PN-ABA-593

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON DC 20523

DATE: 7/29/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 5.240
PR#4

Attachment

PN-ABA-543 5.240

PROGRESS REPORT NO. 4

NATURAL INSECTICIDES FOR *SPODOPTERA LITURA* FABR.

A RESEARCH PROJECT

USAID/PSTC PROGRAM

GRANT NO.936-5542-G-00-5032-00

SUBMITTED BY

PICHAET WIRIYACHITRA

PRINCE OF SONGKLA UNIVERSITY

HAT YAI, THAILAND

Rec'd in SCI JUL 2 0 1988

PROJECT PROFILE

Country	:	Thailand
Grant No.	:	936-5542-G-00-5032-00
Program	:	Program on Science and Technology Cooperation
Project Title	:	Natural Insecticides for *Spodoptera litura* Fabr.
Project Leader	:	Pichaet Wiriyachitra
Organization	:	Prince of Songkla University, Hat Yai.
Co-investigators	:	Prachaval Sukumalanand
Total Project Budget	:	US $ 150,000
Project Duration	:	3 years
Reporting Period	:	1 December 1986 to 31 May 1988
Budget Allocation for This Period	:	1,330,211.79 Baht

BACKGROUND

S. litura Fabr. is a widespread insect pest. It is polyphagous and regarded as one of the most damaging pests in the world agriculture. Pandy and Srivastava (1967) reported that the insect attacks 112 species of cultivated crops all over the world. In Thailand, at least 30 varieties of plants are found to be host plants of this insect[1]. These include vegetables, floral plants, ornamental plants, economic field crops and weeds.

The time required for egg, larval and pupal development are 2-3, 14 and 7-8 days respectively[2]. The adult longevity is 8-10 days in the male and 10-12 days in the female. The larva molts 5 times. It is at this stage that the insect is most damaging. Since the early larval instars are quite vunerable to external damage, it has been chosen as the target organism for this work.

For self-defence, it is not surprising that plants would contain substances which are insect antifeedants, insect repellents and insecticides. Some naturally occurring antifeedants which have been published are: glycosides of steroidal alkaloids, demissine[3], solacauline[4], tomatine[5], leptines I and II[6], ring A enone and/or ring D aromatic steroids, the nicandrenones and other[7,8,9,10]; juglone (5-hydroxynaphthoquinone)[10]; the isoquinoline alkaloid isoboldine[11], phenylpropanoids[12], germacranes sesquiterpenes shiromodiol and shiromol[13,14]; *ent*-clerodane and clerodane diterpenes, clerodendrin[15]; caryoptin and others[16]; the hydroxylated steroid meliantriol[17]; azadirachtin[18]; xylomolin[19]; ajugarins[20]; harrisonin[21]; polygodial, ugandensidial and warburganal[22]; inflexin[23]; and isodomedin[24]; crotepoxide[24] and unedoside[25]. However there is no report of any of these substances being used effectively in agriculture.

A number of naturally occurring compounds have been found to exhibit insecticidal activity. These include alkaloids, terpenoids, phenolics, amines, chromenes, prenylated guinones and sesquiterpenes esterified with phenolic acids[26]. Commercially, the substances which have found their way to agricultural application are nicotine, derris, pyrethrum and the newer product, ryania[27]. Crude extracts of the plant producing these substances, or dried and powdered plant tissue have a long history of use in SE Asia as insecticides. However, none of these natural products is a universal insecticide : Tobacco leaf containing nicotine is subject to attack by several insects, the root of derris-containing plant is attacked by the larvae of a species of beetle, the flower from which pyrethrins are extracted needs synthetic insecticides for protection from insects in the fields. In addition, insects can develop resistance to a certain chemicals after a long exposure. New and better insecticides will, therefore, have to be constantly sought.

Since *S. litura* is one of the major pests in SE Asia, the discovery of a new and more effective insecticide with low mammalian toxicity from local plants would produce enormous benefit to the region as well as to Thailand in general.

OBJECTIVES

1. To isolate and identify natural insecticides and antifeedants, which have low mammalian toxicity for *S. litura*.
2. Extract or synthesizing those compounds on a practical scale and design of industrial extraction procedure.
3. Evaluation of their applicability in the field.

MATERIALS AND METHODS

Plants investigated were collected in the southern region of Thailand. Samples were classified taxonomically and vouchers were deposited in the herbarium in the Department of Biology, Prince of Songkla University.

Ground plant materials were steam distilled or exhaustively extracted with solvents. The extracts/steam distillates were screened for insecticidal and antifeedant activity with *S. litura* and the active extracts resolved into fractions. Each fraction was screened for activity and the active fraction, again, separated into individual constituents using quick column and preparative layer chromatography on silica gel.

Each of the individual components was tested again for activity and the LC_{50} value of the active compound determined. The structure of the compound was elucidated using spectroscopic techniques.

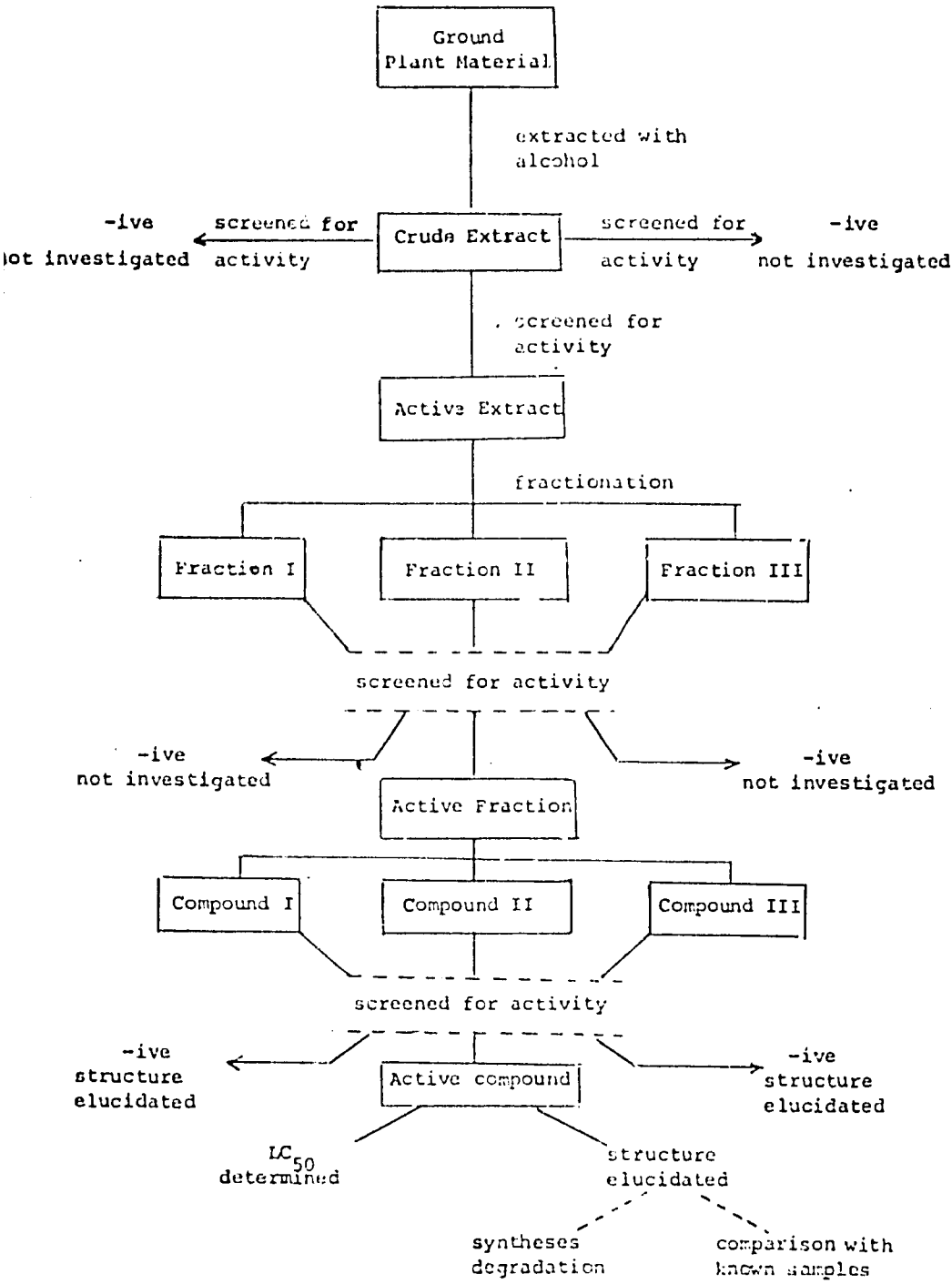


Diagram showing work plan

The leaf dipping (LD) method was used for the detection of stomach poisoning and antifeeding activity, the third instars of *Spodoptera litura* larvae were left to feed on the leaves of *Vigna radiata* which had been immersed in the tested solution then air-dried. Ten larvae were used in each of the four replications (one as control). The number of dead organisms was recorded at the end of each day. The amount of the leaf consumption and the larvae size were also estimated.

The topical application (TA) method was used for the detection of dermal toxicity, the tested solution was dropped on the thorax of the third instar larvae. The organisms were then left to feed on untreated leaves of *Vigna radiata* in a plastic container. Ten larvae were used in each of the four replications (one as control). The number of dead organisms was recorded at the end of each day.

The LD coupled with TA method was used for the detection of the combined effect of stomach poisoning and dermal toxicity. The tested solution was dropped on the thorax of the third instar larvae. The organisms were then left to feed on the leaves of *Vigna radiata* which had been immersed in the same tested solution then air-dried. Ten larvae were used in each of the four replications (one as control). The number of dead organisms was recorded at the end of each day.

RESULTS/DISCUSSION/TABLES

Reinvestigation of the antifeeding activity of 4 *Aglaia* species, e.g. *A. adorata*, *A. adamantica*, *A. argentea*, *A. squamulosa*, revealed the significant activities even at low concentration. Attempt was then made to fully explore the reaction of *S. litura* larvae to these extracts/fractions.

A soy bean antificial diet was admixed with these crude extracts/fractions and used as feed for the larvae. Outstanding results were obtained from extracts/fractions from *A. odorata*. The feeding of larvae was significantly reduced, the larval growth was therefore slow. They eventually died. Table I, Ill. 1 show these results in detail.

Ill. 1

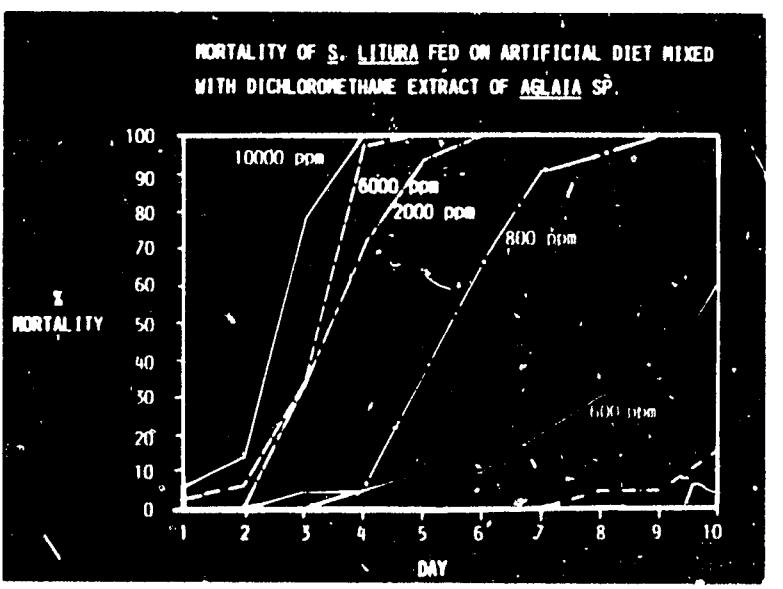


TABLE I

MORTALITY OF *S. LITURA* F. FED ON ARTIFICIAL DIET MIXED WITH EXTRACT OF *AGLAIA* SPECIES

PLANT SPECIES	FRACTION	CONC(PPM)	ACCUMULATIVE % MORTALITY (ABBOTT'S FORMULA)									
			DAY 1	2	3	4	5	6	7	8	9	10
Aglaia odorata	C_6H_{14}	10000	7.14	7.14	7.14	7.14	7.14	7.14	7.14	7.14	7.14	-
	CH_2Cl_2	10000	6.67	13.33	16.67	100.00	-	-	-	-	-	-
		8000	3.37	6.67	33.33	96.67	100.00	-	-	-	-	-
		6000	3.33	6.67	33.33	96.67	100.00	-	-	-	-	-
		4000	0.00	0.00	53.33	100.00	-	-	-	-	-	-
		2000	0.00	0.00	33.33	70.00	93.33	100.00	-	-	-	-
	1) $QC(C_6H_{14})$	6000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	-
	2) $QC(CH_2Cl_2)$	10000	0.00	0.00	3.44	57.14	92.86	96.43	100.00	-	-	-
		8000	0.00	0.00	10.34	42.85	78.57	100.00	-	-	-	-
		6000	0.00	0.00	3.44	24.99	78.57	96.43	100.00	-	-	-
		4000	10.00	10.00	17.23	24.99	39.29	57.14	67.85	85.71	100.00	-
		2000	0.00	0.00	0.00	0.00	14.28	32.14	42.85	75.00	92.60	96.15

TABLE I

MORTALITY OF *S. LITURA* F. FED ON ARTIFICIAL DIET MIXED WITH EXTRACT OF *AGLAIA* SPECIES

PLANT SPECIES	FRACTION	CONC(PPM)	ACCUMULATIVE % MORTALITY (ABBOTT'S FORMULA)									
		DAY	1	2	3	4	5	6	7	8	9	10
Aglaia odorata	3) QC(C_6H_{14}:CH_2Cl_2,3:1)	10000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		8000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		6000	3.34	3.34	3.34	3.34	3.34	3.34	3.34	3.34	3.34	3.34
		4000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		2000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	4) QC(CH_2Cl_2)	10000	0.00	0.00	100.00	-	-	-	-	-	-	-
		8000	0.00	0.00	100.00	-	-	-	-	-	-	-
		6000	0.00	3.33	90.00	100.00	-	-	-	-	-	-
		4000	0.00	0.00	73.34	100.00	-	-	-	-	-	-
		2000	0.00	0.00	30.00	93.11	100.00	-	-	-	-	-
	5) QC (MeOH)	10000	3.34	3.44	65.51	100.00	-	-	-	-	-	-
		8000	0.00	0.00	30.00	93.11	100.00	-	-	-	-	-
		6000	0.00	0.00	62.38	93.10	100.00	-	-	-	-	-
		2000	0.00	0.00	27.58	89.65	96.43	100.00	-	-	-	-

MORTALITY OF *S. LITURA* F. FED ON ARTIFICIAL DIET MIXED WITH EXTRACT OF *AGLAIA* SPECIES

PLANT SPECIES	FRACTION	CONC(PPM)	ACCUMULATIVE % MORTALITY (ABBOTT'S FORMULA)										
			DAY 1	2	3	4	5	6	7	8	9	10	11
Aglaia odorata	MeOH	10000	0.00	6.67	23.33	93.33	100.00	-	-	-	-	-	-
		8000	0.00	3.33	66.67	66.67	89.85	96.55	100.00	-	-	-	-
		6000	0.00	0.00	60.00	66.67	66.20	93.10	96.55	100.00	-	-	-
		4000	0.00	0.00	3.33	3.33	16.67	44.82	62.07	82.76	82.76	96.55	100.00
		2000	0.00	0.00	3.33	3.33	3.44	6.89	24.13	26.58	37.92	55.55	-
	EtOAc (insol)	10000	0.00	0.00	0.00	0.00	0.00	0.00	3.44	27.58	34.47	51.72	-
		8000	0.00	0.00	0.00	0.00	0.00	0.00	3.44	20.68	20.68	27.58	-
		6000	0.00	0.00	0.00	0.00	0.00	0.00	3.44	3.44	10.34	10.34	-
		4000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
		2000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
	EtOAc (sol)	10000	0.00	10.34	48.27	96.55	100.00	-	-	-	-	-	-
		8000	0.00	6.89	55.17	100.00	-	-	-	-	-	-	-
		6000	0.00	6.87	27.58	89.65	100.00	-	-	-	-	-	-
		4000	0.00	0.00	10.34	96.55	100.00	-	-	-	-	-	-
		2000	0.00	6.89	20.69	62.07	89.65	100.00	-	-	-	-	-

TABLE I

MORTALITY OF *S. LITURA* F. FED ON ARTIFICIAL DIET MIXED WITH EXTRACT OF *AGLAIA* SPECIES

PLANT SPECIES	FRACTION	CONC(PPM)	ACCUMULATIVE % MORTALITY (ABBOTT'S FORMULA)												
		Day	1	2	3	4	5	6	7	8	9	10	11	12	13
Aglaia odorata	CH_2Cl_2	800	0.00	0.00	0.00	5.00	35.00	65.00	90.00	95.00	100.00	-	-	-	-
		600	0.00	0.00	5.00	5.00	10.00	10.00	20.00	30.00	35.00	60.00	60.00	65.00	75.00
		400	0.00	0.00	0.00	0.00	0.00	5.00	20.00	30.00	40.00	40.00	40.00	40.00	40.00
		200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.00	5.00	15.00	15.00	15.00	15.00
	MeOH	800	0.00	0.00	0.00	0.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
		600	0.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
		400	0.00	0.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
		200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
	-																	
)	75.00	75.00	84.21	89.47	98.47	89.47	89.47	94.73	100.00	-	-	-	-	-	-	-	-	-
)	40.00	-0.00	78.94	84.21	89.47	94.73	94.73	94.73	94.73	94.73	100.00	-	-	-	-	-	-	-
)	15.00	10.00	15.78	26.31	31.57	31.57	81.57	76.84	42.10	42.10	52.63	52.63	52.63	52.63	-	-	-	-
)	5.00	15.00	15.00	30.00	40.00	40.00	40.00	40.00	50.00	55.00	55.00	60.00	65.00	70.00	70.00	80.00	80.00	85.00
)	5.00	5.00	10.00	10.00	10.00	10.00	10.00	0.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	25.00	30.00
)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00

TABLE 1

MORTALITY OF *S. LITURA* F. FED ON ARTIFICIAL DIET MIXED WITH EXTRACT OF *AGLAIA* SPECIES

PLANT SPECIES	FRACTION	CONC (PPM)	ACCUMULATIVE % MORTALITY (ABBOTT'S FORMULA)									
			Day 1	2	3	4	5	6	7	8	9	10
Aglaia andamanica	C_6H_{14}	10000	0.00	0.00	0.00	3.33	3.33	3.33	3.33	3.33	3.33	3.33
		8000	0.00	0.00	0.00	3.33	3.56	3.56	3.56	3.56	3.56	3.56
		6000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		4000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		2000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH_2Cl_2	10000	0.00	0.00	3.33	10.00	10.71	10.71	10.71	10.71	10.71	10.71
		8000	0.00	0.00	0.00	16.67	16.67	16.67	16.67	16.67	16.67	16.67
		6000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		4000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		2000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	MeOH	10000	0.00	0.00	3.33	10.00	13.34	13.34	20.00	23.34	23.34	23.34
		8000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.33	3.33	3.33
		6000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.33	3.33	3.33
		4000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		2000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

TABLE I

MORTALITY OF S. LITURA F. FED ON ARTIFICIAL DIET MIXED WITH EXTRACT OF AGLAIA SPECIES

PLANT SPECIES	FRACTION	CONC (PPM)	ACCUMULATIVE % MORTALITY (ABBOTT'S FORMULA)									
			Day 1	2	3	4	5	6	7	8	9	10
Aglaia argentea	C_6H_{14}	10000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		8000	3.33	3.33	3.33	3.33	3.33	3.33	3.33	3.33	3.33	3.33
		6000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.33
		4000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		2000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH_2Cl_2	10000	0.00	0.00	0.00	20.00	63.33	70.00	83.33	93.33	100.00	-
		8000	0.00	0.00	0.00	3.33	43.33	63.33	83.33	96.67	100.00	-
		6000	0.00	0.00	0.00	16.67	23.33	46.67	60.00	86.67	93.33	96.67
		4000	0.00	0.00	3.33	10.00	26.67	43.33	63.33	90.00	96.66	100.00
		2000	0.00	0.00	3.33	13.33	20.00	20.00	60.00	73.33	83.33	93.33
	MeOH	10000	0.00	0.00	3.33	10.00	23.33	53.33	70.00	83.33	86.67	93.33
		8000	3.33	3.33	6.67	16.67	36.67	50.00	60.00	80.00	83.33	93.33
		6000	0.00	0.00	0.00	6.67	10.00	30.00	46.67	70.00	83.33	96.67
		4000	0.00	0.00	0.00	0.00	3.33	10.00	23.33	36.67	60.00	83.33
		2000	0.00	0.00	3.33	3.33	3.33	6.66	20.00	40.00	46.66	76.67

TABLE I

MORTALITY OF *S. LITURA* F. FED ON ARTIFICIAL DIET MIXED WITH EXTRACT OF *AGLAIA* SPECIES

PLANT SPECIES	FRACTION	CONC (PPM)	ACCUMULATIVE % MORTALITY (ABBOTT'S FORMULA)									
			Day 1	2	3	4	5	6	7	8	9	10
Aglaia aquamulosa	C_6H_{14}	10000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.67	6.67	6.67
		8000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		6000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		4000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		2000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH_2Cl_2	10000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		8000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		6000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		4000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		2000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	MeOH	10000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		8000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		6000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		4000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		2000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

TABLE I

MORTALITY OF *S. LITURA* F. FED ON ARTIFICIAL DIET MIXED WITH EXTRACT OF *DYSOXYLUM* SPECIES

PLANT SPECIES	FRACTION	CONC (PPM)	ACCUMULATIVE % MORTALITY (ABBOTT'S FORMULA)									
			Day 1	2	3	4	5	6	7	8	9	10
Dysoxylum procatum	C_6H_6	10000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.66	6.66	16.67
		8000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.66	10.00	20.00
		6000	0.00	0.00	0.00	0.00	0.00	0.00	3.33	6.67	10.00	26.67
	CH_2Cl_2	10000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.33	3.33	3.33
		8000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.33	3.33	3.33
		6000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.33	16.67	23.33
	1) crystal	10000	0.00	0.00	0.00	0.00	0.00	0.00	3.33	6.67	6.67	16.67
		8000	0.00	0.00	0.00	0.00	0.00	3.33	3.33	6.67	6.67	16.67
		6000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.33	3.33	10.00
	2) non-crystal	10000	0.00	0.00	0.00	0.00	0.00	0.00	10.00	10.00	16.67	16.67
		8000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		6000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.33	10.00	13.33
	MeOH	10000	0.00	0.00	0.00	0.00	0.00	0.00	13.33	13.33	13.33	13.33
		8000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		6000	0.00	0.00	0.00	3.33	3.33	6.67	6.67	6.67	6.67	6.67

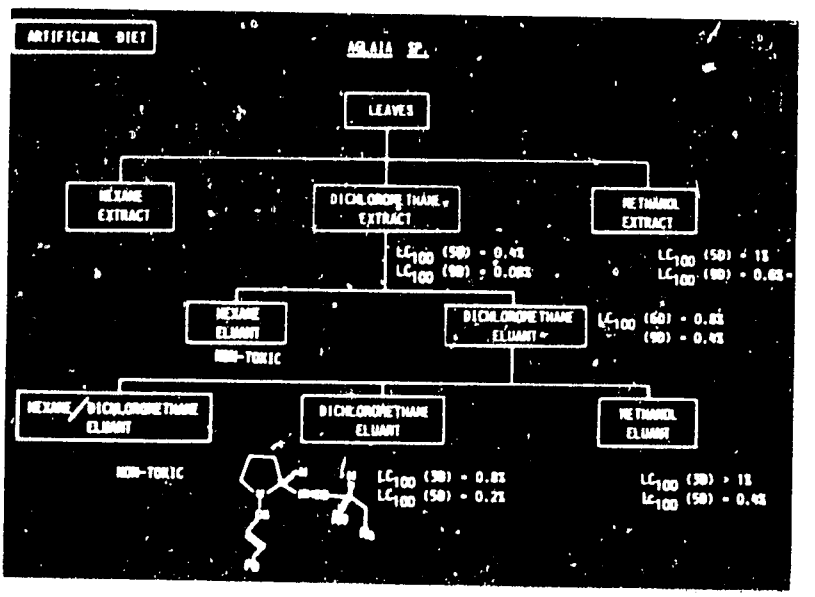
TABLE I

MORTALITY OF *S. LITURA* F. FED ON ARTIFICIAL DIET MIXED WITH EXTRACT OF *DYSOXYLUM* SPECIES

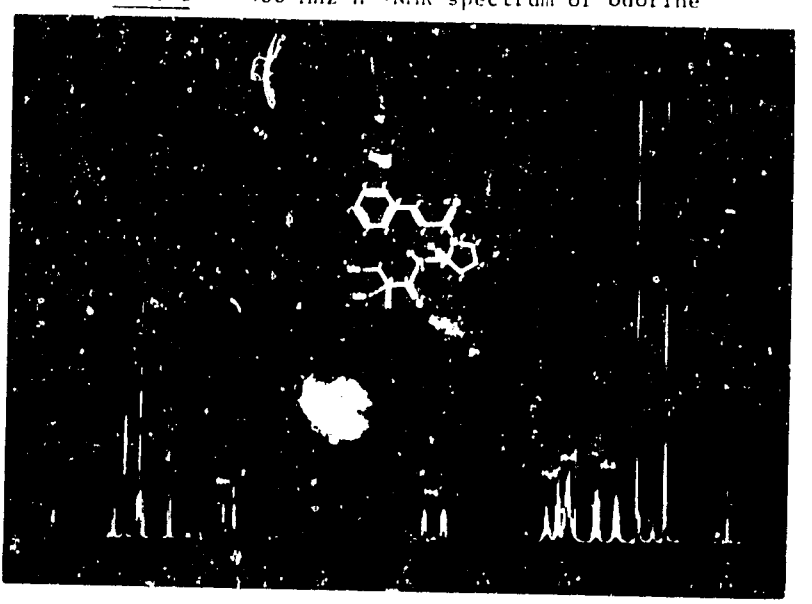
PLANT SPECIES	FRACTION	CONC(PPM)	ACCUMULATIVE % MORTALITY (ABBOTT'S FORMULA)									
			Day 1	2	3	4	5	6	7	8	9	10
Dysoxylum sp.	C_6H_6	10000	3.33	3.33	3.33	3.33	3.33	3.33	3.33	3.33	3.33	3.33
		8000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.67	10.00
		6000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.67	6.67	10.00
	CH_2Cl_2	10000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.33	3.33
		8000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		6000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	MeOH	10000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		8000	0.00	3.33	3.33	3.33	3.33	6.67	6.67	6.67	6.67	6.67
		6000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Separation was carried out to isolate the active constituents from *A. odorata* using the scheme as shown in Ill. 2. From the purified fraction of high activity, a bisamide, odorine(1) was isolated. Its structure was evidence from its NMR spectra (Ill. 3) and the stereochemistry was confirmed by x-ray crystallography (Ill. 4).

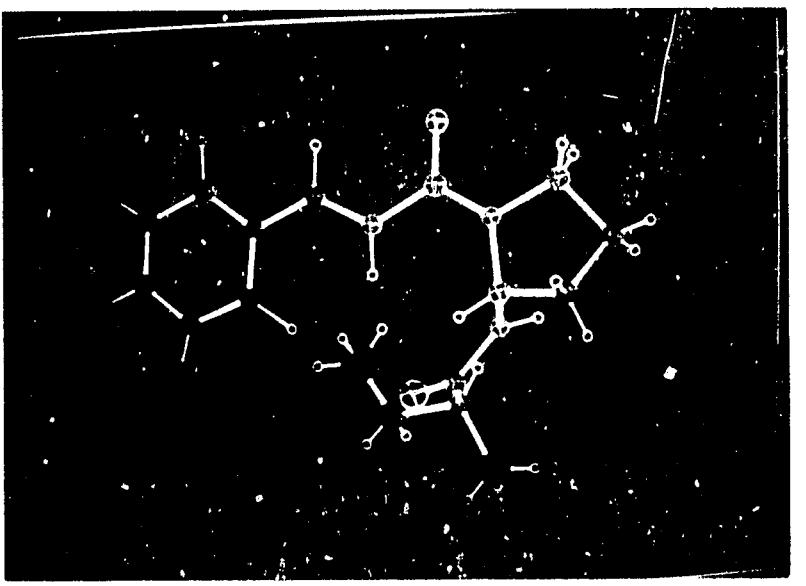
Ill. 2 - Diagram showing separation scheme for odorine



Ill. 3 - 400 MHz H^1-NMR spectrum of odorine

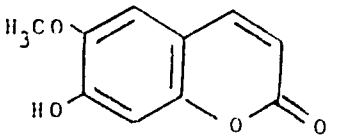


Ill. 4 - Crystal structure of odorine



Upon re-testing the activity of this pure component, no significant antifeeding activity was observed. Therefore this compound cannot be the active ingredient and may only acts as a synergist in the active fraction.

Further attempt was then made to isolate the minor constituents in the active fraction. Finally, with extensive chromatography (quick column, chromatotron) scopoletin (2) was isolated.



(2)

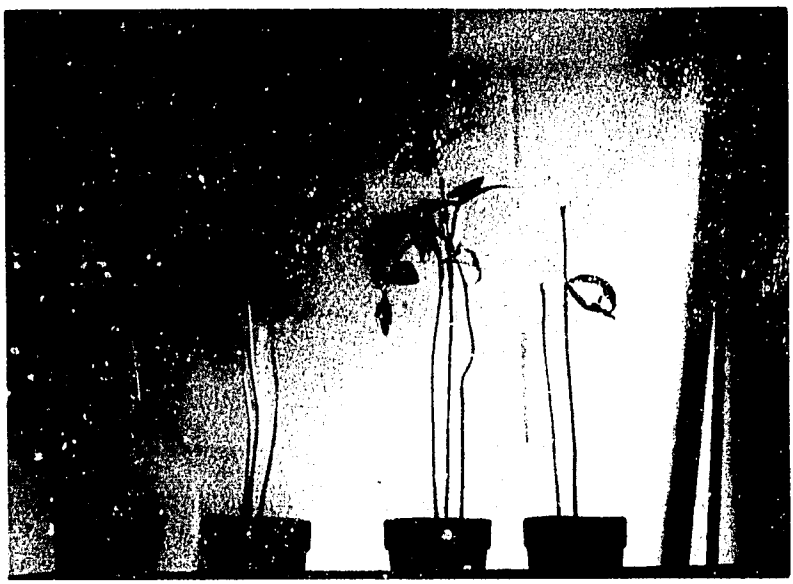
This substance was confirmed as the active ingredient since 100% larval mortality resulted from an LD method using 160 ppm solution of this substance.

Experiment was carried out to pre-determine the formulation and application method for testing the antifeeding efficiency of the crude extract in the field: ten third instar of *S. litura* were left to feed on each of 4 *Vigna radiata* plants in clay pots. The pots were surrounded by water in clay trays to prevent the larvae to leave the plant pots.

Crude methanolic extract was admixed with bentonite in 1:3 by weight : 3 parts of bentonite were added to 1 part of methanolic extract in methanol, the solvent was then evaporated to dryness to give a brown powder.

An aqueous emulsion containing 0.05% Triton and an equivalent of 2% crude methanolic extract was made from the brown powder and sprayed on two of the *Vigna radiata* plants. As a control, the other two plants were sprayed with a solution containing the same concentration of Triton and bentonite. A marked difference in the leave damage between the two sets of plants was observed. After 6 days, the control plants were completely eaten up while the treated plants remained healthy. The results is shown in Ill. 5.

Ill. 5 - *Vigna radiata* 6 days after treatment with crude methanolic extract (treated-control-treated-control).



A small scale field test was then carried out with this formulation. 150 *Vigna radiata* plants were grown in cement pots (Ill. 6). After 30 days, 300 third instar larvae of *S. litura* were left to feed on the leaves in each pot (Ill. 7). The plants in 4 pots were sprayed with the tested solution (Ill. 8). As a control, another 4 pots were sprayed with an aqueous solution containing the same concentration of bentonite and triton. A marked difference in the degree of feeding was observed (Ill. 9, Ill. 10) at 24 hours. Visual estimation of the area of leaf damage and number counts of remaining larvae were done at 24, 48 and 72 hours after spraying, using the following formula :-

Calculation of area of leaf damage by *S. litura*

$$\% \text{ of leaf damage} = \frac{a(5 \times 0.5) + b\,(5 \times 0.38) + c\,(5 \times 0.18)}{5^{*} \times n^{**}}$$

$$= \frac{a(2.5) + b(1.9) + c\,(0.9) \times 100}{5 \times n}$$

a = no. of leaves which were damaged more than 50%

b = no. of leaves which were damaged more than 25%

c = no. of leaves which were damaged more than 10%

(or no. of leaves with continued damaged spots)

d = no. of leaves which were less than 10% damaged

(or no. of leaves with discontinued damaged spots)

e = no. of leaves which were not damaged

5^{*} = approx. area of 5 cm^2, in which degree of damage was estimated

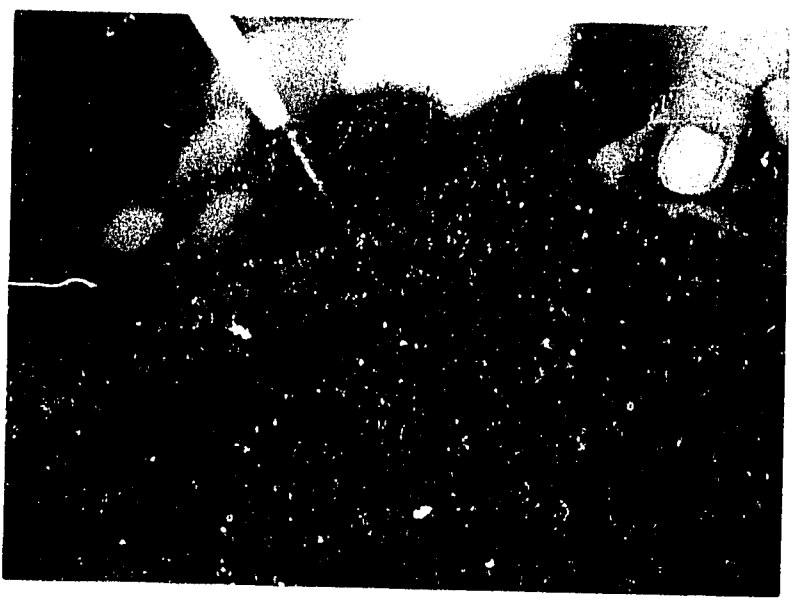
n^{**} = no. of leaves randomly selected for checking in each treatment

Similar test was also carried out with an aqueous extract of 10% (w/w) fresh leave and 0.05% Triton.

The results of both tests were shown in III. 11 and III. 12.

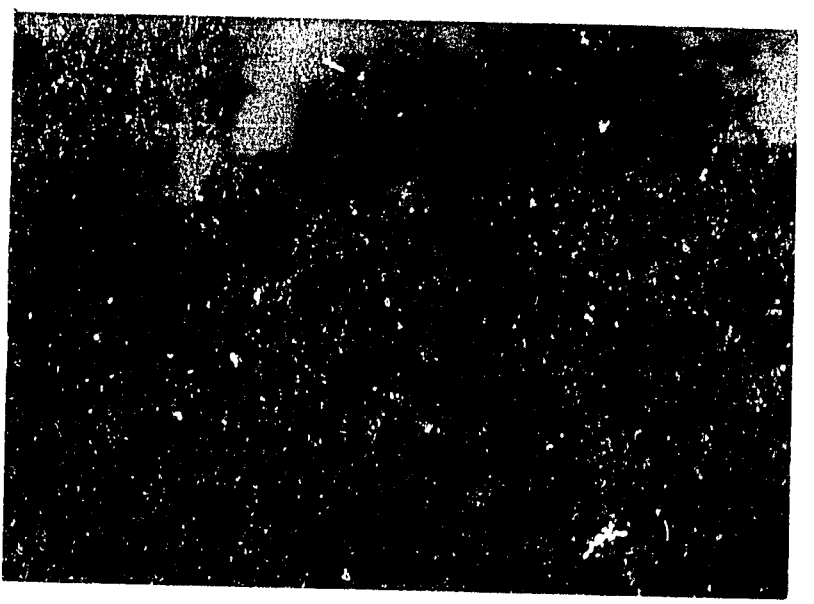
Ill. 6 - *Vigna radiata* in cement pot, 30 days old.

Ill. 7 - *S. litura* were left to feed on *Vigna radiata* leave.



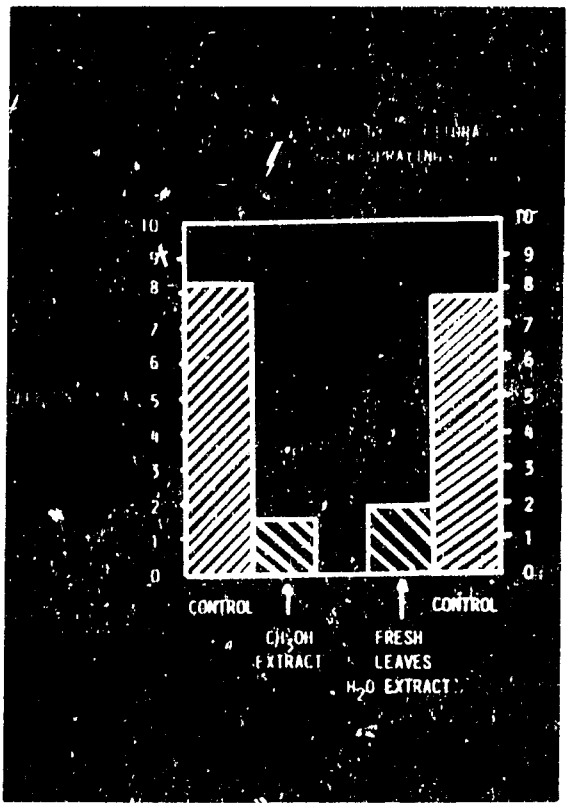
Ill. 8 - Spraying with tested solution.

Ill. 9 - *Vigna radiata* 24 hrs after spraying with control solution.

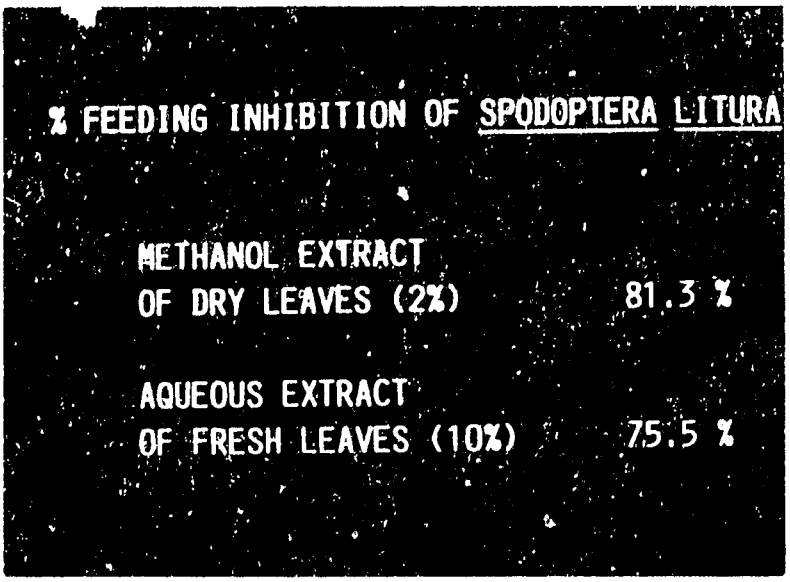


Ill. 10 - *Vigna radiata* 24 hrs after spraying with tested solution.

Ill. 11 - Comparison of feeding area in tested and control experiment.



Ill. 12 - % Feeding inhibition by tested solutions.



The efficacy of the *Aglaia sp* in crop protection was tested in the farmer plots:

Field Test 1, Ill. 13

Date : 10 December 1987

Place : Farmer plots at Ban Mae Tae, Amphur Sun-Sai, Chiang Mai

Crop : Cauliflowers, 45 days after seedling transfer

Number of larva : *S. litura*'s eggs were attached to the cauliflower's leaves (Ill. 14). After 7 days, larvae hatched and grew to third instar (Ill. 15), at which time the number of larvae was counted prior to spraying.

Treatments T_1, sprayed with control solution, 0.05% Triton in aqueous solution

T_2, sprayed with an aqueous suspension containing 8% of methanolic extract-bentonite (1:3) mixture and 0.05% Triton

T_3, sprayed with 10% fresh leaf aqueous extract with 0.05% Triton

T_4, sprayed with 10% dry leaf aqueous extract with 0.05% Triton

Observation - count number of larvae prior to spraying, 12, 24 and 48 hours after spraying

Results - summarized in Ill. 16.

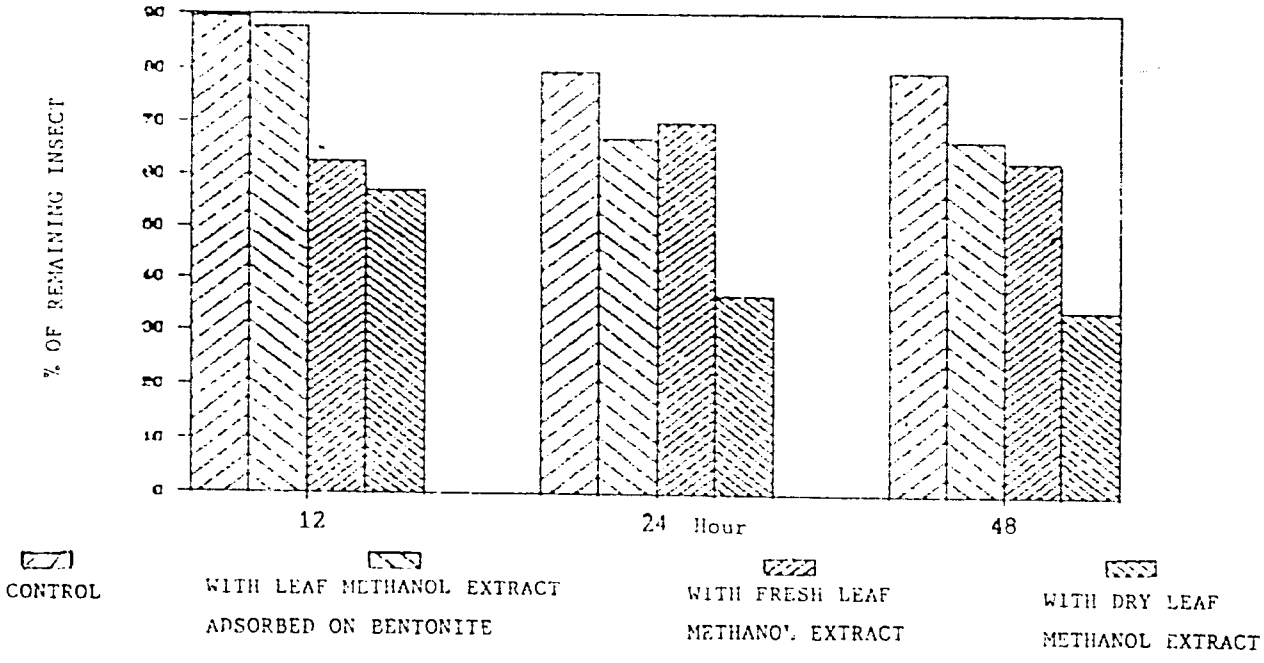
Ill. 13

Cauliflowers plots used in Field Test I.

Ill. 14 Attaching *S. litura'* eggs to cauliflowers' leaves.

Ill. 15 - Third instar larvae from the attached eggs.

Ill. 16 - NUMBER OF INSECT AFTER TREATMENT WITH AGLAIA SP.



Field Test 2

Date : 21 February 1988

Place : Farmer plots beside road leading to Wat Sart Luang, Chiang Mai

Crop : Broccoli, 40 days after seedling transfer

Number of larvae : 200/rep.

Number of replications : 3 replications of 1 x 3 m^2 plot.

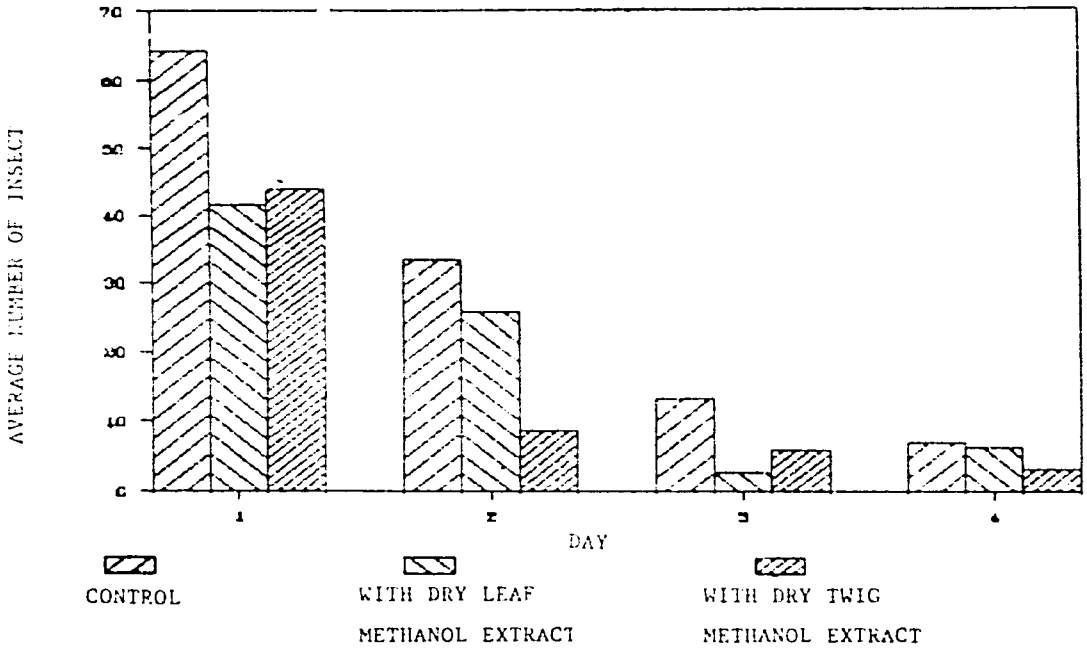
Treatment : T_1, sprayed with control solution, 0.05% Triton in aqueous solution.

T_2, sprayed with 10% dry leaf aqueous extract with 0.05% Triton.

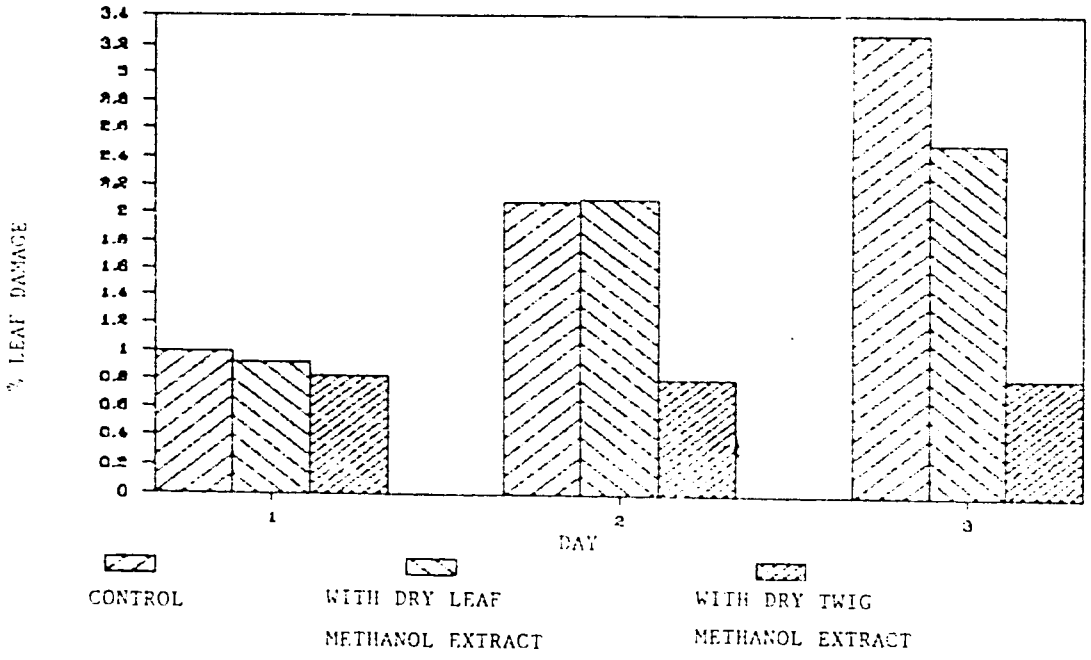
T_3, sprayed with 10% dry twig aqueous extract with 0.05% Triton.

Results summarized in Ill. 17, and Ill. 18.

Ill. 17 - NUMBER OF INSECT AFTER TREATMENT WITH AGLAIA SP.



Ill. 18 - - LEAF DAMAGE AFTER TREATMENT WITH AGLAIA SP.



Although some degree of efficacy of *Aglaia sp.* extract in crop protection was observed, accurate results could not be acheived due to the diminishing numbers of larvae both in the treated and control experiment.

Another experiment was then carried out under the better controlled conditions.

Date : 30 March 1988

Place : Post-harvest Centre, Chiang Mai University

Crop : Broccoli (25 days after seedling transfer) in clay pot surrounded by 1 inch of water in clay tray to prevent the larvae from escaping.

Number of larvae : 20/pot

Replications : 4 replications of 10 pots

Treatment : T_1, sprayed with control solution, 0.05% Triton in aqueous solution

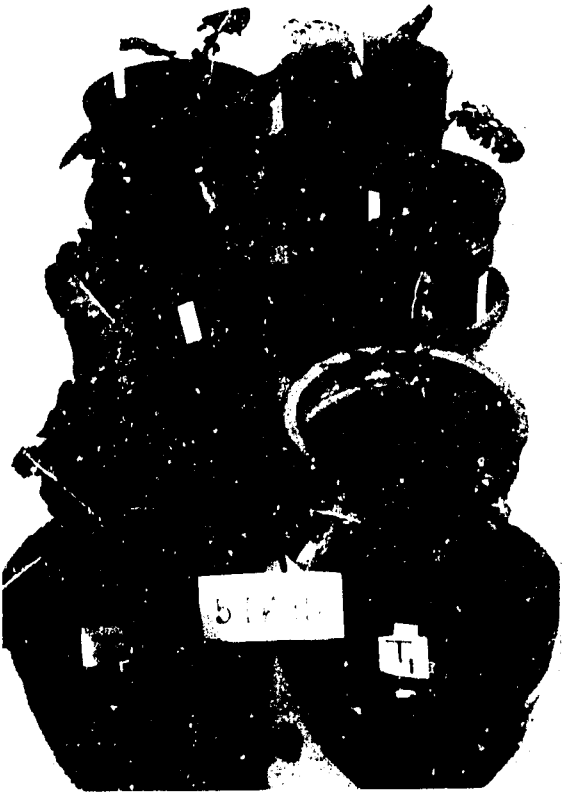
T_2, sprayed with an aqueous emulsion of 2% leaf methanolic extract and 0.05% Triton.

T_3, sprayed with an aqueous emulsion of 2% twig methanolic extract and 0.05% Triton.

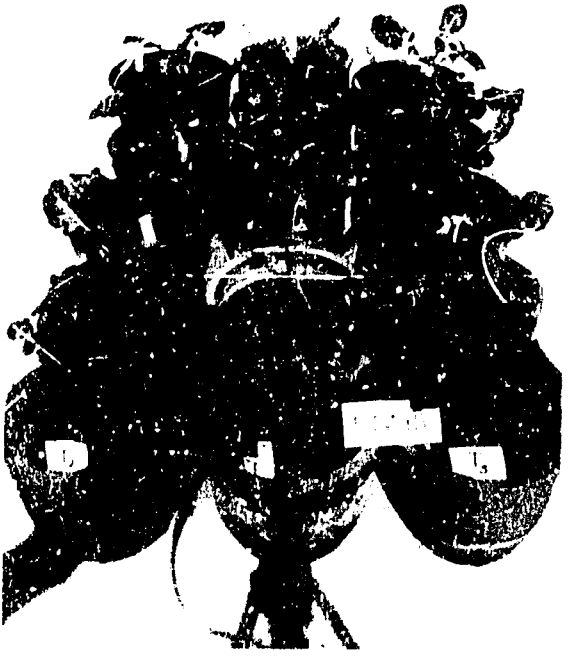
Results : summarized in Ill. 19, 20, 21, 22, 23.

Ill. 19 - Comparison of leaves damage 4 days after treatment with 2% leaf methanolic extract and control.

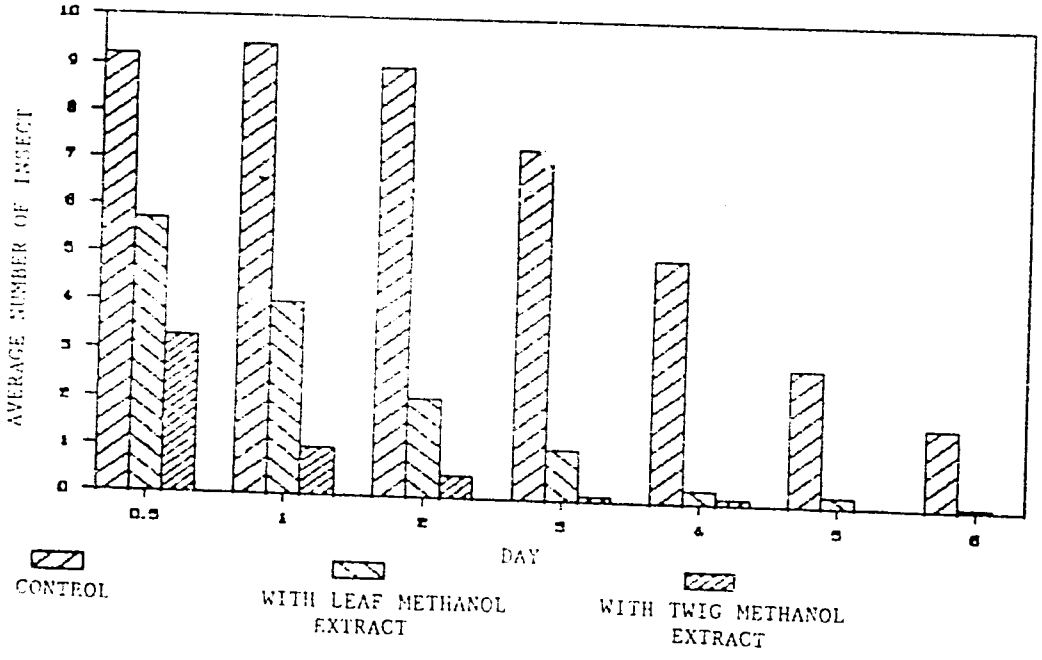
Ill. 20 - Comparison of leaf damage 5 days after treatment with 2% leaf methanolic extract and control.



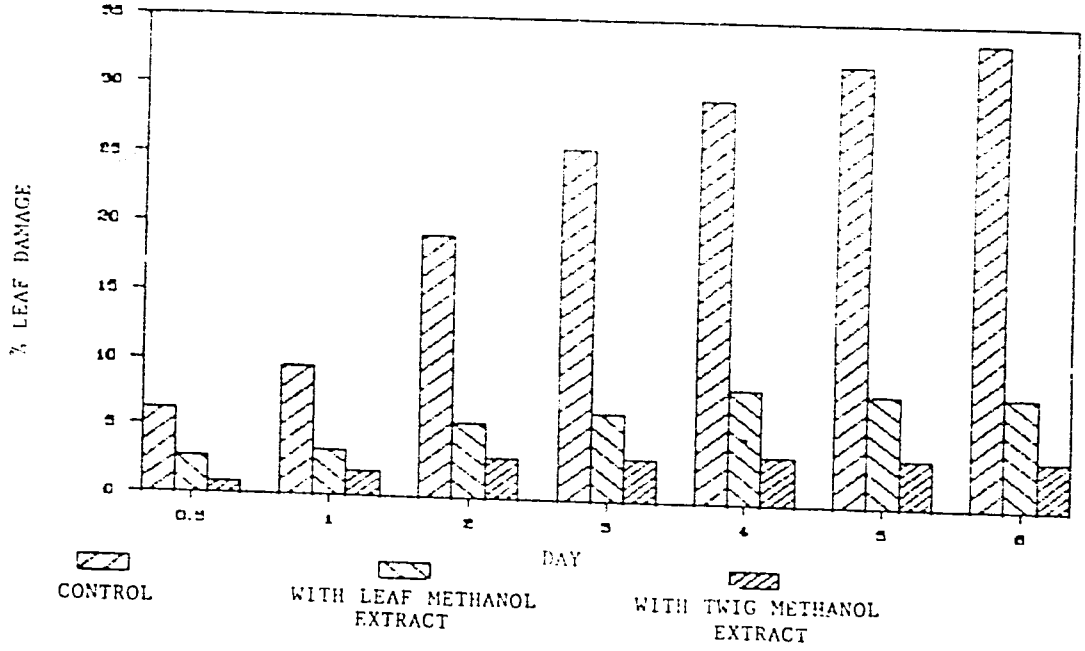
<u>Ill. 21</u> - Comparison of leaf damage 5 days after treatment with 2% leaf methanolic extract (T_2), 2% twig methanolic extract (T_3), and control (T_1).



111. 22 - NUMBER OF INSECT AFTER TREATMENT WITH AGLAIA SP.



Ill. 23 - LEAF DAMAGE AFTER TREATMENT WITH AGLAIA SP.



REMARK/WORK PLAN

It can be concluded from the experiments on *Aglaia sp.* that there exist a possibility of using this plant as an antifeedant for *Spodoptera litura* in agriculture. Plans have been made for the following studies:

1) Further assess other formulations of *Aglai sp.* in the field.

2) Compare the activity of scopoletin, which is the active antifeedant, in this plant, with other analogues.

Toward the end of this reporting period, we had reassessed the insecticidal activity of the plants which had been previously studied and recorded in our earlier report with various formulations. Remarkable results were obtained from the preliminary studies. The final period of this project will then be also spent in evaluating their applicability in the fields.

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