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MEMORANDUM

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

FROM: AID/SCI, Victoria Ose VO

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

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. CS-198
PR#1

Attachment

PROGRESS REPORT NO. I

USE OF LABIATAE PLANT EXTRACTS FOR INSECT CONTROL

A RESEARCH PROJECT

GRANT NO. 936-5544-G-00-8040-00

SUBMITTED BY

DR.SUPANEE PIMSAMARN

PROJECT LEADER

DEPARTMENT OF ENTOMOLOGY

FACULTY OF AGRICULTURE

KHON KAEN UNIVERSITY

KHON KAEN 40002, THAILAND

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Project Profile

Country : Thailand

Grant No. : 936-5544-G-00-8040-00

Peogram : Program on Science and Technology Cooperation

Project Title : Use of Labiatae Plant Extracts for Insect Control

Project Leader : Dr.Supanee Pimsamarn

Organization : Department of Entomology, Faculty of Agriculture
Khon Kaen University

Co-investigators : Dr.Dawan Boonyarat
Dr.Tasanee Jamjanya
Ms.Nutcharee Siri
Mr.Apichai Therdthianwong

Israeli Collabarators : Dr.Raphael Ikan
Department of Organic Chemistry
The Hebrew University of Jerusalem
Dr.Uzi Ravid
Newe Ya'ar Regional Experiment Station

Total Project Budget : $ 200,000

Project Duration : June 1,1988 - June 1, 1992

Reporting Period : June 1,1988 - December 1,1988

Budget Allocation for This Period : $ 45,093.75

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1. **Background/Introduction** : Two major inputs for entomological research on bioassay test for botanical toxic substances evaluation studies are insect test cultures and plant samples to be investigated.

Test insect is an important input which requires good planning to have sufficient number of the right age and sex at the right time. Mass rearing techniques of the diamondback moth, the brown planthopper and the cowpea weevil were reported (Liu and Sum, 1984; Heinrich et al., 1981; Strong et al., 1968). However, simple and less labour-intensive methods is considerably useful for our research.

2. **Objectives** : The overall aim of this research project is to explore the possibility of using phytochemical substances from the common weeds in Labiatae for insect control.

Major attempts focused on the first six-month period are :

1. Establishing insect rearing facilities and cultures.
2. Collecting and planting of Labiatae plants.
3. Laboratory renovation and crude extract preparation.

3. **Materials and Methods** :

3.1 Survey and Species Confirmation of Labiatae Plants. Extensive field survey and collecting of Labiatae plant samples were conducted mainly in Khon Kaen and nearby provinces; Ubol Rajathani, Surin, Buri Ram and Sri Saket etc. Part of each sample collected were sent for species confirmation at the Department of Botany, Faculty of Science, Khon Kaen University. The bulks were propagated in field plots, 1.5m. x 5 m. Plant materials will be available for extract preparation throughout the experiment.

3.2 Mass Rearing of Test Insects. Three insect species are used throughout the studies. They are; the cowpea weevil, *Callobruchus maculatus* Fabr.; the diamondback moth, *Plutella xylostella* Linn.; and the brown planthopper, *Nilaparvata lugens* (Stål). Mass rearing technique for each species is as follows:

3.2.1 The Cowpea weevils (CW). Adults were collected from mungbean seed storage, Khon Kaen University Experimental Farm, in May 1988. Approximately 3000 adults (or 20 ml. by volume) were maintained in 4.5 liter glass jar provided with 1.5 kg. mungbean seeds as food source and ovipositional site. The seeds were freezed for three days prior to use in order to kill other insects or natural enemies. The jar was also covered with blotting paper to prevent natural enemies contamination. Eggs were collected daily and the adults emerged within 20-23 days afterwards. Adults were then sifted and transferred to new mungbean seed jar thus provide the age uniform weevil supply for tested.

Simple technique for adult transferring is implied. Leaving crinkle paper in the rearing jar for a few minutes, adult will aggregate on the paper which can be easily transferred to another container.

3.2.2 The Diamondback Moth (DBM). Larvae and pupae were collected from farmer field within Khon Kaen, mixed and reared in the insectary on 30 to 40-day-old potted chinese kale in larval-rearing cage, 50 cm. x 50 cm. x 80 cm. To have a continuous supply of larval feeds, planted unsprayed Chinese kale are maintained in cement blocks, 1.5 m. x 4 m. nearby the insectary.

Pupae were collected and kept in wired frame egg-laying cage, 25 cm. x 25 cm. x 25 cm., provided with 7 to 10-day-old Chinese kale seedlings for oviposition. Seedlings were renewed daily and maintained in separate larval-cages in order to obtain batches of age-uniform larvae for test.

3.2.3 The Brown Planthopper (BPH). An initial population of paddy field-collected BPH was obtained from Khon Kaen Rice Experiment Station. RD1 rice variety is grown weekly in clay pots. BPH culture was maintained on 30 to 40-day-old plants in 60 cm. x 60 cm. x 100 cm. cages. Plants were changed twice a week for nymphal-rearing cage and daily for adult-rearing cage to obtain the age-uniform nymph. Gravid adults could be obtained about 24 days after the eggs are deposited.

To prevent contamination from BPH parasites and predators and also from other insect pests, lower leaf sheath of the fresh plants were removed and the plants were splashed with water before placing in the cages.

3.3 Crude Extract Preparation. Air dried whole plant samples were powdered to a uniform mesh in a wiley mill. Exact weight of the powdered material was successively extracted with hexane, chloroform, and methanol at room temperature for 45 days. Each filtrate was evaporated to dryness to give a crude material which was stored in the refrigerator until used for bioassay test.

4. Results/Discussion

4.1 Survey and Species Confirmation of Labiatae Plants. Species of collected plant samples are shown in Table 1. According to Chomchalao (1971), there are four *Ocimum* species in Thailand. They are *O. basilicum*, *O. sanctum*, *O. canum*, and *O. gratissimum*. They are edible and widely distributed in every parts of Thailand. *O. basilicum*, *O. canum* and *O. sanctum* are more popular as food flavor. In some places they are grown in small scale for local markets. They are fast growing although few insects, aphid and lepidopterous leaf roller, are sometimes found damaging the plants. *M. cordifolia* is also used as an important food flavor.

O. aristatus is a well-known medicinal plant and now available in the market as an economic crop.

H. suaveolens is completely wild weed. It is found commonly in crop fields and along the roadside. It has very strong smell and shows perfectly natural resistance to insect damage. We considered it as the most prominent species to be tested.

4.2 Mass Rearing of Test Insects. A continuous and ample supply of test insects is essential to our research. Simple and less labour-intensive techniques is also required.

CW number and proper amount of food source in each containmer is important for its mass rearing technique. The insect spent its entire immature life within a single host seed, thus the major density-dependent source of mortality among bruchids is the competition among larvae within a single seed (Umeya et al., 1975). In our laboratory, we use approximately 3000 adults per 1.5 kg. mungbean seeds which is enough for new progeny development.

Liu and Sun (1984) used rape seedlings for DBM rearing. This technique consumed a lot of rape seeds. Rape is not grown in Thailand and other cruciferous seeds are also expensive. In our research, we use 30 to 40-day-old chinese kale plants for larval feed. Planting new seedlings in every two weeks can provide continuous supply of food source. This method is efficient and need no specific growth chamber or environmental room. We can daily obtain enough age-uniform larval culture for test.

Successful BPH rearing technique is already established by the entomologists in the International Rice Research Institute (Heinrichs et al.,1981). We use the same technique except RD1 rice variety is used as food source instead of TN1 which is not grown in Thailand.

4.3 Crude Extract Preparation. Renovation of extracting apparatus is complete. Three crude extract samples of dried *H. suaveolens* has been obtained. Table 2 illustrates its brief extraction.

5. Conclusion and Remarks :

Research work has been progress well correspondingly to the previous scheduled plan. One research assistant with M.S. Entomology majoring in toxicology, which is highly responsible for bioassay test, and three labours have been employed. Two basic needs, plant and insect test cultures have been established. Successively crude extract preparation has been started.

6. Workplan for the Next Period :

Three major activities concerning the next six-month period are :

1. Maintaining of insect and plant cultures.
2. Successively crude extract preparation from each of Labiatae plant sample.
3. Intensive tests for biological toxicity of each extracted sample for each insect species. Standard test methods will be designated before test.

Table 1. List of Labiatae species collected

Scientific name	Common name	Place collected
1. *Ocimum basilicum* Linn.	common basil, sweet basil	Khon Kaen, Ubol Rajathani, Surin.
2. *Ocimum sanctum* Linn.	holy basil	Khon Kaen, Ubol Rajathani, Surin, Sri Saket.
3. *Ocimum canum* Linn.	hairy basil	Khon Kaen, Surin.
4. *Ocimum gratissimum* Linn.	-	Khon Kaen.
5. *Hyptis suaveolens* Poit.	-	Khon Kaen.
6. *Orthosiphon aristatus* Miq.	-	Khon Kaen.
7. *Mentha cordifolia* Opiz.	kitchen mint	Khon Kaen, Ubol Rajathani.

Table 2. Brief extraction of *Hyptis suavedens* (from initial plant sample of 150 gm. using Soxhlet continuous extractor)

Solvent	Volume of solvent (ml.)	Crude material (g.)
Hexane	1000	15
Chloroform	1000	10
Methanol	1000	31.5

7. References

Chomchalao, Narong. 1971. Preliminary studies on genetics of the sweet basil (Ocimum basilicum Linn.). J. Agricultural Science 4(2):133-142 (In Thai).

Heinrichs, E.A.,S. Chelliah, S.L. Valencia, M.B. Arceo, L.T. Fabellor, G.B. Aquino, and S. Pickin. 1981. Manual for Testing Insecticides on Rice. International Rice Research Institute, Laguna, Philippines.

Liu, M.Y., and C.N. Sun. 1984. Rearing diamondbacked moth (Lepidoptera : Yponomeutidae) on rape seedlings by a modification of the Koshihara and Yamada method. J. Econ. Entomol. 77:1608-1609.

Strong R.g., g.J. Partida and D.N. Warner. 1968. Rearing stored-product insects for laboratory studies: bean and cowpea weevils. J. Econ. Entomol. 61:77-751.

Umeya K., T. Kats, and T. Kocha. 1975. Studies on the comparative ecology of bean weevils. VI. Intraspecific larval competition in Callobruchus analis (F.). Jap. J. app. Ent. Zool.19:47-53.

8. Scientific collaboration : Prof. Raphael Ikan has already received the first payment. The work on the first six-month period is focused on laboratory renovation and procurment of equipment. Varieties of Ocimum species, especially O. basilicum originally from Thailand, have been grown for their agronomic evaluations.

Annex / Pictures

Figure 1. Part of PEIC project researchers

A. Dr.Supanee	Pimsamarn	Principle Investigator
B. Dr.Tasanee	Jamjanya	Researcher
C. Ms.Nutcharee	Siri	Researcher
D. Dr.Dawan	Boonyarat	Researcher

Figure 2. A. Insectary and experimental plots

B. Research assistant (left) and helpers are at work.

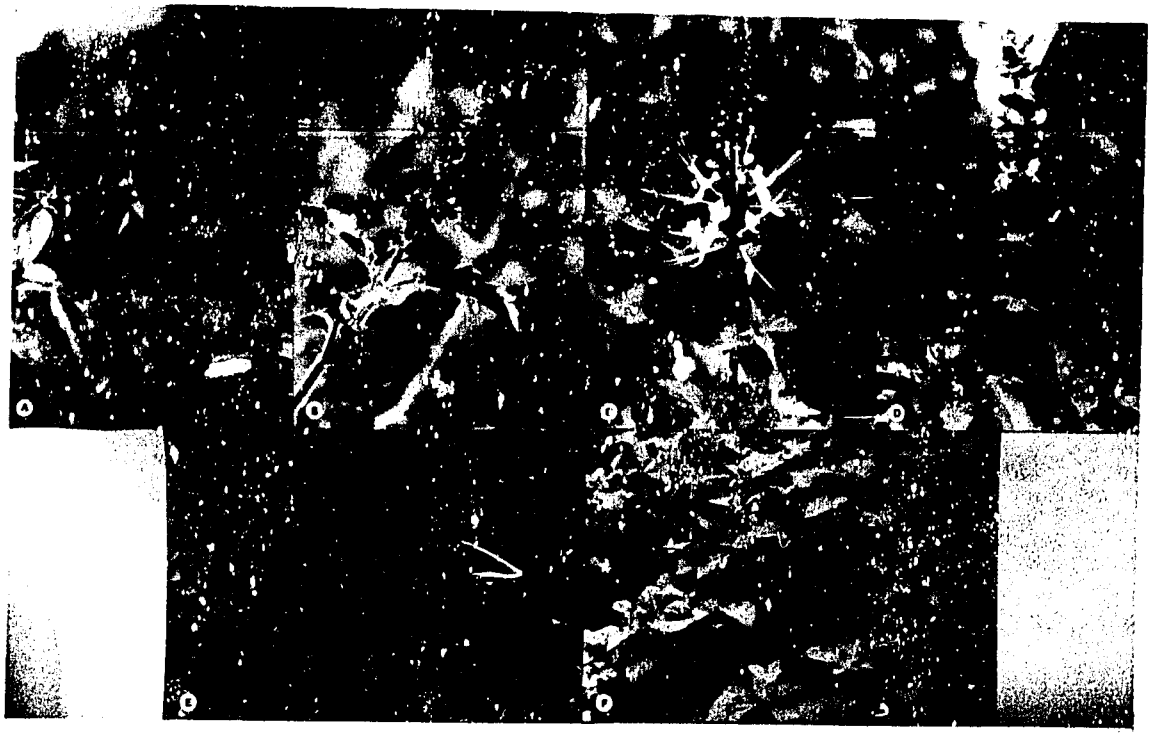

Figure 3. Labiatae species

A. Ocimum basilicum Linn.

B. Ocimum sanctum Linn.

C. Orthosiphon aristatus Miq.

D. Ocimum canum Linn.

E. Mentha cordifolia Opiz.

F. Hyptis suaveolens Poit

Figure 4. A. The cowpea weevil, Callobruchus maculatus Fabr.

B. The brown planthopper, Nilaparvata lugens (Stål)

Figure 5. The diamondback moth, Plutella xylostella Linn.

A. Larva

B. Egg