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"Biorational insecticides as alternatives in pest control"

"المبيدات الطبيعية كبدائل في مكافحة الآفات"

by

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(1): Abstract

. We have establishment mosquito, *Culex pipiens* rearing at our laboratory because this insect play a pivotal role in health importance in this area. Furthermore, this species have been generated to get homogenous and susceptible strain. Next, collection of certain plants from Al-Hassa region for isolation the active crude compounds naturally occurs in the plants. Collected plants were dried using an oven at 50^{o}C for 4 days. Dried plants were ground to fine powder in electrical homogenizer mixed with liquid nitrogen for 1-2 min. Then, the fine powders were transferred in plastic bags tightly closed by thermal electric machine and kept in –20^{o}C until use for extraction. The extraction of chosen plants has been performed using standard methods. The extracted crude compounds were tested against mosquito larvae. The data showed that certain of these extracted material dramatically effective as larvicidal compounds, however, some of them had or had no effect. These data shed the light on promising alternative way in plant derived compounds for pest management would be achieved instead of synthetic insecticides. In addition, ribosome-inactivating protein (RIP) have been isolated from castor bean and separated by sodium dodecyl sulfate polyacrylamide

gel electrophoresis (SDS-PAGE). The analysis of SDS-PAGE showed that the crude protein having the molecular weight of RIP at approximately 30,000 dalton. However, the data showed that RIP at 100 ug/ml had no effect on the hatchability of mosquito egg-masses and had no larvicidal effect.

(2): Introduction and Literature review:

In recent years, Agrochemical companies and research laboratories have been focused on the study of natural products for the development of new insecticides (Addor, 1995). The discovery of active compounds that are more selective and less persistent will be beneficial for both the environment and agriculture product consumers, although natural products cannot automatically be assumed to be without risk.

The protection of agricultural products from pests is essential in many countries suffering from inadequate storage facilities and/or climatic conditions that favor deterioration of food commodities. The application of chemical pesticides apart from environmental concerns may not be sufficiently effective since insects develop resistance to them.

The interaction between plants and insects is chemically mediated by secondary metabolites. Bioactive plant products have been used by man since ancient times, especially in culture with a strong herbal tradition (Secoy and Smith, 1983).

Many authors have undertaken the screening of plant species for pesticidal activity. Hoffmann et al. (1993) evaluated 300 plant species from southwestern USA for antimicrobial activity. Gonzalez-Coloma et al (1994 a,b) detected antifeedant and insecticidal effects in plants from the Canary Islands and Japanese Lauraceae while Cunat et al (1990) and March et al (1991) have studied biocidal and antimicrobial activities in

plants from the Valencian region in Spain. El-Lakwah and El-Kashlan (1999) evaluated neemazal-W (powder of a botanical insecticide containing 10% azadiachtin) for mortality and reduction in F1 progency of stored product insect species at concentration between 20-1000 ppm on wheat grain. They found that the average mortality of *Sitophilus oryze* adults was high and reached 100% at all tested concentrations 14 days post-treatment. However, mortality of *Callosobruchus maculatus* adults reached 38, 92, and 98% at concentrations of 50, 500 and 1000 ppm 1 day after treatment and from 90 to 100% at 50-1000 ppm after 7days. They found also that *C. maculatus* were the most susceptible species , followed by *S. oryzae*, *Rhyzopertha dominica* and *Tribolium. castaneum*. Chander and Ahmed (1988) examined various extracts of the medicinal plant *Embelia ribes* for their insecticidal activity against eggs and larvae of *Musca domestica*. Powdered berries at 15% concentration and their ethyl acetate extracts at 30% concentration reduced percentage pupation of *M. domestica* when added to the larval rearing medium. The ethyl acetate extract and a crystalline fraction both at 4% concentration derived from it produced 90 and 100% mortality, respectively, in 48-hour old larvae. The insecticidal activity of 5 aromatic plants against *Sitophilus granarius* and *Acanthoscelides obtectus* was investigated by Kalionvic et al (1997). They found that for controlling of *S. granarius*, the most effective treatment was dried dust of *Laurus nobilis* under store house

conditions, however, the dust of *R.osmarinus officinalis was* the best insecticidal efficacy against *S. granarius* in milling wheat, while in seed wheat an oil extract of *L. nobilis* was best. Furthermore, a total of 78 different extracts from 20 medicinal plants belonging to 14 plant families were tested against several pests including larvae of mosquitoes (Diallo et al 2001 and Massoud et al 2001)

Anethole is a major insecticidal component of the volatile oil of a number of plant families. Anethole, present in anise oil from *Pimpinella anisum*, was found to be toxic to *Drosophila melanogaster* M., *M. domestica* , and third instar of *Aedes aegypti* L.,(Marcus and Lichtenstein, 1979) as well as the compound was obtained as the principal constituent of star anise, *Illicium verum*, by steam-distillation and it was found that *T. castaneum* adults were more susceptible to both the fumigant and the contact action of anethole than *Sitophilus zeamais* (Ho et al, 1997).

The natural abundance, prolificity and traditional use of neem in Asia and Africa for medical, fertilizer and pest control purposes make it an ideal renewable resource for many and varied biotic agents which have been explored and continued to be detected in it. Neem extracts were reported exhibiting repellent activity (Roomi and Ariquddin, 1977), and acting as a deterrent against rice storage insects (Devi and Mohandas 1982; Rahman and Gupta 1997).

(3): Materials and Methods

1- Establishment of certain insect rearing at our laboratory. We have chose this species of insect for rearing because this insect play a pivotal role in health importance in this area. Furthermore, this species have been generated to get homogenous and susceptible strain as follows:

Rearing mosquito, *Culex pipiens* at laboratory level:

Larvae were collected in plastic box from a static drainage water at Al-Hassa region. Collected larvae were transferred to the Department of plant protection, KFU. Larvae were fed on medium of dried milk mixed with water to reach pupa stages. Pupae were collected daily using Pasteur pipette and placed inside an arrested cage. After emerging to adult stage, female insects were get blood meal from an arrested pigeon to have the ability for laying their eggs, however, adult males were fed on 10% sucrose solution. Then, egg-masses were kept to continue next generation (Pappas 1973 and Singh and Moore, 1985).

(2): Screening and collection of some plants endogenously containing natural insecticides.

We have start to collect some locally plants from Al-Hassa region and this procedure will be continued for the next two months. Collected plants were dried using an oven at 50^{o}C for 4 days. Dried plants were

ground to fine powder in electrical homogenizer mixed with liquid nitrogen for 1-2 min. Then, the fine powders were transferred in plastic bags tightly closed by thermal electric machine and kept in –20^{o}C until use for extraction.

(3): Extraction process for isolating the active components. We extract several crude materials from different collected plants as follows:

1- Extraction from, tooth brush tree roots, *Salvadora presica*: 100 grams of roots were dried and cut down to small pieces. Dried materials were transferred to Soxhelt apparatus in petroleum ether for 6 hours. Then, the extraction was evaporated using rotary evaporator to become semi-dry material. Total yield was 0.205 grams which kept at 10 ^{o}C until used. On the other hand, wet roots (200 gram) of same plant were extracted by soaking in ethanol and acetonitril (1:1) for overnight. The extract was evaporated and concentrated as mentioned above. Total yield by this methods was 6.922 gm which kept at 10 ^{o}C until used.

2- Extraction from camphor leaves, *Cinnamomum Camphora*: 80 grams of roots were dried and cut down to small pieces. Dried materials were transferred to Soxhelt apparatus in petroleum ether for 6 hours. Then, the extraction was evaporated using rotary evaporator to become semi-dry material. Total yield was 2.739 grams which kept at 10 ^{o}C until used.

3- Extraction from nabk leaves, *Ziziphus spina*: 90 grams of leaves were were dried and cut down to small pieces. Dried materials were transferred to Soxhelt apparatus in petroleum ether for 6 hours. Then, the extraction was evaporated using rotary evaporator to become semi-dry material. Total yield was 0.293 grams which kept at 10 ^{o}C until used. On the other hand, dried leaves (90 gram) of same plant were extracted by soaking in petroleum ether for overnight. The extract was evaporated and concentrated as mentioned above. Total yield by this methods was 1.48 gm which kept at 10 ^{o}C until used.

4- Extraction from conocarps leaves, *Conocarpus erectus* leaves: 100 grams of *C. erectus* leaves were dried and cut down to small pieces. Dried materials were transferred to Soxhelt apparatus in petroleum ether for 6 hours. Then, the extraction was evaporated using rotary evaporator to become semi-dry material. Total yield was 2.135 grams which kept at 10 ^{o}C until used. On the other hand, extraction by soaking resulted in an elastic materials have no ability to reconstitute in suitable medium. So, we have canceled this step.

5- Extraction from neem leaves, *Azadirachta indica* : 100 grams of leaves were dried and cut down to small pieces. Dried materials were transferred to Soxhelt apparatus in petroleum ether for 6 hours. Then, the extraction was evaporated using rotary evaporator to become semi-dry material. Total yield was 2.202 grams which kept at 10 ^{o}C until used.

6- Extraction from Anise seeds, *Pimpinella Anisum*: 100 grams of seeds were transferred to Soxhelt apparatus in petroleum ether for 6 hours. Then, the extraction was evaporated using rotary evaporator to become semi-dry material. Total yield was 1.096 grams which kept at 10 ^{o}C until used.

7- Extraction from black pepper seeds, *Piper nigrum*: 100 grams of seeds were transferred to Soxhelt apparatus in petroleum ether for 6 hours. Then, the extraction was evaporated using rotary evaporator to become semi-dry material. Total yield was 1.45 grams which kept at 10 ^{o}C until used.

8- Extraction from clove seeds, *Caryophyllus aromaticus*: 100 grams of seeds were transferred to Soxhelt apparatus in petroleum ether for 6 hours. Then, the extraction was evaporated using rotary evaporator to become semi-dry material. Total yield was 2.33 grams which kept at 10 ^{o}C until used.

9- Extraction from common ginger seeds, *Zingiber officinable*: 100 grams of seeds were transferred to Soxhelt apparatus in petroleum ether for 6 hours. Then, the extraction was evaporated using rotary evaporator to become semi-dry material. Total yield was 2.33 grams which kept at 10 ^{o}C until used.

10- Extraction from lesser cardamom seeds, *Elettaria cardamomum*: 100 grams of seeds were transferred to Soxhelt apparatus in petroleum

ether for 6 hours. Then, the extraction was evaporated using rotary evaporator to become semi-dry material. Total yield was 1.56 grams which kept at 10 ^{o}C until used.

11- Extraction from common cinnamon seeds, *Cinnamomum zeylanicum*: 100 grams of seeds were transferred to Soxhelt apparatus in petroleum ether for 6 hours. Then, the extraction was evaporated using rotary evaporator to become semi-dry material. Total yield was 2.33 grams which kept at 10 ^{o}C until used.

12- Extraction from, *Aloe vera*: 100 grams of seeds were transferred to Soxhelt apparatus in petroleum ether for 6 hours. Then, the extraction was evaporated using rotary evaporator to become semi-dry material. Total yield was 11.562 grams which kept at 10 ^{o}C until used.

13- Extraction from *Garcinia morella* leaves: 60 grams of roots were dried and cut down to small pieces. Dried materials were transferred to Soxhelt apparatus in petroleum ether for 6 hours. Then, the extraction was evaporated using rotary evaporator to become semi-dry material. Total yield was 0.24 grams which kept at 10 ^{o}C until used.

14- Extraction from *Aegle marmelos* seeds : 100 grams of seeds were transferred to Soxhelt apparatus in petroleum ether for 6 hours. Then, the extraction was evaporated using rotary evaporator to become semi-dry material. Total yield was 4.83 grams which kept at 10 ^{o}C until used.

15- Extraction from *Pistacia lentiscus*: 100 grams of crystal glue materials were transferred to Soxhelt apparatus in petroleum ether for 6 hours. Then, the extraction was evaporated using rotary evaporator to become semi-dry material. Total yield was 10.5 grams which kept at 10 ^{o}C until used.

(4): Results and Discussion

(1): Testing the efficacy of extracted components from different plants on a major household insects such as mosquito, *Culex pipiens*.. Several plant extracts have been used against larvae of *C. pipiens.* Different concentration of each extracted materials have been added to small plastic cup contains 10 larvae in 20 ml distilled water with note that each concentration was performed in 3-4 replicates. Certain of these extract showed dramatic effect on *C. pipiens.*as shown in the following Tables. Table (1) summarized the effect of *S. persica* root extract on *C. pipiens* larvae. The data clearly showed that the extract slightly effective at low concentrations in the range of 200-600ppm, however, at higher concentration of 1000ppm gave 63% mortality. In contrast, *C. comphora* leaf extract showed sever larvicidal effect against *C. pipiens* as shown in Table (2). Furthermore, same trend was observed by *C. erectus* leaf extract which showed dramatic larvicidal effect at different concentrations in the range of 200 ppm to 1000 ppm (Table 3). In addition, *Z. spina* leaf extract was also showed strongly larvicidal effect at different concentrations (Table 4), it gave 100% mortality at 1000ppm. Moreover, the data showed that neem seed extract *Azadirachta indica* was most strongly effective compounds compared to other extracts in the range of 40 ppm up to 200 ppm (Table 5) for neem extract and 200 –

1000 ppm for other compounds. However, *Aegle marmelos* was less effective compounds (Table 6).

Table (1): Effect of *Salvadora. persica* root extract on larvae of *C. pipiens* after 24 hours exposure.

Conc. (ppm)	Mortality (%)			Mortality Average (%)
	R1	R2	R3	
control	0.0	0.0	0.0	0.0
200	20	20	10	17
400	10	20	10	13
600	20	50	20	30
800	70	60	60	63
1000	70	60	60	63

Table (2): Effect of *Cinnamomum camphora* leaf extract on larvae of *C. pipiens* after 24 hours exposure .

Conc. (ppm)	Mortality (%)			Mortality Average (%)
	R1	R2	R3	
control	0.0	0.0	0.0	0.0
200	0.0	10	10	7
400	60	50	50	53
600	80	70	70	73
800	90	90	90	90
1000	100	90	100	97

Table (3): Effect of *conocarpus erectus* leaf extract on larvae of *C. pipiens* after 24 hours exposure .

Conc. (ppm)	Mortality (%)			Mortality Average (%)
	R1	R2	R3	
control	0.0	0.0	0.0	0.0
200	60	40	60	53
400	80	70	80	77
600	80	90	70	80
800	90	100	80	90
1000	100	100	100	100

Table (4): Effect of *Zizyphus spina* leaf extract on larvae of *C. pipiens.* after 24 hours exposure .

Conc. (ppm)	Mortality (%)			Mortality Average (%)
	R1	R2	R3	
control	0.0	0.0	0.0	0.0
200	30	40	60	43
400	80	70	60	70
600	80	80	90	83
800	90	100	90	93
1000	100	100	100	100

Table (5): Effect of *Azadirachta indica* leaf extract on larvae of *C. pipiens* after 24 hours exposure .

Conc. (ppm)	Mortality (%)			Mortality Average (%)
	R1	R2	R3	
Control	0.0	0.0	0.0	0.0
40	40	20	30	30
50	40	90	70	67
80	100	80	70	83
120	100	100	80	93
200	90	100	90	93

Table (6): Effect of *Aegle marmelos* seeds extract on larvae of *C. pipiens* after 48 hours exposure.

Conc. (ppm)	Mortality (%)				Mortality Average (%)
	R1	R2	R3	R4	
Control	0.0	0.0	0.0	0.0	0.0
200	0.0	0.0	0.0	0.0	0.0
400	0.0	0.0	0.0	0.0	0.0
600	10	20	0.0	10	10
800	10	20	10	20	15
1000	20	10	20	20	18

Certain of these extract showed dramatic effect on *C. pipiens.*as shown in the following Tables. The data showed that seed extract of two compounds; *Elettaria cardamomum,* and *Piper nigrum,* were strongly effective against larvae of *C. pipiens.* These compounds showed completely larval mortality after 4 hours exposure at all tested concentration. However, less exposure of larvae of these compounds gave various mortality (Table 7 and 8). Furthermore, *Aloe vera* extract exhibiting dramatic effect after 4 hours exposure (Table 9). However, *Zingiber officinable* was less effective compounds compared to other compounds when exposed for 24 hours and when exposure time was increased to 48 hours, the mortality was reached to 93% at concentration of 800 and 1000 ppm (Table 10 and 11).

Table (7): Effect of *Elettaria cardamomum* extract on larvae of *C. pipiens* after 2 hours exposure.

Conc. (ppm)	Mortality (%)				Mortality Average (%)
	R1	R2	R3	R4	
control	0.0	0.0	0.0	0.0	0.0
200	10	10	10	0.0	8
400	40	40	50	40	43
600	80	70	70	70	73
800	80	80	80	90	83
1000	100	100	90	100	98

Table (8): Effect of *Piper nigrum* extract on larvae of *C. pipiens* after 3 hours exposure.

Conc. (ppm)	Mortality (%)				Mortality Average (%)
	R1	R2	R3	R4	
control	0.0	0.0	0.0	0.0	0.0
200	50	70	60	60	60
400	60	60	60	60	60
600	70	60	60	60	62.5
800	90	90	70	70	80
1000	90	90	90	80	88

Table (9): Effect of *Aloe vera* extract on larvae of *C. pipiens* after 4 hours exposure.

Conc. (ppm)	Mortality (%)				Mortality Average (%)
	R1	R2	R3	R4	
control	0.0	0.0	0.0	0.0	0.0
200	20	30	30	30	28
400	40	30	40	40	38
600	40	40	40	40	40
800	50	50	50	60	53
1000	100	100	95	100	98

Table (10): Effect of *Zingiber officinable* extract on larvae of *C. pipiens* after 24 hours exposure.

Conc. (ppm)	Mortality (%)				Mortality Average (%)
	R1	R2	R3	R4	
control	0.0	0.0	0.0	0.0	0.0
200	40	30	30	30	33
400	40	40	70	30	45
600	60	50	60	50	55
800	50	60	90	50	63
1000	80	60	50	60	63

Table (11): Effect of *Zingiber officinable* extract on larvae of *C. pipiens* after 48 hours exposure.

Conc. (ppm)	Mortality (%)				Mortality Average (%)
	R1	R2	R3	R4	
control	0.0	0.0	0.0	0.0	0.0
200	40	30	70	60	50
400	60	70	70	70	68
600	90	70	80	90	83
800	90	90	100	90	93
1000	100	80	100	90	93

Table (12) Effect of *Capsicum conicum* extract on larvae of *C. pipiens* after 24 hours exposure.

Conc. (ppm)	Mortality (%)				Mortality Average (%)
	R1	R2	R3	R4	
control	0.0	0.0	0.0	0.0	0.0
200	20	20	0.0	0.0	10
400	20	10	0.0	0.0	10
600	10	10	20	20	15
800	50	60	60	50	55
1000	80	70	60	60	68

Table (13): Effect of *Capsicum conicum* extract on larvae of *C. pipiens* after 48 ours exposure.

Conc. (ppm)	Mortality (%)				Mortality Average (%)
	R1	R2	R3	R4	
control	0.0	0.0	0.0	0.0	0.0
200	20	20	10	0.0	13
400	20	20	10	0.0	12
600	10	30	30	20	23
800	50	50	80	100	70
1000	80	80	70	90	80

Table (14): Effect of *Pimpinella anisum* extract on larvae of *C. pipiens* after 24 hours exposure.

Conc. (ppm)	Mortality (%)				Mortality Average (%)
	R1	R2	R3	R4	
control	0.0	0.0	0.0	0.0	0.0
200	0.0	0.0	0.0	0.0	0.0
400	0.0	10	30	30	18
600	40	30	10	10	23
800	60	70	60	60	63
1000	70	80	50	50	63

Table (15): Effect of *Pimpinella anisum* extract on larvae of *C. pipiens* after 48 hours exposure.

Conc. (ppm)	Mortality (%)				Mortality Average (%)
	R1	R2	R3	R4	
control	0.0	0.0	0.0	0.0	0.0
200	0.0	0.0	0.0	0.0	0.0
400	10	20	30	30	23
600	40	30	10	10	23
800	60	70	70	60	65
1000	80	80	60	70	73

Table (16): Effect of *Pistacia lentiscus* extract on larvae of *C. pipiens* after 48 hours exposure.

Conc. (ppm)	Mortality (%)				Mortality Average (%)
	R1	R2	R3	R4	
control	0.0	0.0	0.0	0.0	0.0
200	0.0	0.0	0.0	0.0	0.0
400	0.0	0.0	0.0	0.0	0.0
600	0.0	0.0	0.0	0.0	0.0
800	10	0.0	0.0	0.0	3
1000	10	10	10	0.0	8

Isolation and characterization of Ribosome-inactivating protein(RIP):

One-hundred grams of castor bean seeds were ground to fine powder in liquid nitrogen and homogenized in 500 ml 5mM Tris-HCl buffer pH 7.5. The homogenate was stirred over night at 4^{o}C and filtered through double layers of cheesecloth. The filtrate was centrifuged at 10,000 rpm at 4^{o}C for 30 min. The clear supernatant was kept at –20 ^{o}C until used for electrophoresis analysis and inhibitory effect against some insects.

Electrophoretic analysis of ribosome-inactivating protein:

Sodium dodecyl sulfate ployacrylamide gel elcrophoresis (SDS-PAGE) of above containing-RIP supernatant was performed according to Laemmli (1970), using 12% polyacrylamide gel. Different amount proteins were mixed with SDS-sample buffer then, boiled for 5 min at 95 ^{o}C. The samples were loaded on the gel for 60 min at constant voltage of 200 V. The gel was stained in staining solution containing 40% methanol, 10% acetic acid and 0.1% Coomassie brilliant blue R-250, next, gel was distained in 40% methanol and 10% acetic acid for couple of hours until background of gel became clear. Fig. (1) shows the pattern analysis of RIP protein in crude homogenate isolated from castor bean. Lane 1 represent standard molecular weight protein and lane 2 and 3 were different amount of RIP (20 and 50 μg proteins) respectively. The data

showed that lane 2 and 3 significantly contained major band of RIP approximately 30,000 dalton as marked by arrow.

Larvicidal and ovicidal effect of RIP :

Different concentrations of RIP were tested against larvae and egg-masses of C. pipiens as shown in Table (17 and 18). The showed that RIP had no mortality effect on larvae of C. pipines and had no effect on the hatchability of juvenile larvae from egg-mass of mosquito.

Table (17) Effect of RIP on *C. pipiens* larvae after 48 hours exposure.

Conc. (µglml)	Mortality (%)				Mortality Average (%)
	R1	R2	R3	R4	
control	0.0	0.0	0.0	0.0	0.0
20	0.0	0.0	0.0	0.0	0.0
40	0.0	0.0	0.0	0.0	0.0
60	0.0	0.0	0.0	0.0	0.0
80	0.0	0.0	0.0	0.0	0.0
100	0.0	0.0	0.0	0.0	0.0

Table (18): Effect of RIP on *C. pipiens* egg-masses after 48 hours exposure.

Conc. (µglml)	Hatchability (%)				Hatchability Average (%)
	R1	R2	R3	R4	
control	100	100	100	100	100
20	100	100	100	100	100
40	100	100	100	100	100
60	100	100	100	100	100
80	100	100	100	100	100
100	100	100	100	100	100

Although the use of plant species to control insect pests has been in practice for centuries to a limited extent, it has been only recently that interest has renewed in the pest management potential of natural products. These products are the compounds that have evolved in plants for defense against phytophagous insects. Interestingly, a quite convincing case has been proposed suggesting that these secondary plant products have actually co-¼evolved with insects that would potentially exploit them as a food resource (Berenbaum 1982). One can imagine the plant kingdom striving to slow down the attack of the herbivores over evolutionary time by formulating novel compound after novel compound and tirelessly conducting bioassays in order to find out what works and what does not. The herbivores in return develop new strategies over evolutionary time to break down some of these chemical defenses to exploit the plants if they can. It is because of this never ending back and forth struggle that these vast number of chemical compounds have evolved in the plant kingdom. The modern researcher now has the technology to exploit the toxic properties of some of these compounds and use them against organisms that were never originally intended.

One group of compounds that has demonstrated significant toxic effects on some pests of modern man have been discovered in the neem tree (Azadiricta indica) (A. Juss.). The most active constituent, azadiractin (AZA), a triterpenoid, has been shown to have properties including

feeding and ovipositional deterrence, repellency, growth disruption, reduced fitness, and sterility in a number of species of hemimetabolous and holometabolous insects (Ascher and Meisner 1989; Shmutterer 1990). Research has been focused on controlling agricultural pests as well as medically important arthropods with products derived from neem. Perhaps the most medically important arthropod world wide is the mosquito which transmits diseases such as the malarias, dengue, and yellow fever to name but a few. Because mosquitoes and many other insects have become resistant to pesticides, heavy and frequent applications are required leading to problems of toxic residues contaminating the environment and adversely affecting non-¼target organisms. This dictates the need to develop safe, less expensive, and preferably locally available materials for pest control. In this vain, NeemAzal, a product derived from the neem seed kernel, was evaluated as a potential means of control for Aedes aegypti (Boschitz and Grunewald 1994). Boschitz and Grunewald used the larval stage of the mosquito to measure mortality rates depending on concentration and then looked at the effects of sub-lethal doses on the fecundity of the surviving larvae. They hypothesized that if sterility could be proven, then the advantage of sub-lethal doses would be the obvious reduction of risk of damage to non-target flora and fauna. Other control projects that have been conducted with neem tree products have also targeted other

medically important mosquito vectors such as Anopholes stephensi, Culex quinqefasciatus, Culex pipiens, Aedes togoi and Aedes aegypti (Attri & Pradas 1980; Chavan & Nikam 1988; Zebitz 1984). These studies reported strong variations in susceptibility of a muito species towards neem tree products.

Neem and its products have also been the focus of agricultural pest control research as well. Dimetry et al. (1995) have been working with neem azal-F to inhibit the growth and reproduction in the cowpea aphid (Aphis craccivora). This particular product contains 5% azadiractin and produced an antifeedent effect which hindered larviposition of the adults and decreased the reproductive period as well as the longevity of the adults. In addition they were able to show an aphicidal effect as the concentration increased. In another application, Naumann and Isman (1995) used three concentrations of an oil-free neem seed extract to deter oviposition in noctuid moths. They found that most commercial neem-based products are not effective noctuid oviposition deterrents. Their studies suggest that the results demonstrated in other research using neem-based deterrents were effective because the compounds were removed by a higher level of processing and thus not found in commercial products. Another approach using neem products has involved integrating neem with endomychorrhiza to control root knot

nematodes on tomato plants (Rao et al. 1995). In this effort researchers planted mychorrizal seedlings of tomato into soil that was treated with neem cake. Vesicular arbuscular mycorrhizal fungi (VAM) has been established as a reducer of nematode parasites of many plant species. By combining neem cake in the soil with the VAM the investigators sought to elevate the level of protection for the seedlings. Yet another application for this product has been tested by a group of researchers in Winnipeg, Canada on three stored-product beetles (Xie et al. 1995). The beetles in question; the rusty grain beetle (Cryptolestes ferrugineus) (Stephens), the red flour beetle (Tribolium casteneum) (L.), and the rice weevil (Sitophilus oryzae were exposed in the laboratory to several extracts of neem. In this case the researchers were looking for repellency effects as well as toxic effects. The variance in susceptibility between the species was expected as several investigators working with mosquitoes observed similar phenomena.

Neem is not the only plant derived chemical that has demonstrated arthropocidal and toxic effects however. Several species of Juniper have also been studied and the active constituents isolated. A broad spectrum study was conducted using extracts from twelve different species of Juniper to look for termiticidal activity and then to isolate the active components (Adams et al. 1988). Oda et al. 1977 conducted a systematic survey of Juniper species across the United States and reported

the isolation of two sesquiterpene alcohols, cedrol and widdrol, as the most active ingredients. Other research, involving Lyme disease vectoring ticks (Ixodes scapularis) (Acari: Ixodidae) (Say) has demonstrated that two species of Juniper; western and eastern, (J. occidentalis) and (J. virginiana) respectively, had considerable acaricidal activity (Panella et al. submitted). Moreover it was shown that these two species had toxic effects on the Oriental rat flea (Xenopsylla cheopis) (Insecta: Syphonaptera) an important vector of the plague bacterium Yersinia pestis, in laboratory (Panella et al. submitted). In addition to species of Juniper, Panella et al. (submitted) demonstrated significant biological activity in several different plant extracts against ticks and fleas.

Discussion

It is clear by the review of recent research listed above that there is an unlimited number of applications for secondary plant compounds and their derivatives and many different methods have been used to study their biological activity. Boshitz and Gruwenwald (1994) placed larval. aegypti mosquitoes in beakers containing deionized water with differing concentrations of NeemAzal. This bioassay technique sought to simulate real ecological parameters that exist in nature to a certain extent. Probably the best bioassys are conducted in the field under real life circumstances, but when that is not possible the researcher must use

creativity and resourcefulness to obtain a high quality bioassay. Perhaps the most interesting observation made by Boschitz and Grunewald was that the toxic effect of the product decreased as the larval stage increased. That is 1st instar larvae were more susceptible to the NeemAzal than were 3rd and 4th instar. Boschitz and Grunewald also reported that there was not a significant reduction in fecundity among the mosquitos that were exposed to sub-lethal concentrations and allowed to molt into adults. These results were inconsistent with results published by other researchers that claimed a significant reduction in fecundity after neem exposure (Mordue 1985; Schmidt 1986;Feder et al. 1988; and Schmutterer 1986). This variation could be due to the difference in bioassay techniques. Boschitz and Grunewald put the NeemAzal directly into water allowing the larvae to come into contact with it by normal locomotion whereas the other studies mentioned above administered the toxin by either blood meal or direct injection into the hemocoel. It was clear in either case though that NeemAzal inhibited growth of the early instar larvae.

Detrimental physiological effects have also been observed in insect agricultural pests such as aphids. Dimetry et al. (1995) tested various concentrations of Neem Azal-F (a commercial product of the neem seed kernel extract containing 5% azadiractin) on the cowpea aphid (Aphis craccivora) to measure inhibition in growth and reproduction.

Lowery and Isman (1994) also tested neem seed oil on several species of aphids to measure the same effects. The results obtained by Dimetry and Hawary (1995) demonstrate that Neem Azal-F was effective at reducing fecundity in the adults and inhibiting successful molting. They also determined that the aphicidal effect is concentration dependent, which is consistent with the published results of other studies done with aphids (Schmutterer 1985; Rembold 1989; Koul et al. 1990). Lowery and Isman (1994) in an earlier study also found similar results among the species they tested, but also reported that susceptibility varied among the life stages. Both studies employed bioassays that required the larval aphids to ingest treated plant materials. It is still unclear whether or not direct contact with neem will induce the same results (Lowery & Isman 1994). Another interesting observation made by Dimetry and Hawary (1995) was that when the Neem Azal-F was not in lethal concentration it caused the surviving larval aphids to increasingly molt into the winged form as concentrations increased. This suggests that the Neem Azal-F even in non-lethal doses can trick the aphids physiology into thinking that conditions at its present location are unfavorable and develop the winged form to perhaps leave and look for a more suitable host plant.

In contrast to the fecundity reducing effect neem has on aphids, Naumann and Isman (1995) found that their same neem seed oil extract had little or no effect on three species of noctuid moths: *Peidroma saucia*,

and *Spodoptera litura*. In this experiment captive moths were given cabbage plants treated with the three extracts of varying concentrations (10,50 and 100ppm of azadiractin) and then oviposition was measured for the life span of the female which is 8-13 days. There was no significant difference in the amount of eggs laid by the female on the plants of varying treatments. When the researchers presented the female moths with a choice of treated and non-treated cabbage plants to oviposit on there were no differences observed in the number of eggs laid. According to Naumann and Isman (1995) this is in contrast to what has been suggested in many reports, including those for S. litura. Again the question of processing and formulations of both the products and extracts is raised. The pesticidal power of neem is not just limited to phytophagous insects. Roa et al. (1995) were able to achieve control of the root knot nematode on tomato plants by integrating a known nematocide, vesicular arbuscular mychorrhiza, with neem cake. The bioassay used in this study could easily be applied in the real world because plants don't move much so changes are usually easily observable to the trained eye. Rao et al. (1995) placed tomato seedlings with the (VAM) already growing on the roots into soil amended with neem cake. They then removed a sample of five plants at pre-determined time intervals to look for infestations of nematodes. What they found was quite remarkable. Not only were the root knot nematodes virtually

wiped out, but the soils amended with neem cake caused an increase in plant growth parameters and an increase in the mychorrizal population on the roots, thus affording the plants even greater protection. The other class of plant-derived chemicals that have been shown to demonstrate biological activity in arthropods are those found several plant species in North America (Forlines et al. 1992). Research conducted with extracts from Juniper species have proved to be effective in controlling termites. Termites were exposed to the heartwood, bark/sapwood, and leaves initially and then to a methanol and hexane extract of twelve different species of Juniper found throughout the United States (Adams et al. 1988). Adams et al. (1988) found that the bioassay they used for the raw materials was 100 percent effective for all species after 4 weeks of exposure eartwood. This bioassay consisted of placing 1.5g of raw materials in a zipper case with 50g of sand, 7ml of distilled water and 100 termites. Mortality was checked every 7 days and in all instances 100 percent mortality was achieved noted after 4 weeks. Adams et al. (1988) fail to mention what kind of control they ran so it is hard to be sure that it was the plant material that was exhibiting all the activity. With the Hexane and Methanol extracts, the investigators treated filter paper with a 1mg/ml solution and placed 25 termites on it. These bioassay trials yielded mixed results. The Hexane extracts only produced 100 percent mortality after the 4 weeks in 5 species, while the Methanol extracts were

100 percent effective in only 4 species. From these results Adams et al. (1988) concluded that perhaps the Hexane and Methanol extracts were not extracting all the antitermitic properties of the compounds found in the heartwood.

Other research using the extracts of Juniper as well as other plant species has been focusing attention on two medically important arthropods: ticks and fleas (Panella et al. submitted). In these studies several extracts of heartwood, bark/sapwood and leaves have been evaluated as to their biological effectiveness against the afore mentioned arthropods. The bioassay technique used for this research is known as the disposable pipet method first developed by Barnard et al. (1982). This method consists of serially diluting the extract in acetone solvent into as many concentrations as desired. Once the formulations are complete the solutions are sucked into the pipette a number of times to ensure complete coating. The pipettes are left for 24 hours to dry and then the ticks or fleas are introduced via a vacuum pump. After 24 hrs the arthropods are checked for mortality. Thus far, it appears that two of the crude extracts show a lot of promise. Those extracts being from Alaska yellow cedar (*Chamaecyparis nootkatensis*) and Eastern red cedar (*Juniperus virginiana*). Both of these extracts have exhibited impressive values and could potentially be commercially important. It is worth noting that these extracts are much more toxic to the larval stage of ticks than the nymphal

stage, which is current with what other researchers have encountered while testing other compounds such as neem on different stages of insects. The active chemical compounds in these extracts has yet to be elucidated, but that is the focus of current research.

To understand how these compounds are working on the physiological level, investigations into the behavioral and sensory effects have been carried out on some neem based products. Three extracts; toosendanin, salanin, and azadirachtin, from plants of the genus Melia were compared in their ability to deter feeding and evoke neurophysiological responses with Maragosan-OR, a commercial product based on an ethanolic extract of seeds from Azadirichta indica (Lin-er et al. 1995). The anti-feedant bioassay consisted of placing treated and untreated cabbage discs in a petri dish and then introducing caterpillars of the species Pieris brassicae. The discs were checked at intervals to measure the amount the larvae consumed of each disc over a 4 hour period. The degree of material consumed was then converted to an anti-feedant index established by the authors. They found that Margosan was the best anti-feedant, but the other extracts were not significantly worse. To measure sensory responses in the insects the investigators used the tip technique which recorded the action potentials of the two sensilla styloconica when stimulated with various concentrations of the 4 compounds mentioned above. By doing this the authors were able to get a picture of what was

happening on the cellular level in specific sensory cells. They noted that some of the compounds were causing a change in the insects'_ sensory code. They were able to observe if the compounds were inhibiting the sugar cell, glucosinalate cell or the amino acid cell. At higher concentrations the responses were greater for all compounds. As research continues in the rapidly growing field of plant derived chemicals, many more applications will arise that have not been discussed in this article. As researchers gain more understanding as to how these compounds affect organisms on the behavioral and physiological level the potential for friendly products to control pests is enormous. Future research in this field should expand out of the laboratory and into the real world. In this way these novel products can be evaluated as viable alternatives to the persistent, less environmentally friendly products on the market currently.

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The Project PI

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