between paranitrophenyl acetate and catalytic of esterase enzyme (Mackness *et al.*, 1983).



paranitrophenyl acetate acetic acid paranitrophenol

Detection of glutathione S-transferase activity

The 1,150 µl of 0.1 M potassium phosphate buffer pH 7 was sucked in a cuvette by a micropipette, followed by 130 µl of 0.01 M GSH in 0.1 M potassium phosphate buffer pH 7, 20 µl of enzyme solution and 10 µl of CDNB. The reference standard contained 1,150 µl of 0.1 M potassium phosphate buffer pH 7, 130 µl of 0.01 M GSH in 0.1 M potassium phosphate buffer pH 7, 20 µl of 0.001 M of EDTA, 0.01 M of GSH in potassium phosphate buffer pH 7.5 and 10 µl of CDNB.

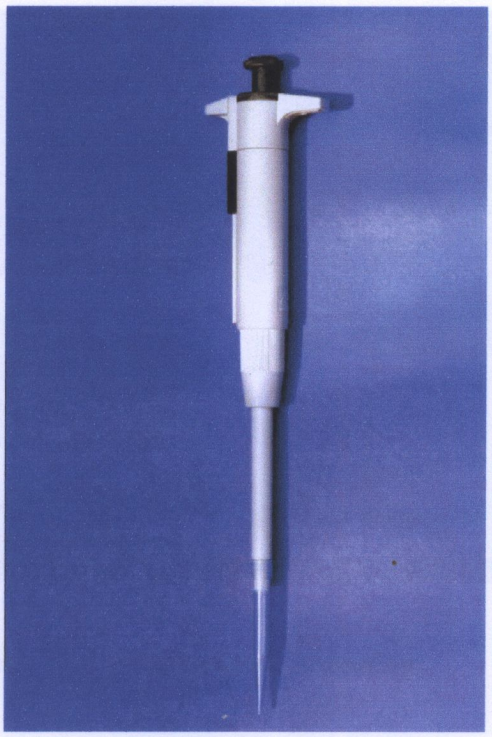


Figure 24 The supernatant liquid sucked by a micropipette for the detection of detoxification enzymes

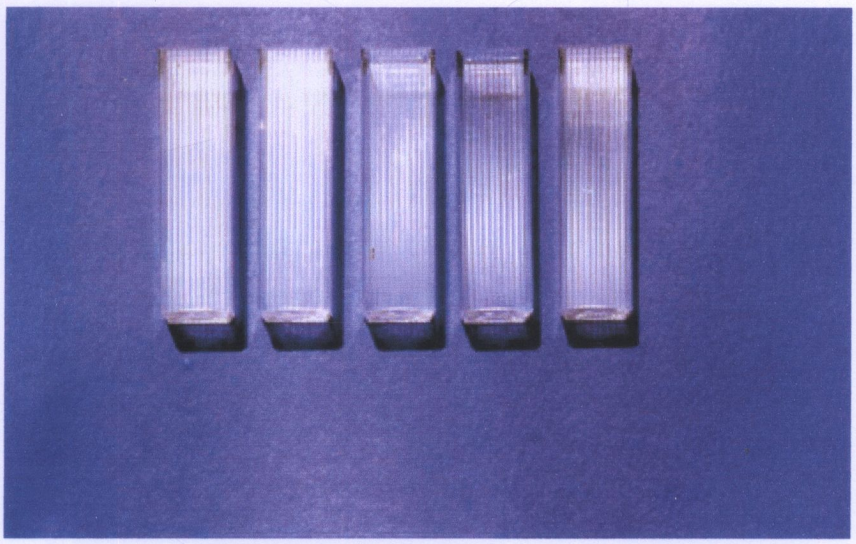
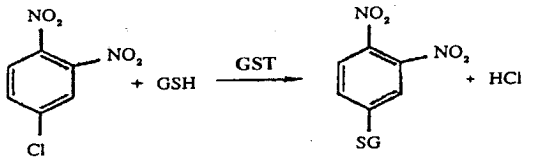


Figure 25 The cuvettes containing the supernatant liquid

Figure 26 Spectrophotometer used for absorbance measuring

Each cuvette was sealed by parafilm, made the solution mix together quickly and took to detect glutathione S-transferase activity. Absorbance examining method of the product of monochloronitro-benzene-glutathione was read by spectrophotometer at wavelength of 340 nanometer. The product was formed by conjugation reaction of chlorodinitrobenzene with glutathione and catalytic of glutathione S-transferase (Chotimanothum, 2000).



CDNB
(chlorodinitrobenzene)

CDNB-Conjugated product

3.1 Study on number of African red mites per homogenization for esterase activity

The number of African red mites from Hat Yai strain were varied from 113-900 individuals/batch. Each batch was homogenized and paranitrophenol activity was recorded. Paranitrophenol assays were performed at pH 7.5. Preparation and experimental methods of esterase enzyme was conducted in the same manner as described earlier.

3.2 Study on incubation pH of African red mite per homogenization for esterase activity

The appropriate number of African red mites from Hat Yai strain according to experiment 1 were used for this study. Paranitrophenol assays were performed at pHs 6.5, 7, 7.5 and 8. Preparation and experimental methods of esterase enzyme was conducted in the same manner as described earlier.

3.3 Study on the amount of esterase activity in susceptible and field strains

The appropriate number and incubation pH of African red mites from field

and susceptible strains according to experiment 1 and 2 were used for this study. Preparation and experimental methods of esterase enzyme was conducted in the same manner as described earlier.

3.4 Study on the amount of glutathione S-transferase activity in susceptible and field strains

The appropriate number and incubation pH of African red mites from field and susceptible strains according to 1 and 2 were used for this study. Preparation and experimental methods of glutathione S-transferase was conducted in the same manner as described earlier.

4. Study on toxicity of some candidate acaricides for controlling the African red mite

Thung Khru Strain (susceptible strain) and the highest resistant strain was tested with the other candidate acaricides such as propargite 20%EC, fenbutatin oxide 55%SC, pyridaben 20%WP and tebufenpyrad 2%EC, by the same manner as described earlier. The data were recorded and analyzed by probit analysis. The toxicity of candidate acaricides were shown by a value of LC_{50} and LC_{95}.

Places and Duration

Places

1. Laboratory of Mite and Spider Research Group, Entomology and Zoology Division, Department of Agriculture, Ministry of Agriculture and Cooperatives

2. Laboratory of Biotechnology Research and Development Institute, Department of Agriculture, Ministry of Agriculture and Cooperatives

3. Laboratory of Toxic Substances Division, Department of Agriculture, Ministry of Agriculture and Cooperatives

4. Department of Entomology, Faculty of Agriculture, Kasetsart University

Duration

This experiment was carried out from January, 1997 to December, 1999.

RESULTS

1. Study on acaricide resistance in African red mite by leaf-dip bioassay technique

The monitoring of the acaricide resistance to African red mites were carried out by survey and collecting population from various tangerine orchards in Thailand. Name of strains, codes, locations and regions were shown in Table 1. Laboratory toxicity of amitraz 20%EC and dicofol 18.5%EC were tested against the adult females of the mite at various concentrations.

The results of toxicity of amitraz to African red mites were shown in Table 2 for susceptible strain of TKS (Thung Khru Strain), Table 3 for SPS (Suphan Buri Strain), Table 4 for NSS (Nong Sua Strain), Table 5 for NKS (Nong Khae Strain), Table 6 For OKS (Ongkharak Strain), Table 7 for BMS (Ban Mi Strain), Table 8 for NCS (Nakhon Chaisi Strain), Table 9 for PPS (Pob Phra Strain), Table 10 for FAS (Fang Strain), Table 11 for HYS (Hat Yai Strain) and Table 12 for PSS (Phrasaeng Strain). The values of 95% fiducial limits, slope and chi-square of all strains were shown in Table 24.

The LC_{50} and LC_{95} values of amitraz for susceptible strain and field strains of African red mites from tangerine orchard were shown in Table 26. The LC_{50} values of PSS (Phrasaeng Strain), HYS (Hat Yai Strain), NSS (Nong Sua Strain), OKS (Ongkharak Strain), FAS (Fang Strain), SPS (Suphan Buri Strain), NCS (Nakhon Chaisi Strain), NKS (Nong Khae Strain), BMS (Ban Mi Strain), PPS (Pob Phra Strain) and TKS (Thung Khru Strain) were 274.35, 176.00, 168.76, 131.29, 113.32, 100.72, 97.44, 91.69, 88.15, 61.83 and 39.26 ppm, respectively. The LC_{95} values of PSS (Phrasaeng Strain), HYS (Hat Yai Strain), NSS (Nong Sua Strain), BMS (Ban Mi Strain), NKS (Nong Khae Strain), PPS (Pob Phra Strain), FAS (Fang Strain), SPS (Suphan Buri Strain), OKS (Ongkharak Strain), NCS (Nakhon Chaisi Strain) and TKS (Thung Khru Strain) were 1430.73, 829.07, 786.40, 702.52, 596.06, 538.87, 536.06, 510.79, 438.27, 408.32 and 252.33 ppm, respectively.

Table 1 Name of strains, codes of African red mite, *E. africanus*, in tangerine orchards in Thailand

Strains	Codes	Locations	Regions [1/]
Thung Khru Strain	TKS	Thung Khru, Bangkok	C
Suphan Buri Strain	SPS	Muang, Suphan Buri	C
Nong Sua Strain	NSS	Nong Sua, Pathum Thani	C
Nong Khae Strain	NKS	Nong Khae, Saraburi	C
Ongkharak Strain	OKS	Ongkharak, Nakhon Nayok	C
Ban Mi Strain	BMS	Ban Mi, Lop Buri	C
Nakhon Chaisi Strain	NCS	Nakhon Chaisi, Nakhon Pathom	C
Pob Phra Strain	PPS	Pob Phra, Tak	N
Fang Strain	FAS	Fang, Chiang Mai	N
Hat Yai Strain	HYS	Hat Yai, Songkhla	S
Phrasaeng Strain	PSS	Phrasaeng, Surat Thani	S

[1/] C = Central, N = North, S = South

Table 2 Toxicity of amitraz 20%EC at various doses against African red mite, *E. africanus*, from TKS at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	6.50	0.80	60	5	3.13	0.0521
2	12.50	1.10	60	10	9.35	0.1558
3	25.00	1.40	60	18	20.70	0.3450
4	50.00	1.70	60	34	35.08	0.5846
5	100.00	2.00	60	43	47.75	0.7958
6	200.00	2.30	60	60	55.50	0.9250

[1/] TKS = Thung Khru Strain

[2/] ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 1.76 + 2.04 x ; LC_{50} = 39.26 ppm

Table 3 Toxicity of amitraz 20%EC at various doses against African red mite, *E. africanus*, from SPS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	25.00	1.40	60	7	4.74	0.0790
2	50.00	1.70	60	13	14.34	0.2390
3	100.00	2.00	60	30	29.83	0.4971
4	200.00	2.30	60	40	45.39	0.7565
5	400.00	2.60	60	56	55.13	0.9188
6	600.00	2.78	60	60	57.88	0.9647

1/ SPS = Suphan Buri Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 0.33 + 2.33 x ; LC_{50} = 100.72 ppm

Table 4 Toxicity of amitraz 20%EC at various doses against African red mite, *E. africanus*, from NSS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	25.00	1.40	60	3	1.24	0.0206
2	50.00	1.70	60	8	5.81	0.0968
3	100.00	2.00	60	15	17.28	0.2880
4	200.00	2.30	60	24	34.32	0.5720
5	400.00	2.60	60	50	49.31	0.8218
6	600.00	2.78	60	60	54.74	0.9124

1/ NSS = Nong Sua Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = -0.48 + 2.46 x ; LC_{50} = 168.76 ppm

Table 5 Toxicity of amitraz 20%EC at various doses against African red mite, *E. africanus*, from NKS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	12.50	1.10	60	4	2.40	0.0400
2	25.00	1.40	60	10	7.61	0.1268
3	50.00	1.70	60	14	17.83	0.2971
4	100.00	2.00	60	31	31.81	0.5304
5	200.00	2.30	60	39	45.21	0.7534
6	400.00	2.60	60	56	54.13	0.9022
7	600.00	2.78	60	60	57.04	0.9506

[1/] NKS = Nong Khae Strain

[2/] ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 1.03 + 2.02 x ; LC_{50} = 91.68 ppm

Table 6 Toxicity of amitraz 20%EC at various doses against African red mite, *E. africanus*, from OKS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	25.00	1.40	60	2	0.71	0.0118
2	50.00	1.70	60	6	5.63	0.0939
3	100.00	2.00	60	18	21.31	0.3551
4	200.00	2.30	60	41	43.03	0.7171
5	400.00	2.60	60	57	56.15	0.9358
6	600.00	2.78	60	60	58.86	0.9809

[1/] OKS = Ongkharak Strain

[2/] ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = -1.66 + 3.14 x ; LC_{50} = 131.29 ppm

Table 7 Toxicity of amitraz 20%EC at various doses against African red mite, *E. africanus*, from BMS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	12.50	1.10	60	6	3.65	0.0608
2	25.00	1.40	60	11	9.54	0.1590
3	50.00	1.70	60	18	19.60	0.3266
4	100.00	2.00	60	25	32.39	0.5398
5	200.00	2.30	60	45	44.52	0.7419
6	400.00	2.60	60	52	53.08	0.8847
7	600.00	2.78	60	60	56.14	0.9357

1/ BMS = Ban Mi Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

◊ Y = 1.45 + 1.82 x ; LC_{50} = 88.15 ppm

Table 8 Toxicity of amitraz 20%EC at various doses against African red mite, *E. africanus*, from NCS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	25.00	1.40	60	4	3.55	0.0592
2	50.00	1.70	60	12	13.31	0.2218
3	100.00	2.00	60	32	30.71	0.5119
4	200.00	2.30	60	48	47.73	0.7955
5	400.00	2.60	60	55	56.85	0.9475
6	600.00	2.78	60	60	58.89	0.9815

1/ NCS = Nakhon Chaisi Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

◊ Y = -0.26 + 2.64 x ; LC_{50} = 97.44 ppm

Table 9 Toxicity of amitraz 20%EC at various doses against African red mite, *E. africanus*, from PPS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	2	0.00	0.0000
1	12.50	1.10	60	9	6.74	0.1123
2	25.00	1.40	60	16	14.74	0.2457
3	50.00	1.70	60	23	26.15	0.4359
4	100.00	2.00	60	36	38.55	0.6425
5	200.00	2.30	60	45	48.83	0.8138
6	400.00	2.60	60	60	55.32	0.9220

[1/] PPS = Pob Phra Strain

[2/] ppm = Calculated from commercial formulation ; Probit(p) = a + bx

◊ = 1.87 + 1.75 x ; LC_{50} = 61.83 ppm

Table 10 Toxicity of amitraz 20%EC at various doses against African red mite, *E. africanus*, from FAS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	25.00	1.40	60	6	3.29	0.0548
2	50.00	1.70	60	10	11.60	0.1932
3	100.00	2.00	60	24	26.84	0.4474
4	200.00	2.30	60	42	43.57	0.7262
5	400.00	2.60	60	54	54.54	0.9091
6	600.00	2.78	60	60	57.67	0.9612

[1/] FAS = Fang Strain

[2/] ppm = Calculated from commercial formulation ; Probit(p) = a + bx

◊ = -0.01 + 2.44 x ; LC_{50} = 113.32 ppm

Table 11 Toxicity of amitraz 20%EC at various doses against African red mite, *E. africanus*, from HYS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	25.00	1.40	60	3	1.15	0.0192
2	50.00	1.70	60	6	5.45	0.0908
3	100.00	2.00	60	14	16.46	0.2743
4	200.00	2.30	60	29	33.24	0.5540
5	400.00	2.60	60	45	48.49	0.8082
6	600.00	2.78	60	60	54.21	0.9035

[1/] HYS = Hat Yai Strain

[2/] ppm = Calculated from commercial formulation ; Probit(p) = a + bx

◊ = -0.49 + 2.44 x ; LC_{50} = 176.00 ppm

Table 12 Toxicity of amitraz 20%EC at various doses against African red mite, *E. africanus*, from PSS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	50.00	1.70	60	5	2.70	0.0450
2	100.00	2.00	60	10	9.45	0.1574
3	200.00	2.30	60	20	22.59	0.3765
4	400.00	2.60	60	30	38.78	0.6464
5	600.00	2.78	60	46	46.93	0.7821
6	800.00	2.90	60	59	51.41	0.8568

[1/] PSS = Phrasaeng Strain

[2/] ppm = Calculated from commercial formulation ; Probit(p) = a + bx

◊ = -0.59 + 2.29 x ; LC_{50} = 274.35 ppm

The result in Table 26 showed that PSS (Phrasaeng Strain) exhibited the highest resistance to amitraz with resistance ratio (RR at LC_{50} value of 6.99-fold followed by HYS (Hat Yai Strain), NSS (Nong Sua Strain), OKS (Ongkharak Strain), FAS (Fang Strain), SPS (Suphan Buri Strain), NCS (Nakhon Chaisi Strain) NKS (Nong Khae Strain), BMS (Ban Mi Strain) and PPS (Pob Phra Strain) with resistance ratio value of 4.48, 4.30, 3.34, 2.89, 2.57, 2.48, 2.34, 2.25 and 1.57-fold, respectively. The result in Table 26 showed that PSS (Phrasaeng Strain) exhibited the highest resistance to amitraz with resistance ratio (RR at LC_{95}) value of 5.67-fold and followed by HYS (Hat Yai Strain), NSS (Nong Sua Strain), BMS (Ban Mi Strain), NKS (Nong Khae Strain), PPS (Pob Phra Strain), FAS (Fang Strain), SPS (Suphan Buri Strain), OKS (Ongkharak Strain) and NCS (Nakhon Chaisi Strain) with RR values of 3.29, 3.12, 2.78, 2.36, 2.14, 2.12, 2.02, 1.74 and 1.62-fold, respectively.

The results from the study of toxicity of dicofol to African red mites were shown in Table 13 for susceptible strain of TKS (Thung Khru Strain), Table 14 for SPS (Suphan Buri Strain), Table 15 for NSS (Nong Sua Strain), Table 16 for NKS (Nong Khae Strain), Table 17 for OKS (Ongkharak Strain), Table 18 for BMS (Ban Mi Strain), Table 19 for NCS (Nakhon chaisi Strain), Table 20 for PPS (Pob Phra Strain), Table 21 for FAS (Fang Strain), Table 22 for HYS (Hat Yai Strain) and Table 23 for PSS (Phrasaeng Strain). The values of 95% fiducial limits, slope and chi-square of all strains were shown in Table 25.

The LC_{50} and LC_{95} values of dicofol for susceptible strain and field strains of African red mite from tangerine orchards were shown in Table 27. The LC_{50} values of HYS (Hat Yai Strain), OKS (Ongkharak Strain), FAS (Fang Strain), PPS (Pob Phra Strain), NSS (Nong Sua Strain), PSS (Phrasaeng Strain), NKS (Nong Khae Strain), BMS (Ban Mi Strain), SPS (Suphan Buri Strain), NCS (Nakhon Chaisi Strain) and TKS (Thung Khru Strain) were 76.75, 44.19, 32.23, 16.98, 14.79, 14.61, 11.15, 7.76, 5.37, 2.80 and 2.42 ppm, respectively. The LC_{95} values of HYS (Hat Yai Strain), OKS (Ongkharak Strain), FAS (Fang Strain), PSS (Phrasaeng Strain), NKS (Nong Khae Strain), NSS (Nong Sua Strain), PPS (Pob Phra Strain), BMS (Ban Mi Strain), NCS (Nakhon Chaisi Strain), SPS (Spuhan Buri Strain) and TKS (Thung Khru Strain) were 519.05, 240.97, 169.98, 122.19, 109.44, 104.69, 98.60, 52.33, 37.47, 23.73 and 17.48 ppm, respectively.

Table 13 Toxicity of dicofol 18.5%EC at various doses against African red mite, *E. africanus*, from TKS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	0.72	-0.14	60	12	9.40	0.1566
2	1.45	0.16	60	19	20.10	0.3350
3	2.89	0.46	60	32	33.52	0.5587
4	5.78	0.76	60	42	45.93	0.7655
5	11.56	1.06	60	57	54.20	0.9033
6	23.13	1.36	60	58	58.19	0.9698
7	46.25	1.67	60	60	59.57	0.9929

1/ TKS = Thung Khru Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 4.26 + 1.92 x ; LC_{50} = 2.42 ppm

Table 14 Toxicity of dicofol 18.5%EC at various doses against African red mite, *E. africanus*, from SPS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	1.45	0.16	60	7	4.43	0.0737
2	2.89	0.46	60	15	14.80	0.2466
3	5.78	0.76	60	25	31.96	0.5326
4	11.56	1.06	60	49	48.12	0.8020
5	23.13	1.36	60	58	56.82	0.9470
6	46.25	1.67	60	60	59.49	0.9914

1/ SPS = Suphan Buri Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 3.14 + 2.55 x ; LC_{50} = 5.37 ppm

Table 15 Toxicity of dicofol 18.5%EC at various doses against African red mite, *E. africanus*, from NSS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	5.78	0.76	60	16	12.89	0.2148
2	11.56	1.06	60	24	25.07	0.4179
3	23.13	1.36	60	35	38.79	0.6464
4	46.25	1.67	60	49	49.86	0.8310
5	92.50	1.97	60	57	56.30	0.9383
6	185.00	2.27	60	60	58.99	0.9831

1/ NSS = Nong Sua Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 2.74 + 1.94 x ; LC_{50} = 14.79 ppm

Table 16 Toxicity of dicofol 18.5%EC at various doses against African red mite, *E. africanus*, from NKS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	1.45	0.16	60	5	4.25	0.0709
2	2.89	0.46	60	10	9.92	0.1654
3	5.78	0.76	60	21	19.08	0.3180
4	11.56	1.06	60	29	30.62	0.5103
5	23.13	1.36	60	38	42.02	0.7003
6	46.25	1.67	60	50	50.83	0.8472
7	92.50	1.97	60	59	56.17	0.9362

1/ NKS = Nong Khae Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 3.26 + 1.66 x ; LC_{50} = 11.15 ppm

Table 17 Toxicity of dicofol 18.5%EC at various doses against African red mite, *E. africanus*, from OKS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	11.56	1.06	60	7	5.80	0.0967
2	23.13	1.36	60	14	15.90	0.2651
3	46.25	1.67	60	33	31.06	0.5176
4	92.50	1.97	60	44	45.79	0.7631
5	185.00	2.27	60	54	55.05	0.9175
6	370.00	2.57	60	60	58.82	0.9803

1/ OKS = Ongkharak Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

◊ = 1.33 + 2.23 x ; LC_{50} = 44.19 ppm

Table 18 Toxicity of dicofol 18.5%EC at various doses against African red mite, *E. africanus*, from BMS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	1.45	0.16	60	5	4.44	0.0740
2	2.89	0.46	60	11	11.83	0.1971
3	5.78	0.76	60	25	23.97	0.3996
4	11.56	1.06	60	36	38.05	0.6342
5	23.13	1.36	60	52	49.60	0.8266
6	46.25	1.67	60	54	56.28	0.9380
7	92.50	1.97	60	60	59.02	0.9837

1/ BMS = Ban Mi Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

◊ = 3.23 + 1.98 x ; LC_{50} = 7.76 ppm

Table 19 Toxicity of dicofol 18.5%EC at various doses against African red mite, *E. africanus*, from NCS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	2	0.00	0.0000
1	0.72	-0.14	60	13	11.70	0.1956
2	1.45	0.16	60	18	20.32	0.3387
3	2.89	0.46	60	35	30.51	0.5084
4	5.78	0.76	60	40	40.64	0.6774
5	11.56	1.06	60	43	48.95	0.8159
6	23.13	1.36	60	55	54.59	0.9098
7	46.25	1.67	60	60	57.74	0.9623

1/ NCS = Nakhon Chaisi Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

◊Y = 4.35 + 1.46 x ; LC_{50} = 2.80 ppm

Table 20 Toxicity of dicofol 18.5%EC at various doses against African red mite, *E. africanus*, from PPS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	5.78	0.76	60	10	9.41	0.1568
2	11.56	1.06	60	22	21.58	0.3596
3	23.13	1.36	60	33	36.82	0.6137
4	46.25	1.67	60	54	49.54	0.8256
5	92.50	1.97	60	54	56.61	0.9435
6	185.00	2.27	60	60	59.23	0.9872

1/ PPS = Pob Phra Strain

/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

◊Y = 2.35 + 2.15 x ; LC_{50} = 16.98 ppm

Table 21 Toxicity of dicofol 18.5%EC at various doses against African red mite, *E. africanus*, from FAS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	11.56	1.06	60	11	9.32	0.1553
2	23.13	1.36	60	25	22.29	0.3714
3	46.25	1.67	60	31	38.38	0.6396
4	92.50	1.97	60	51	51.09	0.8515
5	185.00	2.27	60	59	57.48	0.9581
6	370.00	2.57	60	60	59.53	0.9921

[1/] FAS = Fang Strain

[2/] ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 1.56 + 2.28 x ; LC_{50} = 32.23 ppm

Table 22 Toxicity of dicofol 18.5%EC at various doses against African red mite, *E. africanus*, from HYS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	11.56	1.06	60	5	3.10	0.0516
2	23.13	1.36	60	8	9.06	0.1509
3	46.25	1.67	60	20	19.89	0.3314
4	92.50	1.97	60	31	33.83	0.5637
5	185.00	2.27	60	44	46.53	0.7756
6	370.00	2.57	60	58	54.73	0.9121

[1/] HYS = Hat Yai Strain

[2/] ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 1.26 + 1.98 x ; LC_{50} = 76.75 ppm

Table 23 Toxicity of dicofol 18.5%EC at various doses against African red mite, *E. africanus*, from PSS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	5.78	0.76	60	18	14.19	0.2364
2	11.56	1.06	60	24	25.69	0.4281
3	23.13	1.36	60	36	38.34	0.6391
4	46.25	1.67	60	45	48.84	0.8140
5	92.50	1.97	60	57	55.41	0.9235
6	185.00	2.27	60	60	58.52	0.9754

1/ PPS = Phra Saeng Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 2.92 + 1.78 x ; LC_{50} = 14.61 ppm

Table 24 Responses of eleven strains of African red mite, *E. africanus*, to amitraz 20%EC at 48 hrs after treating

Codes	LC_{50} (ppm)	95% confidence intervals (ppm)	Slope(±SE)	Chi-square(df) 1/
TKS	39.26	26.56-59.40	2.04(±0.18)	9.03*(4)
SPS	100.72	85.44-118.05	2.33(±0.20)	6.33(4)
NSS	168.76	103.44-286.90	2.46(±0.21)	16.97*(4)
NKS	91.68	65.50-128.10	2.02(±0.16)	10.42*(5)
OKS	131.29	114.73-150.12	3.14(±0.26)	4.91(4)
BMS	88.15	61.74-125.20	1.82(±0.15)	10.06*(5)
NCS	97.44	83.60-112.93	2.64(±0.22)	2.62(4)
PPS	61.83	39.26-96.06	1.75(±0.17)	8.83*(4)
FAS	113.32	96.80-132.24	2.44(±0.21)	5.87(4)
HYS	176.00	116.82-272.73	2.44(±0.21)	12.53*(4)
PSS	274.35	166.22-454.54	2.29(±0.21)	16.11*(4)

1/ Significance of Chi-square for goodness of fit : *, P > 0.10. Since goodness of fit of Chi-square is significant, a heterogeneity factor is used in the calculation of confidence intervals.

Table 25 Responses of eleven strains of African red mite, *E. africanus*, to dicofol 18.5%EC at 48 hrs after treating

Codes	LC_{50} (ppm)	95% confidence intervals (ppm)	Slope(±SE)	Chi-square(df) 1/
TKS	2.42	1.98-2.91	1.92(±0.17)	4.48(5)
SPS	5.37	4.59-6.26	2.55(±0.22)	5.93(4)
NSS	14.79	11.92-17.89	1.94(±0.19)	3.34(4)
NKS	11.15	9.15-13.60	1.66(±0.14)	4.21(5)
OKS	44.19	37.17-52.17	2.23(±0.20)	2.58(4)
BMS	7.76	6.48-9.25	1.98(±0.16)	3.68(5)
NCS	2.80	1.78-4.05	1.46(±0.14)	8.27(5)
PPS	16.98	14.06-20.19	2.15(±0.20)	6.30(4)
FAS	32.23	26.89-38.08	2.28(±0.22)	6.25(4)
HYS	76.75	64.16-92.34	1.98(±0.18)	4.76(4)
PSS	14.61	11.55-17.89	1.78(±0.18)	5.67(4)

1/ Since goodness of fit of Chi-square is not significant, no heterogeneity factor is used in the calculation of confidence intervals.

Table 26 Resistance ratio value of ten strains of African red mite, *E. africanus*, to amitraz 20%EC at 48 hrs after treating

Regions [1/]	Codes	amitraz 20%EC			
		LC_{50} (ppm)	RR[2/]	LC_{95}(ppm)	RR[3/]
Standard	TKS	39.26	1.00	252.33	1.00
C	SPS	100.72	2.57	510.79	2.02
C	NSS	168.76	4.30	786.40	3.12
C	NKS	91.69	2.34	596.06	2.36
C	OKS	131.29	3.34	438.27	1.74
C	BMS	88.15	2.25	702.52	2.78
C	NCS	97.44	2.48	408.32	1.62
N	PPS	61.83	1.57	538.87	2.14
N	FAS	113.22	2.89	536.06	2.12
S	HYS	176.00	4.48	829.07	3.29
S	PSS	274.35	6.99	1430.73	5.67

[1/] C = Central, N = North, S = South, Standard = Susceptible strain of African red mite came from Thung Khru district, Bangkok province for standard check

[2/] RR = Resistance Ratio = LC_{50} of field strain/ LC_{50} of susceptible strain

[3/] RR = Resistance Ratio = LC_{95} of field strain/ LC_{95} of susceptible strain

The results in Table 27 showed that HYS (Hat Yai Strain) exhibited the highest resistance to dicofol with resistance ratio (RR at LC_{50}) value of 31.71-fold followed by OKS (Ongkharak Strain), FAS (Fang Strain), PPS (Pob Phra Strain), NSS (Nong Sua Strain), PSS (Phrasaeng Strain), NKS (Nong Khae Strain), BMS (Ban Mi Strain), SPS (Suphan Buri Strain) and NCS (Nakhon Chaisi Strain) with RR values of 18.26, 13.32, 7.02, 6.11, 6.04, 4.61, 3.21, 2.22 and 1.16-fold, respectively. The result in Table 27 showed that HYS (Hat Yai Strain) exhibited the highest resistance to dicofol with resistance ratio (RR at LC_{95}) value of 29.69-fold followed by OKS (Ongkharak Strain), FAS (Fang Strain), PSS (Phrasaeng Strain), NKS (Nong Khae Strain), NSS (Nong Sua Strain), PPS (Pob Phra Strain), BMS (Ban Mi Strain), NCS (Nakhon Chaisi Strain) and SPS (Suphan Buri Strain) with RR values of 13.79, 9.72, 6.99, 6.26, 5.99, 5.64 2.99, 2.14 and 1.36-fold, respectively.

The mapping of resistance ratios (RR) obtained by divided LC_{50} value of various field strains with LC_{50} value of the susceptible strain (TKS) of amitraz and dicofol were shown in Figure 27.

Table 27 Resistance ratio value of ten strains of African red mite, *E. africanus*, to dicofol 18.5%EC at 48 hrs after treating

Regions [1/]	Codes	dicofol 18.5%EC			
		LC_{50} (ppm)	RR[2/]	LC_{95}(ppm)	RR[3/]
Standard	TKS	2.42	1.00	17.48	1.00
C	SPS	5.37	2.22	23.73	1.36
C	NSS	14.79	6.11	104.69	5.99
C	NKS	11.15	4.61	109.44	6.26
C	OKS	44.19	18.26	240.97	13.79
C	BMS	7.76	3.21	52.33	2.99
C	NCS	2.80	1.16	37.47	2.14
N	PPS	16.98	7.02	98.60	5.64
N	FAS	32.23	13.32	169.98	9.72
S	HYS	76.75	31.71	519.05	29.69
S	PSS	14.61	6.04	122.19	6.99

[1/] C = Central, N = North, S = South, Standard = Susceptible strain of African red mite came from Thung Khru district, Bangkok province for standard check

[2/] RR = Resistance Ratio = LC_{50} of field strain/ LC_{50} of susceptible strain

[3/] RR = Resistance Ratio = LC_{95} of field strain/ LC_{95} of susceptible strain

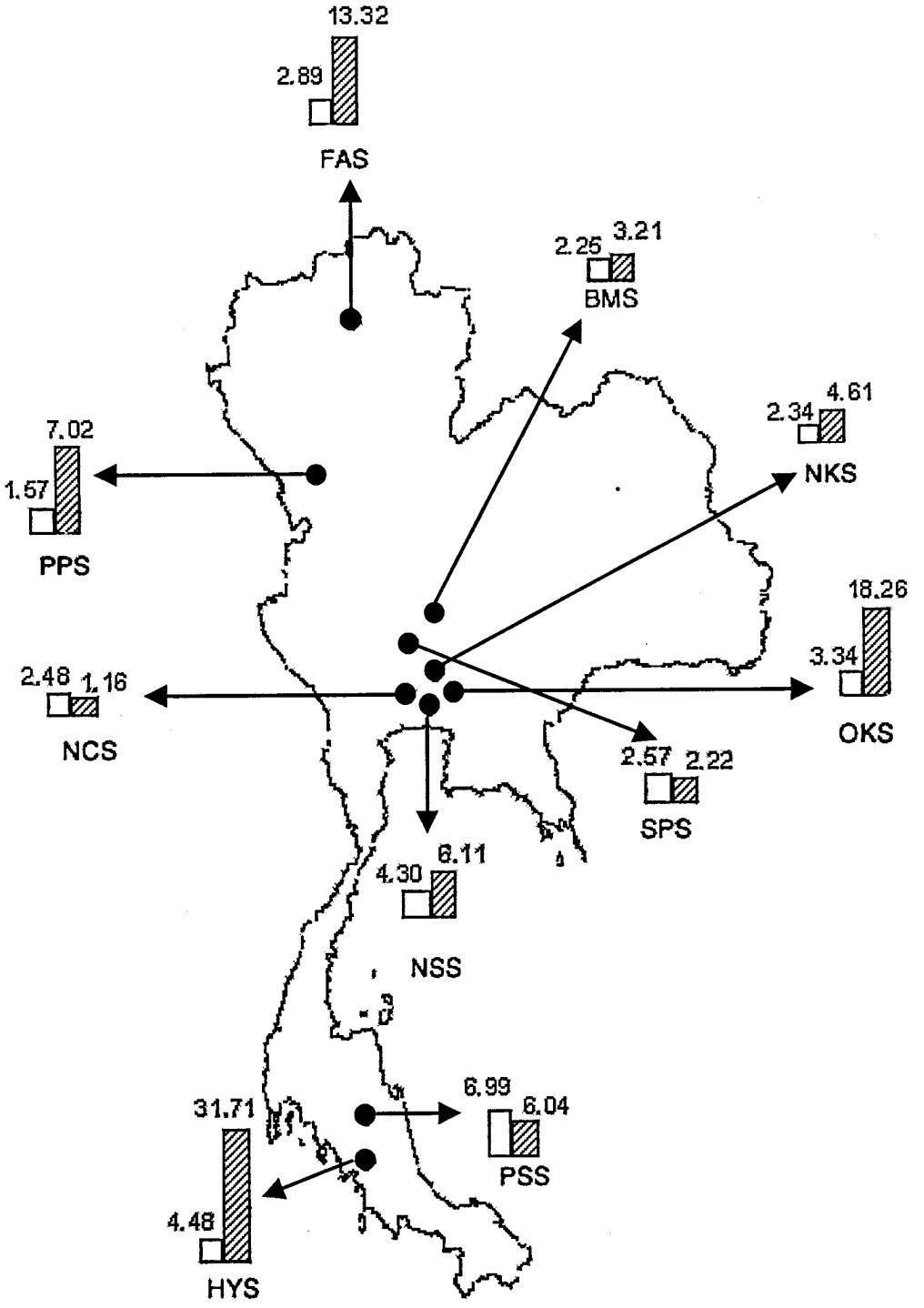


Figure 27 Mapping of resistance ratio (at LC_{50}) of field strains of African red mite, *E. africanus*, against acaricides, amitraz and dicofol

2. Study on developmental stages, female longevity and fecundity of susceptible and field strains of African red mite

Field and susceptible strains of *E. africanus* were reared on Indian coral leaflet in the laboratory. The developmental stages of them were shown in Table 28, life cycle, female longevity and fecundity of them were shown in Table 29.

All eggs of field and susceptible strains hatched in 4.35 days (in average) and no statistically significant differences were detected on their embryonic development.

The larval development of Thung Khru strain (1.71 days), the susceptible strain of *E. africanus* was not significantly different as compared to all field strain, but Pob Pra strain (1.53 days) developed significantly faster than those on Nong Khae strain (1.83 days) and Fang Strain (1.87 days)

The developmental period of protonymph of susceptible and field strains developed in 1.73 days (in everage), were not significantly different, however, protonymphal stage lasted longer (1.90 days) on Nong Khae and Fang strains (field strain) than those on Thung Khru strain (susceptible strain) and other field strains (1.62–1.76 days).

The developmental period of deutonymph on field and susceptible strains developed in 1.38 days (in average), were not significantly different, however, deutonymphal stage lasted longer (1.58 days) on Fang strain (field strain) than those on Thung Khru strain (susceptible strain) and other field strains (1.17–1.55 days).

The longest life cycle of *E. africanus* on Fang strain (field strain) in 9.74 days was significantly different when compared to life cycle of susceptible strain and the rest field strains (8.93 – 9.26 days). The shortest fertilized female longevity of *E. africanus* on Thung Khru strain (susceptible strain) was 7.32 days, significantly different when compared to fertilized female longevity (10.00 days) on field strain (Ongkharak strain), but not significantly different. The life cycle of susceptible strain and other field strain were not significantly different from the rest field strains. However, fertilized female longevity on all field strains were not significantly different.

Table 28 Developmental stages of susceptible and field strains of African red mite, *E. africanus*, under laboratory condition

Strains	Developmental stages (days)			
	E[2/]	LV	PN	DN
Pob Phra Strain	4.30	1.53 a[1/]	1.75	1.55
Phrasaeng Strain	4.39	1.58 ab	1.72	1.24
Ban Mi Strain	4.36	1.58 ab	1.62	1.51
Nakhon Chaisi Strain	4.24	1.67 ab	1.76	1.40
Hat Yai Strain	4.50	1.67 ab	1.63	1.33
Ongkharak Strain	4.24	1.70 ab	1.73	1.44
Thung Khru Strain	4.47	1.71 ab	1.64	1.25
Nong Sua Strain	4.33	1.72 ab	1.74	1.47
Suphan Buri Strain	4.27	1.80 ab	1.63	1.17
Nong Khae Strain	4.27	1.83 b	1.90	1.27
Fang Strain	4.47	1.87 b	1.89	1.58
CV(%)	10.20	9.20	13.60	16.60

[1/] Means followed by the same letters are not significantly different at 0.05 level as determined by DMRT.

[2/] E= EGG, LV = LARVA, PN = PROTONYMPH and DN = DEUTONYMPH

Table 29 Life cycle, female longevity and fecundity of susceptible and field strains of African red mite, *E. africanus*, under laboratory condition

Strains	Life cycle (days)	Female longevity (days)	Eggs/fertilized female
Thung Khru Strain (susceptible strain)	9.03 a	7.32 a	20.33 a
Pob Phra Strain	9.19 a	7.83 ab	27.78 ab
Ban Mi Strain	9.06 a	8.70 ab	33.00 abc
Nakhon Chaisi Strain	9.07 a	8.10 ab	33.48 abc
Hat Yai Strain	9.13 a	8.40 ab	34.13 abc
Fang Strain	9.74 b	8.76 ab	35.01 bc
Phrasaeng Strain	9.01 a	9.01 ab	35.69 bc
Nong Sua Strain	9.26 a	8.81 ab	39.17 bc
Suphan Buri Strain	8.93 a	9.20 ab	41.07 bc
Nong Khae Strain	9.23 a	8.80 ab	42.80 c
Ongkharak Strain	9.11 a	10.00 b	43.21 c
CV(%)	21.31	13.20	21.47

1/ Means followed by the same letters are not significantly different at 0.05 level as determined by DMRT.

The least number of eggs/fertilized female of *E. africanus* on susceptible strain (20.33 eggs) was not significantly different when compared to some field strains were Pob Phra, Ban Mi, Nakhon Chaisi and Hat Yai (27.78-34.13 eggs) but significantly different from the rest field strains of Fang, Phrasaeng, Nong Sua, Suphan Buri, Nong Khae and Ongkharak (35.01-43.21 eggs). However, the number of eggs/fertilized female on all field strains, except from Pob Phra were not significantly different.

3. Study on acaricide resistance in African red mite by biochemical technique

3.1. Study on number of African red mites per homogenization for esterase activity

The African red mite of Hat Yai Strain in batches of between 113-900 mites were homogenized. Lower number of them (113,225 mites per batch) were non detection (ND). Higher number of them (450 mites per batch) gave about 44.29% paranitrophenol activity per mg of mite (0.031 nanomole paranitrophenol produced/min/mg mite) compared to a batch size of 900 mites. The highest number of African red mite (900 mites per batch) gave the highest nanomole (paranitrophenol produced/min/mg mite) (Table 30). Hence, batch of 900 mites was used for subsequent experiments. This number (ca 50 mg) was used for all other strains.

3.2. Study on incubation pH of African red mite per homogenization for esterase activity

The enzymes will show the highest activity when they are in appropriate incubation medium. Potassium phosphate buffer 0.1 M and Hat Yai strain were used in this study for comparing various incubation media of pH 6.5, 7.0, 7.5 and 8. The highest activity of 0.079 nanomole paranitrophenol produced/min/mg mite at pH 7.5 (Figure 28).

Table 30 Effect of number of African red mites, *E. africanus*, per homogenization on esterase activity [a/] from Hat Yai strain

No. of African red mites	Nanomole paranitrophenol produced/min/mg mite
113 (6.25 mg)	ND [b/]
225 (12.50 mg)	ND
450 (25.00 mg)	0.031
900 (50.00 mg)	0.070

[a/] = Homogenizing media was 0.1 potassium phosphate buffer, pH 7.5, containing 1 mM EDTA, 50% (w/w) PVPP.

[b/] = Non-detection

3.3 Study on the amount of esterase activity in susceptible and field strains

Form study of 1 and 2, 900 mites and incubation media of pH 7.5 were use in this study. The amount of esterase activity of all strains were shown in Table 31. Thung Khru Strain (susceptible strain) had the activity of 0.032 nanomole (paranitrophenol produced/min/mg mite). Ongkharak strain had the highest activity of 0.152 nanomole. Fang, Hat Yai, Phrasaeng, Nong Khae, Ban Mi, Nong Sua, Pob Phra, Nakhon Chaisi, Suphan Buri strains had the activities of 0.099, 0.079, 0.069, 0.061, 0.055, 0.044, 0.041, 0.035 and 0.016 nanomole, respectively (Figure 29). The enzyme ratio values of Ongkharak, Fang, Hat Yai, Phrasaeng, Nong Khae, Ban Mi, Nong Sua, Pob Phra, Nakhon Chaisi and Suphan Buri strains were 4.75, 3.09, 2.47, 2.16, 1.91, 1.72, 1.38, 1.28, 1.09 and 0.5-fold, respectively higher than Thung Khru strain.

3.4 Study on the amount of glutathione S-transferase activity in susceptible and field strains

The amount of glutathione S-transferase activity of all strains were shown in Table 31. Thung Khru strain (susceptible strain) had the lowest activity of 3.666 nanomole (CDNB conjugated/min/mg mite). Hat Yai strain had the highest activity of 17.006 nanomole. Ongkharak, Pob Phra, Fang, Nong Khae, Phrasaeng, Nong Sua, Ban Mi, Suphan Buri and Nakhon Chaisi strains had the activities of 13.239, 6.925, 6.772,

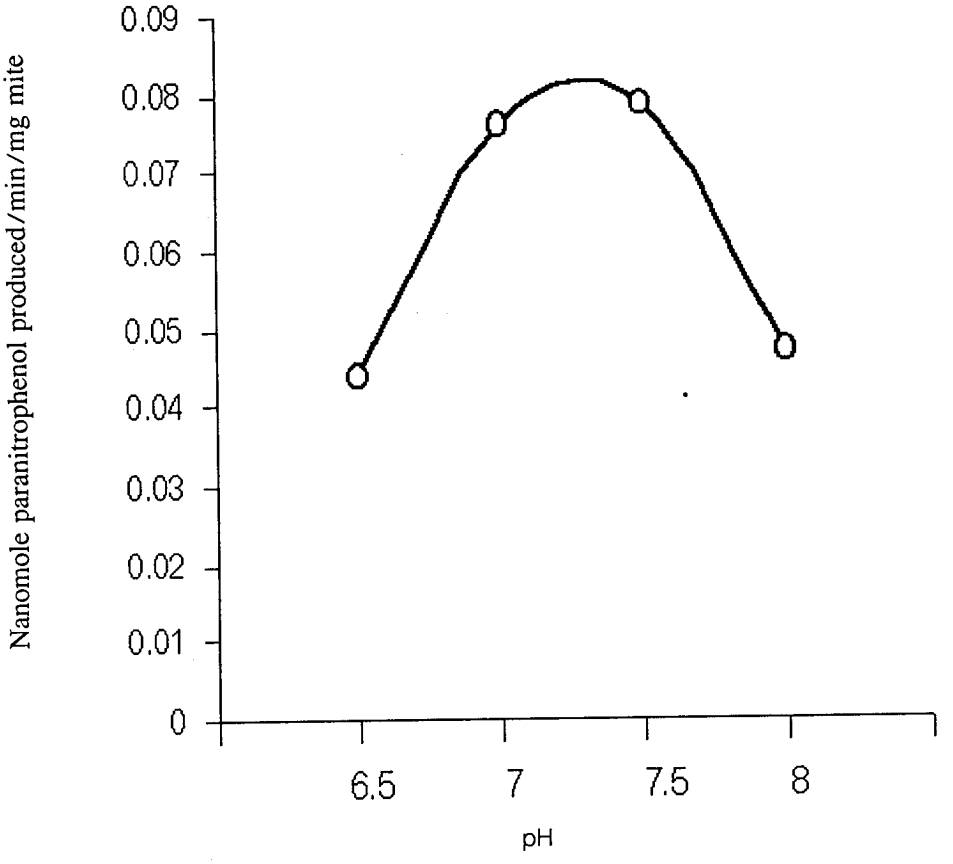


Figure 28 Effect of incubation pHs of African red mite, *E. africanus*, per homogenization on esterase activity in Hat Yai strain

Table 31 Comparison between enzyme quantities in various strains of African red mite, *E. africanus*, and RR value (at LC_{50}) of amitraz and dicofol

Codes	RR of amitraz	RR of dicofol	Esterase quantity (nanomole paranitrophenol produced/min/mg mite)	GST quantity (nanomole CDNB conjugated/ min/mg mite)
TKS	1.00	1.00	0.032	3.666
SPS	2.57	2.22	0.016	4.888
NSS	4.30	6.11	0.044	6.212
NKS	2.34	4.61	0.061	6.314
OKS	3.34	18.26	0.152	13.239
BMS	2.25	3.21	0.055	5.296
NCS	2.48	1.16	0.035	4.073
PPS	1.57	7.02	0.041	6.925
FAS	2.89	13.32	0.099	6.772
HYS	4.48	31.71	0.079	17.006
PSS	6.99	6.04	0.069	6.232

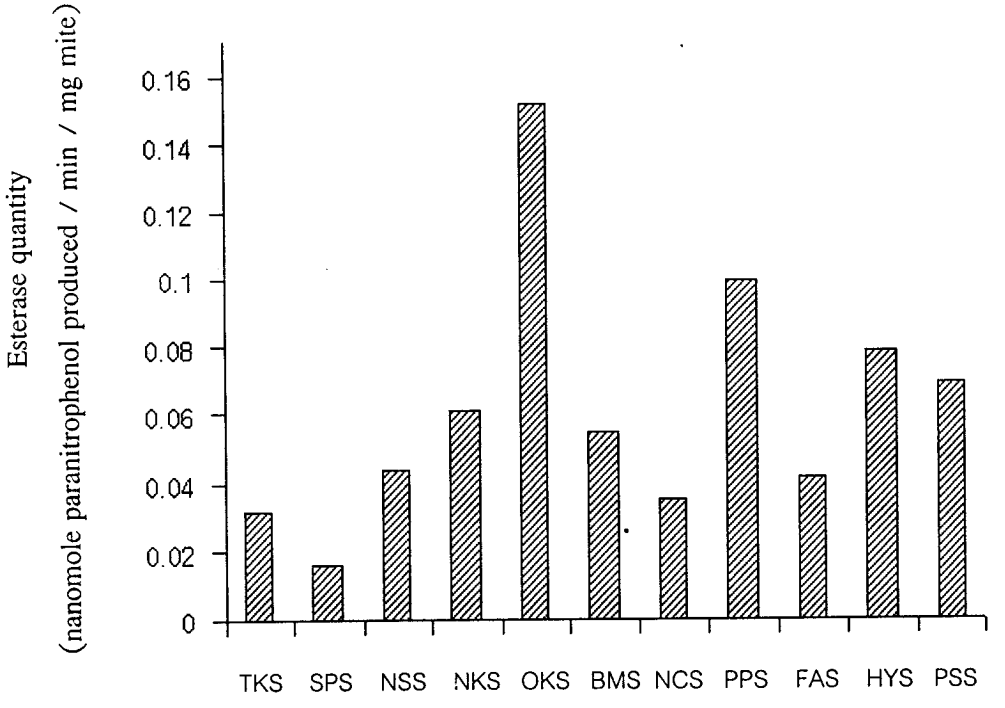


Figure 29 The esterase quantity found in different strains of *E. africanus*

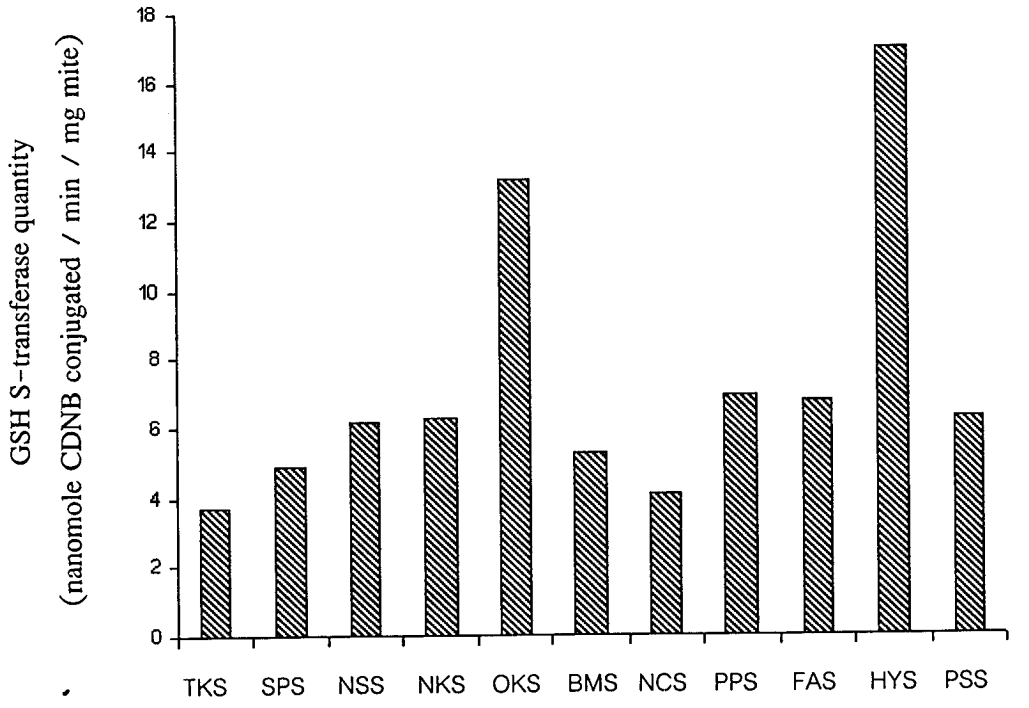


Figure 30 The glutathione S-transferase quantity found in different strains of *E. africanus*

6.314, 6.232, 6.212, 5.296, 4.888 and 4.073 nanomole, respectively (Figure 30).

The enzyme ratio values of Nokhon Chaisi, Suphan Buri, Ban Mi, Nong Sua, Phrasaeng, Nong Khae, Fang, Pob Phra, Ongkharak and Hat Yai strains were 1.11, 1.33, 1.44, 1.69, 1.70, 1.72, 1.85, 1.89, 3.61 and 4.64-fold, respectively higher than Thung Khru strain.

Stepwise regression techniques were used to find which factors from 5 factors such as X_1 = esterase(Table 31), X_2 = glutathione S-transferase (Table 31), X_3=life cycle, X_4 = fertilized female longevity and X_5 = eggs laid by fertilized female (Table 28,29) were related with RR of Y_1 = amitraz or Y_2 = dicofol.

For amitraz, the correlation was used to find the relationship between Y_1 and X_1, X_2, X_3, X_4 and X_5. The result showed that the correlation coefficient (r) between Y_1 and X_1, Y_1 and X_2, Y_1 and X_3, Y_1 and X_4 and Y_1 and X_5 were 0.30^{NS}, 0.35^{NS}, -0.06^{NS}, 0.47^{NS} and 0.39^{NS}, respectively. These values revealed that RR of African red mite to amitraz were not significantly correlated with esterase, glutathione s-transferase, life cycle, fertilized female longevity and number of eggs laid by fertilized female. The ultimate result showed that all factors had no influence to the increasing or decreasing of RR of African red mite to amitraz.

For dicofol, the correlation was used to find the relationship between Y_2 and X_1, X_2, X_3, X_4 and X_5. The result showed that the correlation coefficient (r) between Y_2 and X_1, Y_2 and X_2, Y_2 and X_3, Y_2 and X_4 and Y_2 and X_5 were 0.64^{*}, 0.97^{**}, 0.25^{NS}, 0.26^{NS} and 0.19^{NS}, respectively. The results indicated that the RR values of the African red mite to dicofol were not significantly correlated with life cycle, Fertilized female longevity and fecundity, but the RR of this mite to dicofol was highly significant correlated with the quantity of esterase and glutathione S-transferase. The use of stepwise regression gave an ultimate conclusion that there was only one factor that had an influence upon the RR of African red mite to dicofol. It was glutathione S-transferase. This can be shown in the following linear regression equation:

$\hat{Y} = 2.2022X - 7.5353$ ($r = 0.97^{**}$ $r^2 = 0.94$)(Figure 31)

$F_{cal} = 130.89 > F_{0.01(1)(9)} = 10.56$

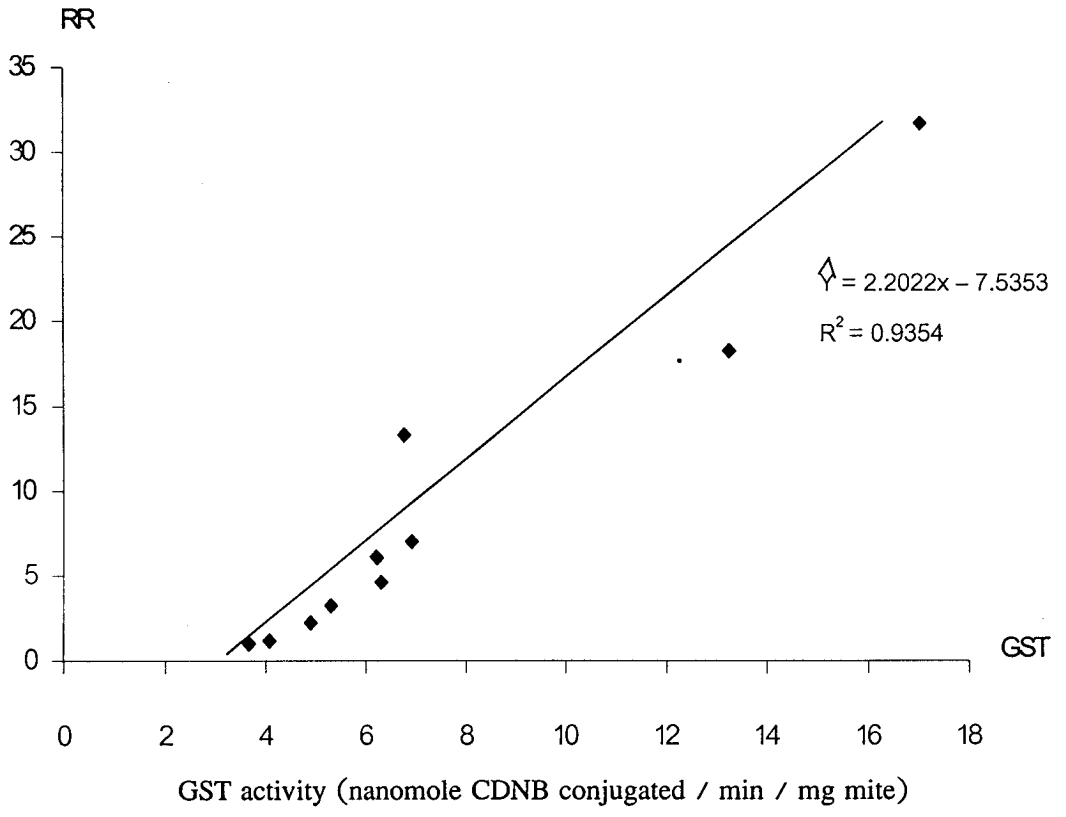


Figure 31 Relation between RR of dicofol (at LC_{50}) and glutathione S-transferase activityin African red mite, *E. africanus*.

This equation means that , RR of the African red mite to dicofol will increase 2.20 units if GST increases 1 unit, GST had the influence upon RR of African red mite to dicofol at 94% and other factors had influences upon RR of this mite to dicofol at 6% and RR value was estimated by the earlier mentioned linear regression equation.

4. Study on toxicity of some candidate acaricides for controlling the African red mite

In this study, the toxicity of some candidate acaricides such as, propargite 20% EC, fenbutatin oxide 55%SC, pyridaben 20%WP and Tebufenpyrad 2%SC for controlling African red mite on Thung Khru Strain (susceptible strain) and Hat Yai Strain (the highest resistant strain of dicofol) were shown in Tables 32-39.

Probit analysis of LC_{50} value for the toxicity test of four acaricides against African red mite were given at 6-7 concentrations. The results were shown in Table 32-39.

The results indicated that the tested acaricides for susceptible strain, pyridaben is the highest effective acaricide for controlling African red mite with LC_{50} value of 1.45 ppm (Table 32), followed by tebufenpyrad, propargite and fenbutatin oxide with the LC_{50} values of 3.04 ppm (Table 33), 7.23 ppm (Table 34) and 48.23 ppm (Table 35), respectively.

The toxicity test of 4 acaricides of African red mite for Hat Yai strain gave the same result as the susceptible strain. Pyridaben is the highest effective acaricide for controlling African red mite with LC_{50} value of 3.76 ppm (Table 36). followed by tebufenpyrad, propargite and fenbutatin oxide with LC_{50} values of 9.19 ppm (Table 37), 32.29 ppm (Table 38) and 76.01 ppm (Table 39), respectively. The results revealed that all acaricides are highly toxic (LC_{50}'s < 100 ppm) to African red mite of both strains (Table 40).

The resistance ratios of *E. africanus* from Hat Yai strain to the tested candidate acaricides were very low as compared to the susceptible strain (Table 41).

Table 32 Toxicity of pyridaben 20%WP at various doses against African red mite, *E. africanus*, from TKS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	6	0.00	0.0000
1	0.39	-0.41	60	10	6.61	0.1101
2	0.78	-0.11	60	16	16.90	0.2817
3	1.56	0.19	60	28	31.68	0.5280
4	3.13	0.50	60	42	45.88	0.7646
5	6.25	0.80	60	57	54.86	0.9143
6	12.50	1.10	60	60	58.69	0.9781

1/ TKS = Thung Khru Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 4.65 + 2.15 x ; LC_{50} = 1.45 ppm

Table 33 Toxicity of tebufenpyrad 2%EC at various doses against African red mite, *E. africanus*, from TKS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	8	0.00	0.0000
1	0.78	-0.11	60	10	6.91	0.1151
2	1.56	0.19	60	15	16.70	0.2783
3	3.13	0.50	60	29	30.64	0.5106
4	6.25	0.80	60	41	44.28	0.7370
5	12.50	1.10	60	54	53.65	0.8941
6	25.00	1.40	60	60	58.12	0.9686

1/ TKS = Thung Khru Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 4.02 + 2.03 x ; LC_{50} = 3.04 ppm

Table 34 Toxicity of propargite 20%EC at various doses against African red mite, *E. africanus*, from TKS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	3.13	0.50	60	16	7.75	0.2959
2	6.25	0.80	60	28	27.78	0.4629
3	12.50	1.10	60	43	38.24	0.6373
4	25.00	1.40	60	47	48.21	0.7868
5	50.00	1.70	60	50	53.55	0.8924
6	100.00	2.00	60	57	57.23	0.9539
7	200.00	2.30	60	60	59.00	0.9833

1/ TKS = Thung Khru Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 3.73 + 1.48 x ; LC_{50} = 7.23 ppm

Table 35 Toxicity of fenbutatin oxide 55%SC at various doses against African red mite, *E. africanus*, from TKS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	3	0.00	0.0000
1	9.38	0.97	60	7	4.63	0.0772
2	18.75	1.27	60	13	12.34	0.2056
3	37.50	1.57	60	23	24.80	0.4134
4	75.00	1.88	60	33	38.97	0.6495
5	150.00	2.18	60	50	50.29	0.8381
6	300.00	2.48	60	60	56.64	0.9440

1/ TKS = Thung Khru Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 1.63 + 2.00 x ; LC_{50} = 48.23 ppm

Table 36 Toxicity of pyridaben 20%WP at various doses against African red mite, *E. africanus*, from HYS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	0	0.00	0.0000
1	0.78	-0.11	60	5	2.59	0.0432
2	1.56	0.19	60	10	10.24	0.1687
3	3.13	0.50	60	20	25.24	0.4207
4	6.25	0.80	60	42	42.61	0.7101
5	12.50	1.10	60	55	54.29	0.9048
6	25.00	1.40	60	60	58.83	0.9805

1/ HYS = Hat Yai Strain

2/ ppm = Calculated from commercial formulation ; Probit (p) = a + bx

$\hat{Y}$ = 3.56 + 2.51 x ; LC_{50} = 3.76 ppm

Table 37 Toxicity of tebufenpyrad 2%EC at various doses against African red mite, *E. africanus*, from HYS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	5	0.00	0.0000
1	1.56	0.19	60	7	3.84	0.0640
2	3.13	0.50	60	10	10.65	0.1776
3	6.25	0.80	60	20	22.22	0.3703
4	12.50	1.10	60	32	36.24	0.6040
5	25.00	1.40	60	46	48.28	0.8047
6	50.00	1.70	60	60	55.62	0.9270

1/ HYS = Hat Yai Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 3.10 + 1.98 x ; LC_{50} = 9.19 ppm

Table 38 Toxicity of propargite 20%EC at various doses against African red mite, *E. africanus*, from HYS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	7	0.00	0.0000
1	12.50	1.10	60	15	12.63	0.2104
2	25.00	1.40	60	21	24.85	0.4141
3	50.00	1.70	60	40	38.68	0.6446
4	100.00	2.00	60	50	49.87	0.8312
5	200.00	2.30	60	55	56.34	0.9390
6	400.00	2.60	60	60	59.02	0.9836

1/ HYS = Hat Yai Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 2.05 + 1.95 x ; LC_{50} = 32.29 ppm

Table 39 Toxicity of fenbutatin oxide 55%SC at various doses against African red mite, *E. africanus*, from HYS[1/] at 48 hrs after treating under laboratory condition

N	Dose (ppm)[2/]	Dose (log)	No.of subjects	Observed responses	Expected responses	Prob
-	0.00	-	60	8	0.00	0.0000
1	37.50	1.57	60	17	18.41	0.3068
2	75.00	1.88	60	34	29.77	0.4962
3	150.00	2.18	60	40	41.19	0.6864
4	300.00	2.48	60	48	50.20	0.8367
5	600.00	2.78	60	54	55.81	0.9301
6	900.00	2.95	60	60	57.68	0.9613

1/ HYS = Hat Yai Strain

2/ ppm = Calculated from commercial formulation ; Probit(p) = a + bx

$\hat{Y}$ = 1.90 + 1.65 x ; LC_{50} = 76.01 ppm

Table 40 Comparative toxicity of some candidate acaricides to African red mite, *E. africanus*, at 48 hrs after treating

Some candidate acaricides	TKS [1/]		HYS [2/]	
	LC_{50} (ppm)	LC_{95} (ppm)	LC_{50} (ppm)	LC_{95} (ppm)
Pyridaben	1.45	8.40	3.76	17.01
Tebufenpyrad	3.04	19.58	9.19	62.48
Propargite	7.23	94.10	32.29	224.57
Fenbutatin oxide	48.23	319.74	76.01	759.38

[1/] TKS = Thung Khru Strain (susceptible strain)

[2/] HYS = Hat Yai Strain

Table 41 Resistance Ratio value of HYS [1/] of African red mite, *E. africanus*, to four acaricides at 48 hrs after treating

Acaricides	RR[2/]	RR[3/]
Fenbutatin oxide	1.58	2.37
Pyridaben	2.59	2.03
Tebufenpyrad	3.02	3.19
Propargite	4.47	2.39

[1/] HYS = Hat Yai Strain

[2/] RR = Resistance Ratio = LC_{50} of field strain/LC_{50} of susceptible strain

[3/] RR = Resistance Ratio = LC_{95} of field strain/LC_{50} of susceptible strain

DISCUSSION

In Thailand the information concerning the resistance of mites to various groups of pesticides are limited. Most of the works have been done on the resistance of insects to certain groups of insecticides. The most important factor for studying acaricide resistance is having a susceptible strain for comparing with field strains to find RR values. First I had contacted to Dr. Meyer, an expert taxonomist of the spider mite in South Africa, but she had no susceptible strain of African red mite. A strain of the African red mite that had not been exposed to pesticides was collected from Indian coral leaves in the mixed crop orchard at Thung Khru district, Bangkok province. It has been reared on Indian coral leaves in the laboratory for 2 years (approximately 56 generations) before bioassays. Ayad (1997) obtained a susceptible strain of an insect for his studying of insecticide resistance by rearing the collected strain in the laboratory for many generations away from chemical use. Significant differences among LC_{50} for populations of each strain with each compound were detected by non overlapping of 95% confidence intervals of the LC_{50} (Knight *et al.*, 1990). The LC_{50} of amitraz and dicofol of a susceptible strain were the lowest level when compared with field strains. Significant differences among LC_{50} for populations of each strain by non overlapping 95% confidence intervals of the LC_{50}.

Sriratanasak and Tayathum (1999) reported that the comparison of LC_{50} of insecticides among 11 WBPH-population implied that the tested populations could not represent a susceptible strain because no populations of Whitebacked Planthopper showed the lowest LC_{50} to nine pesticides. Goka (1998) used the susceptible strain of the Kanzawa spider mite, *Tetranychus kanzawai* Kishida, collected from along period of non-pesticide-sprayed mulberry in the city of Ube, Yamagushi perfecture, Japan. The susceptible strain had also reared on potted beans under laboratory condition for many generations within one year before he used it as a susceptible strain for a study of mode of inheritance of resistance to three new acaricides. Campos *et al.* (1995) reported that two-spotted spider mite collected from greenhouse roses in April 1991 showed an initial resistance ratio of 658 at the LC_{95} relative to the RU-s population. These mites were first cultured in the laboratory without selection pressure and the resistance ratio dropped from 759-to 23-fold (LC_{95}) in 2 weeks (approximately two generations). The resistance ratio decreased to 13-fold over the next 4 weeks. The resistance ratios are obtained by dividing the value of the LD_{50} of the resistant strain by the value of the LD_{50} of the

susceptible strain of the same species (or the laboratory strain reared for many generations away from chemical use (Ayad, 1997). In this study a susceptible namely Thung Khru strain could represent a susceptible strain for studying acaricide resistance of the African red mite according to the result above.

In choosing a method of evaluating the practical significance of resistance in population of spider mites to acaricides, the technique should be simple, provide reproducible results, and simulate as closely as possible the conditions under which the acaricide will be used for mite control (Walker *et al.*, 1973). Leaf-dip bioassay was used in these resistant tests of acaricides according to the method for used on spider mites of White *et al.* (1994) by using the edible leaves for the mites. The simplest method to do these were by dipping the leaves in various concentrations of the acaricides, then the leaves were dried. The mites were released on the leaves when they dried. This method is advised for spider mite not able to use the slide-dip method or slide-spray method (Helle and Overmeer, 1985).

The resistant tests of acaricides against the African red mite in the laboratory in this study cannot be used the slide-dip methods (dipped slide or sprayed slide) because the application on the mites caused quick death within 24 hours the same as Chaiphet's experiment (1995) on the *Eutetranychus orientalis* (Klein). She reported that slide-dip method could not be used for *E. orientalis* because the mites also caused quick death within 24 hours. Slide-dip method could be used for some spider mites such as *Tetranychus fijiensis* Hirst (Phanfak *et al.*, 1975). Huimin and Xinhu (1994) reported that by means of the slide-dip method recommended by FAO, the baseline of *Tetranychus viennensis* Zacher to 13 kinds of insecticides and acaricides were tested for studying of insecticide-resistance of the mite population. The result showed that the mite displayed moderate resistance to dicofol and amitraz. Two-spotted spider mites were evaluated for their susceptibility to abamectin with a leaf residual or leaf-dip assay (Campos *et al.*, 1995).

Study on the monitoring of resistance ratio at LC_{50} by using leaf-dip bioassay against the African red mite from ten tangerine orchards in various parts of Thailand indicated that PSS showed the highest resistance to amitraz with RR value of 6.99 - fold followed by HYS, NSS, OKS, FAS, SPS, NCS, NKS, BMS and PPS with RR values of

4.48, 4.30, 3.34, 2.89, 2.57, 2.48, 2.34, 2.25 and 1.57-fold, respectively. HYS showed the highest resistance to dicofol with RR values of 31.71-fold and followed by OKS, FAS, PPS, NSS, PSS, NKS, BMS, SPS and NCS with RR values of 18.26, 13.32, 7.02, 6.11, 6.04, 4.61, 3.21, 2.22 and 1.16-fold, respectively. Acaricide resistance of African red mites were determined by RR values. Populations with RR of 10 and <10 were considered resistant strain and tolerant strain; RR = LC_{50} of field-collected colony/ LC_{50} of the susceptible colony (Campos *et al.*, 1995; Ayad, 1997; Sriratanasak and Tayathum (1999). The predaceous mite, *Typhlodromus pyri* for which 10-to 100-fold resistance is generally recommended in integrated control programs for controlling *Panonychus ulmi* (Koch) and *Tetranychus urticae* Koch (Croft, 1990).

It was found that the African red mites from all tangerine orchards in the North, the Central and the South of Thailand were tolerant to amitraz because the RR<10 and the mites from HYS in the South, OKS in the Central and FAS in the North showed a resistant tendency to dicofol.

In this study, it was the first report for atendency dicofol resistance of African red mite in Thailand. Dicofol resistance of African red mite was caused by the frequent applications of dicofol, especially in tangerine orchards at Hat Yai district, Songkla province, Ongkharak district, Nakhon Nayok province and Fang district, Chiang Mai province. There were many reports about resistance to the pesticide development that supported this study. For many pest species, the more frequently the pesticide is used, the more frequently the resistance to the pesticide develops (Crow, 1957). A higher frequency of spraying was likely to induce a higher frequency of resistant mites for acaricides (Goka, 1998). Resistance was correlated with the number of applications and the total time of abamectin use (Campos *et al.*, 1995). Several studies indicated that the two-spotted spider mite populations developed resistance to an acaricide by repeated treatment (Jeppson *et al.*, 1975).

Niemczyk *et al.* (1995) reported that the results of laboratory tests indicated the main life parameters of two examined strains of *Typhlodromus pyri* female, were very similar. Both resistant and unresistant females to fenitrothion and phosalone lived throngh about the same time and deposited about the same number of the eggs. These the females of both strains lived about 60 days. Although unresistant females lived at the average of 3

days longer, this difference was not significant. Number of egg laying by females of both populations were very similar. Female of both populations deposited during their life span at average about 43 eggs. Slightly higher mean fecundity of resistant females (about 3 eggs) was not significant. In this study, the main life parameters or biological activities such as life cycle, fertilized female longevity and fecundity of ten field strains to amitraz and dicofol and the susceptible strain were both similar and significantly different. Life cycle of the susceptible strain (9.03 days) was mostly similar when compared with those resistant and tolerant strains (8.93–9.26 days) except that of the resistant strain (from Fang) (9.74 days). Fertilized female longevity of the susceptible stain (7.32 days) was also similar to most of the resistant and tolerant strains (7.83–9.20 days) except that of the resistant strain from Ongkharak (10.00 days). The fecundity of the fertilized female of the susceptible strain (20.33 eggs) was similar when compared with those four resistant and tolerant strains from Pob Phra, Ban Mi, Nakhon Chaisi and Hat Yai. (27.78–34.13 eggs) but significantly different when compared with other six resistant and tolerant strains (Fang, Phrasaeng, Nong Sua, Suphan Buri, Nong Khae and Ongkharak) (35.01–43.21 eggs). However, these biological activities were not correlated or less correlated with the RR of amitraz and dicofol.

Mackness *et al.* (1983) used α–naphthylacetate and β–nitrophenylacetate (PNPA) substrates for general esterase activity in homogenates of three strains of the rust red flour beetle, *Tribolium castaneum* (Herbst). These substrates were hydrolysed by homogenates of all strains. Visetson (1991) found that PNPA activity was measured in three strains of *T. castaneum* with various concentrations of enzymes. PNPA activity was reasonably stable, although the enzyme solution was stored in liquid nitrogen for up to 28 days and the result from both substrates were similar. For the esterase activity in this study, PNPA was used according to Visetson's study and the result was satisfactory.

Visetson (1991) used DCNB and CDNB as the substrates for glutathione S-transferase activity in all experiments except in the using of protecting agents where only DCNB conjugation was employed; this is because DCNB is more straight-forward an easier method to use than CDNB and also trends in activity with both substrates should be similar. CDNB was used as the substrate in this study.

To obtain the specific activity from the calculation, the amount of glutathion S-transferase activity was measured as the rate of change in absorbance and converted to nanomole per minute per milligram of tissue using an extinction coefficient or converted to nanomole per minute per milligram of protein using another extinction coefficient (Rose and Wallbank, 1988; Visetson, 1991). In this study I used the amount of mite (mg of mite) in stead of the amount of protein (mg of protein).

Knowles and Hamed (1990) used 250 (ca 50 mg) bulb mites, *Rhizoglyphus echinopus* (Fumouze and Robin) for propargite metabolism study. 113, 225, 450 and 900 (50 mg) mites were used for esterase activity study in this experiment. 113 and 225 mites were non-detection and 900 mites gave the best effective, so 900 mites were used for esterase activity study. They also used potassium phosphate buffer pH 7.5. Visetson (1991) used potassium phosphate buffer pH 7.5 in esterase activity study of *T. castaneum*. The potassium phosphate buffers were varied into pH 6.5, 7.0, 7.5 and 8 for the study of esterase activity and the pH 7.5 gave the best effective. Two detoxification enzymes : esterase and glutathione S-transferase were detected from enzymes extract.

Stepwise regression test was used to find the relationship of one or both enzymes or biological factors with RR of the African red mite to acaricides, amitraz and dicofol. The result showed that only glutathione S-transferase influenced RR of the mite to dicofol.

The correlation tests were used to find the relationship between the life cycle, the fertilized female longevity, fecundity of the fertilized female, the quantity of esterase and glutathione S-transferase and RR of amitraz and dicofol. The objective of these tests were to select some factors that correlated with RR of amitraz and dicofol to use in the stepwise regression test. The result revealed that there were no correlations between the earlier mentioned factors with RR of amitraz. These meant all factors did not influence the amitraz resistance of African red mite and there were some correlations between the quantity of esterase and glutathione S-transferase with RR of dicofol. The stepwise regression test was used to find what factors that influenced the RR of dicofol. The result revealed that the quantity of glutathione S-transferase increased while the dicofol resistance of African red mite populations increased (r^2 = 0.94, r= 0.97**). It meant that

glutathione S-transferase was a major factor influenced the dicofol resistance of African red mite at 94%.

Our biochemical or physiological studies revealed that glutathione S-transferase was a major mechanism of resistance to dicofol because there was a positive correlation between the amount of GST and RR value of the African red mite to dicofol. Fourniers *et al.* (1985) concluded that glutathione S-transferase activity was significantly linear correlated with the resistance level ($r=0.81$; $P<0.01$). Thus, each strain became resistant due to the increased activity of glutathione S-transferase activity, the sole biochemical mechanism of resistance found in the resistant strain. Motoyama and Dauterman (1977) reported that glutathione S-transerase activity is important in resistance to organochlorine insecticides. Degradation of dicofol by a breakdown enzyme may well be the common mechanism in other species (Cranham and Helle, 1985). The mechanism of resistance to amitraz may be caused by increasing lipids of the cuticle of mites to decrease the absorption of insecticides or acaricides, altering behavior to avoid the lethal effect of toxicant, biochemical reaction with mite body such as increasing monooxygenase enzyme, accelerating excretion to destroy toxicant or developing fat tissue synthesis to absorb more toxicant and make the toxicant of insecticides or acaricides decrease and altered target sensitivity.

Biochemical or physiological resistance studies in African red mite populations involve the detoxification enzymes. The aim is to determine what detoxification enzymes are involved in resistance. The possibility of adequate mite control by future acaricides are not very high when there is not this knowledge. Although biochemical resistant mechanisms study requires complex techniques and many kinds of equipment. This knowledge is very important in acaricide resistance management. There has been no study performed on the biochemical basis of acaricide resistance in African red mite populations. It is convenient to study in the laboratory. This is a new method for biochemical resistance study in African red mite populations in Thailand. The problem in this method are : 1. It requires adequate knowledge in both toxicology and biochemistry and 2. the chemicals for biochemical detection are very expensive. From the toxicity test of some acaricides are derived RR of African red mite, while the quantity of detoxification enzymes in detoxification enzymes extracting method is determined. If researchers are able to carry out proper and accurate experiments using detoxification enzyme extracting

method, the quantity of detoxification enzymes can be determined to match with RR values of African red mite as standard. Studies on biochemical or physiological mechanism could also help in designing resistance detection tests. When the mechanisms have been clearly defined, analyses can be used to characterize specific enzymatic reactions responsible for resistance (Vidal and Kreiter, 1995).

Study on toxicity of some candidate acaricides, propargite, fenbutatin oxide, pyridaben and tebufenpyrad revealed that all acaricides exhibited highly toxic to African red mite of Hat Yai strain (LC_{50}'s<100 ppm). Compounds with LC_{50}'s<100 ppm were considered highly toxic, those with LC_{50}'s ranging from 100 to 1,000 ppm as moderately toxic and those with LC_{50}'s>1,000 ppm as non-toxic (Knowles *et al.*, 1988). Propargite (Omite 20%EC) have already been registered in Thailand for controlling African red mite in tangerine orchard and it was found that this mite was not resistant to propargite (RR <10) in tangerine orchard on Hat Yai strain, the highest resistant strain of dicofol. It is used for controlling African red mites that were resistant to dicofol or used for rotation with dicofol for reducing the problem of acaricide resistance of African red mite. Bruce-Oliver and Grafton-Cardwell (1996) reported that propargite was rotated with dicofol in cotton fields for controlling tetranychid densities. They suggested that propargite helped to reduce *T. urticae* resistance to dicofol. Smith and Papacek (1993) reported that propargite is one of the most commonly used acaricide in tangerine orchard the same as dicofol and amitraz. It is generally non disruptive the most beneficials including phytoseiid mites. Fenbutatin oxide has been registered for controlling this mite in pummelo and durian orchards, pyridaben has been registered for controlling *Phyllocoptruta oleivora* (Ashmead) in pummelo orchard and for controlling *E. africanus* in durian orchard and tebufenpyrad has been registered for controlling *E. africanus* in durian orchard. Hat Yai strain of *E. africanus* which showed the highest resistant to dicofol, was non-resistant to these acaricide. It indicated that dicofol showed no cross-resistant to these acaricides.

Thwaite *et al.* (1996) reported that laboratory bioassays also showed that tebufenpyrad was toxic to the predatory mite, *Typhlodromus pyri*, but it was approximately 70 times less toxic to the predator than to *P. ulmi* at the LC_{50} . Field trials in 4 commercial apple orchards in New South Wales using 4 concentrations of tebufenpyrad (10, 7.5, 5 and 2.5 g a.i./100 liters) confirmed that it was selectively more toxic to the pest.

In the near future, if these acaricides; Tebufenpyrad, pyridaben and fenbutatin oxide are going to be registered for controlling *E. africanus* in tangerine, they will have been used with the resistant strains to replace dicofol or used together with dicofol and amitraz for rotation to delay acaricide resistance development of the African red mite. Propargite can be used together with dicofol and amitraz or used instead of dicofol for the control of resistant African red mite. Propargite has already been registered and the African red mite has not yet developed resistance to this acaricide.

CONCLUSION

The African red mite, *E. africanus*, is widely distributed in Thialand on the tangerine. It feeds on the upper leaf surface and on the fruit. The monitoring of acaricide resistance of the mites were carried out during January 1999–December 1999. Ten strains of the mites which were heavily sprayed with amitraz and dicofol were collected from various tangerine orchards in the North, the Central and the South of Thailand. Resistance bioassays were studied in laboratory condition of 26.4±1.3°C, 65.3±2.3%RH and uder the light from fluorescent lamps for 9 hrs per day. Adult female mites of all strains including susceptible strain were tested with amitraz and dicofol by leaf-dip bioassay technique to obtain LC_{50} (ppm) values. This study revealed that the resistance ratio (RR) of LC_{50} (ppm) value of various field strains compared to the LC_{50} (ppm) value of the susceptible strain showed that the populations of African red mite from Pob Phra, Ban Mi, Nong Khae, Nakhon Chaisi, Suphan Buri, Fang, Ongkharak, Nong Sua, Hat Yai and Phrasaeng strains were non resistant to amitraz (RR ranged from 2.25-6.99-fold) and the populations of African red mite from Fang, Ongkharak and Hat Yai strains showed some tendency to be resistant to dicofol (RR ranged from 13.32-31.71-fold) and from Nakhon Chaisi, Suphan Buri, Ban Mi, Nong Khae, Phrasaeng, Nong Sua and Pob Phra strains were tolerant to dicofol (RR ranged from 1.11-6.38-fold).

Studies on the life cycle, the fertilized female longevity and fecundity of the fertilized female of resistant and tolerant strains (10 strains) and susceptible strain found that all strains of *E. africanus* could develop well on the same host plant. The life cycle of susceptible strain (9.03 days) was significantly shorter than that of the resistant strain from Fang (9.74 days). The fertilized female longevity of susceptible strain (7.32 days) was significantly shorter than that of the resistant strain from Ongkharak (10.00 days). The fecundity of the fertilized female of susceptible strain (20.33 eggs) was significantly lower than those of some resistant and tolerant strains from Fang, Phrasaeng, Nong Sua, Suphan Buri, Nong Khae and Ongkharak (35.01-43.21 eggs).

Stepwise regression analysis was used to find out which biological activities (life cycle, fertilized female longevity and fecundity of fertilized female) or biochemical activities (two detoxification enzymes, esterase and glutathione S-transferase) has an influence upon the resistance ratio (RR). For resistance ratio of amitraz, the biological

activities and the biochemical activities were not proportional to the RR of amitraz. For resistance ratio of dicofol, the biological activities were not correlated with the RR of dicofol and for the biochemical activities; only the quantities of glutathione S-transferase influenced the RR of dicofol at 94%. There was a significant positive correlation between the quantities of glutathione S-transferase and RR of dicofol. The simple linear regression was $\hat{Y}$ =2.2022 X -7.5353, R^2=0.94.

Toxicity test of some candidate acaricides, pyridaben, tebufenpyrad, propargite and fenbutatin oxide showed highly toxic (LC_{50}'s<100 ppm) to the highest resistant strain of African red mite and showed the low levels of resistance ratio to this mite.

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APPENDICES

Appendix A

Appendix Table 1 LC_{50} analysis of acaricide for African red mite

Probit analysis (Finney, 1971) was used to obtain LC50 value of the acaricides toxicity to African red mite. SPSS program was used in this analysis. For example; Probit : Mitac, Hat Yai strain

	DOSE	TOTAL	RESPONSE
1	0	60	0
2	25.00	60	3
3	50.00	60	6
4	100.00	60	14
5	200.00	60	29
6	400.00	60	45
7	600.00	60	60

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DATA Information

6 unweighted cases accepted.

0 cases rejected because of missing data.

1 case is in the control group.

0 cases rejected because LOG-transform can't be done.

MODEL Information

ONLY Normal Sigmoid is requested.

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Parameter estimates converged after 12 iterations.

Optimal solution found.

Parameter Estimates (PROBIT model : (PROBIT(p))= Intercept + BX):

	Regression Coeff.	Standard Error	Coeff./sE

DOSE	2.44376	.21053	11.60773
	Intercept	Standard Error	Intercept/S.E.
	-5.48749	.47412	-11.57410

Pearson goodness of fit of Chi-square = 12.533 DF = 4 P = .014

Since goodness of fit of Chi-square is significant, a heterogeneity factor is used in the calculation of confidence intervals.

PROBIT ANALYSIS

Appendix Table 2 Observed and expected frequencies

DOSE	Number of Subjects	Observed Responses	Expected Responses	Residual	Prob
1.40	60.0	3.0	1.150	1.850	.01917
1.70	60.0	6.0	5.450	.550	.09084
2.00	60.0	14.0	16.456	-2.456	.27427
2.30	60.0	29.0	33.238	-4.238	.55396
2.60	60.0	45.0	48.493	-3.493	.80821
2.78	60.0	60.0	54.209	5.791	.90348

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Appendix Table 3 Confidence intervals for effective dose

Prob	DOSE	95% confidence intervals	
		Lower	Upper
.01	19.65868	3.81086	40.04099
.02	25.41577	5.89066	48.43793
.03	29.91420	7.75503	54.73031
.04	33.81568	9.52876	60.04976
.05	37.36158	11.25923	64.79920
.06	40.67115	12.97041	69.17473
.07	43.81336	14.67647	73.28872
.08	46.83228	16.38658	77.21256
.09	49.75812	18.10718	80.99506
.10	52.61266	19.84298	84.67146
.15	66.28173	28.85437	102.22368
.20	79.63646	38.58248	119.57199
.25	93.21869	49.15888	137.74389
.30	107.37922	60.66144	157.55156
.35	122.41440	73.13937	179.83224
.40	138.62344	86.62733	205.57051
.45	156.34584	101.16356	236.00065
.50	175.99793	116.81705	272.73068
.55	198.12023	133.72397	317.93173
.60	223.44903	152.13300	374.66341
.65	253.03619	172.46443	447.46814
.70	288.46617	195.40255	543.51906
.75	332.28607	222.07076	675.01708
.80	388.95842	254.41654	864.79977
.85	467.32744	296.19615	1161.81770
.90	588.74179	356.13639	1696.36805
.91	622.51700	372.01635	1860.41151
.92	661.40864	389.94605	2057.30935
.93	706.98244	410.51621	2298.74910
.94	761.60307	434.60595	2603.05597

Appendix Table 3 (Contd.)

Prob	DOSE	95%confidence intervals	
		Lower	Upper
.95	829.06749	463.60358	3000.92744
.96	916.00333	499.88624	3548.63784
.97	1035.47044	548.03114	4363.79212
.98	1218.74228	618.68063	5749.95906
.99	1575.65354	747.63438	8897.40648

Appendix B

1. Chemical preparation for detoxification enzymes experiment

1.1 Reagents for esterase enzyme extraction

- 0.1 M KH_2PO_4 preparation

  136.09 gm of KH_2PO_4 was weighed and dissolved in 1,000 ml of distilled water for proparing 1 M KH_2PO_4 solution. 400 ml of 0.1 M KH_2PO_4 was prepared by diluting 40 ml of 1 M KH_2PO_4 solution with 360 ml of distilled water. pH of the solution was adjusted to 7.5 and 7.

- 0.001 M EDTA in 0.1 M KH_2PO_4 solution preparation

  0.09 gm of EDTA was weighed and dissolved in solution of 200 ml of 0.1 M KH_2PO_4. The solution was adjusted by saturated NaOH solution until a buffer reached pH 7.5 by using the least volume of saturated NaOH solution.

- 0.01 M GSH preparation

  0.092 gm of GSH was weighed and dissolved in solution of 30 ml of 0.001 M EDTA in 0.1 M KH_2PO_4.

- PNPA preparation

  0.1 gm of PNPA was weighed and dissolved in 5 ml of ethanol.

1.2 Reagents for GST enzyme extraction

- 0.1 M KH_2PO_4 preparation

  0.1 M KH_2PO_4 solution was prepared and adjust pH 7.

- 0.001 M EDTA preparation

  0.001 M EDTA was prepared in KH_2PO_4.

- 0.01 M GSH preparation

  0.092 gm of GSH was weighed and dissolved in 30 ml of KH_2PO_4. The solution was adjusted pH 7.5. 0.092 gm GSH was weighed and dissolved in 30 ml of 0.001 M EDTA in KH_2PO_4 (pH 7).

- CDNB preparation

  0.15 gm of CDNB was weighed and dissolved in 5 ml of ethanol.

2. Calculation of specific activities for spectrophotometric assays

2.1 Esterase with PNPA

Nanomole paranitrophenol produced/min

= OD/min/mg African red mite x 58.8235 x total volume of assay (ml)

Tolal volume of assay = $\frac{50 \text{ ml}}{1{,}000}$ (Mackness *et al.*, 1983)

2.2 Glutathione S-transferase with CDNB

Milimole CDNB conjugated/min

= $\frac{\text{OD} \times 1.31}{9.6 \times 1{,}000}$

Nanomole CDNB conjugated/min

= $\frac{\text{OD} \times 1.31 \times 10^6}{9.6 \times 1{,}000}$ (Kitchin, 1983)