

BIOLOGICAL EFFECTS OF ALIPHATIC SESQUITERPENE ANALOGUE ON THE DEVELOPMENTAL STAGES OF THE FLESH FLY, *PARASARCOPHAGA RUFICORNIS* FABR. (DIPTERA : SARCOPHAGIDAE)

NOUWARATN SUKHAPANTH^a AND CHITAPA KETAVAN^b

^a Department of Biology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand.

^b Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

(Received March 15, 1995)

ABSTRACT

Aliphatic sesquiterpene analogue (ASA) was tested for its biological activity against egg hatching and the various developmental stages of flesh flies (Parasarcophaga ruficornis Fabr.) utilizing the topical drop and dipping methods. Morphological studies were made of all stages of treated and untreated flies using stereomicroscopic and SEM methods. ASA exerted the same effects on all stages tested, and a single dose of application affected all subsequent stages of the life cycle. Eggs exhibited shrinkage, malformation, breakage, and shell decomposition. In the early stages, new larval cuticle layers were not fully formed which interfered with molting. ASA also affected the reproductive system, resulting in failure of mating, interference with oogenesis and malformation of eggs in ovariole sacs, and low fecundity. In addition, it affected the body size, body color, and the development of the eyes and wings. In the treated adults, ASA shortened longevity, lowered the rates of mating and oviposition and inhibited the growth of ovaries and ovarioles.

INTRODUCTION

Synanthropic flies are medically important insects which serve as vectors of many pathogenic organisms which cause both acute and chronic diseases to people and livestock. The flesh fly (*Parasarcophaga ruficornis* Fabricius: Sarcophagidae) is found indoors and outdoors, in garbage piles, feces and organic material near human dwellings and animal sheds¹. It occurs in many tropical and subtropical regions^{3,4}, including Thailand. *P. ruficornis* is a large grayish flesh fly with a grayish-black checkered abdomen and black-striped thorax. A few species are brownish-black in color, but the thoracic stripes are always present and the body is metallic⁵. Adults feed on nectar, sap, fruit juices, and honey-dew. Larvae are frequently found on garbage piles, decaying organic matter and at animal wounds or sores. The life cycle of flesh flies, from egg to adult, takes 22 days at 32-36°C, and approximately 4 weeks at 29°C. The females lay eggs which usually develop in skin pustules, carrion or feces. Occasionally adults are larviparous and oviparous with relatively few maggots⁶⁻⁸. The flesh flies may act as vectors and carriers of cholera, acute diarrhea, dysentery, typhoid,

paratyphoid, other enteroviral infections, poliomyelitis, yaws, conjunctivitis, ophthalmia, tuberculosis, shigellosis etc. The methods of transmission are both direct and indirect. The larval stages of *P. ruficornis* in certain areas of the tropics cause myiasis in humans and domestic animals¹.

The rapid selective evolution of resistance to chemical pesticides by pests has caused failure in many vector control campaigns⁹. Since the flies are closely associated with humans, chemical insecticides used in any control program could be hazardous to humans and cause certain environmental problems. In spite of these problems, the prospects for safer chemical control are still encouraging. It would be desirable to find chemical agents with toxicity its more specific to insect and arthropod pests.

The insect growth regulator substances having aliphatic sesquiterpene lactone groups which act as juvenile hormone analogs have attracted a great deal of attention due to their selective activities specific to target pests and their non-persistence in the environment. Much progress has been made and some insect growth regulators have exhibited enough promising selective properties to be introduced for practical use, either for vector control or as a complementary measure in integrated control operations.

Admas (1974)¹⁰ reported that insect growth regulators caused a wide range of morphogenic and biological activity changes in ovarian follicle morphogenesis in house flies, snipe flies and horn flies. Exposure of eggs to juvenile hormone analogue affected all the developmental stages of flies and finally caused death to larvae and adults^{11,12}.

However, research on synthetic insect growth regulator substances (aliphatic sesquiterpene analogues) to control pests in Thailand has lagged due to our limited knowledge of both the insects and the biological activity of the compounds. Both laboratory and field studies of aliphatic sesquiterpene analogues (ASA) for insect pest control have recently been carried out in this region. This investigation was performed mainly to analyze the effects of ASA on all development stages of the flies, and determine the percent mortality, using the topical drop and dipping methods. The ultimate goals were thus to find the mode of action of ASA substances, and to develop a method to control these flies which are among our most serious pests.

According to Roller (1975)¹⁵ and Gringrich and Hapkins (1978)¹⁶, flies have developed mild resistance to many types of insecticides, and moderate resistance to insect growth inhibitors such as methoprene, or Altosid[®]. Schwarz and Miller (1979)¹⁷ reported that the compounds related to juvenile hormone, arylterpenoid and 9-(p-isopropylphenyl)-2-ethoxy-2,6-dimethyl-nonane, showed outstanding activity against the house fly, face fly and stable fly, and that they inhibited up to 97-100% of the development from pupal to adult stage. The synthetic aliphatic sesquiterpene lactone analogue is no doubt one of the most effective compounds and will be soon introduced into control programs for important insects and other arthropod pests.

MATERIALS AND METHODS

Rearing the Flesh Fly: The rearing media were placed in a petri dish in each cage, and in addition meat soaked in water was provided as a site of oviposition. The larval medium used in this experiment was that used by Sukhapanth and Ketavan (1984)⁸ and Sukhapanth, *et al.* (1986)¹⁸. The eggs, first, second, and third instar larvae, puparia and adults were collected from the rearing cages and placed in 300 ml glass bottles containing meat soaked in water to serve as their diet.

Laboratory tests used 2-methoxyl-9-(4-isopropylphenyl)-2, 6-dimethylno-nane. This aliphatic sesquiterpene analogue (ASA), arylterpenoid compound was of technical grade.^{a/}

ASA was diluted to appropriate concentrations by adding 1 ml of stock solution in 99 ml of acetone. The concentrations of ASA used for laboratory tests were 0.001, 0.01, 0.1, 1 and 10 mg/l. The solutions were tested against eggs, larvae, puparia and adults of *P. ruficornis* by means of topical drop and dipping methods. There were 5 replications of each treatment plus an untreated check (Tables 1-6).

In the topical drop method, each specimen is exposed to 3 μ l of aliphatic sesquiterpene analogue at given concentration with a microdispenser (DrummondÆ CAT # 510). The eggs, larvae and adults were anesthetized with CO₂ for 3 min prior to application, and each treatment group (100 flies) was placed in a separate plastic box at room temperature. The treated specimens were checked daily for survival and morphological changes.

In the dipping method, 20 specimens of each stage (eggs, first, second, third instar larvae, puparia and adults) were soaked together in a 100 ml beaker containing 60 ml of the compound at a given concentration. After two minutes, the specimens were transferred to plastic boxes and kept at room temperature. Sarcophagid larvae

were transferred to glass bottles covered with nylon cloth to prevent them from crawling out. Five replications of each stage and concentration of ASA were made in this experiment.

The treated specimens (both topical drop and dipping methods) were checked for survival daily for 5 days. The dead specimens were kept in 5% formalin for morphological study. The number of dead flies in each concentration was corrected by using the Abbott's formula and the LC₅₀ values of the compound were determined through log-concentration-probit mortality regression lines. Data in these experiments were analyzed with probit analysis program on a computer.

Biological studies: Additional records were kept on percentage of hatching, the molting of each stage of larvae, pupation and adult emergence together with adult fecundity.

Morphological studies: Abnormal specimens treated with ASA were studied under a stereo microscope to determine their morphological changes in comparison with the normal form.

^{a/}Aliphatic sesquiterpene analogue with 93.3% active ingredient, 80.2% para, and 13.3% ortho.

RESULTS AND DISCUSSION

The effectiveness of insect growth regulator substance (aliphatic sesquiterpene analogues: ASA) against the eggs, larvae, puparia and adults will be reported in two parts. The effects on survivorship of the different stages will be described first followed by discussion of the morphological aberrations obtained.

Survival of Eggs and Larvae

The percentage hatching of the treated flesh fly eggs were significantly lower than that for the untreated eggs at an ASA concentration of 0.01 mg/l with the dipping method and 0.1 mg/l with the topical drop method (Tables 1 and 2). When the eggs of *P. ruficornis* were exposed to the 0.1 mg/l ASA the percentage of hatching was 55% by the topical drop method but 63% in the dipping method (Table 1).

At the lowest concentration of 0.001 mg/l all developmental stages of the flesh flies survived, and many reached the adult stage. The percentages of pupation and adult emergence obtained from the two methods of application are presented in Table 1. The percentages obtained from both methods of treating eggs with various concentrations of ASA compound were significantly different from the check (Tables 1-4 and Figures 1-2).

The first instar larvae of flies were treated with 0.1 mg/l of ASA compound. The percentage mortalities of the first instar larvae in the treatments with 0.1 mg/l of ASA topical drop and dipping methods were 56 and 86% respectively. It should be noted here that some of them could not survive and died in the second instar. The death in pupal-adult intermediate form of *P. ruficornis* died in the mature first larval stage (Tables 1 and 2).

After exposure of second instar larvae to 1 mg/l ASA, the percentage mortalities obtained in topical drop and dipping methods respectively, were 80 and 100%. In the treatments of the third instar larvae, the percentage mortalities were 55 and 93% (Tables 2-3).

Exposure of second instar larvae of *P. ruficornis* to a concentration of 1 mg/l of ASA compound resulted in percentages of pupation and adult emergence of 42 and 26% by the topical method but 24 and 6% by the dipping method respectively (Table 1). At the highest concentration 10 mg/l, only 19% of larvae pupated but no adults emerged (Table 1). A pupal-adult intermediate form was found inside most puparia. Puparial cases were brownish-black. These abnormal forms will be described in the next chapter (Figure 4C).

The effects of ASA on the percentage mortalities of the second and third instar larvae of *P. ruficornis* when exposed to the various concentrations are summarized in Tables 1-3 and Figure 1. More toxic effects were found after treatment of the second than the third instar larvae when ASA was applied by both methods. At concentrations of 0.1 and 1 mg/l, 0-3% of the larvae died or pupal-adult intermediate forms. (Tables 2-4). Some died in a stage of partial adult emergence. With treatment of third instars, The percentage mortalities observed in the topical drop and dipping methods were 55 and 93%, respectively. At 1 mg/l of the compound in the dipping method, the percentage of pupation and adult emergence were 34 and 22%. The percentage of pupation was only 21% and only 3% of adults emerged (Tables 2 and 3). The ASA exerted more toxic effects in the early larval stages

than in later ones. The percentage mortalities of the second and the third instar larvae varied directly with the concentration of ASA (Tables 2 and 3).

When third instar larvae of flies were treated with 0.1 mg/l, more larvae survived to the adult stage. The adult emergence of *P. ruficornis* observed from the treatments using topical drop and dipping methods were 56 and 42%, respectively (Table 2).

The comparative potencies of ASA tested against each larval stage are shown in Figure 5. The LC50 values of ASA are summarized in Figure 1 and Table 5.

There was no significant difference among the replications ($P > 0.01 = 4.43$). However, there was a significant difference between the methods of application. The percentages of pupation and adult emergence were very low in eggs and first instar larvae exposed to 0.1 mg/l of ASA by both methods. When the eggs were dipped in 0.1 mg/l of ASA only four adults emerged (Tables 1 and 2).

The LC50 values of ASA for the eggs of *P. ruficornis* were determined from log-concentration-probit mortality regression line using a probit analysis program on computer. The LC50 values were 1 and 0.09 mg/l for the topical drop and dipping methods, respectively (Figures 1 and 2).

The effects of ASA and the percentage mortalities of the fly larvae after exposure to ASA are shown in Tables 2 and 3, respectively. The mortality of the test larvae varied directly with concentration. The mortality was high for larvae of all ages were dipped in the compound at 0.1 mg/l. At 0.001 mg/l, survival of first instar larvae was 83% for the topical drop method and 71% for the dipping method (Figure 1).

No adults emerged when first or second instar larvae were dipped in 1 mg/l of ASA. When 1 mg/l was applied by topical drop method on the first instar larvae, adult emergence was 32% (Table 2), and for second instar larvae, it was 26%.

Survival of Pupae and Adults

The percentage mortality was relatively high when the pupal stage was exposed to ASA by topical drop and dipping methods. At 0.1 mg/l of ASA, the percentage mortality found in the topical drop and dipping methods was 46 and 74%, respectively. When 1 mg/l of ASA was used, only 13% of treated puparia emerged, and when the puparia were dipped in the compound at the highest concentration (10 mg/l), the percent adult emergence was only 3% (Table 3).

The abnormalities induced in adult flies obtained from this experiment were classified by larval stage. At the lowest concentration (0.001 mg/l) of ASA applied by both methods, the percentages of adult emergence of the treated puparia were significantly different from those emerging from the untreated puparia (figure 5). Some adult flies were found to be sterile while some were inactive and did not copulate. Some females laid only a few eggs per batch. The percentage of hatching was very low when compared with the eggs deposited by normal females (Table 4).

No significant differences among the replicates ($P > 0.01 = 4.43$) were found in the percentage of pupation or adult emergence. There were significant effects caused by mode

TABLE 1 Effects of aliphatic sesquiterpene analogue on first instar larvae *Parasarcophaga ruficornis* Fabr. (100 individuals per treatment).

Concentration of ASA (mg/l)	Method of treatment	Percentage mortality at various stages of development									Total mortality (%)	Corrected % mortality ¹
		L1	L2	L3	L(P)	L-P	P	P-A	A	F		
Check	T	8	1	0	0	0	1	0	1	0	11	-
	D	12	1	1	0	0	1	0	1	1	17	-
0.001	T	15	2	0	0	0	1	0	1	1	20	10.11
	D	23	3	2	1	0	3	0	3	2	37	24.09
0.01	T	24	4	1	0	0	2	0	2	2	31	22.47
	D	36	7	3	1	2	5	2	4	4	64	56.63
0.1	T	35	7	3	1	1	3	1	3	2	56	50.56
	D	44	13	7	0	2	7	0	6	7	86	83.13
1	T	42	12	5	2	0	5	0	5	5	77	74.16
	D	57	19	12	0	3	9	0	0	0	100	0
10	T	54	19	9	0	2	8	2	6	0	100	0
	D	72	23	5	0	0	0	0	0	0	100	0

TABLE 2 Effects of aliphatic sesquiterpene analogue on second instar larvae *Parasarcophaga ruficornis* Fabr. (100 individuals per treatment).

Concentration of ASA (mg/l)	Method of treatment	Percentage mortality at various stages of development								Total mortality (%)	Corrected % mortality ¹
		L2	L3	L(P)	L-P	P	P-A	A	F		
Check	T	6	1	0	0	0	0	1	0	8	-
	D	12	3	1	0	2	0	1	1	20	-
0.001	T	12	3	0	0	2	0	2	1	20	13.04
	D	20	6	0	2	4	2	3	2	39	23.75
0.01	T	22	6	1	0	2	1	3	2	37	31.52
	D	31	13	3	0	7	2	7	4	67	58.75
0.1	T	31	10	2	2	5	0	6	4	60	56.52
	D	40	19	3	2	10	0	12	6	92	90.00
1	T	42	14	0	2	8	2	6	6	80	78.26
	D	49	23	0	4	15	3	6	0	100	0
10	T	58	20	3	0	12	3	4	0	100	0
	D	67	30	0	0	0	0	0	0	100	0

¹/Percentage mortality were corrected by using Abbott's formula.

TABLE 3 Effects of aliphatic sesquiterpene analogue on third instar larvae of *Parasarcophaga ruficornis* Fabr. (100 specimens per treatment)

Concentration of ASA (mg/l)	Method of treatment	Percentage mortality at various stages of development							Total mortality (%)	Corrected % mortality ¹
		L3	L(P)	L-P	P	P-A	A	F		
Check	T	6	0	0	1	0	1	0	8	-
	D	10	0	0	2	0	1	2	15	-
0.001	T	12	0	0	2	1	1	1	17	9.76
	D	21	0	0	2	1	2	1	28	15.29
0.01	T	23	1	0	2	1	2	3	32	26.09
	D	34	2	1	4	3	3	4	51	42.35
0.1	T	33	2	1	4	2	3	3	48	43.48
	D	45	2	2	5	3	5	4	66	60.00
1	T	40	2	0	4	1	4	4	55	51.01
	D	58	4	4	9	3	10	5	93	91.76
10	T	53	2	2	5	2	4	3	71	96.48
	D	73	4	4	12	4	3	0	100	0

TABLE 4 Effects of aliphatic sesquiterpene analogue on the pupal stage of *Parasarcophaga ruficornis* Fabr. (100 specimens per treatment)

Concentration of ASA (mg/l)	Method of treatment	Percentage mortality at various stages of development				Total mortality (%)	Corrected % mortality ¹
		P	P-A	A	F		
Check	T	7	0	2	1	10	-
	D	13	1	5	3	22	-
0.001	T	17	0	3	2	22	13.13
	D	25	1	8	5	39	21.33
0.01	T	23	1	4	2	30	22.22
	D	36	2	11	5	54	41.03
0.1	T	33	2	7	4	46	40.00
	D	47	3	16	8	74	66.66
1	T	46	2	9	5	62	57.78
	D	57	3	18	9	87	83.33
10	T	54	3	11	7	75	72.22
	D	71	1	23	2	97	96.15

¹Percentage mortality were corrected by using Abbott's formula.

TABLE 5 Effects of aliphatic sesquiterpene analogue on the adult stage of *Parasarcophaga ruficornis* Fabr. (100 specimens per treatment)

Concentration of ASA (mg/l)	Method of treatment	Percentage mortality at various stages of development		Total mortality (%)	Corrected % mortality ¹
		A	F		
Check	T	7	2	9	-
	D	10	2	12	-
0.001	T	15	6	21	13.19
	D	24	4	28	18.18
0.01	T	25	5	30	23.08
	D	35	7	42	34.09
0.1	T	32	6	38	31.87
	D	52	15	67	62.50
1	T	43	10	53	48.35
	D	69	18	87	85.23
10	T	51	13	64	60.44
	D	86	13	99	98.86

TABLE 6 Relationship between concentration of ASA compound and mortality of eggs, first, second, and third instar larvae, puparia and adults of *Parasarcophaga ruficornis* Fabricius, in the topical drop and dipping method.

Developmental stages	Method of treatment	ASA LC50 values (mg/l)	Regression equation Y=a+bX
Egg	T	1.00	Y=4.997+0.402X
	D	0.09	Y=5.924+0.847X
First instar larva	T	0.78	Y=5.164+0.686X
	D	0.40	Y=5.438+0.662X
Second instar larva	T	4.66	Y=4.816+0.464X
	D	0.65	Y=5.314+0.694X
Third instar larva	T	5.60	Y=4.775+0.417X
	D	0.37	Y=5.488+0.679X
Puparium	T	2.30	Y=4.935+0.461X
	D	0.32	Y=5.499+0.680X
Adult	T	6.00	Y=4.858+0.439X
	D	0.09	Y=5.735+0.710X

1/Percentage mortality were corrected by using Abbott's formula.

Abbreviation in Table 1-6

E=eggs; L1=first instar - late first instar larvae; L2=second instar - late second instar larvae; L3 = third instar - late third instar larvae; L(P)=larvae puparium form; L-P=late puparia and incomplete puparia; P=puparia; P-A =pupal - adult intermediate form; A=adult mating and female lay a few egg; AE=adult emergence; H=hatching; F=sterile - uncopulation; T=topical drop method; D=dipping method.

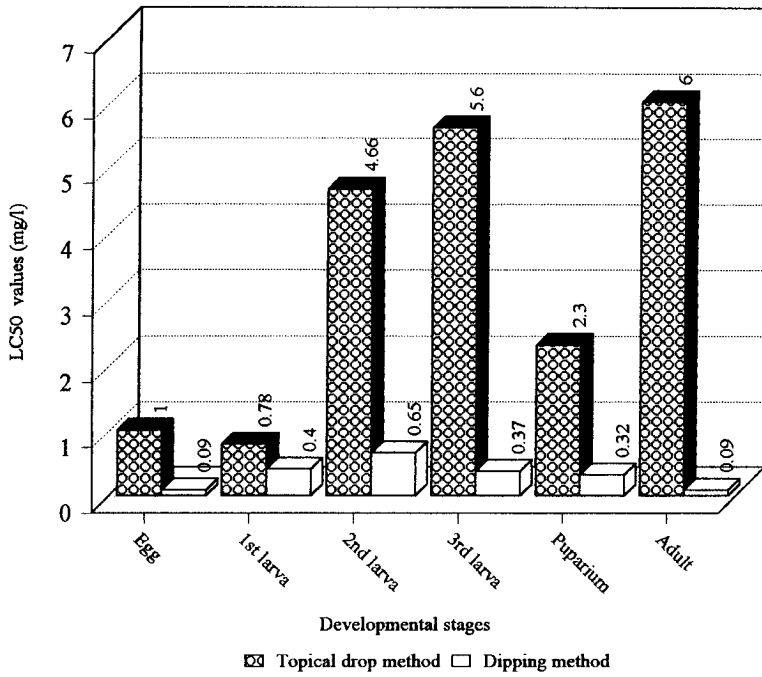


Fig. 1. LC₅₀ values (mg/l of aliphatic sesquiterpene analogue) after tested on each stage of the flesh fly, *P. ruficornis* Fabr.

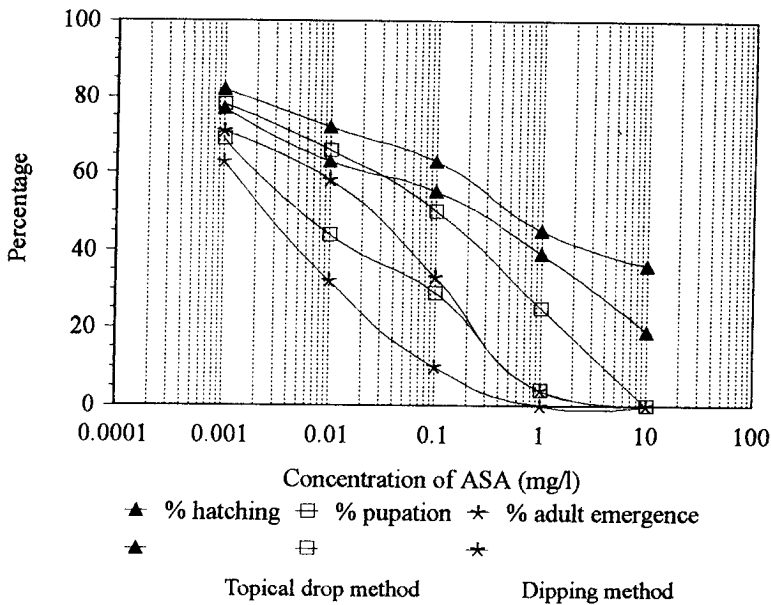


Fig. 2. The percentage of egg hatching, pupation and adult emergence of the flesh fly, *P. ruficornis* Fabr.

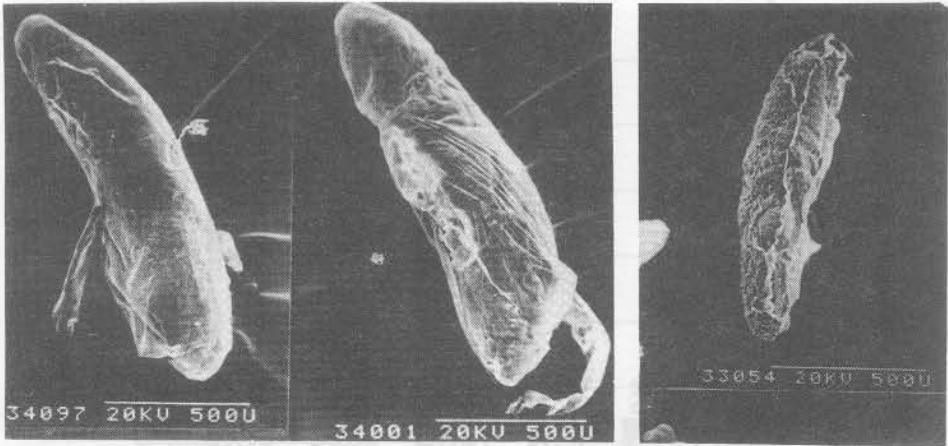


Fig. 3. Scanning electron microscopic photograph of eggs of *P. ruficornis* Fabr. after treatment with ASA compound at 1 and 10 mg/L

- A : general view of a normal egg.
- B : undeveloped treated egg with wrinkled chorion.
- C : undeveloped first instar larva showing decomposition of embryonic cuticle, with 1 mg/l ASA.

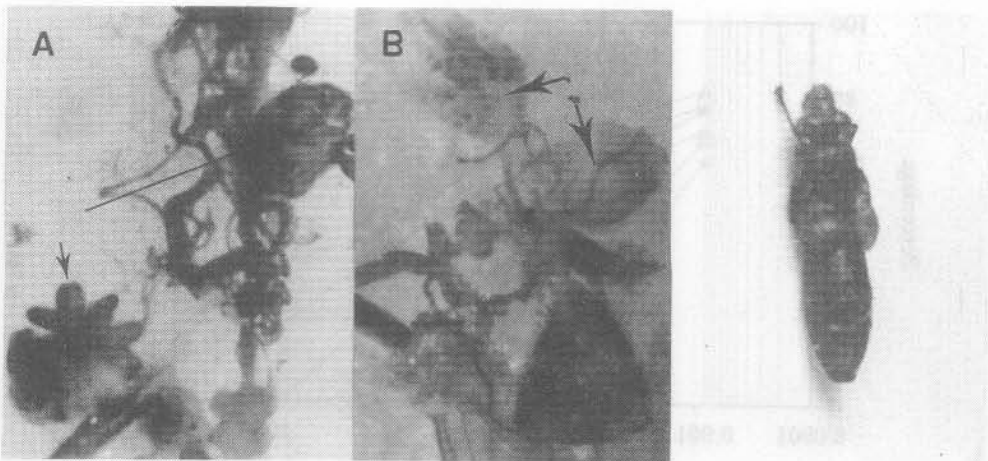


Fig. 4. Reproductive organs of female adult flies, *P. ruficornis* Fabr.

- A : Untreated female fly showing normal reproductive organ with ovarioles developing in ovary sac
- B : Undeveloped ovarioles in ovary sac of treated puparia with ASA at 1 mg/L (100X)
- C : Malformed of puparium (pupal-adult intermediate form).

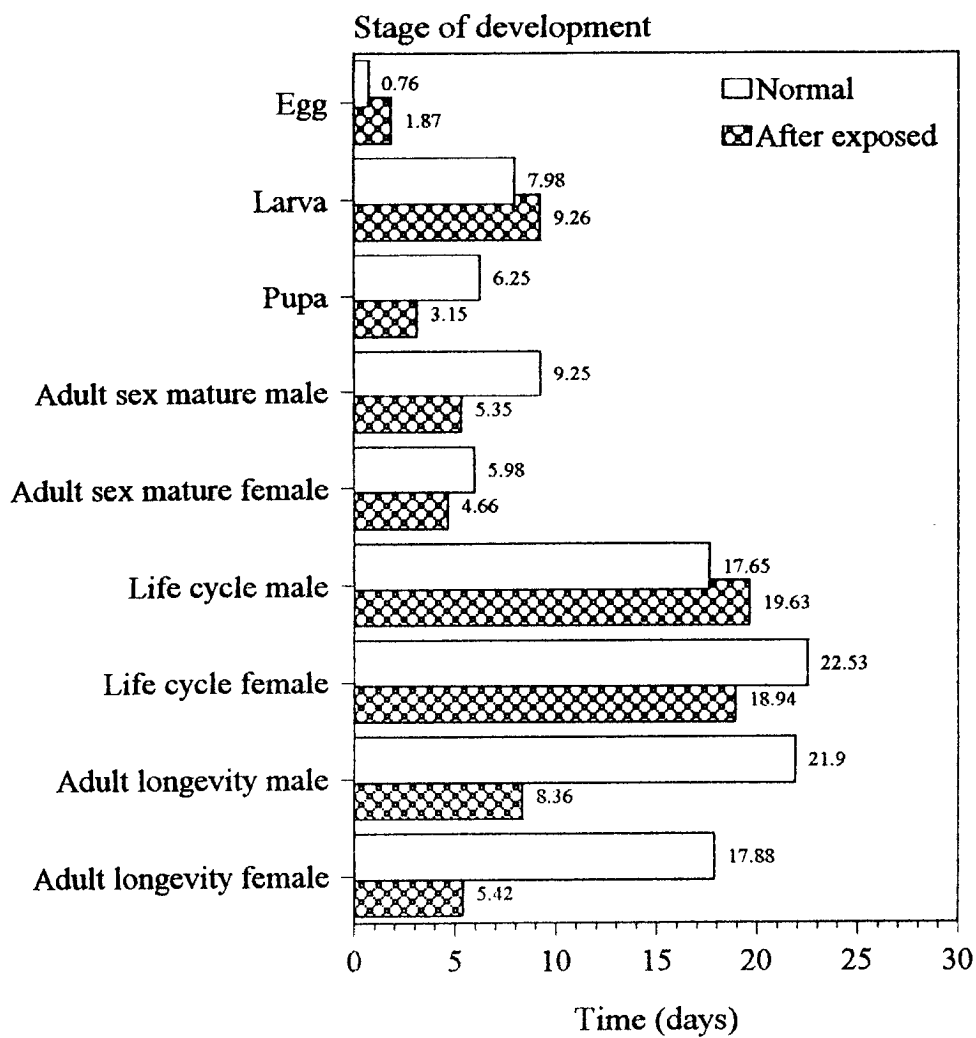


Fig. 5. Comparison of average duration of life stages of normal and treated flies exposed to 1 mg/ml of aliphatic sesquiterpene analogue, reared at $27\pm 4^{\circ}\text{C}$ and $78\pm 4\%$ RH.

of application and the dosage of ASA ($P < 0.01 = 4.10$). Highly significant differences among the various stages were obtained between the lowest and highest concentrations (Table 5).

The LC50 values for puparia of *P. ruficornis* obtained from topical drop and dipping methods were 2.30 and 0.32 mg/l (Figures 1 and 2). The relative potencies of the compound against the puparia by the two methods are shown in Figures 1 and 2.

Adults: The mortality of adult males and females treated at 1 mg/l of ASA were high. The percentage mortality at the highest concentration (10 mg/l) obtained from topical drop and dipping methods were respectively, 64 and 99%. When the adults were dipped in 0.1 mg/l and 1 mg/l of ASA, the percentage mortalities were 67 and 87%, respectively (Table 4).

The LC50 values for adult flies obtained by topical drop and dipping methods were 6.00 and 0.09 mg/l, respectively. The effects of ASA on adult flies are shown in Figures 1 and 2.

ASA induced some effects on the behavior of the flies such as the inertial rate activity or movement. The rate of copulation was also less than the normal rate. Some flies deposited only a few eggs after copulation and the eggs were smaller than those of normal flies. The dead or inactive female flies were dissected and the morphogenetic aberrations seen are shown in Figure 3.

Adult females born from the eggs treated with ASA they were smaller and paler in color than normal females. They were mostly sterile although some could lay a few eggs (less than 20 eggs per batch), none of which hatched. The females were inactive and the rate of copulation was low.

ASA also reduced longevity of egg and larval stages. Hatching periods of *P. ruficornis* were prolonged to 1.87 ± 0.90 days. Molting periods in parasarcophagid larval stages were delayed and each stage required a longer time for completion of development (Figure 5).

Effects of ASA on larval stages varied depending on age and instar of treatment. In the first instar, the molting processes failed.

The second and third instar, showed various different abnormal morphological characteristics including giant size, incomplete puparia, pupal-adult intermediate form and the malformed adults. The latter was exhibited when legs and wings stuck to the pupal case and became detached from the body, small size, pale coloration of body and compound eyes. ASA also affected the reproductive system so that mating was impaired, fecundity was low and egg viability was reduced. The longevity of the adult stage was reduced to 8.36 ± 4.95 days (Figure 5). Protruding genital organs from terminal-abdominal segments, accompanied by incomplete development of the ventro-abdominal integument, were common abnormalities observed.

ASA has biological activities which resemble juvenile hormone, which results in an imbalance of between juvenile hormone and ecdysone. These interactions result in the blocking of molting process and larval cuticular formation, so that larval-pupal and pupal-adult intermediate forms occur. ASA compound is able to penetrate through the pupal case and exert its effects on the developing adult. Adults emerging from treated puparia show the morphological features of larvae.

The effects of ASA on body size, color, malformations of the genital organs, optic lobes, compound eyes, brain, and prothoracic gland, were similar to those obtained by Moribayashi and Ohtaki (10), Pappas and Fraenken (12), and Buei *et al.*, (3).

Adult treated with ASA showed low rate of mating and oviposition, and slowing down of activity in general. Their life span was shortened to an average of 5.42 ± 2.76 days. Most deposited eggs failed to hatch and larvae which emerged from these eggs did not survive beyond the second instar (17)

ASA compound is therefore a powerful chemical which affects all stages of development of the these parasarcophagid flies. It is possible to use as a contact food additive stray of mix with the medium in their habitats to allow the contact to occur which makes possible ASA to penetrated into their coelom bodies. It may thus be unful in controlling populations of the flies, and may be unful in controlling other types of noxious insects in the future.

ACKNOWLEDGEMENTS

We wish to thank Prof.Dr.Warren Brockelman for review the manuscript ; and Mr. Somphob Leetchewa for analyzing the data and typing.

REFERENCES

1. Adams, T.S. (1974). The role of juvenile hormone in house fly ovarion follicle morphogenesis. *J. Ins. Physiol.* **20**, 263-76.
2. Berridge, M.J. (1980). Hormone Action : A Search for Tranducing Mechanisms. Academic Press, New York. 15p.
3. Buei, K., T. Niki and T. Ohtaki. (1979). Effect of a juvenile hormone mimic, methoprene, aganist synanthropic files. *J. Pastic. Sci. (Nimon Noyakugaku Kaishi)*. **4(4)**, 481-86.
4. Chamberlain, W.F. (1975). Review article : Insect growth regulating aganist for control of arthropods of medical and veterinary importance. *J. Med. Ent.* **12**, 395-99.
5. Gillot, C. (1980). Entomology. 1st ed., Plenum Press, New York. 729 p.
6. Gringrich, A.R. and D.E. Hopkins. (1978). Stages of the horn fly susceptible to methoprene. *Inter. J. Ins. Morpho. Physiol.* **70**, 107-8.
7. Harwood, R.F. and M.T. James. (1979). Entomology in Human and Animal Health. 7th ed., Macmillan Publishing Co. Inc., New York. 463 p.
8. Miller, J.A., M. Schwarz and J.E. Wright. (1977). Methoprene for control of horn flies : A suppression program on the island of Molakai, Hawaii. *J. Econ. Ent.* **7(4)**, 417-23.
9. Mordue, W., G.J. Goldsworthy and W.M. Blaney. (1980). Insect Physiology. Blackwell Scientific Publication, Oxford. 108 p.
10. Moribayashi, A.F. and T. Ohtaki. (1978). Inactivation of ecdysone and the possible feed back control of the titre during pupation of *Sarcophaga peregrina*. *J. Ins. Physiol.* **24**, 279-84.
11. Pont, C.A. and J.P. Dear. (1980). Catalogue of the Diptera of the Afrotropical Region. British Mueseum (Natural History), London. 9 p.
12. Pappas, C. and G. Fraenken. (1978). Hormonal aspects of oogenesis in the flies, *Pharmia regina* and *Sarcophaga bullata*. *J. Ins. Physiol.* **24**, 75-80.

13. Roller, H. (1968). The chemistry and biology of juvenile hormones. *Recent Progress in Hormone Research*. **24**, 551-651.
14. Schwarz, M. and R.W. Miller. (1979). Compounds related to juvenile hormone : Activity of analogues of A13-36206 against the house fly (Dipter : Muscidae). *J. Med. Ent.* **15(3)**, 300-1.
15. Sukhapanth, N. and C. Ketavan. (1984). Rearing flies for experimental purposes. Symposium of Entomology-Zoology and Plant Sciences. *Proceeding of the 22nd Annual Conference* **22(1)**,271-83.
16. Sukhapanth, N., E.S. Upatham and C. Ketavan. (1988). Effects of food and media on egg reproduction, growth and survivorship of flies (Diptera : Calliphoridae, Muscidae and Sarcophagidae). *J. Sci. Soc. Thailand* **14**, 41-50.
17. Sukhapanth, N., P. Prempree and C. Ketavan. (1992). Biological activity and toxicology effects of a juvenile hormone analogue on the various developmental stages of blow fly. 18th Congress on Science and Technology of Thailand, Queen Sirikit National Convention Centre, October 27-29, 352-3.

บทคัดย่อ

การทดสอบหาความเป็นพิษของสาร (อลิฟาติก เซสควิเทอร์พีน อนาร์ลอค) ที่แสดงฤทธิ์เกิดปฏิกิริยาต่อการฟักไข่และการเจริญเติบโตในระยะเวลาต่างๆ ของแมลงวันหลังลาย *P. ruficornis* Fabr. โดยใช้วิธีแบบหยดลงบนส่วนอกและแบบชุบตัวให้เปียก เพื่อหาความเป็นพิษของสารนี้ที่ระดับ LC50 (ภาพที่ 1 และ 2) ผลการทดลองแสดงว่าสารนี้มีบทบาททางชีววิทยา ต่อรูปร่าง สันฐาน เป็นสาเหตุให้เกิดการยับยั้งการเจริญต่อเนื่องของวงชีวิตการของระยะตัวหนอนวัยต่างๆ ทำให้มีผนังลำตัวเจริญไม่สมบูรณ์แบบและการลอกคราบช้ากว่าปกติ บางตัวไม่สามารถลอกคราบได้ จากการศึกษาทางเนื้อเยื่อวิทยาด้วยกล้องจุลทรรศน์ และกล้องแผ่ขยาย เอสอีเอ็ม (SEM) พบว่าส่วนประกอบของเยื่อหุ้มไข่บางเปราะ ฉีกขาดง่าย สารนี้มีผลกระทบต่อระบบสืบพันธุ์และความผิดปกติของการสร้างไข่ในรังไข่ ทำให้แมลงวันออกไข่ลดปริมาณลง อีกทั้งมีผลทำให้ขนาดรูปร่างเล็กลงกว่าเดิม สีของลำตัว ปีก และดาวรวมขีดกว่าปกติ การทดสอบกับตัวเต็มวัย พบว่าสารนี้ทำให้อัตราการเจริญและแพร่ขยายพันธุ์ลดลง