POTENTIAL OF SELECTED NATURAL PRODUCTS AS REPELLENTS AGAINST VERTEBRATE PESTS OF CROPS.

by

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BIOLOGICAL CONTROL OF VERTEBRATE PESTS

ABSTRACT

Potential of selected natural products as repellents against vertebrate pests of crops.

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There is a need for effective and environmentally sensitive methods of controlling vertebrate pest problems in agriculture and urban environment. Nonlethal natural repellents may meet this need where more traditional methods of control, such as scaring, shooting, and trapping, are either ineffectual or unacceptable. Neem (*Azadirachta indica* A. Juss) extracts: oil, seed and leaf powder and chemicals from cockroach (*Blaberus giganteus* L.) were tested for their repellent properties. In addition defensive volatiles from *B. giganteus* were tested in an arena based on choice by smell (cheese or insect volatiles). This arena test used laboratory rats (*Rattus norvegicus* Berk.); females were more active than males. Both sexes visited the holes with cheese more than holes containing insect's volatiles. However in a feeding test with one choice of food the control did not differ from the treatment. Neem products seem to act as antifeedant on rats: neem seed oil, neem seed powder and neem leaf powder reduced rats feeding respectively at concentration of 15 ml of oil/kg, 15 - 50 g of seed powder/kg, and 25 - 50 g of leaf powder/kg of rat chow. Overall neem leaf powder was less effective than seed powder and oil. Neem and insect products may have potential in controlling rats particularly in storage situation, which could lead to an important reduction of post-harvest loss of grains in Sahelian and Asian countries.

RÉSUMÉ

Pouvoir repulsif de certains produits naturels contre les vertébrés ravageurs des récoltes.

M.Sc. Abdouramane Tilly Gaoh Sciences des Ressource Naturelles

Pour faire face aux problèmes des vertébrés ravageurs des récoltes et des produits entreposés, un besoin se fait sentir pour des techniques éfficaces et respectueuses de l'environnement. Des répulsifs naturels non léthaux pourraient satisfaire ce besoin où les méthodes de controle traditionnelles, telles la chasse et le piégeage sont inéfficaces ou simplement inadéquates. Des extraits de *neem* (*Azadirachta indica* A. Juss): huile, poudre de graines et de feuilles, ainsi que des substances chimiques provenant de blattes (*Blaberus giganteus* L.) ont été testés pour leurs propriétés répulsives. Des rats de laboratoire (*Rattus norvegicus* Berk.) ont alors été exposés d'une part à des odeurs (fromage *versus* sécretions de blattes), et d'autre part à de la nourriture (avec ou sans sécretions de blattes ou extraits de neem). Les resultats démontrent que les rats sont plus attirés par les odeurs de fromage que par les secrètions de blattes, tandis qu'aucune préfèrence n'a été démontré pour la nourriture avec ou sans secrètions de blattes. Quant aux tests avec ou sans extraits de neem, ils suggèrent fortement que ceux-ci inhibent l'appetit des rats: l'huile de neem, la poudre de graines, et la poudre de feuilles de neem ont respectivement réduit la consommation des rats en nourriture à une concentration de 15 ml d'huile/kg, 15-50g de poudre de graines/kg, et 25-50g de poudre de feuilles/kg de nourriture. Finalement, les propriétés apparemment répulsives des extraits de neem et des secrètions de blattes pourraient être exploitées dans le controle des rats pour réduire les pertes post-recoltes de grains dans les pays Sahéliens et Asiatiques.

Dedicated in loving memory of my father, El. Hadj Tilly Gaoh, who encouraged me to pursue graduate studies.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

I . INTRODUCTION

Food security issues have been the subjects of numerous international meetings and debates for a number of years. As we enter the 21st century, there is a great concern about feeding the world's increasing population. The most important demographic phenomenon is human fertility, particularly in Africa and other third world countries, where by the year 2050 the population may increase by 160% and 72%, respectively (Henripin, 1997). According to an FAO estimate, the third world food carrying capacity is about 11 billion (Henripin, 1997), assuming moderate soil conservation measures and little change in agrarian methods. Africa and other third world countries have great potential food production, but the most sensitive determinants of poor countries' future food supply are related to their economic and political organization and agricultural techniques (Henripin, 1997). As the earth's population increases, so does the demand for plant products of every kind (Oerke *et al.*, 1994), however every crop is infested by an array of pests. Among cereal crops such as wheat, corn, oats, barley and rice, rice is one of the world's most important food crops. It is a staple food for more than two billion people in Asia, the world's most densely populated region (Heinrichs *et al.*, 1991), and for hundreds of millions of people in Africa and Latin America. Because of the large number of people that depend on rice for their sustenance, annual production must increase by five million tons a year just to keep pace with population growth (Heinrichs *et al.*, 1991).

Animal pests are one of the major constraints that limit cereal production. For example, rodents (rats) attack all cereals from the seedling to maturity, and feed on all parts of the plant including roots, stems, leaves, and seeds. Rat damage decreases the yield

and lowers grain quality. Thus rodent pest management is a major component of most cereal crop protection programs, particularly dealing with post-harvest losses. Emphasis in crop pest management programs in the past has been placed on pesticides (Heinrichs *et al.*, 1991). Because the use of pesticides can be costly and dangerous to workers, can contaminate the environment, and encourages pests (rats) to develop resistance, there is much interest in the development of alternative tactics for crop rodent pest management, e.g. biological means, using pathogens, predators or parasitoids and natural products. Many plants, insects and animals are endowed with natural defense mechanisms (Prakash and Rao, 1997; Eisner, 1966, 1970; Epple et al., 1993), investigations in those areas could be a great opportunity of finding repellents which could be used in crop protection at possibly low cost.

During the era of pesticides, laboratory rats, such as the albinos (*Rattus norvegicus* Berkenhou), have been used and are still used to assess the efficacy and the behavior of chemicals and repellents tested (Barnett, 1963; Carlberg, 1981, 1985 a, b,). Numerous experimental methods such as feeding tests (one choice or two choices of food) and arena tests, etc., have also been used to evaluate repellents (Zungoli et al., 1988; Rollo et al., 1995; Shumake et al., 1997; Bouchard et al., 1997). For 30 years psychologists extended their work on how clever rats are to learn complicated puzzles, studying how they learned mazes (Silverman, 1978). To perform most of the experiments dealing with rats and choice preferences of smells and tastes, many studies suggest that novelty in rat environment is certainly important, but the rate of habituation to repeated stimuli depends on the response as well as the stimuli (Silverman, 1978; Galef and Whiskin, 1998). According to Ramirez

(1993) rats prefer dilute nutritive solutions and suspensions over a vehicle, this applies to a diverse array of nutrients. Animals possess, to a varying degree, an innate ability to recognize and ingest the appropriate food for their suvival (Valsecchi et al., 1992). Animal behaviour should be taken in to consideration for these tests using insect and plant biochemicals and assessing rat preferences of smells and tastes.

The objectives of this research project were (1) to determine the responses of rats to defensive secretions from cockroaches, and (2) to evaluate the effectiveness of neem (*Azadirachta indica* A. Juss) products as repellents against rats. The assessment of these two objectives and the implementation of the findings could initiate the reduction of post-harvest losses especially at farm level, and overall enhance pest management and environmental health in Sahelian countries.

II. LITERATURE REVIEW

A. Food Loss Due to Pests and Means of Control

It is estimated that crop losses (rice, wheat, corn, etc.) due to pests range from 15 to 25% (FAO, 1979); this includes losses from insects, weeds, plant pathogens, rodents and birds. Damage is unquestionably evaluated in hundreds of millions and perhaps billions of dollars annually. For example, it is estimated on an annual basis that in the United States alone, crops worth $9.1 billion are lost to diseases, $7.7 billion to insects and $6.2 billion to weeds (Agrios, 1997). Direct damage from rodents in U.S. probably exceeds $500 million each year (Timm, 1994, cited in Common Sense Pest control quarterly) In west Africa, particularly in Senegal, several ploceid species of birds are responsible for crop losses of at least US $4 - 5 million annually (Bruggers and Ruelle, 1981).

Recent extension of cereal production and the introduction of large-scale farming in Africa has resulted in the need to protect fields against grain-eating birds (Manikowski, 1987); the black-faced dioch or Quelea (*Quelea quelea* L.) causes the most severe losses in at least 22 African countries (Ruelle, 1983; Manikowski, 1988). In 1953, Quelea damage was so intense in West Africa, that the red-billed bird was proclaimed a public calamity (Mallamaire, 1959). Consequently, massive Quelea control strategies were introduced in the region in 1954 using deadly poisons (parathion, fenthion and cyanophos) (Manikowski, 1987, 1988). The harm to non target species and environmental concerns of intensive applications of avicides, has caused researchers to reorient control strategies from population reduction objectives to crop protection techniques (use of chemical

repellents, agronomic methods and nets) (Elmahdi et al., 1985; Bruggers and Ruelle, 1982; Ruelle, 1983).

Throughout the world, there are conflicts between rodents and the economic interests or well-being of humans; disease transmission, food destruction, reduced timber, damage to forage crops and storage facilities are some of the most important cases (Elias, 1988). Rodents can inflict severe damage to crops; for example, Wood (1971) estimated that rats were responsible for yield reductions of more than 60% in rice, and Fall (1977) reported that rat damage was a limiting factor in rice production in areas of the Philippines. Maize, sorghum, millet and wheat are other important cereal crops affected. Worldwide, and especially in developing countries, different control methods and techniques, mostly chemical and physical, have been used (Fall, 1980, 1982). Many kinds of rodenticides have been used over the past three decades and concerns of environmental security have been raised. Nowadays, the concept of eradication has been replaced by that of management, where the goal is to reduce pest populations to levels that are uneconomical to control (Rampaud and Richards, 1988).

Presently, more than 40% of the world crops are under intensive cultivation (Vincent and Coderre, 1992). Thus, monoculture has induced significant attacks of insects and became more exposed to plant diseases (Vincent and Coderre, 1992). According to Pimentel (1981), the cost of controlling nuisance organisms in crops could ensure adequate food for the world population. Vincent and Coderre (1992) stated that losses incurred by insects, pathogens and weeds account for 35% of agricultural production. If

losses due to vertebrates (birds and rodents) were to be considered, the total losses would be estimated at 45% (Vincent and Coderre, 1992).

Pesticidal resistance can be acquired by many pests due to increased use of synthetic pesticides. In the United States alone, Pimentel et al. (1980) evaluated the costs associated to chemical pesticide resistance at US $118 million per year.

Large scale use of synthetic pesticides in developing countries is attributable to rice and cotton cultivation. According to Bouguerra (1985), cotton protection alone accounted for 20% of the pesticides used globally. There are few alternatives to chemical control of pests. Biological control and integrated control methods could be feasible ecological approaches for pest control. For example, the concept of trap cropping has received much attention and the application of this new approach for controlling crop pests has already been developed (Pedigo, (1989), Javaid and Joshi, (1995), Agrios, 1997).

B. Economic Impact of Damage due to Rats and Means of Control

Food losses due to rats are encountered from the field to the storage facilities. They can inflict serious damage, not only to stored products, but also to packaging and even to storage buildings (FAO, 1994). Losses are caused by consuming grains and by contaminating far more than they consume. Rodents are also vectors of diseases (rabies, leptospirosis) which may be transmitted to humans (FAO, 1985).

Following is an assessment of rat damage in pre-harvest situations. During the wet season, in Andhra Pradesh (India), for example, *Bandicota bengalensis* (Gray), *Millardia meltada* (Gray) and *Rattus rattus* (L.) damaged 10 to 61% of rice plants during the wet season and 0.5 to 31% during the dry season. In other parts of India, other species, particularly *Rattus*

argentiventer (Robinson and Kloss) predominated, and caused losses up to 60% (Oerke et al., 1994). In Bangladesh, various rat species were a constant threat to the growing crop; *B. bengalensis* and *B. indica* (Bechstein) were the predominant species causing damage in 33 to 83% of the fields, and were responsible for an average loss of 5.7% (Wood (1971), Oerke et al., 1994).

In many developing countries of Africa, Asia and Latin America, overall post-harvest losses of cereals and grain legumes of 10 to 15% are fairly common; losses may reach 50% in some regions (FAO, 1994). Food contaminated by rats could be a health hazard in many places; Leslie (1942) quotes instances where contamination of food by mouse droppings was responsible for the death of humans from *Salmonella* poisoning.

Food grain is liable to suffer heavy losses both in the field and in storage as a result of infestation by insects and the action of rodents and birds (Bencini, 1991; Greeley, 1987). Rodent pests appear less frequently than insects, but can cause heavy losses. Several species attack the crop in the field, at harvest, and during drying and storage (Bencini, 1991; FAO, 1994; Greeley, 1987). Ways of controlling rodents are described by the following list of control methods.

1) Chemical Control of Rodents

In the vast majority of cases, rodent infestations are controlled by the use of rodenticidal baits, with fumigants and contact formulations (such as dusts) playing a minor and more occasional role (Greaves, 1982). Hunting and trapping methods have been replaced by these easier operated and more effective methods. Other chemical control

methods have been tried experimentally, including the use of rodent repellents, toxic ground sprays and chemosterilants, but none has yet been successful (Greaves, 1982).

Rodenticidal baits are generally the most effective and widely used means of controlling rodents (FAO/WHO, 1979; Greaves, 1982). There are two categories of formulations: firstly, acute (quick-acting or single dose applications) rodenticides which can cause death after a single dose is consumed during a short period not longer than few hours. These rodenticides are usually used at relatively high concentrations (0.1% to 10%) depending upon their toxicity (FAO/WHO, 1979; Greaves, 1982; Rampaud and Richards, 1988). Secondly, chronic (slow-acting or multiple dose) rodenticides (also called anticoagulants) cause death after a series of doses have been consumed over a period of days. These potent materials are used generally at low concentrations in baits (0.005% and 0.1%) (FAO/WHO, 1979, Greaves, 1982). Examples of acute rodenticides include alphachloralose (4% in bait), norbormide (0,5 to 2.0% in bait), scilliroside (0,015% and 10.0% in bait), and crimidine (0.25 to 1.0% in bait). There are other acute rodenticides which have restricted usage as they are too dangerous for humans: arsenic trioxide, thallium sulphate, alphanaphthylthiourea, barium carbonate, sodium arsenite, strychnine, yellow phosphorus, phosacetim, pyrinuron, silatrane, sodium fluoroacetate, and fluoroacetamide (Greaves, 1982, FAO, 1979).

Despite the apparent disadvantage of slowness of action, the introduction of dicoumarol, the first chronic rodenticide, revolutionized rodent control and today it is thought that chronic poisons account for at least 90% of all rodent control treatments that are undertaken (Greaves, 1982; FAO, 1979). For example, Klerat 20 g wax blocks (0.005

percent brodifacoum) were found to be both attractive and palatable to squirrels, in West Africa (Smith and Nott, 1988).

2) Non-Chemical Methods

Traps. Numerous non-chemical techniques can contribute to rodent control; the application of such methods minimizes the need to use rodenticides and can make their use more efficient. In some cases, non-chemical methods alone may provide the answer to a rodent problem, though normally it is not possible to avoid the use of rodenticides completely. Trapping is an age-old method of rodent destruction and many traditional types, including pitfall, deadfall, snare and box or cage type traps, are still in limited use (Greaves, 1982).

Rodent Barriers. Buildings and food storage containers can be made rodent proof (Greaves, 1982). Rat guards are often advocated as a means of preventing access by rodents to outdoor stores and storage cribs (traditionally used in Niger, Mali, Senegal etc.). More efficient barriers have been used in crop protection; these include metallic and electrified fences (around rice fields in the Philippines), flexible plastic fences (in Indonesia), and wire mesh fences (around oil palm trees in Africa) (Appert and Deuse, 1982).

C. Repellents

There are three major chemical senses in animals: olfaction (smell), gustation (taste) and chemical irritation or pain (touch); their basic functions are to respond to positive or negative stimuli.

1) Olfactory Stimuli as Repellents.

Olfactory perception comprises a great number of unique sensory experiences. Although the attractiveness or repellency of most odors is learned during nursing (Beauchamp, 1995), there may be a few cases where attractiveness or repellency is partially innate. According to Mattina et al. (1991), Andelt et al. (1991) and Epple et al. (1993), animals are inclined to avoid odors that denote danger, such as predator odors which can be good candidates for repellents.

2) Gustatory Stimuli as Repellents.

Unlike olfaction, few stimuli are involved; they include: sweet, sour, salty, bitter and the exceptional taste of monosodium glutamate (Bartoshuk and Beauchamp, 1994). Although these classes of tastes are derived from human studies, a few of them also apply to mammals; however, species differences appear in the sensitivity to the taste compounds (Beauchamp and Mason, 1991). Beauchamp et al. (1977) reported that some species of the family Felidae (Bobcat, Lion, etc.) prefer amino acids (e.g., L-proline) over carbohydrate sweeteners. Salts (e. g., NaCl) are preferred especially by herbivores, this is perhaps due to the role of Na^{+} ion in animal physiology. Limited studies have been conducted with acids (sour), but they are known to be avoided by animals. However, some studies with inbred mouse strains showed very high preference for citric acid (no irritation) (Beauchamp, 1995). Bitter substances are possible candidates for repellents; but some contradictions have been reported due to the bitter-rejection threshold exhibits (Beauchamp, 1995). Nolte et al. (1994) confirmed that the insensitivity of guinea pigs to bitter stimuli was consistent across different compounds.

3) Irritating Stimuli as Repellents.

Tingling, itching, burning, pain, and cooling are the sensations transmitted over the trigeminal (fifth cranial nerve). Such signals may imply danger, for example, pain which is a very unpleasant sensation. Beauchamp (1995) reported that the use of a multisensory array of substances could be the most effective strategy in developing repellents for vertebrates. Although previous work has concentrated exclusively on chemical signals, it is obvious that auditory (propane exploders, distress call amplifiers, shouting and clapping) and visual (scarecrows, reflection tapes) stimuli should not be ignored (Beauchamp, 1995; Belant et al., 1996)

D. Natural Products.

a) Natural Products of Animal Origin (Insects)

Insects have been used traditionally by humans as food and medicine (Read, 1982; De Conconi, 1982; Comby, 1990). However, many insects are also known to possess special glands that contain defensive secretions (Eisner, 1966, cited in Sondheimer and Simeone, 1970; Blum, 1981; Evans and Schmidt, 1990) to repel potential predators.

In nature, preeminent among chemically defensive arthropods are the beetles (Coleoptera) (Dettner, 1987; Evans and Schmidt, 1990).

1) Types of Chemical Defenses.

Arthropod defensive compounds are derived from a variety of glandular and non glandular sources; these can be readily classified by their location within the body and their overall functional morphology (Evans and Schmidt, 1990; Eisner, 1970; Blum, 1981).

a) Glandular Defenses. These glands include eversible glands in caterpillars of Papilionidae butterflies. Other glands are oozing glands in beetles and bugs, and spraying glands in carabid beetles. Other beetles such as the bombardier beetle rely on the hot mixture of their reactor glands to repel enemies. Particularly well known are *Eleodes* beetles (Eisner, 1970; Evans and Schmidt, 1990) that lower their heads and discharge an obnoxiously odorous and irritating spray from the tip of their abdomen. The secretion, which contains benzoquinones (Eisner, 1970; Evans and Schmidt, 1990; Blum, 1981), is the chief line of defense of these beetles, and has been shown to be effectively repellent to a diversity of predators (Eisner, 1966, cited in Sondheimer and Simeone, 1970). However, some species of *Elodes* have taken chemical defense to an extreme; appropriately called "bombardier beetles", they generate 1,4 quinones in the abdominal reaction chamber; this hot (100 $^{\circ}C$) and toxic mixture is explosively ejected with a loud pop from the abdomen as a vapor. A flexible abdominal turret allows the bombardier to discharge this cannonade directly into the face of an attacking predator (Eisner, 1970; Evans and Schmidt, 1990; Blum, 1981).

Insects such as the African grasshopper *Poekilocerus bufnius* (Klug) and the cockroach *Eurycotis floridana* (Walker) are equipped with tracheal glands to protect themselves (Eisner, 1970; Blum, 1981; Evans and Schmidt, 1990).

b) Non glandular Defenses. Numerous arthropods such as the Mexican bean beetle (*Epilachna varivestis* Mulsant) are endowed with defensive constituents in their blood and they may have active control over the release of the fluid when they are being disturbed (Eisner, 1970; Blum, 1981; Evans and Schmidt, 1990). Some insects (grasshoppers)

defend themselves using enteric discharges when disturbed or under stress (they regurgitate or defecate). Experimentally, Jays have exhibited a very elegant way of circumventing the regurgitative defenses of grasshoppers; when given *Romalea*, the birds pulled the head, then ate part of the body leaving the head and crop behind (Eisner, 1970; Blum, 1981).

2) Chemistry of Defensive Substances.

Insects manufacture an extraordinarily diverse array of defensive compounds; more than 600 have been catalogued by Blum (1981) and since then, the number has grown substantially to few thousands (Evans and Schmidt, 1990). In general, the active principle components of most defensive substances released from glands are known to be relatively low in molecular weight, highly volatile and are strongly odorous. In fact, it is often the odor given off by the animal when handled that provides the first clue of its possession of defensive glands. Dozens of compounds have been isolated and identified; included are normal alkanes, terpenes, alcohols, ketones, esters, aldehydes, organic acids, phenols and quinones. They are secreted as blends containing both polar and non polar constituents (Eisner, 1970; Blum; 1981; Evans and Schmidt, 1990). In contrast to exocrine allomones, defensive substances that fortify blood and internal organs are typically more complex; these include steroids, tricyclic alkaloids and complex amines (Blum, 1981; Dettner, 1987 cited in Evans and Schmidt, 1990).

3) Cockroaches as a Source of Repellents.

Robinson (1969) categorized insect defense systems as follows: a primary defense system that lowers the possibility of an attack (e.g. camouflage, swift running, flying,

burrowing, mimicry, nocturnal behavior, diving into water and swimming under water), and a secondary system that comes into effect after an attack is initiated (e.g. defensive secretions, antipredator displays and active fighting). Many insects are known to possess both types of defense systems, including stick insects (Carlberg, 1986; Bouchard et al., 1997) and particularly cockroaches (Guthrie and Tindall, 1968; Roth, et al., 1958; Stay, 1957).

Among the secondary defense mechanisms encountered in cockroaches, chemical secretions have been recorded in more than 20 species (Waterhouse and Wallbank, 1967; Wallbank and Waterhouse, 1970; Blum, 1964, 1981). Wallbank and Waterhouse (1970) went as far as to say that the species that have been recorded as producing defensive secretions all belong to two (Blaberidae and Blattidae) of the five families that constitute the order of Blattodea. Furthermore, within the Blattidae those species with defensive scents appear to belong, with one exception to only one of the four subfamilies (Polyzosteriinae).

4) Identification of the defensive volatiles.

Many insects possess defensive chemicals such as acids, aldehydes, ketones, quinones, terpenes, alkaloids and other compound. The substances released by insects are complex mixtures and require the use of sophisticated techniques (GC-MS, HPLC). The GC-MS analysis enabled the identification of many substances such as those in stink bugs (Aldrich et al., 1993; Aldrich et al., 1994) and stick insects (Bouchard et al., 1997). For the identification of the cockroach (*B. giganteus*) secretions, a novel technique Microwave Assisted Process (MAP™) was used for extraction and GC/MS for separation and

identification (Paré et al., 1994; Yaylayan and Keyhani, 1998). The uniqueness of this process is that under the stress of the heat due energy released by excited H_2O molecules, the insect released all its volatile compounds in the microwave vessel during extraction. Six major compounds were identified (Table 3) from the live cockroach. According to Blum (1981), the lack of aromatic hydrocarbons in arthropod defensive secretions is conspicuous. The fact that these aromatic hydrocarbons were detected only during the extraction from the live insect might indicate their relevance as components of defensive secretions of the insect. Those compounds termed as polycyclic aromatic compounds (dibenz[a,h]anthracene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[ghi]perylene, indeno[1,2,3,-cd]pyrene, etc.) are known since 1930 as carcinogenic compounds (Hieger, 1961; Vo-Dinh, 1989) and could be toxic. There is very little information available about the function of the defensive secretions of cockroaches in nature or about the identity of the natural enemies that are actually repelled (Wallbank and Waterhouse, 1970). In some genera of cockroaches (*Eurycotis*, *Polyzosteria*, *Euzosteria*, *Zonioploca*, *Megazosteria*, *Desmozosteria*) all species examined secreted hex-2-enal which is reported to cause vertigo and nausea in humans (Wallbank and Waterhouse, 1970) and would appear to be particularly effective against vertebrates if discharged into their eyes. Similarly, *B. giganteus* secretions caused allergies (eyes and skin) to students while taking care of the culture in our laboratory. However, Blum (1961) records the lizard, *Anolis cristatellus* D. & B., as eating *Pelmatosilpha coriacea* Rehn (head first) in nature; thus, inhibiting the cockroach from secreting defensive chemicals. On the other hand, Eisner et al. (1959)

found in laboratory tests that a bird, a lizard, a frog, and several arthropod predators were repelled by chemicals from the cockroach *Eurycotis floridana*.

b) Natural Products of Plant Origin

Plants have evolved over a period of 400 million years and have acquired effective defense mechanisms during their evolution which allow them to survive under harsh environmental conditions and against enemies. Protective means include thick cuticular waxes, thorns and sticky hairs. Some plants can produce toxins that protect them from attacks by insects, diseases and other herbivorous animals. The understanding of these natural defense mechanisms might provide evidence for the development of new ecologically acceptable biopesticides. Thus, a great number of different plant species contain natural chemical compounds, and some of these have been used as pesticides since very early times (Prakash and Rao, 1997). From early Roman times to the mid-20th century, only a few natural products were widely used as insect repellents and toxicants in the western hemisphere (pyrethrum, rotenone, nicotine, sabadilla and quassin) (Jacobson, 1989). Most botanical pesticides were largely abandoned during the era of synthetic pesticides, but since these synthetics have been proven to be unduly toxic and/or ecologically disastrous, the study of natural pesticides today contributes to novel approaches in pest control strategies.

According to Jacobson (1989), the most promising botanicals for use at this time and the immediate future are species of the families Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae, and Canellaceae. Most of the work done on botanicals were related to insect control; few cases are for rodent control. Two major effects resulted from the

action of botanicals on agricultural pests: antifeedant and repellent effects which are sometimes confounded by people. Antifeedants are substances which prevent the pests from destroying the plant, they belong to several chemical groups and exert their action through various mechanisms; the bitter taste of this large group of natural products may account in part for these properties (Bernays, 1983; Marini Bettolo, 1983). For example Azadirachtin obtained from the neem leaves has proved to be the most powerful antifeedant known. Although it is difficult to make a sharp distinction between antifeedants and repellents in plants, the key point is that some plants contain active principles which prevent attack and infestation by pests (e. g. insects). Repellents are also defined by substances in plants or a preparation which can discourage a pest from attacking, by driving back, causing aversion, etc. (Marini Bettolo, 1983)

1) Uses of Botanicals

In North America, although the most spectacular advances in chemical pest control are attributable to native species (e.g., nicotine from tobacco), the flora has been largely ignored phytochemically (Berenbaum, 1989). According to Berenbaum (1989), about 20,000 plant species native to North America have potential that has been ignored and are currently unexploited; this review is based on the ethnobotanical literature of native Americans, in which many plants are anthelmintic (toxic to 'worms').

Although synthetic pesticide use has been prevalent throughout Africa, many countries have been experimenting with traditional botanicals as possible replacements for the costly pesticides.

In Zanzibar and Tanzania, lemongrass (*Cymbopogon citratus* Stapf) is cultivated; it contains 75 to 80% citral, an essential oil that kills gram-negative bacteria. Lemongrass, (*C. flexuosus* (Nees ex. Steud) Wats. var. *flexuosus* Hack.), a native of East India, grows wild in many parts of Africa and is harvested for the extraction of citral and camphene (Simonetti, 1990 cited in Grossman, 1993). Grainge and Ahmed (1988) listed seven *Cymbopogon* species that are pesticidal against fungal plant pathogens *Alternaria*, *Botrytis*, *Fusarium*, *Verticillium*, nematodes, insects and spider mite pests.

In some African countries, particularly in Niger (West Africa), lack of sufficient synthetic chemicals for pest control causes farmers to use traditional methods based on the use of plant materials such as neem (*Azadirachta indica* A. Juss) (Barkiré, 1996). Thus, about 25 plant species have been used and are still used in Niger and other countries for the protection of cowpea (*Vigna unguiculata* L. Walp.), a proteineous plant under constant insect attack from the seedlings to the stored grains. In the Sahelian region, plant materials are also used as fumigants (by burning the fresh leaves to produce smoke) or by the technique called 'sandwich', which consists of alternating layers of plant materials and grains in granaries (made of mud) (Barkiré, 1996). In rural areas, striga plants (*Striga hermonthica* (Del.) Benth) are good for fumigants in repelling mosquitoes, and today neem oil is gaining popularity in the post harvest protection of cowpea. The introduction of the neem tree into the Sub-Sahelian region of northern Nigeria has been hailed as the greatest boon of the century, because previously very little grew in that rainfall-deficit area, and because of the multitude of applications of the neem tree (e.g.

reforestation, windbreaks) (Ketkar, 1976; Jain, 1983; Ahmed and Grainge, 1985; Schmutterer et al., 1984; Prakash and Rao, 1997; Maydell Von, 1986).

Pyrethrum, a low toxicity botanical derived from species such as *Chrysanthemum cinerariaefolium* (Trevir) Vis., has been used by the Chinese for over 2000 years against biting flies (Grossman, 1993).

The Chinaberry (*Melia azedarach* L.), has received particular attention due to the pesticidal effects of it extracts, and also because it grows widely in warm temperate and tropical regions. This is in contrast to neem, which grows primarily in India, Africa and Mexico (Olkowski and Olkowski, 1988; Prakash and Rao, 1997).

Research in China has shown the effectiveness of *M. azedarach* as a feeding deterrent against insects in many cultivated crops (Chiu, 1989). According to Stoll (1986, cited in Olkowski and Olkowski 1988), on-farm preparation of powdered dry seeds of *M. azedarach* protected stored wheat from insects. Similar to neem, *M. azedarach* contains mixtures of active ingredients, and azadirachtin is considered the most powerful constituent (Jacobson, 1981). Kraus *et al.*, (1981) showed that five active compounds (ohchinolid A and B, nibolinin A and B, and nimbolidin B) from extracts of *M. azedarach* exhibited antifeeding activity against the Mexican bean beetle (*Epilachna varivestis* Mulsant).

2) Neem

The neem tree (*A. indica* A. Juss; synonym: *M. azadirachta* Linn.), a native of India and Burma, is very common in Pakistan, Sri Lanka, Thailand and Cambodia where it is recognized as an important medicinal plant (Quarles, 1994; Jain, 1983; Ketkar, 1976;

Prakash and Rao, 1997; Maydell Von, 1986; Schmutterer 1990; Tewari, 1992). In the last 60 years, it was introduced to some African and Latin American countries, mainly to provide shade but also for firewood. It thrives in southern Florida, Oklahoma, Southern California, Arizona, and Australia, it was recently planted in Saudi Arabia, the Philippines, the Caribbean islands, and the Virgin Islands (Stoll, 1986; Quarles, 1994; Ketkar, 1976; Ahmed and Grainge, 1985).

The uses of neem are very well known in India and are mentioned in the earliest Sanskrit medical writings (Jain, 1983; Ahmed and Grainge, 1985; Maydell Von, 1986). Due to its high versatility, the neem tree in the Indo-Pakistan region is regarded as the 'village dispensary' in many villages, because of its numerous medicinal applications (Ketkar, 1976; Schmutterer, *et al.*, 1981). Leaf juice and decoction, which possess anthelmintic, antiseptic, diuretic, emmenagogic, emollient and purgative properties, are also used traditionally for the treatment of eczema and ulcers. Leaves and flowers are used to treat boils and headaches; the bark, which has antiperiodic and astringent properties is used to treat fever, leprosy and scrofula. Neem oil, traditionally used in medicinal hair oils, is also used for rheumatism. The neem tree is used for the treatment of blood disorders, hepatitis and eye diseases; it is also attributed to be antimalarial and a cure for syphilitic conditions (Ketkar, 1976; Ahmed and Gainge, 1985; Jain, 1983; Tewari, 1992).

Neem also has many non-pharmacological uses. Traditionally, neem leaves are mixed with food grain for pest control in storage, and its twigs are commonly used in South Asia for cleaning the teeth (a neem toothpaste is available in India). The tree's hard

termite-resistant wood is highly prized for construction and its resin is a gum substitute (Maydell Von, 1986).

3) Chemistry of Neem.

Influenced by the traditional folk-lore medicinal properties of neem, the pharmaceutical chemists were the first to attempt the isolation of the active ingredients from neem oil (Jain, 1983). Tremendous breakthroughs in understanding the chemistry of neem constituents were made only after 1960 with the development of sophisticated spectroscopic and chromatographic techniques (Ketkar, 1976; Jain, 1983; Tewari, 1992). A number of organic compounds have been isolated from various parts of the neem tree, and the various compounds have been grouped according to their occurrence in different parts of the tree.

Other than the bitter components such as nimbin and nimbolides, the fresh leaves are reported to contain quercetin and *B*-sitosterol. Quercetin, a polyphenolic flavonoid, is known to have antibacterial and antifungal properties (Jain, 1983; Tewari, 1992).

The flowers contain mainly flavonoids. Three glucosides, namely quercetin-3-galactoside, kaempferol-3-glucoside, and melicitrin were reported to occur in the flowers (Subramanian and Nair, 1972 cited in Jain, 1983). Melicitrin was the first natural myricetrin-pentoside to be reported.

Nimbin, nimbidinin, nimbinin and nimbidic acid have been identified from the trunk bark of both *Melia* species (*A. indica* and *M. azedarach*) (Jain, 1983). Fraxinellone and nimbolin A and B were detected in the bark of *A. indica* (Ekong et al., 1969 cited in Jain,

1983). Kraus et al. (1981) further isolated nimbinene and 6-desacetyl- nimbinene from the bark. In total, eight limonoids have been identified from these sources (Jain, 1983).

The head space volatile constituents from neem seeds were analyzed by capillary Gc-mass spectroscopy, and a total of 25 volatile compounds were identified (Tewari, 1992). The major components of neem bitters are salannin and nimbin; others occurring in smaller quantities include azadirachtin, azadirone, azadiradione and its derivatives, gedunin and its derivatives, salannol, vilasinin and its derivatives nimbin, nimbolide, meliantriol, nimbolidin, meldenin, vepinin and nimbinene. Only three of these have been implicated with biological activity of the crude seed cake, e.g., azadirachtin and meliantriol as locust antifeedants, and salannin as an antifeedant for houseflies (Ketkar, 1976; Jain, 1983; Schmutterer, 1990; Tewari, 1992; Quarles, 1994; Prakash and Rao, 1997).

The isolation and identification of the active ingredients like melantriol and azadirachtin (Lavie, et al., 1967; Butterworth and Morgan, 1968, 1971; Zanno, et al., 1975 and Jacobson, et al., 1978 cited in Jain, 1983) have now attracted worldwide attention, and tests with these chemicals have demonstrated the potential of neem in pest control (Jain, 1983; Tewari, 1992).

The discovery of the growth-disrupting nature of the antifeedant azadirachtin by Gill and Lewis (1971) and Ruscoe (1972) has added another dimension to the biological activity of the neem products, and prompted the beginning of a broad research program by the United States Department Of Agriculture (USDA) in 1975 (Jain, 1983). As a result of extensive investigations on chemical, biological and toxicological aspects of neem products; the Environmental Protection Agency (EPA) has granted the registration of

many neem based pesticides since 1994 (e.g., Bioneem, Neemix and Azatin) (Quarles, 1994).

c) Justification of Cockroach and Neem Usage

The cockroaches (*Blaberus giganteus*) (Dictuoptera: Blattodea: Blaberidae) were originally confined to the American tropics, but now extend into temperate South America (Cornwell, 1968). Few studies have been done with regard to defense mechanisms. Is it possible that cockroach defensive secretions be used in pest control? Little research have been done in the area of pest control. Despite the advantage of being such large insects (65-73 mm) and easy to rear as a laboratory material, their slow rate of development (140-200 days of nymphal growth) and cryptic behavior has restricted the amount of research compare to the Blattidae (*Blatta orientalis* L., and *Periplaneta americana* L.) (Cornwell, 1968; Guthrie and Tindall, 1968).

The geographical distribution of the neem tree is now in Niger and many African countries, where women have a thorough knowledge of the traditional way of seed oil extraction, which could popularize the use of neem extracts. Thus, farmers could enhance their crop protection systems by producing their own natural pesticides at low cost. Furthermore, in Niger, the National Tree Day (August 3rd) enables the annual planting of thousands of neem trees throughout the country, which could increase the potential production of neem products. For example in India, it is estimated that there are about 13, 800, 000 neem trees with the potential to produce over 83, 000 mT of neem oil and 330, 000 mT of neem cake from 413, 000 mT of seeds (Koul, et al., 1990).

The exploitation of insect defense secretions and the bitter compounds of certain tropical plants such as neem could be a great opportunity to reduce crop losses due to vertebrate pests (rat and bird) especially in storage situation where rats caused the greatest losses throughout the world.

III. MATERIALS AND METHODS

Laboratory rats were subjected to two kinds of natural products, insect secretions and neem products, in order to assess their behavioral responses. Feeding tests and Arena tests have been designed to assess the effectiveness of the insect volatiles as natural repellents and two-choice feeding tests were used to determine the repellency of neem products.

A. Experiments Testing Insect Secretions

1. Sample Analysis (insect secretions).

a) **Materials.** Specimens of *Blaberus giganteus* (Linnaeus) (Fig. 1) from a colony originally obtained from the Insectarium of Montreal (Montreal, Canada) were reared on rabbit chow pellets and oranges (*Citrus aurantifolia* L.). The cockroaches were maintained in glass cages (46 X 32 X 42 cm) at room temperature (28 ^{0}C), 12 hr light: 12 hr dark photoperiod and had free access to water.

Soxwave ^{TM}100 (focused microwave extraction system at atmospheric pressure) was obtained from Prolabo (Fontenay-Sous-Bois Cedex, France). The apparatus consists of a command box and a microwave module. It operates with an emission frequency of 2450 MHz and a 300 W power. It is equipped with a 250 ml quart vessel, a refrigerant column, Graham type (400 mm long) and a bent extraction tube.

b) **Sample preparation.** One live insect (*B. giganteus*) was placed in the extraction vessel of the microwave; petroleum ether (50 ml) was then added, and the vessel was subjected to irradiation in the Soxwave (microwave) at 100% power as follows: 50 s ON, 60 s OFF, 90 s ON, pausing when the solvent got high in the condenser.

1.) The extract was filtered and dried over anhydrous sodium sulfate and analyzed by gas chromatography coupled with mass spectrometry (GC-MS). After the extraction, the insect was dried in an oven (at 35 ^{0} C) for 15 min. and then placed in a domestic microwave for 1 min. at full power (900 W) to ensure that the insect was completely dry. The dried insect was powdered in a mortar and placed in the extraction vessel with 8 ml of water and 1.5 ml of NH_4 OH and then irradiated for 2 min. at 20% power. After cooling and addition of 5 ml ethanol, the solution was extracted with 24 ml of 50% ethyl ether and petroleum ether in a separatory funnel. 2.) The yellow organic phase was dried over anhydrous sulfate and analyzed by GC-MS.

c) **GC-MS analysis.** This method was previously described by Yaylayan and Keyhani (1998). A Hewlett-Packard GC-mass selective detector (5890 GC/5971B MSD) was used for the analysis. The GC column flow rate was 0.8 ml/min. for a split ratio of 92 : 1 and the septum purge was 3 ml/min. The injector temperature was set at 250 ^{0} C. Capillary direct MS interface temperature was 180 ^{0} C; ion source temperature was 280 ^{0} C. The ionization voltage was 70 eV, and the electron multiplier was 1682 V. The mass range analyzed was 50-550 atomic mass unit (amu). The column was a fused silica DB-5 column (30 m long X 0.25 mm ID. X 25 um film thickness; Supelco, Inc.). The column initial temperature was 25 ^{0} C and was increased to 250 ^{0} C at a rate of 7.5 ^{0} C/min.; the temperature was then further increased to 300 ^{0} C at the rate of 25 ^{0} C/min. and kept at 300 ^{0} C for 10 minutes. Products were identified by spectral library search. Each sample was analyzed in duplicate.

2. Tests With Rats.

a) **Materials.** Forty rats (*Rattus norvegicus* Berkenhou), strain CD (20 males and 20 females), weight ranging from 100 to 125 g, were purchased from Charles Rivers (St Constant, Quebec, Canada) and maintained in the Department of Animal Science Animal Facility, under conditions of controlled temperature (23^0 C), 50-60% relative humidity, and 12 hr light : 12 hr dark cycle (light onset at 0600 hr). They were individually caged on wood chip bedding (changed every three days) in clear plastic cages (35 X 23 X 17 cm), with wire mesh on top and bottom, and given free access to rat chow and tap water. Prior to testing, rat chow was ground and the animals were allowed to acclimatize.

Forty-eight feeders (6.5 cm diameter X 8 cm height) with leads (perforated rings which inhibit spillage) and covers were purchased from Allentown Caging Equipment Co. (Allentown, New Jersey, USA). Forty small jars (4.5 cm diameter X 6.7 cm height) with covers were used. Fifteen small holes were drilled in the small jar covers to allow the insect volatiles to flow out. Two chronometers were used to time feeding.

b) **Feeding tests.** Ten male (weight 400-500 g) and ten female (weight 250-350 g) laboratory rats (*R. norvegicus*) of the strain CD were fed with powdered Purina rat chow and fresh water was provided daily. Rats were kept in individual cages and each was provided with a feeder attached with a small jar which contained the insects or was empty. Rats were randomly assigned to 4 treatment groups (N=5 rats of the same sex/group) and adapted to an 16-hr (1700-0900 hr) food-deprivation schedule. All experiments commenced at 0900 hr and lasted 2.5 hours. The adaptation was followed immediately by four days of treatment (one day/group). One group male and female group were treated

with insects and one group male and female group were treated with the control. The experiment was performed as follows: for each group (one/day), each rat was removed from its cage, the feeder (containing food) was placed in the cage, and a small jar containing two *B. giganteus* or nothing (control) was attached to the feeder (Fig. 2) and the rat was returned to its cage. Immediately following this, the number of visits of the rat to the feeder and the duration of each visit were recorded for 30 minutes, and the amount of food consumed was measured. In 1996 the experiment was performed once, whereas in 1997, it was repeated.

c) **Arena test.** Ten male (weight 400-500 g) and ten female (weight 250-350 g) laboratory rats (*R. norvegicus*) of the CD strain were fed Purina rat chow pellets; tap water was provided daily. Rats were maintained individually. One rat was placed in a circular arena (72 cm in diameter) and observed for 30 minutes. The arena was supplied with a layer of wood chip bedding as in the cages (Fig. 3). As previously described by Bouchard et al. (1997) six holes, each 2.3 cm diameter, were drilled in the wall equidistant from one another and 6.5 cm from the floor of the arena. For each of the holes, a horizontal plastic bottle (4 cm diameter X 8.5 cm height) was tightly fitted into the holes to allow the rat inside the arena to insert its nose in the holes when investigating. During the control tests, the horizontal bottles were filled either with distilled water (3 holes: 10 ml/hole) or Swiss cheese (3 holes: 10 g/hole).

A visitation was recorded whenever the nose of the rat went beyond one of the six openings in the wall of the arena. The control tests were conducted twice with twenty rats at one -month intervals (Fig. 3).

Tests with the insects were performed in the same manner as the controls, except that each of the 3 bottles with distilled water was replaced with bottles each containing four live adult cockroaches (*B. giganteus*). The plastic bottles were cut horizontally to insert the insects and the cut was covered with scotch tape. Each of the other 3 holes contained 10 g of Swiss cheese. Visitations of rats to the holes were recorded. The same rats were used in experiments 1 and 2, but different rats were used in 1996 and 1997. Rats were given ample time to investigate, and a choice of two holes were present in both control and live insects tests. Rats were freely fed so that they were not hunger driven during their investigations.

B. Experiments Testing Neem Tree Materials

a) **Biological materials.** In 1996, neem leaves were collected in Niamey (Niger), dried inside at room temperature and subsequently powdered; dried neem seeds were collected from Konni (Niger), and one liter of neem seed oil was obtained from the Department of Plant Protection (Niamey, Niger). All plant materials, including 2 kg of powdered neem leaves, 4 kg of neem seed powder and 1 liter of neem oil were shipped to the laboratory at McGill University, Montreal, Canada. In 1997, 1 liter of fresh neem oil was obtained from the same location in Niger; neem leaf and neem seed powders were available from the previous year. A small quantity (50 ml) of commercial neem oil (CNO), 0.08% concentration of azadirachtin, was obtained from the Fundacion Agricultura y Medio Ambiente, at San Cristobal in the Dominican Republic.

Twenty male and twenty female (weight 100-125 g) rats were purchased from Charles River (St. Constant, Quebec Canada). The rats were individually caged, kept

under the same conditions as in section 2., a) (temperature, relative humidity, and light and dark cycle), and fed powdered Purina rat chow, with access to tap water. Ten pairs of rats were tested each year (1996 and 1997).

b) **Experiments with the two choice feeding test.** Prior to the trials, each cage received 2 feeders separated by 10 cm and attached to the edge of the cages. One feeder contained food treated with neem product, and the other feeder contained food without neem. In 1996, ten male (weight 400-500 g) and ten female (weight 250-350 g) rats were randomly tested against 3 different neem products: (i) 4 different doses of neem seed powder (NSP) (50, 25, 15, and 5 g per kilogram of powdered rat chow); (ii) 3 different doses of neem seed oil (NSO) (15, 10, and 5 ml per kilogram of powdered rat chow); (iii) 3 doses of neem leaf powder (NLP) (50, 25, and 15 g per kilogram of the same food). Dosages were tested in sequence from the highest to the lowest, and each was tested on all the rats at the same time (one dose/day). The order of testing was NSP, NSO, NLP. Prior to each dose assignment from the three series of tests, the rats were starved overnight. They were allowed to rest one day after each test. The rats were subjected to two choices of food (with and without neem product) for all the trials and the quantity of food consumed by each rat was weighed after 5 hours (0600-1100 hrs). All three sets of tests were separated from one another by 2 weeks to allow the rats to recover from eating bitter food. In 1997, a trial with the commercial neem oil, and the previous experiments were conducted following the same protocol. The chemical analysis of the neem materials have not been done as the equipment available in the lab could not analyzed substances which the atomic mass unit is more than 550.

C. Statistical Analyses

a) **Experiments involving live insects.** An analysis of variance using the Proc GLM procedure (SAS., 1989) was first performed for the feeding test to assess the presence of effects between treatments, sexes, and to identify any interaction between treatment and sexes; a t-test was then performed using Proc TTEST procedure (SAS., 1989). In this case, the variables of interest were: the number of visits, total duration of the visits in seconds, number of seconds/visit, total food intake in mg, food intake/visit, and food intake/second.

For the arena test (control and treatment tests) a Student t test for paired samples was performed in the first year using the S.P.S.S. (S.P.S.S., 1989-1995) program, then the results were verified using Chi-square test (Snedecor and Cochran, 1967). The following year, as the data consisted of simple counts, Chi-square (X^2) test analyses were performed for each sex group (Snedecor and Cochran, 1967).

b) **Experiments involving neem tree materials.** Prior to any analysis all the data were subjected to a Bartlett test to determine homogeneity of variances, and then to ANOVA in order to assess the presence of effects for treatment, sex, and treatment-sex interaction. In the first year, all series of tests were analyzed with the t- test for paired samples using the S.P.S.S. program; an analysis of variance using the Proc Univariate procedure (SAS., 1989) was performed the following year. In that case, the variable of interest was the difference between the control and the treatment.

IV. RESULTS

A. Trials Involving Insect Secretions

a) **Chemical composition**. Table 1 lists the compounds identified in the live *B. giganteus* Microwave Assisted Process (MAP) extract; they were mostly fatty acids and their ethyl esters. Palmitic acid was the main fatty acid, followed by oleic acid, which is known to be involved in insect metabolism. The compounds identified after drying the extracted live cockroach (*B. giganteus*) are listed in Table 2. This second MAP extraction revealed the presence of linoleic acid and cholesterol. In addition, numerous long chain alkanes such as hexacosane, nonacosane and octacosane that were not detected in the previous extraction, were also identified. Fused polycyclic aromatic compounds were not present. Table 3 lists the suspected toxic compounds from the cockroach repugnatory secretions; these compounds were not previously reported in any insect species.

b) **Feeding test.** For these particular tests, the ANOVA test showed an effect of sex with regard to some variables such as total duration and total food intake (Appendices 1 and 2). For the subsequent analysis, (TTEST Procedure) male and female data were analyzed separately. In 1996, there were no significant differences between the controls and insect treatment of all variables for the females or males (number of visits, total duration of visits in second, number of seconds/visit, total food intake in mg, food intake/visit and food intake/sec) (Table 4). In the same Table, the mean number of visits to the treated food for females was lower than that of non treated food, but not low enough to be statistically significant.

Similarly, the males exhibited for variable average duration (total duration/number of

visits) a lower mean value for the treated food than that of the control food, but not statistically significant.

In 1997, ANOVA performed prior to the t-test revealed sex effect, treatment effect, and both effects for some variables (for sex effect, number of visits, total duration; for treatment effect, total food intake; for sex and treatment effects respectively, food intake/visit) (Appendix 3 to 6). Thus, for the subsequent t-test, data were analyzed separately with regard to sex. In this 1997 study, the results of the first feeding tests were not statistically significant for all sexes (Table 5); although the expected results were not obtained, some trends could be observed among the mean. For example, the mean of the total duration of the visits (sec) of the control was higher than that of the insect treatment for both males and females; similarly, the same trend appeared when the total food intake (mg) was considered, and overall, males ate more food than females; the same results applies to the variable food intake/visits (mg) (Table 5). In the second feeding test, the results for variables number of visits and total duration for females were highly significant; their visits to the controls were higher than those to the insect treatments. The analyses for the remaining variables of the females and all those of males were not significant (Table 6). In the males, the mean number of visits and the mean of the total duration of visits for the controls were higher than those of the insect treatments, but not enough to be statistically significant. Overall the results of these analyses were inconsistent (Table 6).

c) **Arena test.** In the control test involving rats (1996), the mean number of visits to the holes with distilled water and holes with cheese did not differ statistically for either male or female rats (Chi-square test, $P>0.05$); however, the ranges were widely separated

(especially for females). Few rats that showed a high number of visits to cheese holes exhibited the same behavior for holes with distilled water. When the distilled water was replaced with the live cockroaches releasing secretions which might be containing the six suspected chemicals previously referred to (Table 3), the mean number of visits by both male and female rats increased for cheese holes (except for males in the repeat) and strongly decreased for holes containing the live insects when compared with the control (Table 7). Thus, for the two repeated experiments with sexes combined, the differences between the number of visits to holes with cheese and holes with live insects were highly significant ($P<0.01$). Similarly, in 1997, using a different set of rats and performing the Chi-square test, the same trend confirmed previous results. In this last trial, the mean number of visits to the holes with cheese and to holes with distilled water did not differ statistically for either male or female rats ($P>0.05$). But when the distilled water was replaced with the live insects, the mean number of visits by both males and females increased for the holes with cheese and greatly decreased for holes with the live insects. Thus the differences between the number of visits to the holes with cheese and to the holes with live insects were strongly significant for both replicates ($P<0.01$) (Table 7). The number of visits by females was greater than those by males in all trials. According to Table 7, males seemed to be more affected by the live insects' secretions than the females, because less visitations were recorded for those holes during the 30 minute observations. The females visited holes with the insects' secretions up to 36 and 34 times in 1996 and 1997 respectively, and visited the holes with cheese up to 46 and 48 times in the same years.

B. Trials Involving Neem Tree Materials

1. 1996 studies. Similar to the previous experiments, the ANOVA test performed prior to statistical analysis revealed some effects (sex, treatment, or both) among the variables. Thus, in this study, the variable for paired differences for neem seed oil, showed a treatment effect; for neem seed powder, treatment and sex effects were both shown respectively; similarly, neem leaf powder also showed both effects (treatment and sex) (Appendices 7 to 9). The subsequent analyses were performed without pooling male and female data.

a) **Neem Seed Oil.** For this series of tests, the data were analyzed using the t-tests for paired samples. When only males were considered, the differences between controls and treatments under the two highest doses were significant (15 ml and 10 ml/kg of rat chow) (Appendix 10) . In the same way, when data for female rats were analyzed, only the difference between treated food and control for the highest dose was significant (Appendix 10). The same results were obtained when the means of the treated food eaten vs. means of the untreated food eaten were analyzed (Table 8); thus, in the figure of the same data there was a tremendous feeding reduction of the treated food for both sexes under the two highest doses, in favor of the non treated food which consumption increased when the dose of neem oil was increased in the treated food (Fig. 4).

b) **Neem Seed Powder.** When the data for male or female rats were taken separately, the differences between food with neem seed powder and one without neem seed powder of the variable doses were significant. In this case the mean differences between control food and treated food were highly significant with all doses, and for male

b) **Neem Seed Powder.** When the data for male or female rats were taken separately, the differences between food with neem seed powder and one without neem seed powder of the variable doses were significant. In this case the mean differences between control food and treated food were highly significant with all doses, and for male and female rats (Appendix11). The analysis of the means of food with neem seed powder eaten vs. means of food without neem seed powder exhibited a great reduction of feeding on the treated food for both male and female rats for all doses (Table 9 and Fig. 5). The highest feeding reduction of the treated food for both sexes was achieved with 15 and 25 g of neem seed powder/kg of rat chow, but with 50 g of neem seed powder/kg of rat chow, the feeding of the treated food increase slightly and the feeding of the control food decreased greatly for both sexes (Fig. 5).

c) **Neem Leaf Powder.** Using the t-tests for paired samples, and when data from males or females were treated separately, different results were obtained. For males, there were no significant differences among the paired differences for all three doses; for females, the paired differences for the two highest doses were significant; for the lowest dose (15 g of neem leaf powder/kg of rat chow) there was no significant difference for the paired differences, and the rats ate more the food with neem leaf powder than the control food. (Appendix 12). Taking into account the means of the original data (Table 10 and Fig. 6), the same results were obtained, in that only females significantly reduced their feeding of the treated food with regard to the two highest doses. Male behaviour was more clearly shown in Figure 6, where no significant decrease was seen with the treated food, whereas an increase in feeding on the control food.

2. 1997 studies. For all three series of tests the Proc Univariate procedure was used and the variable of interest was the difference between control and treatment. Similar to the previous year, ANOVA test was performed in the same way as stated previously. Both treatment and sex effect were exhibited for neem seed powder, and for neem leaf powder (Appendices 13 and 14), whereas no effect was shown for neem seed oil. Therefore, the Proc Univariate procedure was performed using male and female data separately.

a) **Neem Seed Oil.** In the test involving the highest dose (15 ml of neem seed oil/kg of rat chow), the differences between control and treatment were highly significant for both male and female. Regarding the middle dose (10 ml of neem seed oil/kg of rat chow), the female responses did not differ significantly, whereas the males responded significantly different. For these two doses (15 ml and 10 ml) males ate more (control food) than females (Table 8), and standard deviations for the males were greater than those of females (Appendix 15). The lowest dose (5 ml) exhibited no significant responses for males, but in the females there was a highly significant difference between control and treatment, but negative, indicating that the females ate more of the treated food (Appendix 15). The means of the original data (food treated vs. control food) (Fig. 7), showed similarity of male and female behaviour, in that feeding of the treated food was reduced when the doses increased, in favor of the untreated food.

b) **Neem Seed Powder.** With regards to the highest dose (50 g of neem seed powder/kg of rat chow), the difference between control and treatment was highly significant for females and males. The middle dose (25 g) also demonstrated significant differences for females and males. For the lowest doses (15 g and 5 g), the differences between control and treatment did not differ significantly for either males or females. For

this bitter material, males ate more of the control food than the females at the two highest doses (Appendix 16, Table 9). The means of the original data (Fig. 8) suggested that male and female behaviour were similar, thus, there was a reduction of feeding in the treated food of the two doses (15 and 50 g of neem seed powder/kg of rat chow), but the feeding was increased with the 25 g dose. The same pattern was shown with the control food.

c) **Neem Leaf Powder.** At 50 g of neem leaf powder/kg of rat chow the differences between control and treatment were highly significant for both male and female rats (Table 10). At 25 g/kg of rat chow, control and treatment did not differ significantly for either sexes, but at 15 g/kg of rat chow, the females exhibited a highly significant difference between control and treated food, but the mean was negative (females ate more treated food than control) (Appendix 17). The male responses did not differ significantly. When the means of the original data were analyzed (Fig. 9), there was a great feeding reduction of the treated food vs. control as the dose increases for both sexes. For the control food the feeding of females increased remarkably, but for males there was a decrease at 25 g dose then an increase in feeding for the highest dose.

d) **Commercial Neem Oil**. In this test, the results at 15 and 5 ml/kg of rat chow for both male and female rats were negative (the rats ate more treated food than control). The Probability (P -values) were not significant at 15 ml, whereas at 5 ml the P- values were highly significant for both sexes. The mean difference between control and treatment for males at 10 ml was positive but not significant, and for females the mean was negative and highly significant which means that the females ate more treated food than control food (Table 11).

TABLE 1. Compounds identified the live cockroach (*Blaberus giganteus* L.) after Microwave Assisted Process of extraction.

Rt (min)	% Area	Compound
21.97	0.44	Tetradecanoic acid (Myristic acid)
24.5	5.37	9-Hexadecenoic acid (Palmitoleic acid)
24.65	1.18	Tridecanoic acid
24.87	25.75	Hexadecanoic acid (Palmitic)
25.1	1.79	Hexadecanoic acid, ethyl ester
26.5	3.67	Benzo[a]pyrene (3,4-Benzpyren)
27	10.8	9-Octadecenoic acid (Z)- (Oleic acid)
27.24	3.05	9-Octadecenoic acid (Z)-, ethyl ester (oleic acid ethyl ester)
30.34	0.5	Nonadecane
30.62	0.34	Octadecane
31.68	1.06	7H-1-Indeno[2,1-a]anthracen-7-one
32.33	1.9	1,2:3,4-Dibenzoanthracene
32.59	2.87	Indeno[1,2,3,-cd]pyrene
32.68	1.78	Dibenz[ah]anthracene
32.88	1.16	Nonacosane
33.35	2.73	Benzo[ghi]perylene
34.21	1.95	Docosane
34.82	2.74	Pentacosane
35.99	0.58	Tricosane
36.32	1.36	Triacontane
36.85	3.15	Dotriacontane
38.97	3.34	Heptacosane
39.43	4.56	Tetratriacontane
40.33	3	Dotriacontane

TABLE 2. Compounds identified in the powdered and dried cockroach (*Blaberus giganteus* L.) after Microwave Assisted Process of extraction.

Rt (min)	% Area	Compound
22.01	0.38	Tetradecanoic acid (Myristic acid)
24.54	6.3	9-Hexadecenoic acid (Palmitoleic acid)
24.94	31.48	Hexadecanoic acid (palmitic acid)
25.12	2.63	Hexadecanoic acid, ethyl ester
27.09	22.72	9-Octadecenoic acid (Z)- (Oleic acid)
27.2	1.25	Ethyl linoleate
227.27	8.2	9-Octadecenoic acid (Z)-, ethyl ester
27.55	0.61	Octadecnoic (Stearic) acid, ethyl ester
30.35	0.48	Docosane
30.63	0.4	Pentacosane
31.11	0.32	Tetracosane
31.83	3.54	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester
32.2	0.34	Hexcosane
32.9	1.06	Nonacosane
34.23	0.68	Octacosane
34.78	3.54	Cholest-5-en-3-ol (3.beta.)
34.85	2.45	Triacontane
39.06	2.66	11-butyl docosane
39.52	3.75	Tricosane

TABLE 3. Suspected toxic compounds identified in live *Blaberus giganteus* L. Microwave Assisted Process extract.

Rt (min)	% Area	Compound
26.5	3.67	Benzo[a]pyrene (3,4-Benzpyrene)
31.68	1.06	7H-1-Indeno[2,1-a]anthracen-7-one
32.33	1.9	1,2:3,4-Dibenzoanthracene
32.59	2.87	Indeno[1,2,3,-cd]pyrene
32.68	1.78	Dibenz[a,h]anthracene
33.35	2.73	Benzo[ghi]perylene

TABLE 4. Effect of *Blaberus giganteus* L. secretions on the feeding of rats (*Rattus norvegicus* Berk.), 1996 experiment (TTEST Procedure).

Variables	Mean		Std Dev		P-value
	Ctrl	Test	Ctrl	Test	
Number of visits					
Male	8.4	11.0	2.7	2.1	0.1290
Female	10.6	6.6	5.7	2.1	0.1775
Total duration (sec)					
Male	836.6	908.2	228.8	262.6	0.6580
Female	537.8	621.8	284.7	440.4	0.7295
Average duration (sec)					
Male	106.7	85.9	40.6	35.1	0.4108
Female	61.5	96.4	47.6	70.1	0.3844
Total food intake (mg)					
Male	5990	6408	1711.1	1712.8	0.7095
Female	2878	3386	1390.6	2241.5	0.6781
Food intake/visits (mg)					
Male	759.7	599.1	302.3	198.3	0.3495
Female	314.6	534.2	218.3	371.5	0.2874
Food intake/sec (mg)					
Male	7.6	7.1	3.0	0.5	0.7344
Female	5.4	6.0	0.8	2.1	0.5688

* indicates that the difference between the control and the treatment are significant at P <0.05.

TABLE 5. Effect of *Blaberus giganteus* L. secretions on the feeding of rats (*Rattus norvegicus* Berk.), 1997 experiment (TTEST Procedure).

First trial.

Variables	Mean		Std Dev		P-value
	Ctrl	Test	Ctrl	Test	
Number of visits					
Male	12.2	12.0	3.6	4.1	0.9361
Female	12.2	12.6	4.3	4.8	0.8936
Total duration (sec)					
Male	677.4	654.2	86.3	81.0	0.6728
Female	449.4	426.8	103.4	100.3	0.7348
Average duration (sec)					
Male	58.1	58.0	11.3	13.7	0.9832
Female	39.4	37.0	9.2	11.0	0.7254
Total food intake (mg)					
Male	5621.8	5544.4	663.4	807.2	0.8725
Female	4429.6	4306.6	917.3	1144.6	0.8559
Food intake/visits (mg)					
Male	486.6	419.8	119.2	124.4	0.9485
Female	391.7	361.7	102.5	68.7	0.6137
Food intake/sec (mg)					
Male	8.3	8.4	0.5	0.6	0.6727
Female	9.9	10.0	0.4	1.1	0.8486

* indicates that the difference between the control and the treatment are significant at P <0.05.

TABLE 6. Effect of *Blaberus giganteus* L. secretions on the feeding of rats (*Rattus norvegicus* Berk.), 1997 experiment (TTEST PROCEDURE).

Second trial.

Variables	Mean		Std Dev		P-value
	Ctrl	Test	Ctrl	Test	
Number of visits					
Male	11.0	8.4	1.6	2.7	0.1004
Female	18.0	7.2	1.6	1.9	0.0000 *
Total duration (sec)					
Male	837.8	643.0	185.7	181.9	0.1324
Female	1101.0	536.6	126.6	293.9	0.0043 *
Average duration (sec)					
Male	78.9	79.7	28.6	23.9	0.9618
Female	61.2	72.1	3.5	29.8	0.4577
Total food intake (mg)					
Male	5650.0	5774.0	1352.0	1233.3	0.8833
Female	2802.0	3434.0	1333.7	2200.1	0.5978
Food intake/visits (mg)					
Male	508.5	726.4	74.3	226.1	0.0748
Female	151.9	502.6	64.7	340.3	0.0825
Food intake/sec (mg)					
Male	7.2	9.9	2.6	4.1	0.2503
Female	0.1	7.5	0.002	6.1	0.0528

* indicates that the difference between the control and the treatment are significant at P <0.05.

TABLE 7. Number of visits by rats (*Rattus norvegicus* Berk.) to holes containing distilled water, cheese or live insects (*Blaberus giganteus* L.) recorded for 30 minutes. 1996 and 1997 experiments.

Test	Sex	Holes	Mean		Sd	
			1996	1997	1996	1997
Control	male	distilled water	22.2	12.4	10.1	4.9
		cheese	19.7	12.6	9.1	5.3
	female	distilled water	31.2	10.3	8.5	4.3
		cheese	31.3	10.2	9.9	4.3
Experimental (1)	male	live insects	13.3 *	13.4 *	5.2	4.9
		cheese	22.1	23.2	12.6	11.5
	female	live insects	24.3 *	23.4 *	8.8	7.9
		cheese	34.5	35.6	11.4	10.8
Experimental (2)	male	live insects	13.1 *	12.4 *	8.6	7.9
		cheese	18.7	19.5	10.3	10.4
	female	live insects	23.5 *	23.1 *	7.5	6.2
		cheese	38.6	39.7	11.2	11.0

N= 10 males and 10 females

* indicates that the mean number of visits to holes with live insects and holes with cheese are significantly different.

TABLE 8. Summary of the effect of neem seed oil on the feeding of rats (*Rattus norvegicus* Berk.), 1996 and 1997 experiments (TTEST Procedure).

Dose (ml)	5		10		15	
Mean difference (Ctrl minus Test) (g)	1996	1997	1996	1997	1996	1997
Male	0.6	-0.4	2.4	2.5	3.2	6.1
Female	0.4	-2.1	0.9	0.1	2.8	3.8
Std Dev						
Male	3.7	2.3	2.8	3.5	2.1	2.5
Female	3.0	2.3	1.6	1.9	1.5	1.9
P-values						
Male	0.641	0.619	0.024 *	0.049 *	0.001*	0.0001*
Female	0.666	0.0163	0.087	0.8483	0.000 *	0.0001*

* indicates that the difference between the control and the treatment are significant at P <0.05.

TABLE 9. Summary of the effect of neem seed powder on the feeding of rats (*Rattus norvegicus* Berk.), 1996 and 1997 experiments (TTEST Procedure).

Dose (g)	5		15		25		50	
Mean Difference (Ctrl minus Test) (g)	1996	1997	1996	1997	1996	1997	1996	1997
Male	8.8	-2.7	12.8	0.8	12.7	5.1	3.8	4.2
Female	5.7	0.7	9.7	-0.1	7.2	4.5	3.3	3.4
Std Dev								
Male	8.1	6.9	3.8	4.2	2.6	4.4	2.7	3.9
Female	6.0	5.4	4.2	1.6	2.3	4.4	2.1	1.7
P-value								
Male	0.007 *	0.2439	0.000 *	0.5382	0.000 *	0.0054*	0.001 *	0.0070 *
Female	0.014 *	0.6867	0.000 *	0.8342	0.000 *	0.0104 *	0.001 *	0.0001 *

* indicates that the difference between the control and the treatment are significant at $P < 0.05$.

TABLE 10. Summary of the effect of neem leaf powder on the feeding of rats (*Rattus norvegicus* Berk.), 1996 and 1997 experiments. (TTEST Procedure)

Dose (g)	15		25		50	
Mean Difference (Ctrl minus Test) (g)	1996	1997	1996	1997	1996	1997
Male	1.6	1.1	2.7	0.8	4.4	5.0
Female	-0.8	-2.9	2.6	0.7	2.2	2.7
Std Dev						
Male	3.5	6.4	6.6	3.3	6.7	3.6
Female	2.8	2.4	3.4	2.7	1.8	2.3
P-value						
Male	0.183	0.5987	0.230	0.4428	0.061	0.0017 *
Female	0.363	0.0111 *	0.038 *	0.4067	0.004 *	0.0047 *

* indicates that the difference between the control and the treatment are significant at $P < 0.05$.

TABLE 11. Summary of the effect of commercial neem oil on the feeding of rats (*Rattus norvegicus* Berk.), 1997 experiments (TTEST Procedure).

Dose (ml)	5	10	15
Mean Difference (Ctrl minus Test) (g)			
Male	-2.1	0.4	-1.9
Female	-3.9	-2.4	-1.2
Std Dev			
Male	2.3	4.4	4.2
Female	2.2	1.8	2.9
P-value			
Male	0.0188 *	0.7502	0.1802
Female	0.0003 *	0.0019 *	0.2139

* indicates that the difference between the control and the treatment are significant at $P < 0.05$.

Figure 1. **Three adult cockroaches (*Blaberus giganteus* L.) feeding on wheat bran. (live insect size).**

Figure 2. *Rattus norvegicus* **Berk. in a cage with a small jar containing cockroaches, and attached to a feeder.**

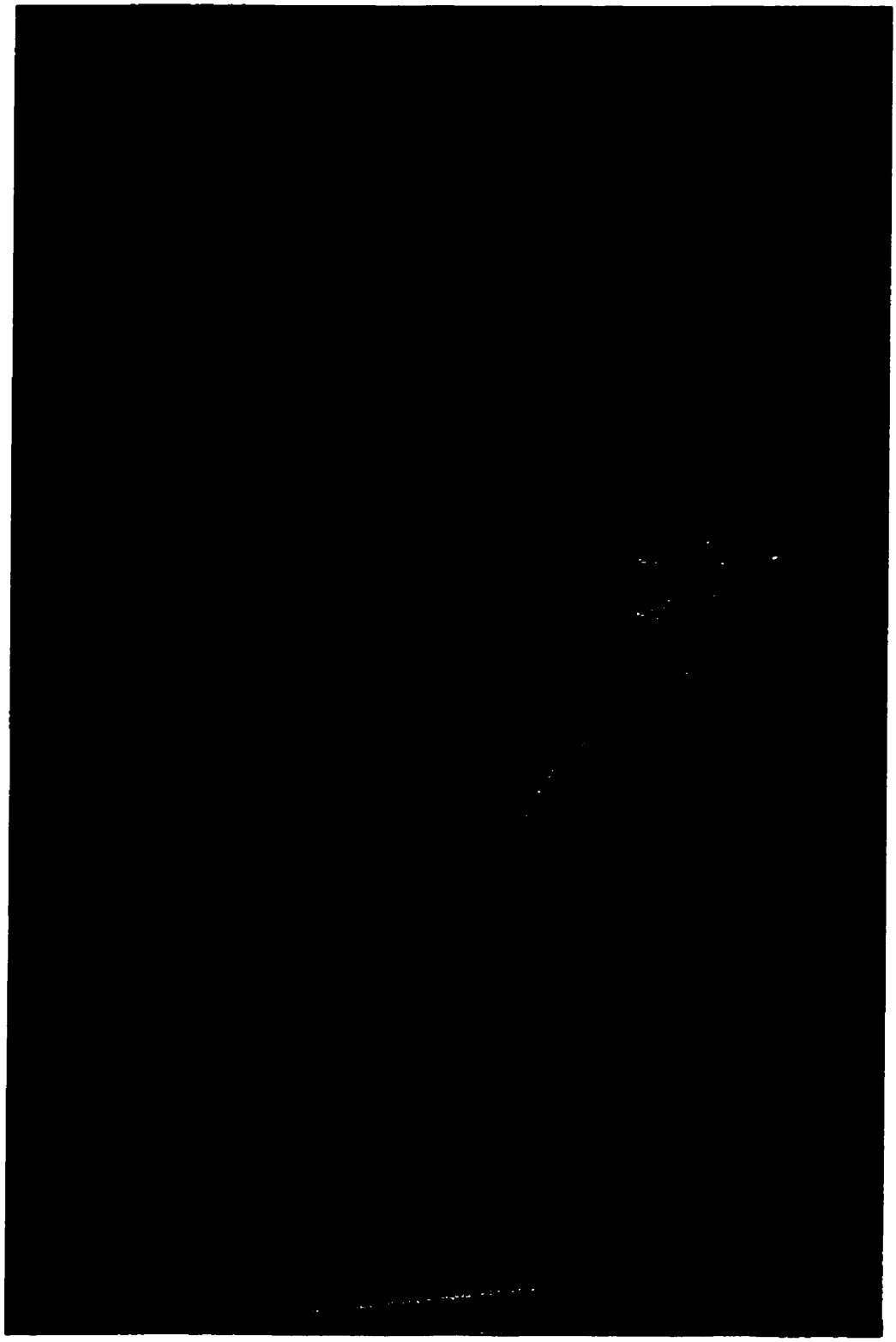


Figure 3. *Rattus norvegicus* **Berk. investigating for smell during the arena test.**

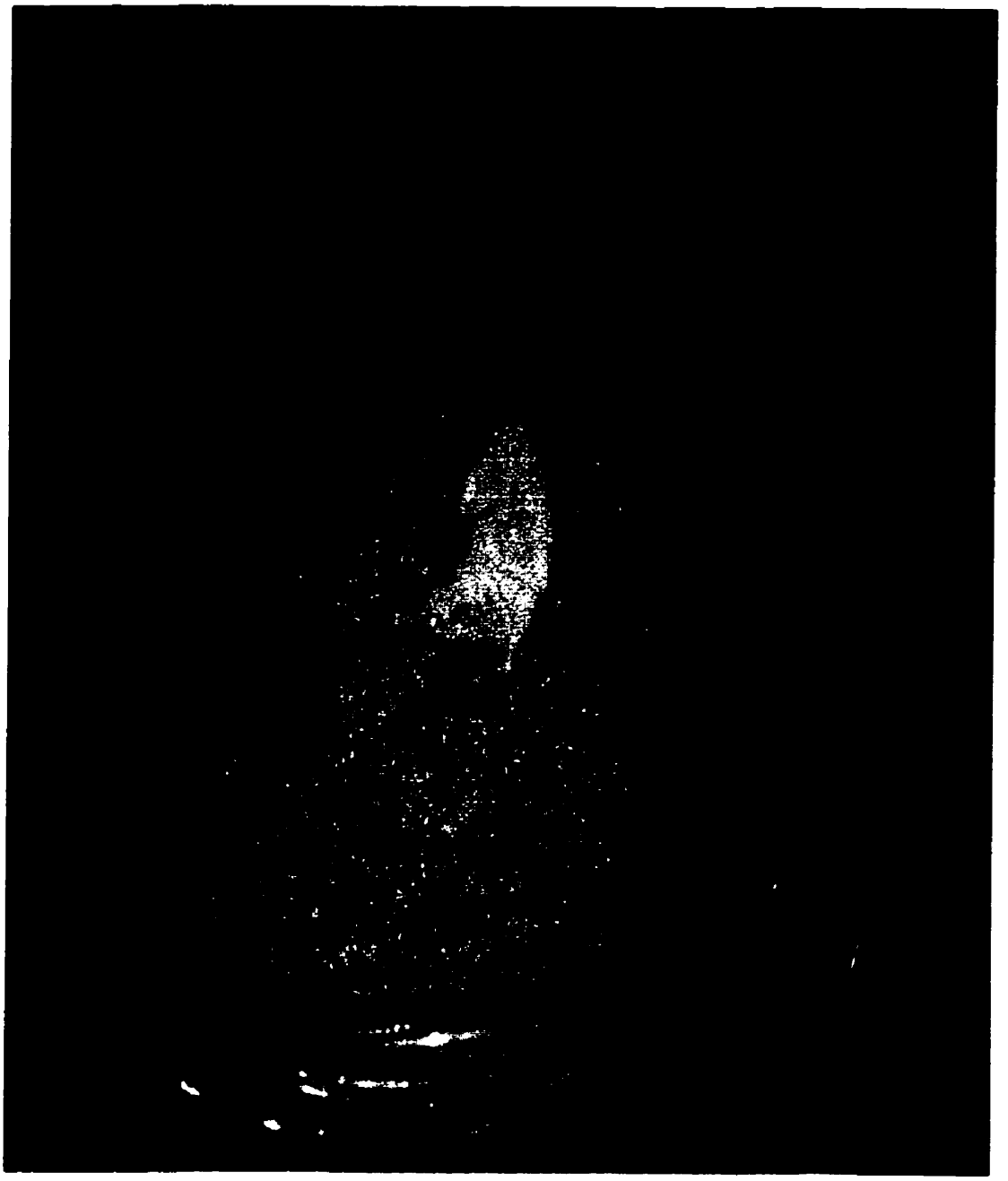


Figure 4. **The effect of neem seed oil on the feeding of rats (*Rattus norvegicus* Berk.), 1996 experiment.**

The lines W neem = with neem, are the treatment associated with the doses.

The lines W O neem = without neem, are the controls associated with each treatment of neem material.

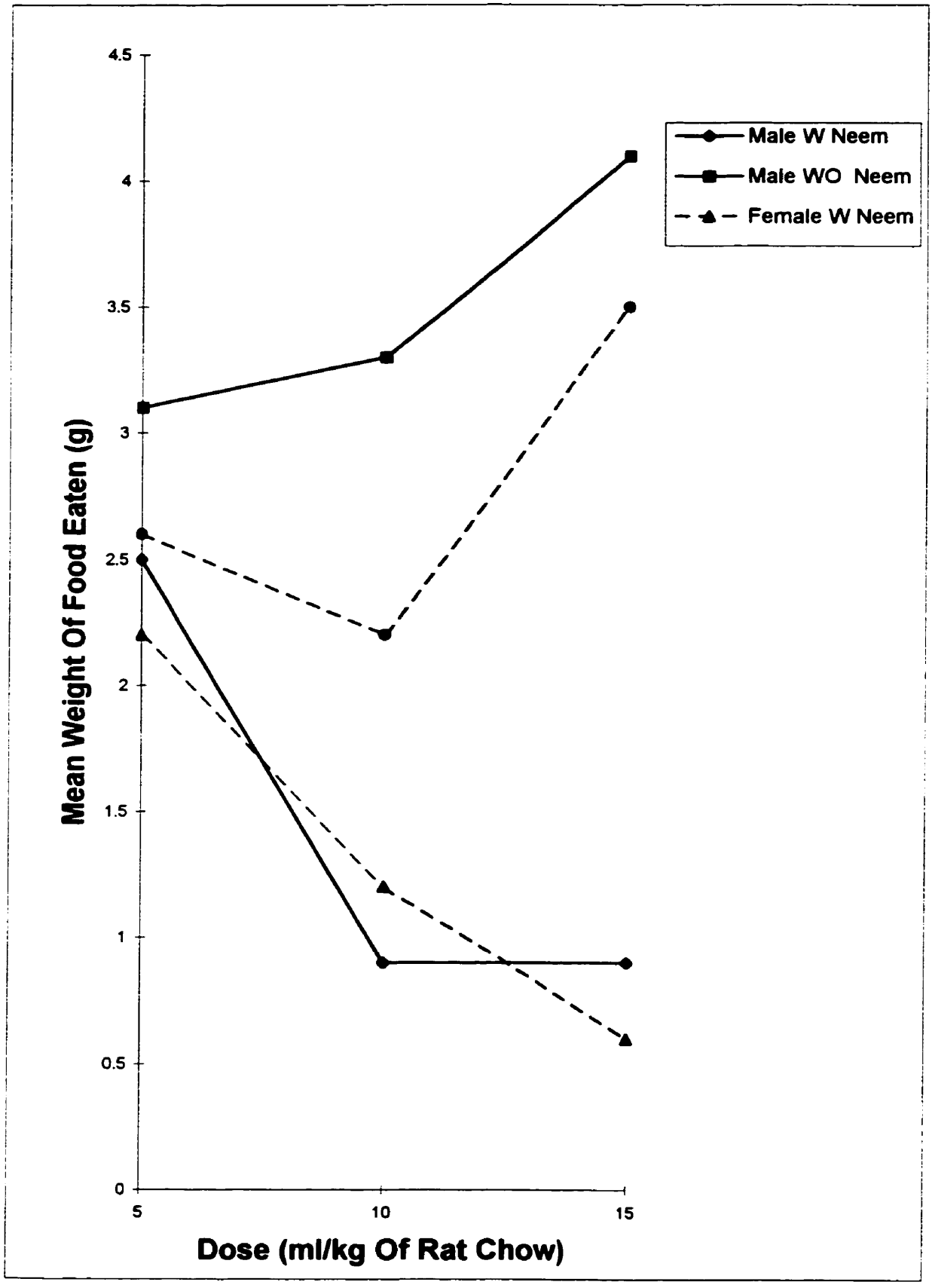

Male W Neem
Male WO Neem
Female W Neem
Mean Weight Of Food Eaten (g)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
5
10
15
Dose (ml/kg Of Rat Chow)

Figure 5. **The effect of neem seed powder on the feeding of rats (*Rattus norvegicus* Berk.), 1996 experiment.**
The lines W neem = with neem are the treatment associated with the doses.
The lines W O neem = without neem, are the controls associated with each treatment of neem material.

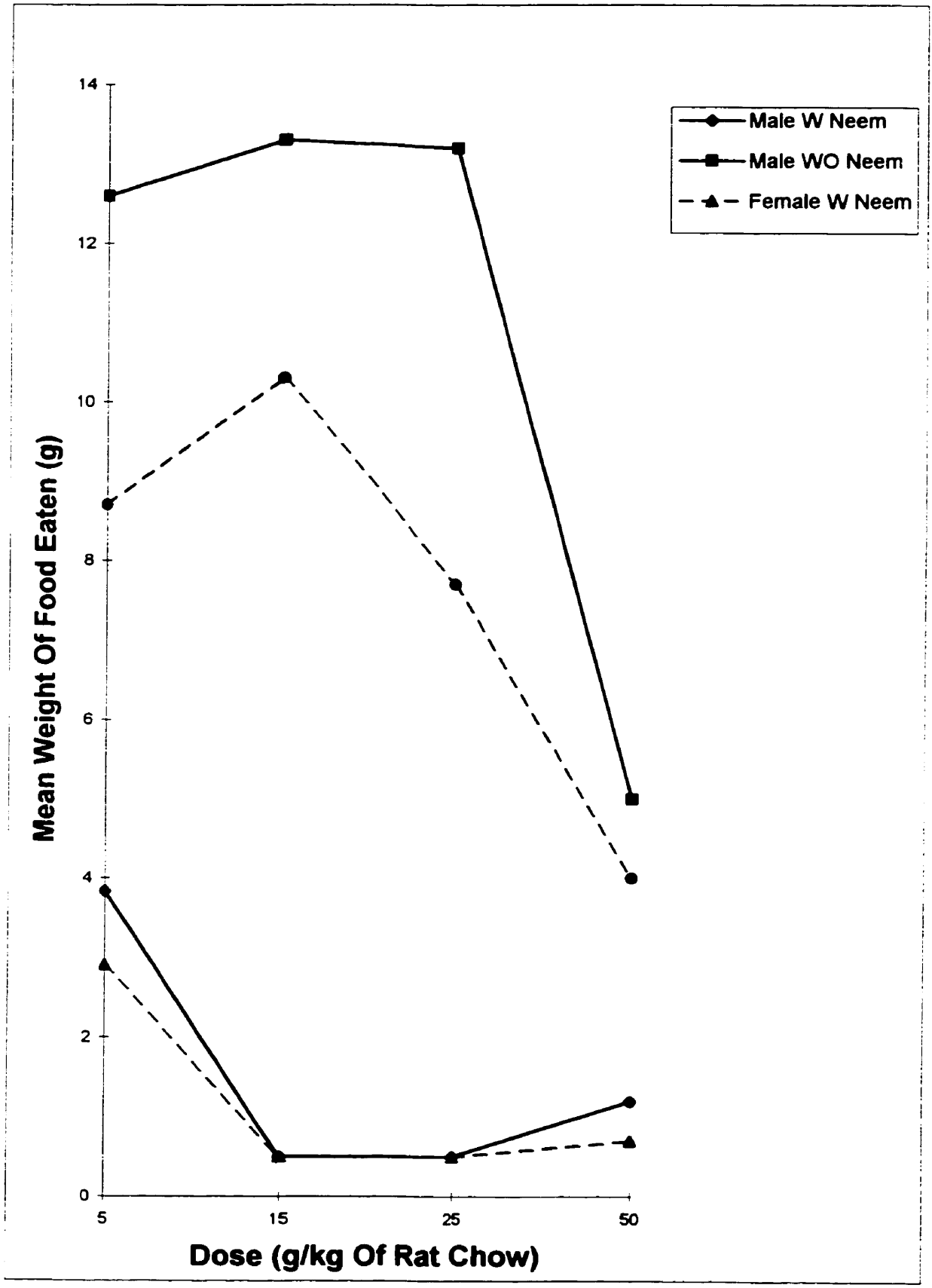

Male W Neem
Male WO Neem
Female W Neem
Mean Weight Of Food Eaten (g)
0
2
4
6
8
10
12
14
5
15
25
50
Dose (g/kg Of Rat Chow)

Figure 6. **The effect of neem seed powder on the feeding of rats (*Rattus norvegicus* Berk.), 1996 experiment.**

The lines W neem = with neem, are the treatments associated with the doses.

The lines W O neem = without neem, are the controls associated with each treatment of neem material.

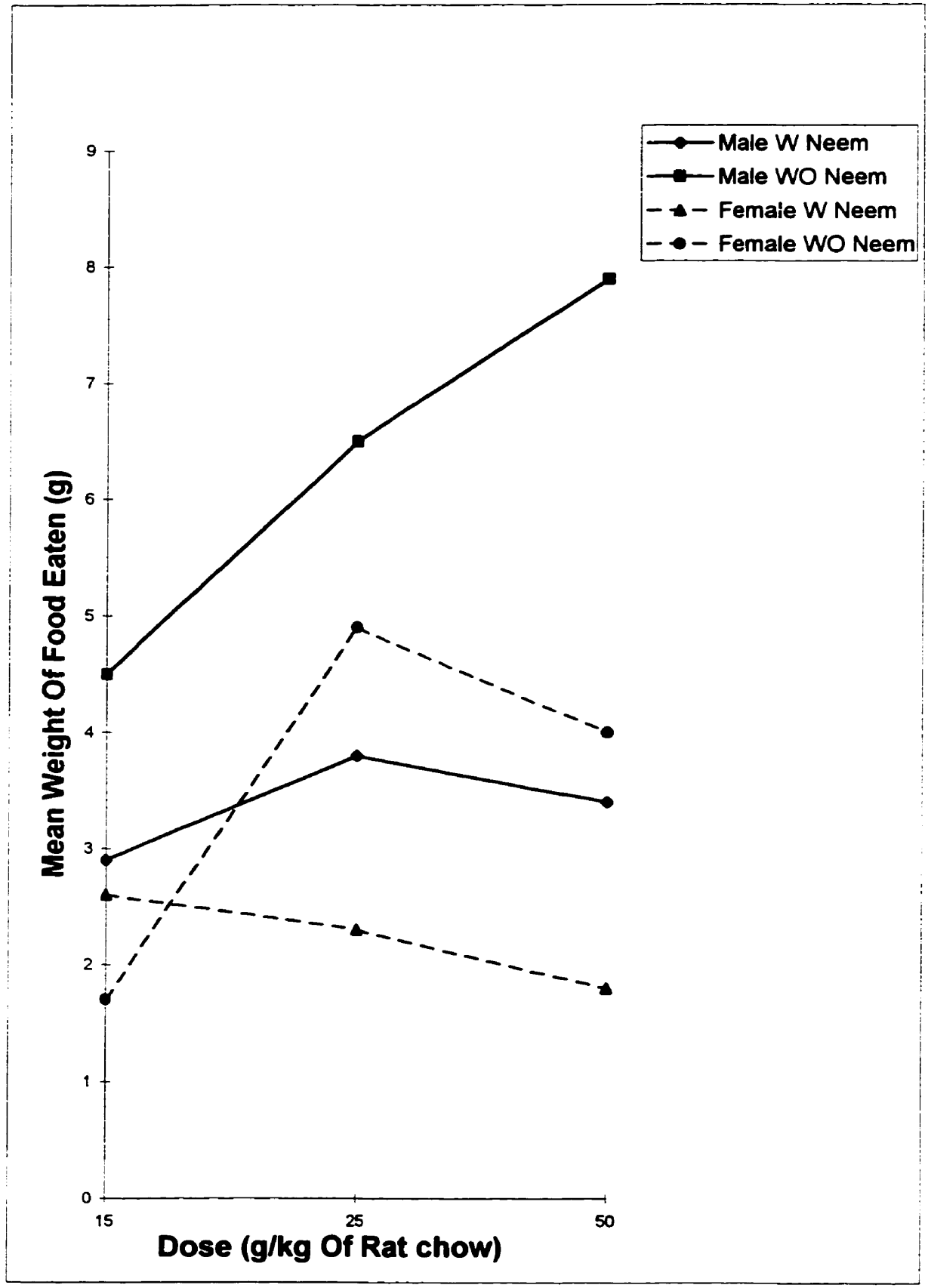

Male W Neem
Male WO Neem
Female W Neem
Female WO Neem
Mean Weight Of Food Eaten (g)
0
1
2
3
4
5
6
7
8
9
15
25
50
Dose (g/kg Of Rat chow)

Figure 7. **The effect of neem seed oil on the feeding of rats (*Rattus norvegicus* Berk.), 1997 experiment.**

The lines W neem = with neem, are the treatments associated with the doses.

The lines W O neem = without neem, are the controls associated with each treatment of neem material.

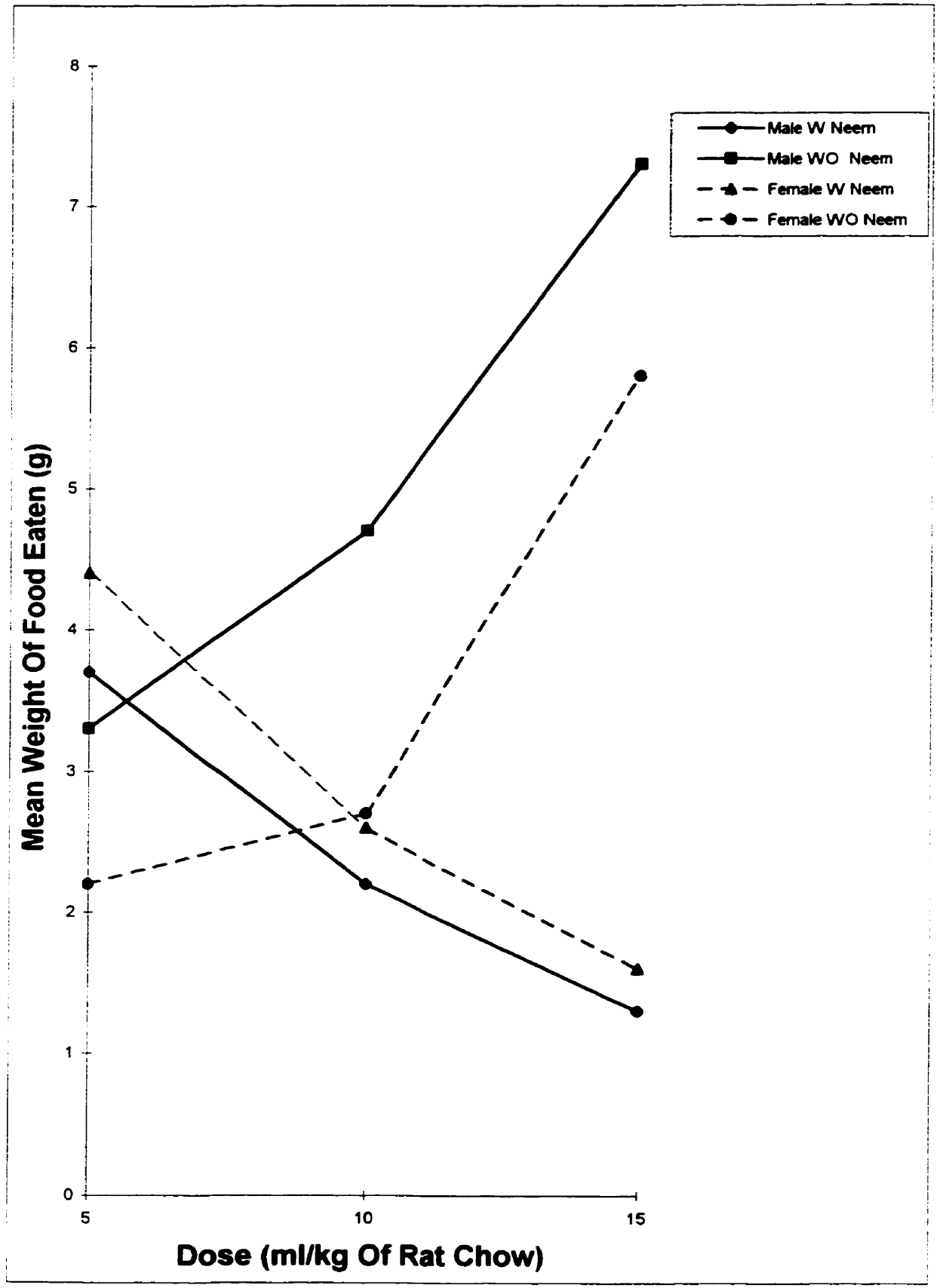

Male W Neem
Male WO Neem
Female W Neem
Female WO Neem
Mean Weight Of Food Eaten (g)
0
1
2
3
4
5
6
7
8
5
10
15
Dose (ml/kg Of Rat Chow)

Figure 8. **The effect of neem seed powder on the feeding of rats (*Rattus norvegicus* Berk.), 1997 experiment.**

The lines W neem = with neem, are the treatments associated with the doses.

The lines W O neem = without neem, are the controls associated with each treatment of neem material.

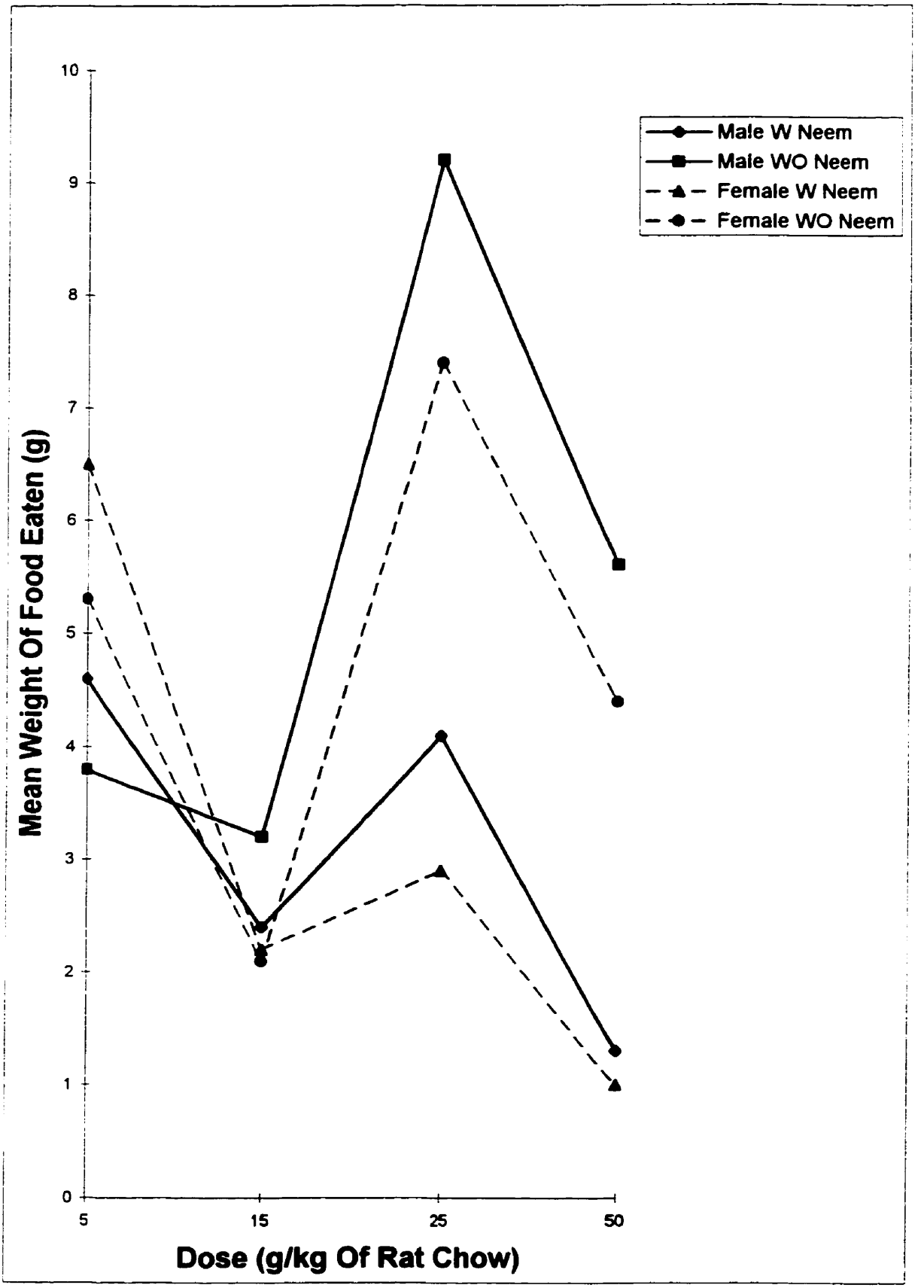
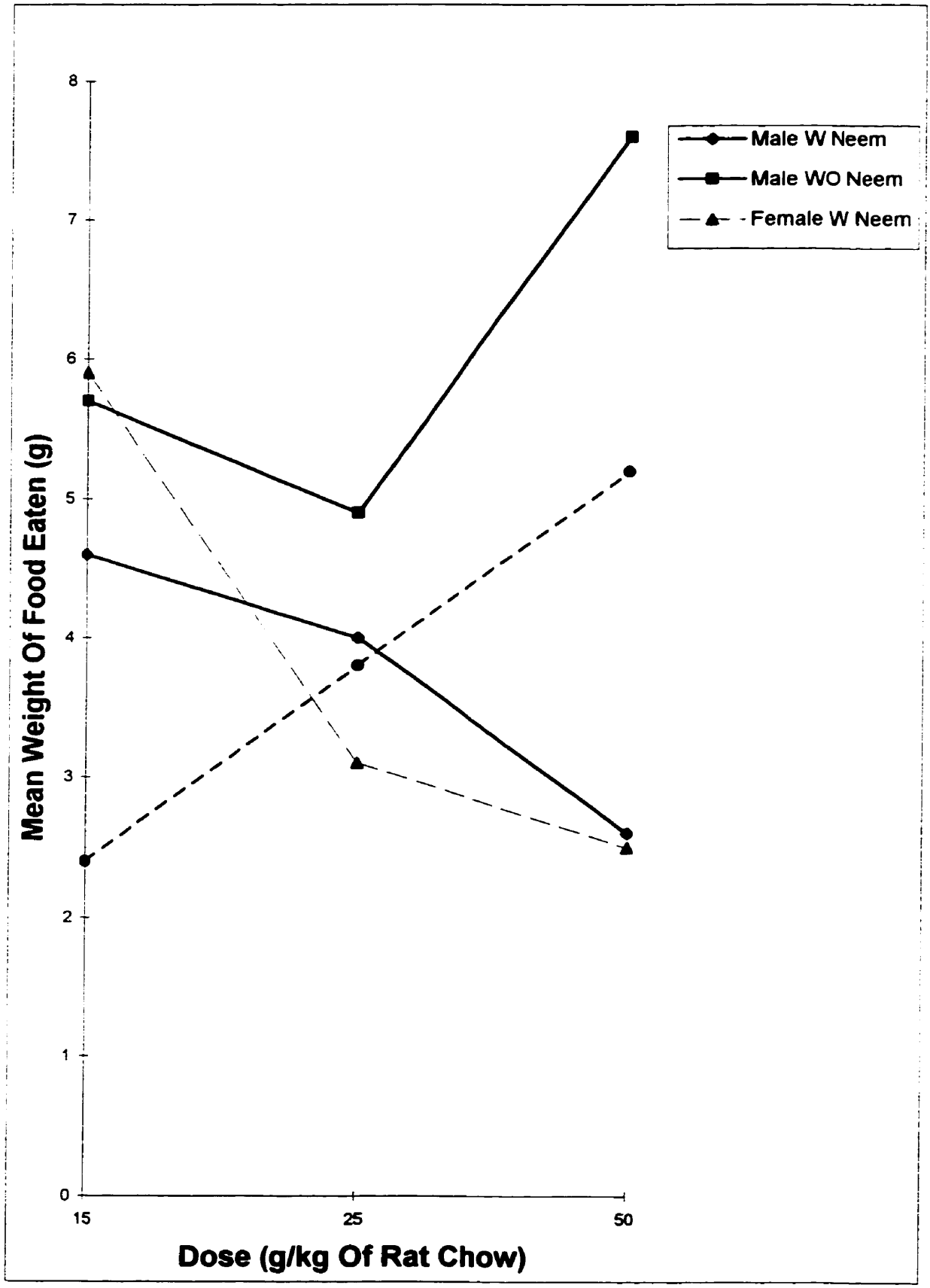

Male W Neem
Male WO Neem
Female W Neem
Female WO Neem
Mean Weight Of Food Eaten (g)
0
1
2
3
4
5
6
7
8
9
10
5
15
25
50
Dose (g/kg Of Rat Chow)

Figure 9. The effect of neem leaf powder on the feeding of rats (*Rattus norvegicus* Berk.) feeding, 1997 experiment. The lines W neem = with neem are the treatments associated with the doses. The lines wo neem = without neem are the controls associated with each treatment of neem material.


Male W Neem
Male WO Neem
Female W Neem
Mean Weight Of Food Eaten (g)
0
1
2
3
4
5
6
7
8
15
25
50
Dose (g/kg Of Rat Chow)

V. DISCUSSION

A. Repellency studies.

a) Feeding test. Not all tests performed were statistically significant; the expected results were not achieved even though male and female were not pooled due to some effects (sex, treatment, or both) revealed by the ANOVA test. But in 1997, the repeated test, with regard to females, the number of visits and total duration of visits were highly significant; females visited the control food more than the treated food. Furthermore, some trends could be observed among the means. For females, the mean number of visits of the control was higher than that of the insect treatment. For males, the same trend was exhibited for the duration of the visits. The number of visits and the duration of visits to the insect treatment were low but not enough to make the other remaining variables (average duration, total food intake, food intake/visit, and food intake/sec) to be statistically significant.

There are several possible causes of the inconsistency of these results. First of all, only a small quantity of the volatiles was released by the two cockroaches in the small jar. Second, as the top of the cages was covered with wire mesh, the volatiles released by the insects flowed out of the jar and diffused in the cages, causing a confounding effect to the rat. Third, the jar was so small that it could not contain more than two insects which could barely move, thus reducing the release of volatiles to such an extent that there was no effect on the rats. During some experiments, the insects stopped moving a few minutes after the beginning of the trial, there were no secretions released (verified by smelling the tubes). This could mean that the 30- minute duration of each test was too long, especially

in a small jar where the insects could not maintain movement which could maintain the continuous flow of volatiles.

It is possible that by using a plastic covered cage (top) with a larger jar and 2 - 4 insects, and reducing the duration of test perhaps to 15 minutes, better and positive results could be achieved.

b) Arena test. In this particular experiment performed in 1996 and 1997, both control tests involving holes with distilled water and holes with cheese did not differ significantly for both sexes (rats were equally attracted to the cheese and to the water). This provided the baseline for the subsequent tests. The experimental tests involving holes with cheese and holes with live insects were highly significant for both sexes. The results of these two sets of (control and experimental) tests are similar to those found by Bouchard et al. (1997), in which case a mixture of the chemical constituents of the insect secretion was used. In Table 7, when 1996 and 1997 data are compared, the same trends are exhibited for control and experimental tests with regards to mean number of visits to holes with distilled water and holes with cheese, and mean number of visits to holes with live insects and holes with cheese. Males seemed more affected by the insects secretions than the females, since in 1996 the mean number of visits to holes with live insects were 13.3 and 13.1 (male), and 24.3 and 23.5 (female) for both experimental tests; similarly in 1997, 13.4 and 12.4 (male), and 23.4 and 23.1 (female) were recorded. These results are also consistent with those of Bouchard et al. (1997).

The choice of 30 minutes for the period of observation could be considered long, especially when volatile compounds are involved. During the experiments with the cheese vs. the live insects, both females and males reduced their mean number of visits to holes

with the live insects in the last 15 minutes of observations for most of the rats; these are similar to the results found by Bouchard et al. (1997). The opposite would have been expected if the insect secretion had evaporated, thus losing its repellent properties. Therefore, I believe that the effect of the insect secretion was retained during the 30 minutes period, especially because the four cockroaches confined in the tube (under stress) were constantly moving, thus releasing repellents. Furthermore, evaporation of volatiles in the holes with live insects (i.e., in contact with air) was reduced by providing only a small vertical hole (2.3 cm diameter) where gas could escape from the tube. The females seemed less affected by the insect secretion, as shown by the higher number of visitations compared to males (Tables 7), but the mean numbers of visits were in the range of 10 - 16 lower compared to visits to the cheese holes. These results were significantly different from those obtained during the control test, showing similar number of visits to the two types of holes (distilled water and cheese). Since there was no significant difference between the number of visits to the distilled water and cheese holes in the control tests, one could conclude that the rats were not especially attracted to either hole. Therefore, when given a choice between cheese and holes with the insect secretion, rats (male and female) visited cheese holes more often. It seems that the rats were repelled by the insect secretion which had elicited some noticeable behavioral responses such as jumping backwards, shaking their heads or cleaning the nose (grooming) when smelling the insect volatiles, which affected also the number of visits. The higher mean number of visits by females in all series of tests might be explained by a difference (gender difference) in physiological state between male and females, for example estrus which could induce the perception of smell (Barnett, 1963). This difference may explain also the greater activity of

females during their investigations in the arena compared to males which spent more time grooming.

Cockroaches in general, have many potential predators, including birds, lizards, frogs, insects (such as ants and praying mantids) and rodents (Roth and Stay, 1958; Eisner et al., 1959; Blum, 1961; Wallbank and Waterhouse, 1970). According to Carlberg (1985a,b, 1986), Bouchard and Hsiung (1996), Bouchard et al. (1997), the use of rats (*R. norvegicus* in this case) as bioassay tools is known to be adequate for this sort of study, as they are known to prey on many insects such as stick insects.

In the experiments of Bouchard et al. (1997), the chemicals identified from the stick insect have been reconstituted with synthetic chemicals in the same ratio they were found by the GC-MS analysis, and the chemical mixture is used as the treatment test, whereas in the present study the chemicals (volatiles) identified from the cockroach (Table 3) were not available for purchase due to their toxicity, which prompted the use of the live insects.

c) Insect defense mechanism. There is a plethora of defense mechanisms in insect defenses against predators (arthropods, birds and mammals). Many insects, such as leaf beetles (*Oreina spp*), are chemically protected by a remarkable diversity of defensive compounds which are either sequestered from plants or synthesized *de novo* (Eggenberger and Rowell-Rahier, 1993; Dobler and Rowell-Rahier, 1994a,b). With regard to defensive secretions, most cockroaches recorded as producing odoriferous secretions belong to two families (Blattidae and Blaberidae) (Wallbank and Waterhouse, 1970). The cockroach *Blaberus giganteus* (Blaberidae), like other insects from the same family, is endowed with abdominal tracheal glands which secrete the chemicals (Blum, 1981). *Diploptera punctata*

Esch. is known to produce p-benzoquinone with their 2-methyl and 2-ethyl homologues (Wallbank and Waterhouse, 1970). The chemicals identified from *B. giganteus* secretions were aromatic hydrocarbons which caused some reactions from the visiting rats, such as drawing the head back, and spending sometime grooming after smelling the potent chemicals. The allergic reactions (swollen eyes and skin rashes) of some colleagues when feeding the cockroaches, suggest that these volatiles could be powerful enough to repel rats especially in storage situation. The repellent effects were consistent with the arena tests involving live cockroach secretions. Another study exhibited toxic sensitivity of few mammals to oral dosages of cardiac glycosides. For example, digitoxin toxicity to mice was demonstrated with *Reithrodonotomys sumichasti* (Saussure) (Chronic LD_{50} > 18.1 mg/kg) and *Peromyscus azectus* (Saussure) (Chronic LD_{50} > 72.2 mg/kg). In these tests intact monarch butterflies were given to the mouse (Glendinning, 1990).

This suggests that there is a need to explore insect defense mechanisms more, to perform additional tests, especially in field situations in order to confirm the results and to study their possible implementation. I recommend that for the feeding test, larger jars, special cages with covered tops and enough rats and cockroaches be obtained in order not to be limited in performing the experiments. The extension of the experimentation to locally available insects could be an important point to consider since the imported species could become pests.

B. Plant Materials (Neem)

a) **Effectiveness of neem oil on rat feeding.** This part of the trial was designed to assess the effects of fresh neem oil acquired from Niger with an azadirachtin content (seeds) of 0.15%, compared to seeds from other countries such as Sudan (0.19%), Togo

(0.4%) and India (0.35%) (Ermel et al. 1986, cited in Yakkundi et al., 1995). These figures of azadirachtin content are subject to change depending on the soil and the neem ecotypes (Kumar and Parmar, 1996). The results obtained for both years (Figures 4 and 7) were consistent, thus, 10 and 15 ml of neem seed oil/kg of rat chow inhibited feeding of male rats whereas 15 ml of neem seed oil/kg of rat chow was inhibitory to female rats. This difference between male and female responsiveness could be explained again by gender difference (Barnett, 1963). Table 8 shows that all the mean differences between control and treatment tests were positive, thus exhibiting a tremendous feeding reduction for males and females as the dose increases. The 5 ml dose of neem seed oil induced both sexes to feed more on tested food than the control in the second year. The feeding reduction in the same table was initiated from 10 ml and increased tremendously at 15 ml of neem seed oil/kg of rat chow. This might be caused by the time period elapsed between the two experiments during which the neem oil compounds might have been reduced. By analyzing the actual data for males and females (means of food with neem oil and means of food without neem oil), Figures 4 and 7 show clearly the feeding reduction as dose increases, and the same figures show that males were affected more than females. According to Ramirez (1993), rats prefer oil suspension, so the animal behaviour would not be affected by the oily rat chow mixture used in this test. Very little work has been done on rodent pest control using plant materials. One possible way to confirm these results would be by conducting the same experiments, but this time with local rats, increasing the sample size (100 rats for example), and with a shorter time of observation.

b) **Effects of neem seed powder on rat feeding.** In this series of experiments, the results exhibited in the 1996 trials were consistent with those found by others (Ketkar,

1976; Schmutterer et al., 1981; Jain, 1983; Tewari, 1992) who found that neem seeds contained the highest amount of azadiractin and other bitter compounds. Thus, the four doses of NSP (50 g, 25 g, 15 g and 5 g/kg of rat chow) strongly reduced the feeding of both male and female rats (Table 9); Figure 5 shows that even though the tests were performed starting with the highest doses (in decreasing order), which exhibited that the highest reduction of feeding was obtained with the following doses (15 g, 25 g, and 5 g) for both sexes. The lowest effect in terms of mean differences was obtained with the highest dose (50 g of neem seed powder/kg of rat chow) that might be explained by the antifeedant property of neem materials. The highest dose (50 g of neem seed powder/kg of rat chow) reduced the feeding of rats (on the food with neem) and also inhibited the rats up taking the control food (without neem), which resulted in a lower mean difference (Table 9). In the second year, only the two highest doses (50 g and 25 g of neem seed powder/kg of rat chow) were inhibitory to male and female rats feeding , whereas 15 g and 5 g/kg rat chow did not affect the feeding of both sexes and at 15 g/kg of rat chow the mean difference was negative, suggesting that the rats ate more food from the test than the control. The dissimilarity of the last two doses (15 g and 5 g) could be explained by the fact that the content of azadirachtin in the neem seeds, since it can be influenced by temperature, humidity and UV light; thus losses of azadirachtin and other bitter compounds could have occurred during the storage of the seeds (1.5 years old) awaiting for the test in 1997 (Yakkundi et al., 1995). According to the same authors neem seeds should be processed as soon as possible, and preferably within 6 months after harvest. For this trial, the analysis of the data (Figure 8) showed a zigzag pattern, from 5 g there was a decrease to 15 g dose, then an increase to 25 g dose, and at last a decrease for 50 g dose.

This unusual pattern might be explained by the lower level of azadirachtin in the lower two doses and the higher level in the two higher doses. In spite of the inconsistency of data in Figures 5 and 8, neem seed powder gave the best results, which are comparable to those of Glendinning (1992) who compared the feeding responses of five species of *Peromyscus* mice (*azectus*, *polionotus* Wagner, *melanotis* J. A. Allen and Chapman, *leucopus* (Rafinesque), and *maniculatus* (Wagner)) to three bitter-tasting cardenolides (ouabain, digoxin, and digitoxin) and demonstrated that two species (*P. azectus* and *R. sumicharsti*) were repelled by the unpleasant taste of cardenolides.

c) **Rats responses to neem leaf powder.** The neem leaf contains the least concentration of azadirachtin and other bitter compounds (Jain, 1983; Tewari, 1992), and the alliaceous odor (organosulfur compounds) of crushed neem leaves diminishes over time (Balandrin et al., 1988). It is not surprising that the results of the two years were inconsistent, due to the time elapsed before each experiment. In the first year, male rats did not respond significantly to 50 g, 25 g, and 15g of neem leaf powder/kg of rat chow doses, in contrast females responded significantly with regard to the two higher doses (50 g and 25 g), but they responded negatively to the lowest dose, thus, the treated food was consumed more than the untreated (control) food (Table 10). Male rats, exhibited a slight increase in feeding from 15 g to 25 g then a decrease at 50 g dose (Fig. 6), but in females the feeding reduction was continuous as the dose increased, and reduced their feeding at the highest dose for the food without neem. Thus, behavior of the rats might be explained by the antifeedant property of neem products. Males feeding on food without neem increased for all doses. In the second year (Fig. 9), at 50 g/kg of rat chow the feeding of both male and female rats was strongly inhibited, and at 25 g/kg of rat chow there were no

significant responses from either sex. Females were highly attracted to the treated food at 15g/kg (Table 10). The reason for this disparity remains obscure and requires additional experimentation. Those experiments could be enhanced by using freshly harvested neem leaves, increasing the sample size (50 males and 50 females), a wider range of doses should be used (6 or more), and that the doses will be assigned at random and with at least 3 days between the trials.

d) **Commercial neem oil.** The results obtained in the trials involving the commercial neem seed oil (from the Dominican Republic), suggest that none of the doses could be recommended as inhibitory to rat feeding (Table 11). This trial was performed only once; other tests are required to confirm the lack of effectiveness of this commercial neem seed oil, which in contrast gave the best results on dermestid beetles (Keeler, 1999). A probable explanation could be the loss of chemical concentration (0.08% of azadirachtin) during the process of extraction; besides this oil is also lacking the organosulfur odor, which is different from the neem seed oil from Niger. It could also be due to the lack of concentration, which might be assessed by performing more trials, using the enhanced protocol.

e) **Plants with biological activity.** Among 1079 plants described in Prakash and Rao (1997) 20 of them were reported to be biologically active against rodents. These plant extracts (leaf, seed, and oil) generally possessed repellency and also showed toxicity to rats and squirrels, and sterilant activity was reported in a few cases. Most of the research on rodents is in the medical fields. According to many authors, neem seeds contain the highest concentrations of azadirachtin, other bitter compounds and organosulfur compounds. Therefore, neem seed powder, neem oil, and neem cake would be the best

materials to use in repelling vertebrate pests. The list of plants with potential pest control activity could be long, as many plants exist in tropical regions such as Niger for example, where about 25 plants have long been used in traditional insect pest management. The results obtained with the neem materials were consistent with those of other studies involving plants possessing similar compounds such as alkloids, flavonoids, steroids, terpenoids and tannins. Ahn et al. (1995) demonstrated that crude oil derived from *Thujopsis dolabrata* S. et Z. var. ***hondai*** sawdust strongly repelled rodents, but the potent activity of its terpenoids (carvacrol, thujopsene, and *B*-thujaplicine) is mostly effective only for a few days, due to their high volatility. Harding (1985) cited in Ahn et al. (1995), reported that thujone oil of *Artemesia absinthium* L. strongly repelled rodents. Many plants from which insects sequester cardenolides (Dobler and Rowell-Rahier, 1994b), pyrrolizidine alkaloids (Nickisch-Rosenegk and Wink, 1993) or aristolochic acids (Nishida et al., 1993) for their own protection, could have potentials for pest control. Capsaicin, a chemical from hot peppers sauce (6.2%) was found to be one of the most effective repellents against captive mule deer (Andelt et al., 1994).

VI. CONCLUSIONS

A. Management Implications.

In summary, the results of the experiments involving the feeding tests with the live cockroaches were negative; among six variables for females, only two (mean number of visits and mean total duration) were significantly different with regard to control food and food with live insects. Results of the arena tests were positive, but should be repeated with an enhanced protocol (use of fresh plant materials, bigger sample size, wider range of doses and a random assignment of the different doses). For the neem tree materials, several doses can be used to repel rats: 15 ml of neem seed oil/kg of rat chow, 25 - 50 g of neem seed powder/kg of rat chow, or 50 g of neem leaf powder/kg of rat chow. Most rat damage occurs in storage situations where the neem material would be more compatible. Depending on the commodities, neem seed oil is the most suitable for use against rodents in food grain storage; the sacks could be sprayed with certain quantity of the oil before or after filling them. The use of double bags will reduce the possibility of altering the food quality and the grain (seeds) could be mixed directly with the neem oil which could ensure a triple protection against rodents, insects, and diseases during storage. After sowing, the treated seeds may retain this triple protection, but this time against field rats, squirrels, and soil inhabiting insects. As the neem seeds contain more chemicals than other parts of the tree (Tewari, 1992), the neem cake (residue after extraction of the oil) contains a considerable concentration of bitter compounds could also be used in certain situations such as in transplanted rice seedlings; the neem cake thrown over the rice seedlings could reduce water birds feeding on newly transplanted rice.

There may be possibilities in using insect defensive secretions based on the study of Rollo et al. (1995), who found that dead cockroaches (*Periplaneta americana*) repelled their conspecifics, and that full repellency was obtained above a dosage of 1.6 cockroach equivalents per shelter (arena). As cockroaches can be easily reared in tropical areas (Guthrie and Tindall, 1968), they may be used in a trial against rats. The theory of "bug juice" in insect pest management suggest that it could be possible to test the repellency of dead cockroaches in storage situations, where freshly killed roaches could be crushed and thrown on plastic bags containing grains or food. But volatile compounds identified in the insect secretion, known as carcinogenic, should be manipulated with caution and tested many times for this matter before using them on food grains.

The repellents (neem tree materials) tested in the present study might also have applications as additives to packaging, plastics, and fabrics where rodent damage is undesirable. These compounds (triterpenoids of neem) might be used as pest control agents in limited spaces, such as storage bins, greenhouses, or buildings, because of their high volatility (Ahn et al., 1995). Neem seed oil could be enhanced for use in rodent pest management in a way that the volatility of its compounds will be reduced; it could be incorporated in paint for walls, floors, and wooden supports for grain bags. According to Harding (1985) and Ahn et al. (1995) it could be interesting to use an appropriate carrier for slow release in the air. For example, a mixture of thujone oil and lacquer (12 : 88), used like paint, has been reported to keep rodents away from the painted area for three to five years (Harding, 1985). The key question is whether insect and plant substances will be effective under field conditions to reproduce the results observed in the laboratory.

B. Public Concerns.

In a few developed countries, concerns for animal welfare, 'humane' treatment and animal 'rights' have extended to rats and the means used to control them (Jackson 1981, cited in Elias, 1988). According to Liss (1995, cited in Anonymous, 1997), Americans have long been in favor of humane treatment of animals, and acceptable wildlife management practices have been dictated by the public. Thus, the quest for benign, environmentally sensitive, yet effective means of vertebrate pest management, has led to an increased interest in the use of nonlethal repellents. Repellents represent an area of much promise, in that many of them have been successfully used against vertebrate pests and are accepted by the public. Cinnamamide (Gill et al., 1994), methyl anthranilate (more effective for birds) (Avery et al., 1995; Liss, 1995), and capsaicin (more effective against mammals) (Mason et al., 1991; Andelt et al., 1994) are chemical repellents, whereas, garlic and garlic oil were found to be great bird repellents (Mason and Linz, 1997), similar to the studies of Mason and Matthew (1996), in which neem has been found to be a promising bird repellent. The organosulfur compounds present in garlic and onion are volatile and cause the repellency. In many mammals, chemosensory detection is an important aspect of predator avoidance, and recent studies have stressed the potential of predator scents as natural repellents. Studies have demonstrated that mountain beaver (*Aplodontia rufa* Nigra) was repelled by secretion from anal glands of minks (*Mustela vison* (Brisson)), and urine from minks, bobcats (*Felis rufus* (Schreber)), and coyotes (*Canis latrans* Ochropus) (Epple et al., 1993). The effectiveness of bobcat urine is probably due to the presence of volatile compounds such as phenol, indole, valerolactam , and palmitic acid identified by Mattina et al. (1991). It is important to point out that there are few studies from an

entomological viewpoint done on neem materials, suggesting that neem materials are environmentally safe for non target species (Spollen and Isman, 1996) and possess insect repellent (Balandrin et al., 1988), antifeedant, and growth-regulating (Govindachari et al., 1996) activities. Scientists investigating on plant extracts have found neem materials to have fungicidal effects on some soil-borne pathogens such as *Fusarium oxysporum* f.sp *ciceri*, *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Sclerotinia sclerotiorum* (Singh et al., 1980), and induce resistance in peas (*Pisum sativum*) against powdery mildew (*Erysiphe psi* DC.) (Singh and Prithiviraj, 1997).

In a study involving side-effects of neem products on insect pathogens and natural enemies of spider mites and insects, Schmutterer (1997) reported that *Bacillus thuringiensis var. israelensis* (Teknar) combined with neem products increased mosquito *Aedes togoi* larval and pupal mortality to the extent that a synergistic effect was apparent. Neem-based pesticides seem to be an ideal candidate for effective and safe pest control. Their effects on many beneficial arthropods, microorganisms, and mammals (low toxicity) appear moderate to benign, suggesting that the use of neem in IPM programs for insects, vertebrate pests and plant pathogens could be effective. In nature, plants, insects and animals possessing chemical defense mechanisms make use of their systems against predators. In this study insect (cockroach) and plant (neem) defense mechanisms were exploited for possible uses in crop protection against rodents.

In conclusion, *Blaberus giganteus* defensive volatiles, including (benzo[a]pyrene (3,4-benzpyren), 7H-1-indeno[2,1-a]anthracen-7-one, 1,2:3,4-dibenzoanthracene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene and benzo[ghi]perylene) showed potential as repellent against *Rattus norvegicus*. Neem tree materials (neem seed oil 15 ml/kg of rat

chow, neem seed powder 15 – 50 g/kg and neem leaf powder 25 – 50 g/kg), have shown much potential as repellents against the same species. These compounds, especially the neem materials, should not pose any hazard to wildlife or the environment. Studies of substances from insects such as cockroaches, grasshoppers, stink bugs, etc., and neem extracts as repellents against rodents are warranted. The prevention of losses due to these pests would represent an important contribution to the reduction of damage to food crops in the developing world.

C. Future Research.

The present results provide rationale for implementing this technology in Africa as a part of an integrated rodent control strategy. Biological control using insect defense mechanisms could be considered impractical due to the perceived cost of the process. The use of the neem tree could be more practical in Third World countries, especially where the tree grows, and because its products can be used effectively to reduce crop losses and to generate additional income for rural farmers. Furthermore, the production of neem pest-control materials at the village level would make rural communities less dependent on very expensive imported pesticides, the supply of which is often discontinuous. The integration of this technique to an overall pest management program requires additional research to learn the benefit of the practices in terms of the impact of the pest, and the constraints to implementation. Follow-up studies are needed to validate, under field conditions, the pest-control effectiveness of the neem materials (neem seed oil, neem seed powder, neem leaf powder, and neem cake), to determine the minimum effective concentrations, the duration of repellency, cost-effectiveness of each product, and to identify the socio-economic conditions which will favor the establishment of neem oil and cake producing facilities.

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APPENDIX 1. Analysis of variance for total duration - Type III sums of squares

(feeding test involving *Blaberus giganteus* chemical secretions in 1996)

Source	Sum of Squares	Df	Mean square	F-Ratio	P-Value
Main Effects					
A: sex	428074.0	1	428074.0	4.59	0.0469 *
B: treat	30264.2	1	30264.2	0.32	0.5764
Residual	1.58545E6	17	93262.0		
Total (corrected)	2.04379E6	19			

All F- ratios are based on the residual mean square error.

* Indicates P<0.05 or at (95.0% confidence level).

APPENDIX 2. Analysis of variance for total food intake - Type III sums of squares

(feeding test involving *Blaberus giganteus* chemical secretions in 1996)

Source	Sum of Squares	Df	Mean square	F-Ratio	P-Value
Main Effects					
A: sex	4.7024E7	1	4.7024E7	15.59	0.0010*
B: treat	1.07185E6	1	1.07185E6	0.36	0.5590
Residual	5.12886E7	17	3.01698E6		
Total (corrected)	9.93929E7	19			

All F- ratios are based on the residual mean square error.

* Indicates P<0.05 or at (95.0% confidence level).

APPENDIX 3. Analysis of variance for number of visits - Type III sums of squares

(feeding test involving *Blaberus giganteus* chemical secretions in 1997)

Source	Sum of Squares	Df	Mean square	F-Ratio	P-Value
Main Effects					
A: sex	235.294	1	235.294	27.46	0.0001*
B: treat	30.4941	1	30.4941	3.56	0.0775
Residual	137.106	16	8.56912		
Total (corrected)	413.158	18			

All F- ratios are based on the residual mean square error.

* Indicates P<0.05 or at (95.0% confidence level).

APPENDIX 4. Analysis of variance for total duration - Type III sums of squares

(feeding test involving *Blaberus giganteus* chemical secretions in 1997)

Source	Sum of Squares	Df	Mean square	F-Ratio	P-Value
Main Effects					
A: sex	745280.0	1	745280.0	14.46	0.0016*
B: treat	17415.8	1	17415.8	0.34	0.5691
Residual	824392.0	16	51524.5		
Total (corrected)	1.60215E6	18			

All F- ratios are based on the residual mean square error.

* Indicates P<0.05 or at (95.0% confidence level).

APPENDIX 5. **Analysis of variance for total food intake - Type III sums of squares**

(feeding test involving *Blaberus giganteus* chemical secretions in 1997)

Source	Sum of Squares	Df	Mean square	F-Ratio	P-Value
Main Effects					
A: sex	665105.0	1	665105.0	0.26	0.6139
B: treat	3.18414E7	1	3.18414E7	12.68	0.0026*
Residual	4.01937E7	16	2.5121E6		
Total (corrected)	7.33137E7	18			

All F- ratios are based on the residual mean square error.

* Indicates P<0.05 or at (95.0% confidence level).

APPENDIX 6. Analysis of variance for food intake/visits - Type III sums of squares (feeding test involving *Blaberus giganteus* chemical secretions in 1997)

Source	Sum of Squares	Df	Mean square	F-Ratio	P-Value
Main Effects					
A: sex	405685.0	1	405685.0	8.99	0.0085*
B: treat	373919.0	1	373919.0	8.28	0.0109*
Residual	722250.0	16	45140.6		
Total (corrected)	1.54768E6	18			

All F- ratios are based on the residual mean square error.

* Indicates P<0.05 or at (95.0% confidence level).

APPENDIX 7. Analysis of variance for food consumed - Type III sums of squares

(feeding test involving neem seed oil in 1996)

Source	Sum of Squares	Df	Mean square	F-Ratio	P-Value
Main Effects					
A: level	12.2645	2	6.13227	2.51	0.0856
B: sex	6.37238	1	6.37238	2.61	0.1090
C: treat	93.0881	1	93.0881	38.12	0.0000*
Residual	280.807	115	2.4418		
Total (corrected)	386.375	119			

All F- ratios are based on the residual mean square error.

* Indicates P<0.05 or at (95.0% confidence level).

APPENDIX 8. Analysis of variance for food consumed - Type III sums of squares

(feeding test involving neem seed powder in 1996)

Source	Sum of Squares	Df	Mean square	F-Ratio	P-Value
Main Effects					
A: level	406.627	3	135.542	13.65	0.0000*
B: sex	2572.33	1	2572.33	259.02	0.0000*
C: treat	136.567	1	136.567	13.75	0.0003*
Residual	1529.4	154	9.93115		
Total (corrected)	4644.93	159			

All F- ratios are based on the residual mean square error.

* Indicates P<0.05 or at (95.0% confidence level).

APPENDIX 9. Analysis of variance for food consumed - Type III sums of squares

(feeding test involving neem leaf powder in 1996)

Source	Sum of Squares	Df	Mean square	F-Ratio	P-Value
Main Effects					
A: level	57.2035	2	28.6017	3.81	0.0251*
B: sex	121.435	1	121.435	16.16	0.0001*
C: treat	146.224	1	146.224	19.46	0.0000*
Residual	856.51	114	7.51325		
Total (corrected)	1176.94	118			

All F- ratios are based on the residual mean square error.

* Indicates P<0.05 or at (95.0% confidence level).

APPENDIX 10. Statistical analyses for neem seed oil tests on the feeding of rats (*Rattus norvegicus* Berk.), 1996 experiments (t-test for paired samples; for the variable 'difference')

Dose (ml)	5	10	15
Mean			
Male	0.6	2.4	3.2
Female	0.4	0.9	2.8
Std Dev			
Male	3.7	2.8	2.1
Female	3.0	1.6	1.5
P-value			
Male	0.641	0.024 *	0.001 * *
Female	0.666	0.087	0.000 * *

* indicates that the difference between the control and the treatment is significant at P <0.05.

* * indicates that the difference between the control and the treatment is significant at P≤0.001.

APPENDIX 11. Statistical analyses for neem seed powder tests on the feeding of rats (*Rattus* nove*gicus* Berk.), 1996 experiments (t-test for paired samples; for the variable 'difference')

Dose (g)	5	15	25	50
Mean				
Male	8.8	12.8	2.7	3.8
Female	5.7	9.7	7.2	3.3
Std Dev				
Male	8.0	3.9	2.6	2.7
Female	6.0	4.2	2.3	2.1
P-value				
Male	0.007 *	0.000 * *	0.000 * *	0.001 * *
Female	0.014 *	0.000 * *	0.000 * *	0.001 * *

* indicates that the difference between the control and the treatment are significant at P <0.05.

* * indicates that the difference between the control and the treatment is significant at P≤0.001.

APPENDIX 12. Statistical analyses for neem leaf powder tests on the feeding of rats (*Rattus norvegicus* Berk.), 1996 experiments (t-test for paired samples; for the variable 'difference')

Dose (g)	15	25	50
Mean			
Male	1.6	2.7	4.5
Female	-0.8	2.6	2.2
Std Dev			
Male	3.5	6.6	6.7
Female	2.8	3.4	1.8
P-value			
Male	0.183	0.230	0.061
Female	0.363	0.038 *	0.004 *

* indicates that the difference between the control and the treatment are significant at P <0.05.

APPENDIX 13. Analysis of variance for food consumed - Type III sums of squares

(feeding test involving neem seed powder in 1997)

Source	Sum of Squares	Df	Mean square	F-Ratio	P-Value
Main Effects					
A: level	7.92075	2	3.96037	0.70	0.5000
B: treat	47.2382	1	47.238	8.32	0.0047*
C: sex	39.1592	1	39.1592	6.90	0.0098*
Residual	653.089	115	5.67904		
Total (corrected)	747.407	119			

All F- ratios are based on the residual mean square error.

* Indicates P<0.05 or at (95.0% confidence level).

APPENDIX 14. **Analysis of variance for food consumed - Type III sums of squares**

(feeding test involving neem leaf powder in 1997)

Source	Sum of Squares	Df	Mean square	F-Ratio	P-Value
Main Effects					
A: level	7.92075	2	3.96037	0.70	0.5000
B: sex	39.1592	1	39.1592	6.90	0.0098*
C: treat	47.2382	1	47.2382	8.32	0.0047*
Residual	653.089	115	5.67904		
Total (corrected)	747.407	119			

All F- ratios are based on the residual mean square error.

* Indicates P<0.05 or at (95.0% confidence level).

APPENDIX 15. Statistical analyses for neem seed oil tests on the feeding of rats (*Rattus norvegicus* Berk.), 1997 experiments (t-test for paired samples; for the variable 'difference')

Dose (ml)	5	10	15
Mean			
Male	-0.4	2.5	5.5
Female	-2.1	0.1	3.8
Std Dev			
Male	2.3	3.5	2.5
Female	2.3	1.9	1.9
P-value			
Male	0.6199	0.0489 *	0.0001 * *
Female	0.0163 *	0.8483	0.0001 * *

* indicates that the difference between the control and the treatment are significant at $P < 0.05$.

* * indicates that the difference between the control and the treatment is significant at $P \leq 0.001$.

APPENDIX 16. Statistical analyses for neem seed powder tests on the feeding of rats (*Rattus norvegicus* Berk.), 1997 experiments (t-test for paired samples; for the variable 'difference')

Dose (g)	5	15	25	50
Mean				
Male	-2.7	0.8	5.1	4.2
Female	0.7	-0.1	4.5	3.4
Std Dev				
Male	6.9	4.2	4.4	3.9
Female	5.4	1.6	4.4	1.7
P-value				
Male	0.2439	0.5382	0.0054 *	0.0070 *
Female	0.6867	0.8342	0.0104 *	0.0001 * *

* indicates that the difference between the control and the treatment are significant at $P < 0.05$.

* * indicates that the difference between the control and the treatment is significant at $P \leq 0.001$.

APPENDIX 17. Statistical analyses for neem leaf powder tests on the feeding of rats (*Rattus norvegicus* Berk.), 1997 experiment

(t-test for paired samples; for the variable 'difference')

Dose (g)	15	25	50
Mean			
Male	1.1	0.8	5.0
Female	-2.9	0.7	2.7
Std Dev			
Male	6.4	3.3	3.6
Female	2.9	2.7	2.3
P-value			
Male	0.5987	0.4428	0.0017 *
Female	0.0111 *	0.4067	0.0047 *

* indicates that the difference between the control and the treatment are significant at P <0.05.