Summary

Title of Project : Effect of azadirachtin on specific tissues of cockroaches- *Periplanata americana*

Institute where research was carried out : Department of Chemistry, University of Kelaniya and Industrial Technology Institute (ITI).

Chief Scientific Investigator : Dr.(Mrs) P.A. Paranagama

Date of award : 15th January 1997

Date of completion : 04th April 1999

Scientific Background and Scope/Objective of Project

Azadirachtin is a potent insecticidal compound present in neem seeds (*Azadirachta indica)*. This was originally identified as the major component responsible for the antifeedant activity of the plant, since then the compound has been shown to have a range of biological activities on metamorphosis and development of insects. The neem extracts containing azadirachtin are of particular interest at present due to the fact that they have low mammalian toxicity and low environmental problems in contrast to the synthetic pesticides.

Many bioassay experiments have been conducted with partially purified active components, their efficacy and levels of sensitivity are in question. Further neem extracts produce a variety of physiological activities in many insects. However their aspects have not been fully investigated.

The purpose of this study was to understand few physiological activities related to azadirachtin towards one common household pest in Sri Lanka, namely *Periplanata americana*

Experimental methods:

1. Extraction and isolation of azadirachtin from neem seeds and neem cake was carried out using flash chromatographic method and MPLC method.
2. Series of concentration (0.5μg-4μg) of azadirachtin were introduced into the haemolymph of the cockroaches. Both male and female cockroaches were used for the experiments. The body weight reduction and fecal production were measured for 12days.
3. Effect of azadirachtin on activity of midgut enzymes of cockroaches was measured *in vivo* and *in vitro.* This study was to determine the effect of azadirachtin on neurohormonal stimulation of the midgut enzymes in cockroaches.
4. Neem seed volatile was isolated by steam distillation and analysed by GC. Neem seed volatile was tested against cockroaches. Behavioral bioassays, EAG (to construct dose response curve) and GC-EAG were performed to determine the repellant activity of neem volatile.

Results obtained:

1. Azadirachtin was isolated from neem seeds and neem cake by flash chromatographic method and MPLC method. Purity of the sample was confirmed by TLC, HPLC and ^{1}H NMR. The yields of azadirachtin were 0.065% from neem seeds and 0.12% from neem cake.
2. Effect of different concentration of azadirachtin and neem seed oil on body weight of adult male and female cockroaches are shown in fig. 7 and 8.

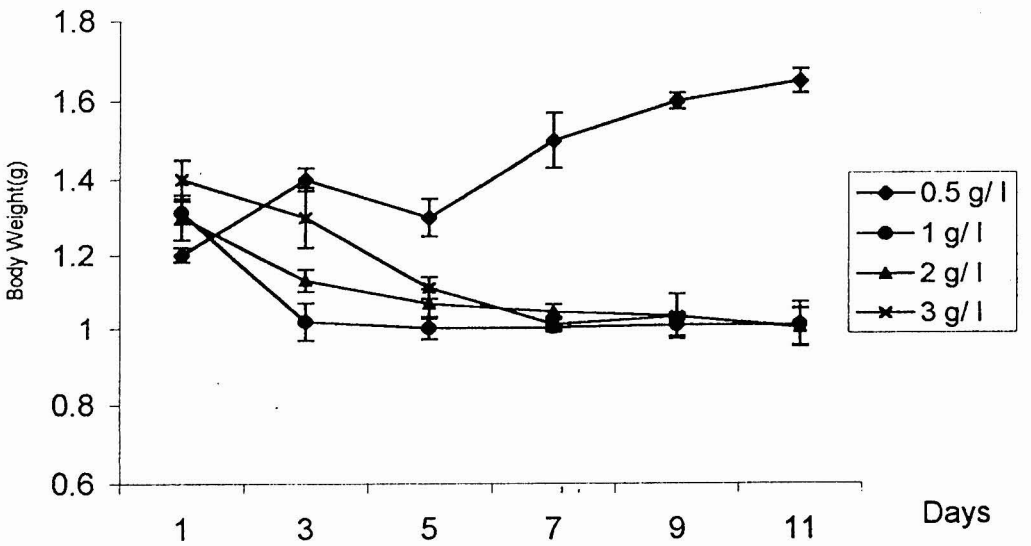

Fig.1.Effect of Different concentration of Azadirachtin on body weight of adult male cockroaches P.americana.

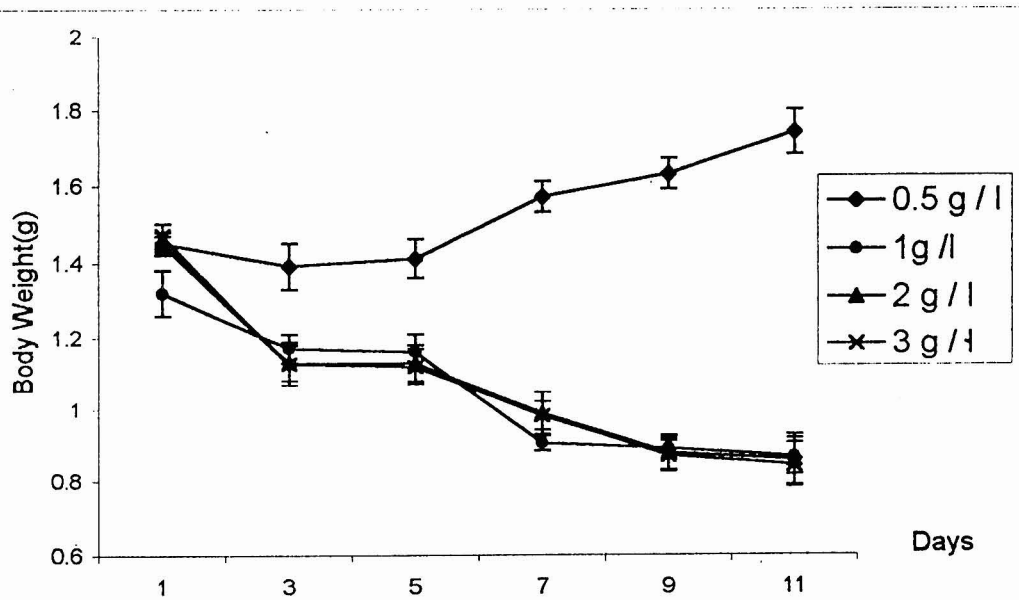

Fig.2.Effect of different Neem Seed Oil concentrations on body weight of adult male cockroaches P.americana.

3. The results of effects of azadirachtin on activity of midgut enzymes are shown in fig. 12 and table 5 shows the data obtained for activity of midgut enzymes in azadirachtin injected cockroaches.
4. EAG results (Table 7) and behavioral bioassay results (Table 8) of cockroaches were obtained after introducing neem volatile.
5. Fig. 22 shows the GC-EAG responses of antennae of cockroaches and FID chromatogram of neem volatile.

Conclusion

1. Two methods were employed to isolate azadirachtin from neem seeds and neem cake. The results reveal that yield was enhanced by MPLC method.
2. The effect of azadirachtin on body weight was dose dependent and the cockroaches treated with azadirachtin showed a continuous weight reduction.
3. Studies on activity of midgut enzymes reveal that more than 50% of the activity was reduced by azadirachtin and the similar results were obtained from the ligatured cockroaches. Hence the toxic effect of azadirachtin is associated with a disruption of endocrine events in cockroaches.
4. Studies on neem volatile against cockroaches demonstrate the repellant properties of neem volatile. GC-EAG results indicate more than five compounds including major compound, are physiologically active against cockroaches.

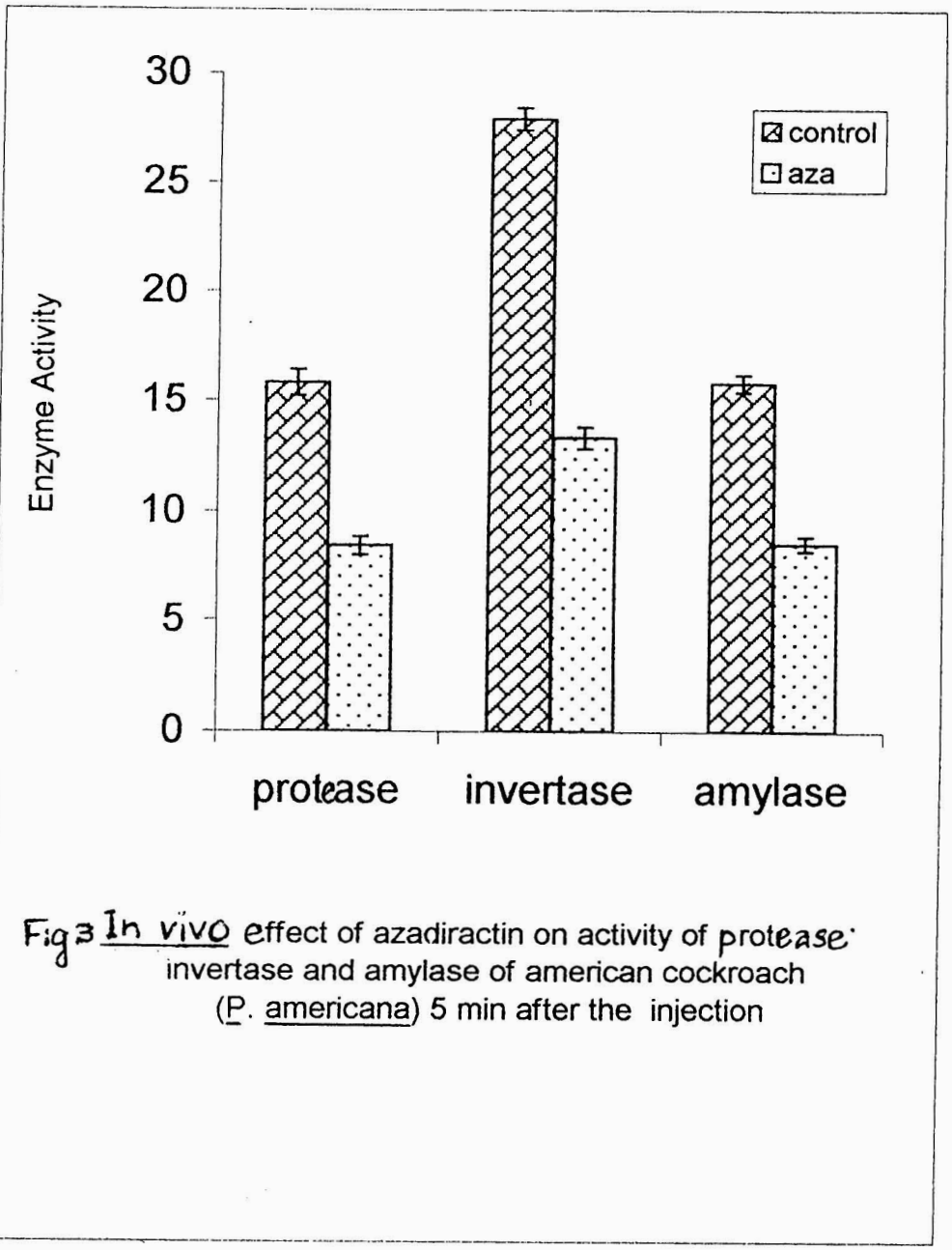

Fig 3 In vivo effect of azadiractin on activity of protease, invertase and amylase of american cockroach (P. americana) 5 min after the injection

Table 1 : Effect of azadirachtin on activity of midgut enzymes *in vivo* and *in vitro* (n=7)

In vivo

Enzyme	Incubation time Mins	Enzyme activity µg/total of protein/hr	
		Treated insects (n=7)	Control insect (n=7)
Protease	5	8.42±.(0.004)	15.79 ±(0.02)
Protease	30	8.13±.(0.03)	14.09±.(0.05)
Amylase	5	8.53±(0.032)	15.79±(0.01)
Amylase	30	8.34±.(0.02)	15.45±.(0.05)
Invertase	5	13.32±(0.005)	27.91±(0.03)
Invertase	30	13.05±.(0.05)	27.45±.(0.05)

In vitro

Protease	5	16.70±(0.04	15.45±(0.04)
Protease	30	15.76±.(0.034)	14.09±.(0.05)
Amylase	5	28.85±(0.05)	28.51±(0.05)
Amylase	30	14.09±.(0.05)	15.85±.(0.06)
Invertase	5	15.76±(0.034)	14.0±(0.005)
Invertase	30	28.51±.(0.01)	30.05±.(0.04)

Table 2 : EAG responses of cockroaches *P americana*

Dose (µg)	**Mean Respones (mV)** (n=7)			
	Adult male	Adult female	Nymph male	Nymph female
0.25	0.09±(0.04)	0.16±(0.05)	0.12±(0.05)	0.16±(0.005)
0.5	0.13±(0.08)	0.18±(0.01)	0.14±(0.01)	0.17±(0.001
1.0	0.126±(0.05)	0.19±(0.03)	0.13±(0.04)	0.21±(0.003
2.0	0.16±(0.07)	0.193±(0.02)	0.13±(0.04)	0.21±(0.002
4.0	0.146 ±(0.05)	0.2± (0.02)	0.16±(0.05)	0.22±(0.002)
5.0	0.17±(0.02)	0.26±(0.05)	0.15 ±(0.05)	0.25±(0.01)
10.0	0.13±(0.01)	0.22±(0.02)	0.13±(0.01)	0.22±(0.006)
15.0	0.11±(0.03)	0.21±(0.03)	0.12±(0.03)	0.19±(0.002)

Table 3: Behavioral bioassay results of cockroach *P. americana* (n=7)

	Adult Male		Adult Female	
concentration (mg/ml)	Baited	non Baited	Baited	non Baited
2	1.71±(0.04)	6.28±(0.05)	0.59 ±(0.06)	7.43±(0.05)
4	0.29±(0.04)	7.71±(0.04)	0.15±(0.04)	7.71±(0.04)
5	1.00±(0.04)	7.00±(0.05)	0.37±(0.04)	7.42±(0.05)
10	1.01±(0.06)	6.99±(0.08)	0.47±(0.06)	7.35±(0.08)
15	0.96±(0.08)	7.10±(0.04)	0.46±(0.08)	7.40±(0.04)

	Nymph male		Nymph Female	
concentration (mg/ml)	Baited	non Baited	Baited	non Baited
2	1.28 ±(0.04)	6.71±(0.05	1.14 ±(0.03)	6.71±(0.08)
4	0.29±(0.04)	7.71±(0.04)	0.28±(0.04)	7.77±(0.04)
5	0.86±(0.04)	7.14±(0.05)	0.71±(0.07)	7.28 ±(0.02)
10	0.85±(0.06)	7.16±(0.08)	0.73±(0.06)	7.27±(0.08)
15	0.85±(0.08)	7.15±(0.03)	0.712±(0.08)	7.32±(0.04)

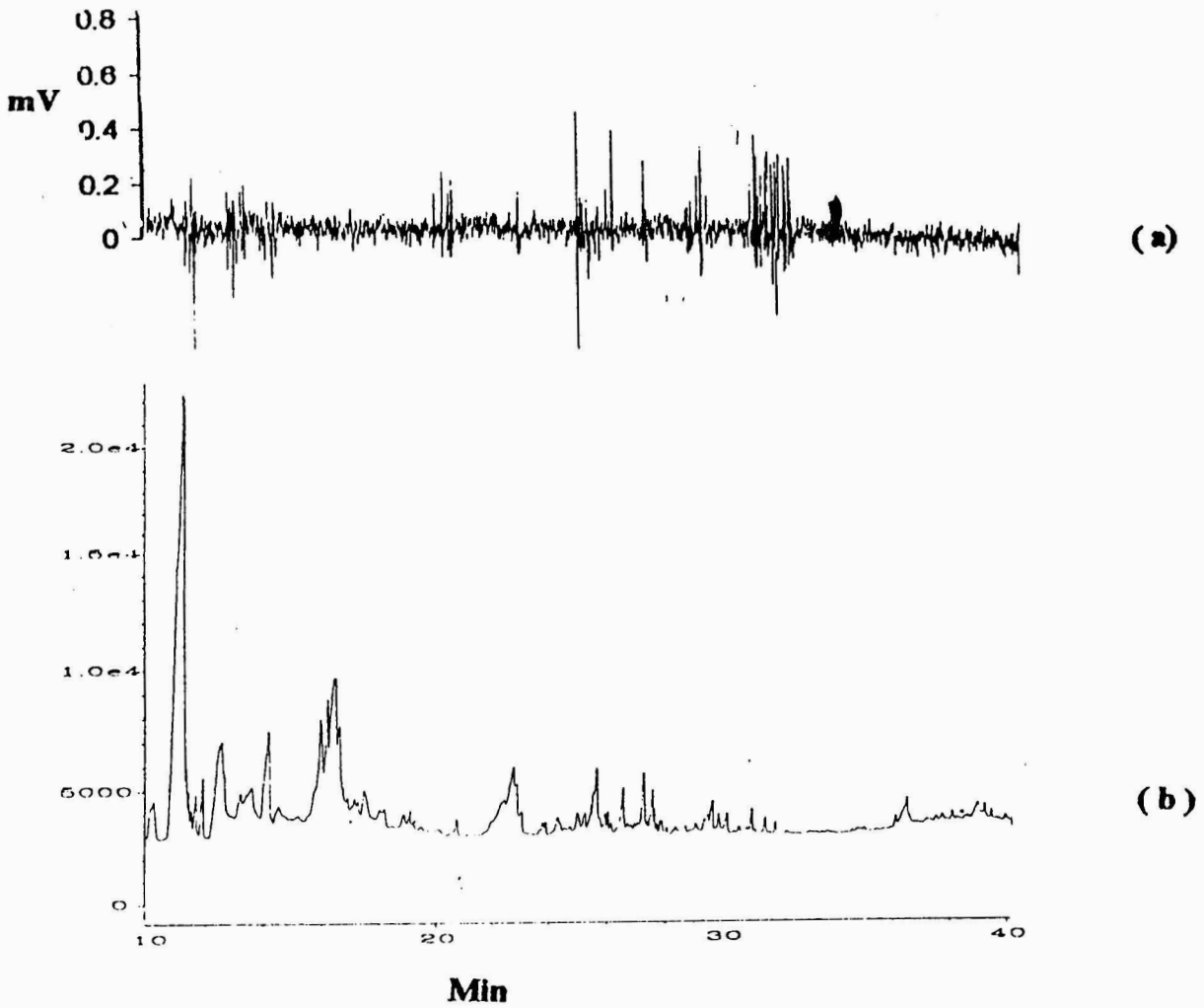

Fig. 4 : Electro Antennogram Detector responses of male and female antennae of the American cockroach *P. americana* (a) and FID chromatogram of neem seed volatiles (b)

Papers published on Work Done under the Contract

1. Effect of azadirachtin on growth of cockroaches, *Periplanata americana*; The paper was accepted for the presentation by Section E-1, SLAAS, 1999.

2. Three papers will be submitted on

   a. Use of MPLC to isolate azadirachtin from neem, *Azadirachta indica* cake.

   b. Effect of azadirachtin on activity of midgut enzymes in cockroaches, *Periplanata americana*

   c. Study on the repellant effect of neem volatile on cockroaches, *Periplanata americana*. .