# ACTIVITY AND BIOLOGICAL EFFECTS OF NEEM PRODUCTS AGAINST ARTHROPODS OF MEDICAL AND VETERINARY IMPORTANCE

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ABSTRACT. Botanical insecticides are relatively safe and degradable, and are readily available sources of biopesticides. The most prominent phytochemical pesticides in recent years are those derived from neem trees, which have been studied extensively in the fields of entomology and phytochemistry, and have uses for medicinal and cosmetic purposes. The neem products have been obtained from several species of neem trees in the family Meliaceae. Six species in this family have been the subject of botanical pesticide research. They are *Azadirachta indica* A. Juss, *Azadirachta excelsa* Jack, *Azadirachta siamens* Valeton, *Melia azedarach* L., *Melia toosendan* Sieb. and Zucc., and *Melia volkensii* Gürke. The Meliaceae, especially *A. indica* (Indian neem tree), contains at least 35 biologically active principles. Azadirachtin is the predominant insecticidal active ingredient in the seed, leaves, and other parts of the neem tree. Azadirachtin and other compounds in neem products exhibit various modes of action against insects such as antifeedancy, growth regulation, fecundity suppression and sterilization, oviposition repellency or attractancy, changes in biological fitness, and blocking development of vector-borne pathogens. Some of these bioactivity parameters of neem products have been investigated at least in some species of insects of medical and veterinary importance, such as mosquitoes, flies, triatomines, cockroaches, fleas, lice, and others. Here we review, synthesize, and analyze published information on the activity, modes of action, and other biological effects of neem products against arthropods of medical and veterinary importance. The amount of information on the activity, use, and application of neem products for the control of disease vectors and human and animal pests is limited. Additional research is needed to determine the potential usefulness of neem products in vector control programs.

KEY WORDS  Meliaceae, neem, azadirachtin, insecticides, mosquitoes, flies, triatomines, cockroaches, fleas, lice, pest control

## INTRODUCTION

The extensive and widespread use of synthetic insecticides during the past half century, since the discovery of DDT during World War II, for the control of household, agricultural, and sylvan pests, as well as human disease vectors has caused some concerns regarding the toxicity and environmental impact of some of these agents. Some inherent features and use patterns of the conventional synthetic insecticides that lead to these concerns are toxicity to mammals including livestock, fish, birds, and beneficial organisms; human poisoning, especially in Third World countries; adverse effects on the environment, causing contamination of soil, water, and air; resurgence of insect pest populations because of the emergence and widespread occurrence of physiologic resistance to conventional insecticides; the high cost of the development of new synthetic insecticides, which puts the new agents out of the reach of pest control programs in Third World countries; and not meeting the modern criteria of use in integrated pest management programs.

Because of these problems and concerns, the search for new, environmentally safe, target-specific insecticides is being conducted all over the world. To find new modes of action and to develop active agents based on natural plant products, efforts are being made to isolate, screen, and develop phytochemicals possessing pesticidal activity. These categories of pesticides are now known as biopesticides (Mulla 1997). Interest in botanical insecticides started in the early 1930s and continued to the 1950s (Campbell et al. 1933; Jacobson 1958), but the interest in their development and use was phased out when synthetic insecticides appeared on the scene. Interest in the development of natural products has been revived during the last 2 decades. Thus far about 10,000 secondary metabolites or phytochemicals have been isolated, but their total number is estimated to be much higher, close to 500,000 (Ascher 1993). More than 2,000 plant species reportedly possess chemicals with pest control properties (Ahmed et al. 1984), and among these about 344 species of plants have been shown to have some degree of activity against mosquitoes (Sukumar et al. 1991). The most prominent phytochemical pesticides studied in recent years are those based on the neem products, which have been researched extensively for their phytochemistry and exploitation in pest control programs. This paper presents a concise review of recent advances in research on the activity, efficacy, and potential uses of neem products for the control of arthropods of medical and veterinary importance.

## OVERVIEW OF NEEM PRODUCTS

### Neem tree

Six species in the family Meliaceae have been studied for pesticidal properties in different parts of the world. They are *Azadirachta indica* A. Juss

(syn. *Melia azadirachta, Antelaea azadirachta*), commonly known as the neem tree, Margosa tree, or Indian lilac; *Azadirachta excelsa* Jack (syn. *A. integrifoliola*), called the Philippine neem tree or marrango; *Azadirachta siamens* Valeton, or the Siamese neem tree or Thai neem tree; *Melia azedarach* L., commonly known as Persian lilac, chinaberry, Australian bead tree, Chinese umbrella tree, or pride of India; *Melia toosendan* Sieb. and Zucc.; and *Melia volkensii* Gürke. Varieties of these named species occur, some of which have been assigned species status in some countries. Generally, the neem tree is referred to *A. indica*. However, herewith we use neem products for all of the bioactive components originating from this group of plants in the family Meliaceae. Plants in the family Meliaceae, especially *A. indica*, contain at least 35 biologically active principles (Rao and Parmar 1984) and these products have many industrial, medicinal, and pesticidal uses. Various components of the tree have potential uses in toiletries, pharmaceuticals, the manufacture of agricultural implements and furniture, cattle and poultry feeds, nitrification of soils for various agricultural crops, and pest control (Koul et al. 1990).

## Active ingredients

A number of bioactive components have been isolated from various parts of the neem tree. These chemical compounds have different designations, among which azadirachtin (AZ) ($C_{35}H_{44}O_{16}$) is the major component. The insecticidal properties of products from the neem tree were first reported by Chopra (1928). Forty years later, AZ, a steroidlike tetranotriterpenoid (limonoid), was isolated by Butterworth and Morgan (1968) from *A. indica*. However, it was not until 1987 that the chemical structure of AZ was completely elucidated by Kraus et al. (1987). Azadirachtin is the predominant insecticidal active ingredient in the seeds of the neem tree. The AZ content in neem oil was highly correlated with its bioactivity against test insects (Isman et al. 1990). A marked difference has been reported in the yield of AZ from neem seeds from different geographical origins (Schmutterer and Zebitz 1984, Chirathamjaree et al. 1997), or even in different seasons in the same geographical area (Sidhu and Behl 1996). Azadirachtin analogs, depending on the minor variations in chemical structure, were given different designations. Azadirachtin-A is the major component of the total AZ (Rembold 1989). Azadirachtin-B (3-tigloylazadirachtol) is present at concentrations of up to 15% of the total AZ. Other AZ analogues such as AZ-C through AZ-G occur at much lower concentrations. Recently, 2 new tetranortriterpenoids, 11-epi-azadirachtin H (Ramji et al. 1996) and AZ-K (Govindachari et al. 1992), have been isolated from neem seeds. In addition to *A. indica*, other plants in the family Meliaceae, such as *M. azedarach*, also

have been reported to contain AZ (Morgan and Thornton 1973). The detailed chemistry of AZ can be found in Ley et al. (1993).

In addition to AZ, a number of other active ingredients have also been isolated and identified from different parts of the neem tree. Compounds such as salannin, salannol, salannolacetate, 3-deacetylsalannin, azadiradion, 14-epoxyazadiradion, gedunin, nimbinen, deacetylnimbinen, 23-dihydro-23β-methoxyazadirachtin, 3-tigloylazadirachtol, and 1-tigloyl-3-acetyl-11-methoxyazadirachtin were isolated from oil extracted from neem seed kernels (Schmutterer 1990). Vilasinin derivatives, meliantriol, azadiradione, 14-epoxyazadiradione, 6-0-acetylnimbandiol, 3-deacetylsalannin, and deacetylazadirachtinol were also recovered from neem seed oil (Schmutterer 1990), whereas nimocinolide and isonimocinolide were recovered from fresh leaves (Siddiqui et al. 1986). Some other compounds, such as alkanes, were isolated from dried leaves (Chavan 1984, Chavan and Nikam 1988), and nonterpenoidal constituents, isocoumarins, coumarins, and saturated hydrocarbons were recovered from fresh, uncrushed twigs (Siddiqui et al. 1988). Although the bark, heartwood, leaves, fruit, and seeds of neem have been investigated chemically for their main biocidal components, the renewable parts (seeds and leaves) received major research attention.

## Mode of action

Neem products are capable of producing multiple effects in insects such as antifeedancy, growth regulation, fecundity suppression and sterilization, oviposition repellency or attractancy, and changes in biological fitness. These aspects are discussed in detail in recently published comprehensive reviews (Ascher 1993; Mordue and Blackwell 1993; Schmutterer 1988, 1990). Most of the information reviewed in these papers was gathered with regard to phytophagous insects. Some of these phenomena are briefly reviewed and discussed below.

*Antifeedancy:* Chemical inhibition of feeding has been studied in detail for a few phytophagous insects. The mechanism of feeding inhibition could be a blockage of the input from chemoreceptors normally responding to phagostimulants, which can be reversed by increasing the amount of phagostimulants, or stimulation of specific deterrent cells or broad spectrum receptors, or through both of these mechanisms (Chapman 1974, Schoonhoven 1982). Azadirachtin and AZ-containing extracts from the neem tree show distinct antifeedant activity, primarily through chemoreception (primary antifeedancy), but also through reduction in food intake due to toxic effects after consumption (secondary antifeedancy) in lethal quantities. However, clear differences exist in the magnitude of these effects, depending on the concentration and formulation of the active principle, the methods of application of the neem products, and the species of test insects.

By applying the neem products in different ways, antifeedant activity of neem products has been found in Orthoptera, Isoptera, Hemiptera, Coleoptera, Lepidoptera, and Diptera (Mordue and Blackwell 1993).

*Growth regulation:* The growth regulatory effects of AZ and other neem-related products are of considerable theoretical and practical interest. Treatment of insects by injection, oral ingestion, or topical application of AZ caused larval growth inhibition, malformation, and mortality. These are effects that are similar to those observed in treating insects with insect growth regulators (IGRs). This activity has been proven in Orthoptera, Hemiptera, Lepidoptera and Diptera (Ascher 1993; Mordue and Blackwell 1993; Schmutterer 1988, 1990). A major action of AZ is to modify hemolymph ecdysteriod and juvenile hormone titers (significant reduction or delays) by inhibiting the release of morphogenetic peptide, prothoracicotropic hormone (PTTH), and allatotropins from the brain–corpus cardiacum complex (Ascher 1993). Extracts from various parts of the neem tree had different degrees of IGR type of activity. For example, with the diamond-back moth (*Plutella xylostella* L.) the order of growth-inhibiting potency of neem extracts was seeds > leaves > bark/twig > wood (Tan and Sudderuddin 1978).

*Fecundity suppression and sterilization:* The interruption of insect reproduction is also an important feature of AZ compounds. Because ecdysteroid is one of the hormones regulating vitellogenesis, and AZ can modify hemolymph ecdysteriod by inhibiting the release of PTTH and allatotropins from the brain–corpus cardiacum complex, adverse effects on ovarian development, fecundity, and fertility (egg viability) occur in Orthoptera, Hemiptera, Heteroptera, Coleoptera, Lepidoptera, Diptera, and Hymenoptera (Ascher 1993; Mordue and Blackwell 1993; Schmutterer 1988, 1990). In addition, the sex behavior of the males and females in mating and response to sexual pheromones (Dorn et al. 1987), and spermatogenesis in males (Shimizu 1988) were affected by AZ treatment.

*Oviposition repellency:* When oviposition sites were treated with AZ or other neem-related products, oviposition repellency, deterrency, or inhibition occurred in Coleoptera, Lepidoptera, and Diptera (Schmutterer 1988, 1990; Dhar et al. 1996).

*Blocking the development of vector-borne pathogens:* This aspect of the mode of action has been studied in the infection of triatomid bugs by trypanosomes. Complete blocking of the development of *Trypanosoma cruzi* in triatomine vectors was observed after a single treatment with AZ (see details below; Garcia 1988; Garcia et al. 1989a, 1989b, 1991, 1992; Rembold and Garcia 1989; Azambuja and Garcia 1992; Gonzalez and Garcia 1992). To date, no similar studies have been reported on other vector–pathogen systems.

*Changes in biological fitness:* The biological fitness of many insect species was substantially reduced even at neem concentrations and dosages below those interfering with the molting process. Changes in biological fitness included reduced lifespan (Wilps 1989), high mortality (Dorn et al. 1987), loss of flying ability (Wilps 1989), low absorption of nutrients (Wilps 1989), immunodepression (Azambuja et al. 1991, Azambuja and Garcia 1992), enzyme inhibition (Naqvi 1987), and disruption of biological rhythms (Smietanko and Engelmann 1989). This means that AZ has subtle effects on a variety of tissues and cells, especially those with rapid mitosis, for example, epidermal cells, midgut epithelial cells, ovary, and testis, which form part of the overall toxic syndrome of poisoning.

## Commercial formulations and persistence

The pesticidal neem products used in practice include those from leaves, whole seed, decorticated seed, seed kernels, neem oil, and neem cake after extraction or extrusion of the oil from the seeds. Large-scale chemical synthesis for pesticidal use is precluded by the extremely high cost of synthesis of the complex structure of AZ and other bioactive neem constituents. Therefore, it is only practical to extract neem bioactive agents from the renewable parts of the tree and to manufacture various pesticidal formulations. The commercial formulations of neem products produced by Indian companies include RD-9 Repelin (ITC Ltd., Andhra Pradesh, India), Neemgard (Gharda Chemicals, Bombay, India), and Neemark (West Coast Herbochem, Bombay, India). These products are used for the control of a variety of pests. In the USA, an AZ-enriched, concentrated neem seed kernel extract formulation, Margosan-O (Vikwood Ltd., Sheboygan, WI, USA), has been granted registration by the U.S. Environmental Protection Agency for the control of pests in nonfood crops and ornamentals. Other formulations of neem, Azatin WP4.5, Azatin EC4.5, Azad WP10, Azad EC4.5, and Neemix EC0.25 by the Thermotrilogy Co. (Columbia, MD); Bioneem and Neemesis by Ringer Corp. (Minneapolis, MN); Neemazal by Trifolio M GmBH (D-6335 Lahnau 2, Germany); and Safer's ENI by Safer Ltd. (Victoria, BC, Canada) are also available. Other companies in Europe are involved in formulating and distributing pesticidal formulations based on neem products.

Like other natural products, the degradation of AZ under field conditions takes place faster than in the laboratory because of the effects of untraviolet (UV) light, temperature, pH, and microbial activity (Stark and Walter 1995, Sundaram 1996, Szeto and Wan 1996). The degradation of AZ at a basic pH is faster than at acidic pH (Szeto and Wan 1996). For increasing the efficacy and longevity of AZ, degradation was shown to be lower with the addition of UV absorbers and protectants such as 2,4-

dihydroxybenzophenone to AZ-A at a rate of 1:1 (w/w) (Sundaram 1996). Under field conditions the residual effect of neem pesticides lasts about 4–8 days, and in the case of systemic effects some days longer.

### Effects on nontarget organisms

Because of a relatively weak contact mode of action in insects and lack of activity against predators and parasitoids, neem products have been shown to be very safe to beneficial organisms such as honeybees, natural enemies of pests, and so forth.

Neem products are relatively safe to mammals and humans according to tests carried out to date. Neem flowers and leaves are eaten as vegetables in Asian countries (India, Myenmar, Thailand). Moreover, some neem products obtained from the fruit, bark, or leaves have played a prominent role in Indian traditional medicine for many centuries. Neem products are also incorporated into a number of toiletry and pharmaceutical products in India, Pakistan, and Germany. However, neem products are not risk free. Adverse effects of neem components were noted in nontarget aquatic invertebrates (Stark 1997), fish (Tangtong and Wattanasirmkit 1997), some mammals (Ali and Salih 1982, Ali 1987), and humans (Reye's syndrome) (Sinniah and Baskaran 1981; Sinniah et al. 1982, 1986). Finally, aflatoxins were usually present in neem extracts if the fungus *Aspergillus* was present during the storage of neem products (Sinniah et al. 1982, 1983, 1986, Hansen et al. 1994). Therefore, caution must be exercised to control the level of these toxins to insure safe use. In general, AZ products are considered to have a good margin of safety to fish, wildlife, and other nontarget biota.

### Development of resistance

No cases of resistance to neem products have been reported. Laboratory selection experiments for resistance to neem products in *P. xylostella* did not result in development of resistance (Völlinger 1987), which may be because the products were mixtures of various related compounds with different modes of action. Development of a high level of resistance to chemicals or products that possess numerous modes of action is somewhat difficult.

### RESEARCH ON NEEM PRODUCTS FOR THE CONTROL OF ARTHROPODS OF MEDICAL AND VETERINARY IMPORTANCE

Relatively little information is available regarding the effects of neem products on arthropods of medical and veterinary importance. Studies, testing, and evaluation started in the early 1970s. Neem products were tested against mosquitoes, flies, tria-

tomines, cockroaches, fleas, lice, sandflies, and ticks. The actions of neem products against these arthropods were considered to include but not be limited to antifeedancy or landing repellency, growth regulation, fecundity reduction, reduced fitness, and blocking of the development of vector-borne pathogens. Information on these aspects of neem products is synthesized and summarized in Table 1. Information for each group of arthropods is presented and discussed in more detail below.

### Mosquitoes

Most of the investigations related to the potential of neem products for the control of insects of medical and veterinary importance were conducted on mosquitoes during the past 20 years, where the neem preparations used were extracts prepared in the laboratory from different parts of the neem tree. The major interest was focused on the IGR type of activity of neem preparations in both the laboratory and the field, even though a few investigations were also conducted to study the repellent activity of neem oil on adult mosquitoes, and the effects on reproductive capacity of mosquitoes.

*Activity against immature stages:* Laboratory tests. Laboratory tests with different neem preparations against the immature stages of mosquitoes have been mostly carried out on the following 7 species, *Anopheles arabiensis* Patton, *Anopheles culicifacies* Giles, *Anopheles stephensi* Liston, *Culex pipiens molestus* Forskal, *Culex quinquefasciatus* Say, *Aedes aegypti* (L.), and *Aedes togoi* Theobald. The IGR activity of neem and related products are discussed below.

The neem tree has received much attention as a source of active agents for mosquito larval control. More than 2 decades ago small-scale tests of neem products against *An. stephensi* were conducted (see Zebitz 1984), which was the 1st attempt in this field. Extracts from *A. indica* seeds such as neem oil and neem seed kernel extracts (NSKEs) provided a good source of pesticidal ingredients. Attri and Prasad (1980) tested the "neem oil extractive," a waste from neem oil refining, against larvae of this species. The complete failure of 1st-stage larvae to develop to the adult stage occurred at the concentration of 0.005% of neem oil. But the material was also toxic to mosquito fish (*Gambusia* sp.) and tadpoles at t his concentration. Continuous exposure of 4th-stage larvae of *Ae. aegypti* in water treated with crude or AZ-enriched NSKEs resulted in noticeable growth disrupting effects. An extreme prolongation of the larval period was attained when 1st-stage larvae were continously exposed in treated water until adult emergence. The effectiveness of the extracts increased with decreasing polarity of the solvents used for extraction. The time necessary for lethal action of NSKEs was similar to that reported for some synthetic IGRs (Zebitz 1984). Singh (1984) found that aqueous extracts from de-

Table 1.　Effects of neem products on feeding, reproduction, and survivorship of arthropods of medical and veterinary importance.[1]

| Species | Stages | Type of action | Neem components | References |
|---|---|---|---|---|
| Antifeedancy or repellency | | | | |
| *Anopheles culicifacies* | Adult | Repellency | Neem oil | Sharma et al. (1993a) |
| *Anopheles and Culex* spp. | Adult | Repellency | Neem oil | Sharma et al. (1993a, 1993b), Sharma and Ansari (1994) |
| *Culex quinquefasciatus* | Larvae | Antifeedancy | AZ formulations | Su and Mulla (1998a) |
| *Cx. tarsalis* | Larvae | Antifeedancy | AZ formulations | Su and Mulla (1998a) |
| *Musca domestica* | Adult | Antifeedancy | Ethanol extract of seeds | Warthen et al. (1978) |
| *Phormia terraenovae* | Larvae | Antifeedancy | AZ | Wilps (1987) |
| *Rhodnius prolixus* | Nymph | Antifeedancy | AZ | Garcia et al. (1984), Garcia and Rembold (1984) |
| *Phlebotomus argentipes* | Adult | Repellency | Neem oil | Sharma and Dhiman (1993) |
| Insect growth regulation | | | | |
| *An. arabiensis* | Larvae | IGR | Fruit kernel extract[2] | Mwangi and Mukiama (1988) |
| *An. culicifacies* | Larvae | IGR | AZ-rich fractions | Rao et al. (1988) |
| *An. stephensi* | Larvae | IGR | NSKEs, AZ, oil extracts | (see Zebitz 1984), Kumar and Dutta (1987), Zebitz (1987) |
| *Cx.* spp. | Larvae | IGR | Lipid/AZ-rich fractions, neem cake | Rao (1987), Rao and Reuben (1989), Rao et al. (1992, 1995) |
| *Cx. pipiens* | Larvae | IGR | AZT-VR-K-E[3] | Zebitz (1987) |
| *Cx. p. molestus* | Larvae | IGR | Acetone extracts[4] | Al-Sharook et al. (1991) |
| *Cx. quinquefasciatus* | Larvae | IGR | Petroleum ether, chloroform, alcohol leaves extracts, alkanes, neem oil extract, NSKEs, AZ, AZ-rich fractions, defatted cake, AZ-based formulations, fruit extracts[2] | Chavan et al. (1979), Attri and Prasad (1980), Chavan (1984), Singh (1984), Zebitz (1987), Chavan and Nikam (1988), Rao et al. (1988), Sinniah et al. (1994), Amorose (1995), Irungu and Mwangi (1995), Sagar and Sehgal (1996), Mulla et al. (1997) |
| *Cx. vishnui* subgroup | Larvae | IGR | AZ-rich/neem oil formulations | Rao (1997) |
| *Ae. aegypti* | Larave | IGR | Neem oil, NSKEs, AZ, nimocinolide and isonimocinolide of fresh winter leaves, extracts of fruits[2] | Zebitz (1984, 1987), Hellpap and Zebitz (1986), Naqvi (1987), Naqvi et al. (1991), Sinniah et al. (1994), Mwangi and Rembold (1987, 1988) |
| *Ae. togoi* | Larvae | IGR | NSKEs, AZ | Hellpap and Zebitz (1986), Zebitz (1987) |
| *Calliphora vicina* | Larvae | IGR | AZ | Bidmon et al. (1987) |
| *Haematobia irritans, Stomoxys calcitrans,* and *M. domestica* | Larvae | IGR | AZ, seed kernel | Wilps (1995), Miller and Chamberlain (1989) |
| *Lucilia cuprina* | Larvae | IGR | AZ | Meurant et al. (1994) |
| *M. autumnalis* | Larvae | IGR | AZ | Gaaboub and Hayes (1984a) |
| *P. terraenovae* | Larvae | IGR | AZ | Wilps (1987) |
| *R. prolixus* | Nymph | IGR | AZ, AZ-A, AZ-B, 7-acetyl AZ-A | Garcia and Rembold (1984), Garcia et al. (1984, 1986, 1987, 1990a, 1990b, 1991) |
| *Triatoma vitticeps*[5] | Nymph | IGR | AZ-A | Garcia et al. (1989a) |
| *Blatta orientalis*[6] | Nymph | IGR | Seed extract | Adler and Uebel (1985) |
| *B. germanica* | Nymph | IGR | AZ | Adler and Uebel (1985), Prabhakaran and Kamble (1996) |
| *Periplaneta americana* | Nymph | IGR | AZ | Qadri and Narsaiah (1978) |
| *Ctenocephatides felis* and *Xenopsylla brasiliensis* | Larvae | IGR | NSKEs | Kilonzo (1991) |

Table 1.   Continued.

| Species | Stages | Type of action | Neem components | References |
|---------|--------|----------------|-----------------|------------|
| *X. cheopis* | Larvae | IGR | AZ | Chamberlain et al. (1988) |
| *Bovicola ovis* | Larvae | IGR | AZ | Heath et al. (1995) |
| **Reproduction suppression and ovicidal activity** | | | | |
| *An. culicifacies* | Adult | Antioviposition, GC impaired, vitellogenesis impaired | Neem oil, volatiles | Dhar et al. (1996) |
| *An. stephensi* | Adult | Antioviposition, GC impaired, vitellogenesis impaired | Neem oil, volatiles | Dhar et al. (1996) |
| *Cx. quinquefasciatus* | Adult | Oviposition repellency | Neem oil, AZ formulations, fruit extract[2] | Zebitz (1987), Irungu and Mwangi (1995), Su and Mulla (1998c) |
| *Cx. quinquefasciatus* | Eggs | Ovicide | Seed extract, AZ formulations | Zebitz (1987), Su and Mulla (1998a) |
| *Cx. tarsalis* | Adult | Ovi-modification | AZ formulations | Su and Mulla (1998b) |
| *Cx. tarsalis* | Eggs | Ovicide | AZ formulations | Su and Mulla (1998c) |
| *Ae. aegypti* | Adult | Oocyte growth reduction | AZ | Ludlum and Sieber (1988) |
| *Glossina morsitans morsitans, G. m. centralis* | Adult | Fecundity reduction | Neem oil, NSKEs | Kaaya (see Wilps 1995) |
| *L. cuprina* | Adult | Antioviposition | Petroleum ether, extracts of ripe fruits | Rice et al. (1985) |
| *L. sericata* | Adult | Antioviposition | Neem oil | Hobson (1940) |
| *M. autumnalis* | Adult | Fecundity reduction | AZ | Gaaboub and Hayes (1984a) |
| *P. terraenovae* | Eggs | Ovicide | NSKEs | |
| *P. terraenovae* | Adult | Fecundity reduction | AZ, NSKEs | Wilps (1995), Wilps (1989) |
| *S. calcitrans* | Eggs | Ovicide | AZ | Gill (1972) (see Wilps 1995) |
| *R. prolixus* | Adult | Fecundity reduction | AZ, AZ-A | Garcia et al. (1987, 1990a), Feder et al. (1988), Moreira et al. (1994) |
| *Amblyomma americanum* | Adult | Fecundity reduction | AZ | Kaufman (1988), Lindsay and Kaufman (1988) |
| **Blocking the development of trypanosomes** | | | | |
| *R. prolixus* | Nymph | Block development of *Typanosoma cruzi* | AZ | Garcia (1988), Garcia et al. (1989a, 1989b), Rembold and Garcia (1989), Azambuja and Garcia et al. (1992) |
| *R. prolixus, T. infestans, Dipetalogaster maximus* | Nymph | Block development of *T. cruzi* | AZ | Gonzalez and Garcia (1992) |
| **Change biofitness** | | | | |
| *G. m. morsitans, G. m. centralis, G. pallidipes* | Adult | Increased mortality | Neem oil, NSKEs | see Wilps (1995) |
| *M. domestica* | Adult | Enzyme inhibition | Petroleum ether fraction of fruits | Naqvi (1987) |
| *M. domestica* | Adult | Splitting of activity rhythm | AZ | Smietanko and Engelmann (1989) |
| *P. terraenovae* | Adult | Changes in metabolism | NSKEs | Wilps (1989) |
| *B. germanica* | Nymphs | Enzyme inhibition | Neutral fraction of leaves | Naqvi (1987) |
| *R. prolixus* | Nymph | Immune depression | AZ | Azambuja et al. (1991), Azambuja and Garcia (1992) |

[1] AZ, azadirachtin; IGR, insect growth regulation; NSKEs, neem seed kernel extracts; GC, gonotrophic cycle.

[2] From *Melia volkensii*.

[3] AZT-VR-K-E was prepared from neem seed kernels in a Soxhlet apparatus using an azeotropic mixture of *tert*-butylmethyl ether and methanol. Fatty components were separated by petrol ether extraction and cooling.

[4] From *M. volkensii* and *Melia azedarach*.

[5] Including the following species: *Triatoma vitticeps, T. pseudomaculata, T. maculata, T. brasiliensis, T. lecticularis, T. matogrossensis, T. infestans, Rhodnius prolixus, R. neglectus, R. robustus, Panstrongylus megistus,* and *P. herrera.*

[6] Including the following species: *Blatta orientalis, B. germanica, Byrsotria fumigata, Gromphadorhina protentosa, P. americana,* and *Supella longipalpa.*

oiled neem seed kernels caused 100% mortality in *Cx. quinquefasciatus* larvae at 62.5, 125, 250, and 500 ppm after 8, 6, 4, and 2 days of exposure, respectively. The reduced concentration of 31.2 ppm still yielded 85% mortality within 12 days of exposure. Another comparable study conducted recently by Sagar and Sehgal (1996) also confirmed the activity of the aqueous extracts of deoiled neem seed kernel, which was more active than those of karanja (*Pongamia glabra* Vent) seed kernel against the same species. Four new AZ-rich fractions prepared from neem seeds with different extraction procedures, designated as nemidin, vemidin, vepcidin, and nemol, were tested for larvicidal activity against the early 4th-stage larvae of *An. culicifacies* and *Cx. quinquefasciatus*. Among these, nemidin, which was found to have a high content of AZ, showed high larvicidal activity against both test species as shown by low median lethal concentration ($LC_{50}$) values of 1.12 and 3.9 ppm, respectively. However, the other fractions showed moderate larvicidal activity only. Nemidin produced rectal prolapse in *Cx. quinquefasciatus* larvae but not in *An. culicifacies* (Rao et al. 1988). Sinniah et al. (1994) tested neem oil extract from crushed seeds against *Ae. aegypti* and *Cx. quinquefasciatus*. At concentrations of 0.02% (relatively high concentration) or more, 100% mortality was attained in 24 h for these 2 species. At a concentration of 0.001%, 59 and 63% mortality, and at a concentration of 0.0005%, 2 and 22% mortality was obtained in 96 h for these 2 species, respectively. Amorose (1995) tested the larvicidal efficacy of neem oil and defatted neem cake on *Cx. quinquefasciatus*. Against 3rd- and 4th-stage larvae, $LC_{50}$s were 0.99 and 1.20 ppm for neem oil, and 0.55 and 0.72 ppm for the deoiled neem cake. Third-stage larvae were more susceptible than were 4th-stage larvae. The growth-modifying effects of the methanolic extract from neem seeds in *Ae. aegypti* often was not correlated with the AZ content, which implied the existence of other principles exhibiting synergistic or antagonistic effects on the active ingredients contained in neem seed extract (Schmutterer and Zebitz 1984). Most likely, different components from the crude extracts of neem, not AZ alone, act together against mosquito larvae.

As to the interaction between the seed extracts of *A. indica* and microbial and chemical insecticides, when 4th-stage larvae of *Ae. togoi* were exposed in water treated with NSKEs, *Bacillus thuringiensis israelensis*, or mixtures of both at different concentrations until adult emergence, the dead larvae to dead pupae ratio was shifted to a higher number of dead pupae in the combination mixtures compared with the ratios obtained after application of each material alone. In most of the combinations, the resultant effect of the 2 substances was the same as the value expected on the basis of the single material, which amounts to an additive action. However, in some combinations the resul-

tant effect was larger than expected and showed a synergistic action (Hellpap and Zebitz 1986). Zebitz (1987) investigated the IGR effects of NSKEs and crude and pure AZ on mosquito larvae and their joint activities with methoprene, a juvenile hormone analog (JHA). The susceptibility of young 4th-stage larvae of 4 species of mosquitoes to NSKEs was studied. Their susceptibility in decreasing order was *Ae. togoi, An. stephensi, Cx. quinquefasciatus,* and *Ae. aegypti*. Azadirachtin and NSKEs exerted a pronounced IGR type of effect during preimaginal development. Joint action of methoprene and NSKEs resulted in pronounced synergistic effects. The results pointed out a strong interference of AZ with hormonal balance, most probably with ecdysteriod titer. The petroleum ether extracts of various parts of neem tree also had synergistic effects with synthetic chemical insecticides, such as phenthoate and fenthion (Kalyanasundaram and Babu 1982).

In addition to *A. indica* seeds, the leaves of this species also contain some mosquitocidal components. Tests by Chavan et al. (1979) showed that extracts of neem leaves with organic solvent were toxic to 4th-stage larvae of *Cx. quinquefasciatus*. Dried leaves were extracted with petroleum ether, ether, chloroform, and ethanol, and the solvents were removed from the extracts in a water bath, yielding residues of the active compounds. The residues were redissolved in a solvent and bioassayed against larvae of this species. At 1% concentration, 100% mortality of mosquito larvae occurred within 24 h when petroleum ether and ether were used as extraction solvents. However, under identical conditions, the residues from extracts with chloroform and ethanol at 1% only yielded 65 and 60% mortality, respectively, within the same period of exposure. The residues from extracts with petroleum ether and ether also exhibited good residual activity (up to 144 h) at the concentrations of 1 and 0.2%, respectively (which are very high by current standards). Residues from petroleum ether extract was further purified with a neutral alumina column and by eluting with different solvent systems. Benzene eluate showed promising results and yielded a material that crystallized and gave 100% mortality at 10 ppm, which also had residual activity producing 100% mortality for 5 days. When used at the very high concentration of 100 ppm, 100% mortality was obtained for 8 days (Chavan 1984). Chavan and Nikam (1988) discovered that the alkanes separated from petroleum ether extracts of neem leaves were effective larvicides against *Cx. quinquefasciatus*. The concentration of 100 ppm yielded 100% mortality; 10 ppm caused 60% mortality in 24 h. Naqvi (1987) and Naqvi et al. (1991) tested 2 new compounds and their parent fractions obtained from fresh winter neem leaves against the larvae of *Ae. aegypti*. The $LC_{50}$s were 0.58 ppm for a neutral fraction of winter neem leaves extracts containing mostly nimocinolide and iso-nimocinolide and

some other tetranotriterpennoids in small quantity, 0.625 ppm for nimocinolide ($C_{28}H_{36}O_7$, melting point 160°C), and 0.74 ppm for its isomer iso-nimocinolide ($C_{28}H_{36}O_7$, melting point 165°C). Thus, it can be concluded that the fresh leaves have more active ingredient than the dried leaves as used by Chavan (1984).

Upon the availability of experimental formulations of AZ, Mulla et al. (1997) tested the larvicidal activity of Azad WP10, Neemix EC0.25, and Neemazad EC4.5 (Thermotrilogy Co.) against *Cx. quinquefasciatus*. The susceptibilities of 2nd-stage larvae and early and late 4th-stage larvae of this species to the test materials were essentially the same. Larvicidal activity was very much a function of the formulation; the wettable powder (WP) was 10 times more active than the emulsifiable concentrate (EC). The cumulative mortality (larvae, pupae, and adults) was greater than 80% when 4th instars were treated at 0.1–1 ppm AZ of different formulations. Neem products are believed to affect the feeding activity of mosquito larvae, which is an additional advantage of AZ formulations when used as larvicides. Antifeedancy of 3 neem formulations (Azatin WP4.5, Azad EC4.5, and Neemix EC4.5, Thermotrilogy Co.) against mosquito larvae was found in *Culex tarsalis* Coquillett and *Cx. quinquefasciatus*. A significant level of antifeeding activity was indicated at 5 and 10 ppm AZ for all test formulations. Within the test concentration range of AZ (1–10 ppm), 5 ppm was the minimum effective concentration for antifeedancy expression in most cases. The *Cx. tarsalis* larvae were more susceptible than were *Cx. quinquefasciatus* larvae to Azad EC4.5 in terms of antifeedancy effects. Formulation-related differences in antifeedancy activity were noted when the concentration of AZ was increased to 10 ppm. At this AZ concentration, in *Cx. tarsalis*, Azad EC4.5 and Neemix EC4.5 were more effective in causing antifeedancy than was Azatin WP4.5. In *Cx. quinquefasciatus*, Azatin WP4.5 and Neemix EC4.5 were more effective than Azad EC4.5 (Su and Mulla 1998a).

In addition to products of *A. indica*, several other species of Meliaceae have been investigated in the laboratory against mosquitoes during the past few years. Kumar and Dutta (1987) tested the larvicidal activity of oil extracts of 10 species of plants in 8 genera against *An. stephensi. Melia azadirachta* ranked 4th with an $LC_{50}$ of 88.5 ppm, with the range of 63.2–113.0 ppm for all tested plants. Extracts from dry fruits of *M. volkensii* exhibited growth-inhibiting activity against the larvae of *Ae. aegypti*. At a concentration of 2 μg/ml in water, the active fraction prolonged the duration of the larval stage. Mortality in treated larvae was high, especially during the molting and melanization processes. The $LC_{50}$ for larval mortality was 50 μg/ml in 48 h. The active compound was not identical to AZ, and had greater acute toxic and growth inhibiting effects on *Ae. aegypti* than the AZ-containing frac-

tion (Mwangi and Rembold 1987, 1988). A fraction of the fruit kernel extract from *M. volkensii* was tested against the larvae of *An. arabiensis*. The $LC_{50}$ in 48 h was 5.4 μg/ml. At low concentrations, this fraction had growth-inhibiting activity, prolonging the duration of larval instars, where lethal effects were exhibited during ecdysis. Further fractionation of this fraction yielded 7 bands in thin-layer chromatography, among which 2 of the most lipophilic bands showed acute toxic effects against the larvae, the next 2 bands had growth inhibiting effects, and the 3 trailing bands had no activity at all. More work is needed to isolate and identify these principles. The acute toxicity and growth-inhibiting effects of the bioactive principles were destroyed by heat during the drying of the fruit (Mwangi and Mukiama 1988). The larvicidal effects of acetone extracts from the seeds of *M. volkensii* and *M. azedarach* were compared with pure AZ-A for their morphogenetic effects against *Cx. pipiens molestus*. The insecticidal activities of the crude extracts were significantly different. Acetone extract of *M. volkensii* was equally toxic for both larvae and pupae, with an $LC_{50}$ of 30 μg/ml. Acetone extract of *M. azedarach* was exclusively larvicidal with an $LC_{50}$ of 40 μg/ml, and had no inhibitory effect on the pupal stage. Like the crude extract from *M. volkensii*, pure AZ-A was also equally toxic to both larvae and pupae, with an $LC_{50}$ of 1–5 μg/ml. The bioactive compounds from *M. volkensii* were thermostable (different from the results reported by Mwangi and Mukiama 1988) and partially soluble in water (Al-Sharook et al. 1991). A chromatographically enriched fraction from dry fruits of *M. volkensii* was purified from a crude methanolic extract by cold precipitation and elution of the precipitate dissolved in a hexane–ethyl acetate solvent system through a silica gel column. Larvae of *Cx. quinquefasciatus* were reared in water containing the fraction at concentrations of between 5 and 200 ppm. The $LC_{50}$ for this fraction was 34.72 μg/ml in 48 h. Second-stage larvae were more susceptible to fraction B when compared to 4th-stage larvae. All 4th-stage larvae that survived the treatment molted into short-lived larval–pupal intermediates (Irungu and Mwangi 1995).

## FIELD TRIALS

A few semifield or field tests of neem materials such as NSKEs and neem cake have been carried out in the breeding sites of culicine mosquitoes, and the efficacy was reported to be promising. In a semifield trial using drums, Zebitz (1987) showed the final concentration of 10–20 ppm of NSKEs to be effective in diminishing populations of preimaginal stages of *Culex pipiens* L. for at least 4 wk. Evaluation of samples of larvae taken from treated and untreated drums revealed that 1st- and 2nd-stage larvae died during molting to the following larval

instar; most 3rd- and 4th-stage larvae died as pupae.

Rao and his colleagues conducted field studies using neem cake powder to control immature rice field-breeding mosquitoes. Based on their studies on the larvicidal activity of neem cake powder in simulation trays planted with rice (Rao 1987), a dosage of 500 kg/ha (a very high dosage) was used in a 6,000 m² field. In these tests 81 and 43% reductions of late larval instars and pupae of culicine mosquitoes were attained, respectively. Subsequently, Rao and Reuben (1989) carried out trials using urea coated with neem powder in rice fields but failed to control immature populations of culicines or to enhance rice yield because of the intrinsic poor quality of the test materials. Therefore, Rao et al. (1992) developed a laboratory bioassay to screen neem cake powder against the larvae of *Cx. quinquefasciatus*. Only samples of neem causing more than 90% bioassay mortality at a 2% concentration within 48 h were used in field trials. When good-quality neem cake powders were applied at the dosage of 500 kg/ha, either alone or coated over urea, a striking reduction occurred in the abundance of late larval instars and pupae. Only 14 pupae were obtained over a period of 13 wk in plots treated with neem cake powder, and 4 pupae were obtained in plots treated with neem coated urea, compared with 101 pupae in control plots. In another field trial, neem cake-coated urea was tested at 500 kg/ka and 250 kg/ha in combination with water management practices. Both rates of application yielded similar results. Larval abundance in plots under water management alone did not differ significantly from the controls, but was significantly reduced when water management was combined with neem products. Two stable formulations, Neemrich-I (lipid rich) and Neemrich-II (AZ rich), gave good suppression of immature culicines. All the treatments with neem also gave higher grain yield than the control.

Considering that neem cake powder is bulky and deteriorates under improper storage conditions, which made large-scale use impractical, Rao et al. (1995) field-tested relatively stable lipid-rich fractions of neem, which were as effective as good-quality crude neem products in the control of culicine vectors of Japanese encephalitis, and produced a slight but significant reduction in populations of anopheline pupae. Neem-based formulations coated over urea significantly increased grain yield, but neem products used alone did not. Combination of neem-coated urea and water management by intermittent irrigation had a greater effect on grain yield than that of water management alone. The neem fractions are relatively cost-effective, and the combined water management and neem-coated urea strategy is acceptable to the farmers, who are already aware of benefits of the use of neem-coated urea, and of water management. Therefore, this technology has considerable promise as an environmentally benign method for rice-field mosquito control that could be self sustaining and implemented by farmers (Rao et al. 1995). Recently, in India, application of AZ-rich and neem oil-based formulations either alone or coated over urea at the time of transplantation in rice paddies was shown to significantly reduce the density of the *Culex vishnui* subgroup, including *Culex tritaenioyhynchus* Giles, *Culex vishnui* Theobald, *Culex pseudovishnui* Colless, and *Culex bitaeniorhynchus* Giles. The neem oil-based formulations were also effective in reducing the pupal density of *Anopheles subpictus* Grassi and *Anopheles vagus* Donitz when combined with intermittent irrigation (Rao 1997).

*Effects on adult mosquitoes:* Landing and blood-feeding repellency were tested in the laboratory and field against anopheline and culicine mosquitoes, but the conclusions were variable. Additionally, the neem products might exert detrimental effects on the reproductive events of mosquitoes, which has been tested in *An. culicifacies, An. stephensi, Cx. tarsalis, Cx. quinquefasciatus,* and *Ae. aegypti.*

Landing and bloodfeeding repellency. The landing and bloodfeeding repellency effect against mosquitoes was investigated under laboratory and field conditions. Sharma et al. (1993a) reported the results of field studies on the repellent action of neem oil against *An. culicifacies* and other anopheline mosquitoes. Even at concentrations as low as 0.5 and 1%, strong repellent action was observed when the material was applied to the skin. At a concentration of 2%, no anophelines bit and the protection provided was 100% during a 12-h period. Furthermore, burning neem oil in kerosene may provide personal protection from mosquitoes (Sharma and Ansari 1994). Kerosene lamps containing neem oil were burned in a living room, and mosquitoes resting on the walls or attached to human bait were collected inside rooms from 1800 to 0600 h. Neem oil (0.01–1.0%) mixed in kerosene reduced biting of human volunteers and catches of mosquitoes resting on the walls in the rooms. Protection was more pronounced for anopheline than for culicine mosquitoes. A 1% neem oil–kerosene mixture may provide economical personal protection from mosquito bites. Considering the effective repellent action of neem oil on mosquitoes, neem oil could be a good substitute for pyrethroids in some mosquito-repellent products such as coils and mats (Sharma et al. 1993b). However, in cage tests with female *Ae. aegypti,* neem oil showed no repellent potency (Zebitz 1987). Azadirachtin fed in bloodmeals to adult female *Ae. aegypti* through an artificial membrane did not cause feeding inhibition over a wide dose range (0–200 ng/female) (Ludlum and Sieber 1988). Different formulations and methods of usage seem to yield different results. Equally important is the species of mosquitoes, which may respond differently to neem or other related products.

Effects on reproduction. The application of neem

products and their uptake through cuticle or ingestion caused detrimental effects on the reproductive events in adult mosquitoes. The gonotrophic cycle of female *An. stephensi* and *An. culicifacies* was impaired by exposure to volatile compounds contained in a neem extract. Vitellogenesis was impaired irreversibly by long-term exposure to neem odor and some extracts. The effect of volatiles seemed to be regulated by absorption through the cuticle, although passage through the spiracles could not be excluded (Dhar et al. 1996). In *Ae. aegypti,* a high dose of ingested AZ failed to inhibit or delay oviposition. However, significant transient retardation of oocyte growth was observed for up to 72 h after feeding. Immature oocytes were observed in 86% of AZ-fed females decapitated 10 h after a blood meal, whereas 96% of decapitated control females contained maturing oocytes (Ludlum and Sieber 1988). This suggests that AZ delays the release of one or more factors from the brain that regulate oogenesis. Adult females were proposed to overcome the effect of AZ by rapid metabolism rather than by excretion of the compound, because as soon as 2 h after ingestion of an AZ-enriched blood meal, only 0.1% of ingested AZ was recovered from excreta and 5% was recovered from the body (Ludlum and Sieber 1988).

Effects on oviposition behavior. A few studies have indicated that neem products act as an oviposition repellent or deterrent or attractant against mosquitoes. For instance, neem oil containing 400 μg AZ/g was an oviposition repellent against *Cx. quinquefasciatus* at various test concentrations. The dilutions of 0.125, 0.25, 0.5, and 1% yielded oviposition activity indexes (OAIs) of −0.2727, −0.4762, −0.9176, and −1, respectively (Zebitz 1987); here the minus values indicated oviposition repellency. In another study, a brief exposure (90 min) to the volatiles from broken neem seed kernels, neem oil, and neem volatile fraction suppressed rather than inhibited oviposition in gravid *An. stephensi* and *An. culicifacies.* Complete inhibition of oviposition was caused by exposure of mosquitoes to odors from neem oil and one fraction containing volatile components for 1–7 days (Dhar et al. 1996). All these neem products tested contained numerous compounds rather than a single material. It will be interesting to determine which specific compounds induce the observed effects. Recently, the effects of experimental AZ formulations Azad WP10 and Azad EC4.5 on oviposition behavior of the mosquitoes *Cx. tarsalis* and *Cx. quinquefasciatus* were investigated by Su and Mulla (1999). In paired tests of distilled water vs. neem suspension, the WP showed significant oviposition attractancy in *Cx. tarsalis* at the concentrations of 0.5, 1, 5, and 10 ppm AZ. The repellency of the EC was noted at higher concentrations of 5 or 10 ppm AZ. In *Cx. quinquefasciatus,* repellency was only found at 10 ppm AZ of both formulations. In multiple tests using distilled water and neem sus

pensions at concentrations of 0.5, 1, 5, and 10 ppm AZ, oviposition attractancy was indicated at 1, 5, and 10 ppm AZ for the WP, and repellency was found at 5 and 10 ppm AZ for the EC in *Cx. tarsalis.* These 2 formulations did not have any effects on the oviposition behavior of *Cx. quinquefasciatus.* In multiple tests for longevity, the aged suspensions of the WP at 0.5 and 1 ppm AZ were more attractant to *Cx. tarsalis.* The attractancy of the suspensions of the WP was lost in 14- and 21-day-old preparations of 0.5 and 1 ppm AZ, respectively. The repellency of the EC suspension at 5 ppm AZ against *Cx. tarsalis* was lost in ≥1-day-old suspensions. In *Cx. quinquefasciatus,* the oviposition repellency of both the WP and the EC suspensions at 10 ppm AZ was lost in ≥4-day-old suspensions. Among other species of Meliaceae, gthe component from *M. volkensii* also has the property of oviposition deterrency against gravid mosquitoes. A chromatographically enriched fraction from dry fruits of *M. volkensii* was purified from a crude methanolic extract by cold precipitation and elution of the precipitate dissolved in a hexane–ethyl acetate solvent system through a silica gel column. This extract was found to be an oviposition deterrent at a concentration of 20 ppm and greater against *Cx. quinquefasciatus* (Irungu and Mwangi 195).

*Effects on egg viability:* Egg rafts of *Cx. quinquefasciatus* oviposited on water treated with NSKEs at concentrations of 2.5, 5, 7.5, 10, and 20 ppm had reduced hatching ability (Zebitz 1987). Recently, Su and Mulla (1998b) conducted a detailed study on ovicidal activity of various formulations of AZ against *Cx. tarsalis* and *Cx. quinquefasciatus.* The formulations tested were Azad WP10, Azad EC4.5, and technically pure AZ. The ovicidal activity of the test neem products was influenced by concentration of AZ, age of the egg rafts, and age of the neem preparations. Other factors such as formulation and mosquito species were also involved in the degree of ovicidal activity. When the egg rafts were deposited directly in fresh neem suspension and left there for 4 h before transfer to untreated water, 1 ppm AZ produced almost 100% mortality in eggs. When 0-, 4-, 8-, 12-, and 24-h-old egg rafts were exposed to 10 ppm neem suspensions for 36 h, ovicidal activity was attained in the egg rafts deposited directly (0 h old) in the neem suspension. On aging, depending on the formulations and mosquito species, the neem suspensions at 1 ppm completely lost ovicidal activity within 7–20 days. The egg rafts of *Cx. quinquefasciatus* were slightly more susceptible to the EC and the technical AZ than those of *Cx. tarsalis.* The formulated neem products were more persistent and slightly more effective than technical AZ. In *Cx. tarsalis,* the WP had greater ovicidal activity than the EC.

## Flies

A variety of neem products have been evaluated and found to have different types of activities against both immatures and adults of several species of flies. The antifeedancy effect was shown in *Musca domestica* L. and *Phormia terraenovae* L. Insect growth regulator activity was found in *Calliphora vicina* Robineau-Desvoidy, *Haematobia irritans* L., *Lucilia cuprina* Wied., *Musca autumnalis* De Geer, *M. domestica*, *P. terraenovae*, and *Stomoxys calcitrans* L. Reproduction suppression occurred in *Glossina morsitans centralis* Machado, *G. m. morsitans* Westwood, and *M. autumnalis*, and oviposition inhibition occurred in *L. cuprina* and *Lucilia sericata* Meigen. Ovicidal activity was seen in *S. calcitrans* and *P. terraenovae*, and finally, reduced biological fitness occurred in *G. m. centralis*, *G. m. morsitans*, *Glossina pallidipes* Austen, *M. domestica*, and *P. terraenovae*.

*Activity against immature stages:* Azadirachtin and other related neem products act as IGRs to inhibit the development of the immature stages of muscoid flies, even at very low concentrations. When 3rd-stage larvae of the face fly *M. autumnalis* were exposed in 0.00001 μg/ml and 0.000039 μg/ml suspensions of AZ in aqueous acetone for 20 min, 2.75 and 11.5% of the larvae died, respectively, and 21.4 and 52.6% of inhibition of adult formation was recorded, respectively (Gaaboub and Hayes 1984b). Exposures for 10 or 20 min to the AZ aqueous acetone suspensions, ranging from 0.00001 to 0.1 μg/ml, caused about 20–83.5% or 22.5–97.6% inhibition of adult formation, respectively. Doses required to inhibit the emergence of 50% of the adults ($IC_{50}$) were very low, 0.0024 μg/ml and 0.000039 μg/ml, for the 10- and 20-min exposures, respectively. The effects on pupae (proportion of undeveloped individuals) and adults (incomplete eclosion, attachment to puparia, or inability to fly) were dose-dependent (Gaaboub and Hayes 1984a).

The addition of AZ to the culture medium of larvae of the blow fly *P. terraenovae* had an antifeedant effect and resulted in a lower growth rate. Intake of this substance, at approximately 3.5 μg/g fresh weight, produced larvae weighing one half as much as controls. A higher intake of AZ was correlated with a further decrease in weight and an increase in mortality. Depending on the AZ quantities consumed, the ratio of the amount of food converted into body weight to the amount of food ingested decreased from 64% in controls to 16% in the treatment when the larvae consumed AZ at 15 μg/g fresh weight. Accordingly, the body weight of the resulting pupae was reduced from 65 mg in the control to 15 mg in treatment. Pupae resulting from the AZ treatment weighing <40 mg failed to complete development and molt to adults. The failure in morphogenesis in the AZ-treated animals was not due to the low body weight, because pupae weighing 15–20 mg resulting from starvation without neem treatment completed eclosion successfully (Wilps 1987). Injection of AZ into larvae of the blow fly led to a number of abnormal biological effects. In a dose- and stage-dependent manner, injected AZ caused a delay in pupariation of larvae when injected in the first one half of the last larval instar (but no effect when injected into late instars), reduction of pupal weight, and inhibition of adult emergence. Adults emerging from AZ-treated larvae were smaller and showed various malformations (Bidmon et al. 1987).

Azadarachtin was also found to impact the immature stages of the horn fly *H. irritans*, the stable fly *S. calcitrans*, and the house fly *M. domestica*. When an ethanolic extract of ground up seed (2.7 mg AZ/g) was blended into cow mankure, the $LC_{50}$ and $LC_{90}$ for the larvae of horn flies were 0.096 and 0.133 ppm AZ, respectively. For the EC formulation of Margosan-O (3 mg AZ/ml), the $LC_{50}$ and $LC_{90}$ were 0.151 ppm and 0.268 ppm AZ in manure, respectively, for the larvae of this species. For larvae of stable flies, the EC formulation had an $LC_{50}$ of 7.7 ppm and an $LC_{90}$ of 18.7 ppm AZ in manure. Against the larvae of house flies, the $LC_{50}$ and $LC_{90}$ were 10.5 and 20.2 ppm AZ in manure, respectively. From this bioassay, among muscoid flies, horn flies clearly are more susceptible than stable flies and house flies to the test EC formulation. When the ethanolic extract was administered daily per os in gelatin capsules at the rate of 0.023–0.045 mg AZ/kg body weight, horn fly development in manure produced by treated cattle was almost completely inhibited. The same level of efficacy was attained under identical conditions when the EC formulation was given at ≥0.03 mg AZ/kg body weight or ground up seeds at ≥10 mg/kg body weight. However, when the ground up seeds were mixed with the feed and fed daily at the high rate of 100–400 mg seeds/kg body weight, inhibition of development of stable flies in the manure was less than 50% (Miller and Chamberlain 1989).

Regarding the mechanism of action of AZ on the development and growth of immature stages of synanthropic and synzootic flies, the key factor is the disruption of endocrinologic events by the treatment with AZ. For example, in *C. vicina*, the treatment of larvae with AZ by injection had a negative dose-dependent effect on the ecdysteroid content of larvae and pupae. Azadirachtin caused a delay in the appearance of ecdysteroid, which triggers puparium formation, in the larvae. The peak of the hormone titer in AZ-treated larvae was lower but lasted longer. The negative effects of AZ on the production of ecdysteroid (biosynthesis and release) were further demonstrated in vitro with isolated brain–ring gland complex. Azadirachtin not only diminished the rate of production of the ecdysteroid but also decreased the rate of its metabolism. The hydroxylation of ecdysteroid at C-20 by

isolated fat body in vitro was decreased in a dose-dependent manner. The changes in the concentration of ecdysteriods observed in the blow fly larvae and pupae may be the result of interference of AZ with several sites of the hormone-producing system. Because ecdysteroids regulate eclosion, the persisting concentration, rather than the normal peaking of ecdysteroids in pupae, likely is the cause of inhibition of adult eclosion (Bidmon et al. 1987). Evidence to further support this point is also available from the structural changes in endocrine glands induced by AZ treatment. Meurant et al. (1994) investigated the effect of AZ-A on the ultrastructure of the prothoracic gland, the corpus allatum, and the corpus cardiacum in the blow fly *L. cuprina*. When early 3rd-stage larvae were fed on 50 ppm AZ-A (high concentration) in the culture medium until pupation, all the endocrine glands within the ring complex exhibited ultrastructural changes during the period of exposure. The effects on the nucleus were the most noticeable, and included the crenulation of nuclear shape, clumping of heterochromatin, and pyknosis. The degenerative changes in the nuclei seemed to precede changes within the cytoplasm. It was concluded that the degeneration of all 3 endocrine glands within the ring complex would contribute to a generalized disruption of neuroendocrine function and thus unsuccessful molting (Meurant et al. 1994).

*Effects on adult flies:* Azadirachtin and other neem products have been shown to influence the feeding, reproduction, oviposition, and metabolism, of adult muscoid flies. Feeding by adult *M. domestica* was deterred when one of the neem components, salannin, isolated from an ethanol extract of neem seeds was incorporated in sucrose at the concentration of 0.1% (Warthen et al. 1978).

Residual or delayed effects of larval treatment on adult reproduction occurred in *M. autumnalis*. If the 3rd-stage larvae were exposed to 0.00001 µg/ml and 0.000039 µg/ml solutions of AZ in aqueous acetone for 20 min, egg production of the resulting adults was reduced by 67.2 and 85.9% respectively, when treated males mated with treated females. Reduction in egg hatching was also observed when untreated males or females mated with treated individuals of the opposite sex. Egg hatchability was decreased more than 30 and 60% by the lower (0.00001 µg/ml) and higher (0.000039 µg/ml) treatment concentrations, respectively (Gaaboub and Hayes 1984b).

When administered to adults topically or orally, neem products also showed toxicity such as mortality, reduced biological fitness, and suppressed reproduction. The $LC_{50}$ of a mixture of triterpenoids from the petroleum ether-soluble neutral fraction of ripe neem fruits against adult *M. domestica* (topical application, 24 h), was 1.4 µg/fly. Inhibition of cholinesterase, acid phosphatase, and alkaline phosphatase were 38, 45, and 48%, respectively (Naqvi 1987). When adults *P. terraenovae* were fed with various concentrations of aqueous extract and AZ-enriched NSKEs between the 2nd and 4th day after eclosion, the intake of either extract shortened lifespan by 4 days, reduced food ingestion and egg deposition, and caused a complete loss of flying ability. Measurement of concentrations of low molecular weight carbohydrates and glycogen in hemolymph showed that NSKEs-fed flies lost the ability to alter carbohydrate concentration and mobilize glycogen content of the abdomen. However, the anabolism of glycogen was not affected (Wilps 1989). When female *P. terraenovae* were injected AZ at 1 µg/fly, egg production was reduced by 85%, weight of ovaries was reduced 40%, and hemolymph and ovary ecdysteroids were reduced 60 and 40%, respectively (Wilps 1995). Research conducted by Kaaya indicated that when neem oil or AZ-riched NSKE was applied topically to female *G. morsitans morsitans* or *G. m. centralis*, delayed larval growth and abortions occurred. Increased mortality in treated females was observed in the above 2 species as well as in *G. pallidipes* (see Wilps 1995).

Neem products acted as an oviposition deterrent against some species of muscoid flies. Three percent emulsions of Margosan oil sprayed on the back of sheep (600 ml/sheep) inhibited oviposition activity of *L. sericata* for 8 days (Hobson 1940). Crude neem oil (CN) and 30% methanolic, dewaxed solvent extract (AZT) acted as a powerful antioviposition agent for *L. cuprina*. The AZT threshold was 0.002–0.005%, with total deterrence at 0.02%. The CN threshold was 0.2–0.5%, with pure neem oil giving 91% deterrence. The AZT extract is 100–1,000 times more potent than CN, probably because its AZ content had been greatly increased (Rice et al. 1985).

In addition to the effects discussed above, the circadian rhythm of locomotor activity was also affected after AZ was injected into adult *M. domestica*. The period was increased by 0.4 h in 83% of the AZ-treated flies, whereas in flies injected with the solvent of AZ, ethanol, the period increased by 0.3 h in 22% only. In 46% of the AZ-injected flies, a splitting of the activity rhythm into 2 components with the same period was induced, whereas only one of the ethanol controls (2%) showed splitting after injection (Smietanko and Engelmann 1989).

*Effects on egg viability:* The ovicidal activity of 80% was obtained when the eggs of *S. calcitrans* were transferred to the filter paper treated with 0.01% AZ (Wilps 1995). An oviposition substrate treated with NSKEs (1.7% AZ) at the rate 50 µl/g substrate exerted significant ovicidal activity against the eggs of *P. terraenovae*, no matter whether the eggs were laid on the treated substrate or transferred to it thereafter (Wilps 1986, 1987; Wilps, unpublished data; see Wilps 1995).

## Triatomine bugs

A number of studies have been carried out on triatomine bugs. Neem active agents had a variety

of effects such as antifeedancy, growth regulation, immune depression, and blockage of the development of trypanosome parasites. Labeled [22, 23-$^3$H$_2$]dihydroazadirachtin A administered orally in a blood meal to *Rhodnius prolixus* Stal, was absorbed and transported in the hemolymph, retained, and then excreted in unmetabolized form through the Malpighian tubules. The highest level of elimination of dihydroazadirachtin A was found during the first 12–24 h after feeding. Thereafter, a relatively constant quantity of dihydroazadirachtin A was recovered from the head, visceral structures, and rest of the body 5 and 10 days after ingestion. The highest amount per milligram of tissue was found in the Malpighian tubules and midgut, the lowest level was in the head and the rest of the body (Garcia et al. 1989c). During the course of feeding, absorption, transportation, retention, and excretion, AZ exerted pronounced effects on feeding behavior, development, reproduction, and development of trypanosomes, of which the triatomine bugs are a transmission vector.

*Antifeedancy:* Azadirachtin-induced antifeedancy was found in a few studies with triatomine bugs. Given through a blood meal, the median effective dose (ED$_{50}$) for the antifeedant effect ranged from 25 to 30 μg/ml of blood for the 3 compounds AZ-A, AZ-B, and 7-acetyl-AZ. Adenosine triphosphate as a phagostimulant to *R. prolixus* (Friend and Smith 1977), reversed the antifeedant action of AZ-A when applied orally. Therefore, AZ-A may block the input of phagostimulant receptors (Garcia et al. 1984). This mechanism also held true for the antifeedant action of precocene (Azambuja et al. 1982). However, Garcia and Rembold (1984) suggested that feeding inhibition was an indirect effect due to interference of AZ with the endocrine system rather than through the inhibition of chemoreceptors. Obviously, more investigations are needed to confirm the antifeeding mechanism. Probably both primary and secondary antifeedancy are involved in AZ-induced antifeedancy effects.

*Growth regulation:* The pronounced IGR type of activity of AZ against triatomine bugs has been well documented. Garcia and Rembold (1984) studied the effects of AZ administered through a blood meal on the development of 4th-stage nymphs of *R. prolixus*. A dose–response relationship of AZ was established using ecdysis inhibition as the effective parameter. The ED$_{50}$ was 0.0004 μg/ml blood for ecdysis inhibition effects. Ecdysone given orally at 5.0 μg/ml blood and JHA applied topically at 70 μg/insect counteracted the AZ-induced inhibition of ecdysis. Subsequently, Garcia et al. (1984) tested the IGR activity of AZ-A, AZ-B, and 7-acetyl AZ-A. The ED$_{50}$ for molting inhibition was 0.04, 0.05, and 0.45 μg/ml blood, respectively. The dosage for molting inhibition was much lower than that for antifeedant action (see above). The structure change from AZ to 7-acetyl-AZ resulted in a great loss of molting inhibition activity. This acetylation

seemed to have blocked a structural element of AZ-A that was important either for absorption through the gut or for the antimolting effect itself. Garcia et al. (1986) established a dose–response relationship of AZ-A using molting inhibition and mortality as effective parameters. Azadirachtin-A, if injected 1–3 days after bloodfeeding, inhibited the molting process. However, if injected during or after the onset of epidemal mitosis, ecdysis was not affected. A single dose of AZ-A was able to block the onset of mitosis in the epidermis, which was associated with the molting cycle and was triggered by molting hormone. Ecdysteriod titers were too low for an induction of ecdysis in the AZ-A-treated nymphs (Garcia et al. 1987, 1991). The inhibition of molting was fully reversed by ecdysone therapy (Garcia et al. 1987). Long-term experiments with 4 feedings on AZ-free blood after ingestion of single dose of AZ were performed with 4th-stage nymphs and adult females. Only in the nymphs treated with low-dose AZ at 0.01 and 0.1 μg/ml blood, was development partially restored; after a single 1.0 μg/ml blood treatment, about 50% of the treated nymphs were still alive 120 days posttreatment without any adult emergence (Garcia et al. 1990a).

The susceptibility of triatomine bugs to AZ varies from species to species. The ED$_{50}$ for molting inhibition by injected AZ-A in 4th-stage nymphs of all the test triatomines, *Triatoma vitticeps* Stal, *Triatoma pseudomaculata* Correa and Spinola, *Triatoma maculata* Erichson, *Triatoma brasiliensis* Neiva, *Triatoma lecticularis* Stal, *Triatoma matogrossensis* Leite and Barbosa, *Triatoma infestans* Klug, *R. prolixus, Rhodnius neglectus* Lent, *Rhodnius robustus* Larrousse, *Panstrongylus megistus* Burmeister, and *Panstrongylus herrera* ranged from 10 to 25 ng/nymph (Garcia et al. 1989b). This mode of administration, although of physiologic interest, has no practical application. The contact mode of entry will be more relevant to control objectives than injection or feeding per os.

Regarding the growth regulation mechanism of action of AZ, Garcia et al. (1990b) proposed the inhibition of PTTH synthesis and release in the treated animals. Azadirachtin-A (1.0 μg/ml), if fed to last-stage nymphs of *R. prolixus* through a blood meal, affected PTTH production, as shown by a reduction or absence of ecdysteroid titers in decapitation experiments. The host nymphs, when decapitated 5 days after feeding, showed a steady decline of ecdysteroid titers in hemolymph, whereas untreated nymphs at this stage maintained their molting hormone titer. Head transplantations from untreated donors 4–5 days after feeding to the headless nymphs sustained hormone production for about 18 h. Heads from AZ-treated donors were unable to sustain a constant ecdysteroid level in this case, and levels declined immediately after transplantation. In converse experiments, head transplantation from untreated donors 5 days after feeding stimulated the production of ecdysteroids in

AZ-treated recipients (40 days after AZ-A treatment) for about 18 h. Therefore, it is suggested that the synthesis and release of PTTH was deficient in the AZ-treated animals.

*Reproduction suppression:* Azadirachtin, like precocene, is an effective inhibitor of reproduction in *R. prolixus,* drastically affecting the oogenesis process and egg deposition. Precocene-induced sterilization was reversed by application of juvenile hormone III; however, the effect of AZ on reproduction was not reversed. Ecdysteriod titers in nymphs and adult females were decreased by the treatments. In vitro analysis suggested that precocene and AZ may act directly on the prothorax and ovaries, which produce ecdysteriod (Garcia et al. 1987). Females treated per os had a lower survival and egg deposition rate than the controls; this activity was dosage-dependent (Garcia et al. 1990a). In a more detailed study (Feder et al. 1988), AZ-A given orally through bloodfeeding to *R. prolixus* caused a reduction in oocyte growth and consequently in egg production in a dose-dependent manner. However, egg viability was not affected. A significant correlation between these effects and the titers of both vitellogenin in the hemolymph and vitellin in the ovaries was observed. Ecdysteroid titers in hemolymph and ovaries, as determined by radioimmunoassay, were decreased by this treatment. In vitro analysis suggested that AZ may directly interfere with the production of ecdysteroid in ovaries.

In a more detailed study, the administration of AZ in bloodmeal (1 μg/ml) inhibited phospholipid transfer to the ovaries in *R. prolixus,* but it did not alter tghe availability of yolk protein in the hemolymph. The lipid composition of lipophorin, its concentration in the hemolymph, and its density were normal in AZ-A-treated females. Partial inhibition of phospholipid transfer was observed when lipophorin from AZ-A-treated females was injected into normal females, or when lipophorin from normal females was injected into AZ-A-treated females. The 2 effects were additive, so that the transfer of phospholipids from the lipophorin of AZ-A-treated females to the oocytes of AZ-A-treated females was nearly eliminated. In combination, these effects of AZ-A on phospholipid transfer to the oocytes limited the capacity of AZ-A-treated females to produce eggs (Moreira et al. 1994).

*Immune depression:* The immune system mediated by the hemocytes themselves or the molecules produced by them has been well documented in a number of insects. Azadirachtin (1.0 μg/ml blood), if fed to last-stage nymphs of *R. prolixus,* affected the immune reactivity, as shown by a significant reduction in numbers of hemocytes and nodule formation following challenge with *Enterobacter cloacae,* a reduction in ability to produce antibacterial activities in the hemolymph when injected with bacteria, and a decrease in the ability to destroy infection caused by inoculation with *E. cloacae*

(Azambuja et al. 1991, Azambuja and Garcia 1992). However, unlike other immune reactions, no evidence was found of interference of AZ with the prophenoloxidase-activating system, because melanin production was not reduced when this system was stimulated by trypsin or by the presence of bacteria in the hemolymph (Azambuja et al. 1991). The immune response was suggested to be deficient in the AZ-treated insects.

*Blocking the development of trypanosomes:* Garcia and his colleagues studied the effects of neem treatment on the development of vector-borne pathogens in triatomine bugs. Development of trypanosomes in triatomine bugs has been blocked in a number of species.

Trypanosomes are ingested by triatomine bugs, and the trypomastigotes transform and differentiate into metacyclic trypomastigotes as the blood meal moves through the midgut, which eventually accumulates in the rectum, from where the trypomastigotes are transmitted to vertebrate hosts. Not much is known about the interaction of the parasite with its invertebrate host and the factors triggering parasite development and differentiation. One such factor may be insect hormone titers, which change during the development and reproduction of the insect host (Garcia 1988). When 4th-stage nymphs were treated with precocene (20 μg ethoxyprecocene II/ml of blood meal) or AZ (1 μg AZ-A/ml blood), the development of *Trypanosoma cruzi* Chagas in *R. prolixus* increased in precocene-treated insects and decreased in AZ-treated ones. The effect of AZ could be partially reversed by ecdysone given to the bugs (Garcia 1988). In several more detailed studies, the administration of a single dose of AZ (1.0 μg AZ A/ml blood meal of *R. prolixus*) was able to block the development of *T. cruzi* if given through the blood meal at different intervals, together with, before, or after parasite infection. The same held true with different triatomine species and different strains of *T. cruzi* (Garcia et al. 1989a, 1989b; Rembold and Garcia 1989; Azambuja and Garcia 1992). A single dose of AZ-A was enough to induce permanent resistance of the insect host against infection by *T. cruzi* (Garcia et al. 1991, Azambuja and Garcia 1992) and to block ecdysis for a long time (Garcia et al. 1991).

Gonzalez and Garcia (1992) carried out a long-term comparative study on the effects of AZ on the course of *T. cruzi* infection in different triatomine vector species. In *R. prolixus,* the development of *T. cruzi* clone Dm28c decreased in a dose-dependent manner, and the $ED_{50}$ of this inhibitory effect was 0.25 μg AZ/ml blood. Using this insect, an experiment showed that treatment by AZ at 1.0 μg/ml blood completely blocked the development of *T. cruzi* from 4 subsequent infective meals taken during the 120 days posttreatment. Similarly, complete elimination of *T. cruzi* in feces and urine was also observed over a period of 50 days after infection in insects treated with AZ. Fifth-stage nymphs

of *R. prolixus, T. infestans,* and *Dipetalogaster maximus* Uhler, infected with different strains of *T. cruzi* displayed drastic inhibition of trypanosome development when treated with AZ (Gonzalez and Garcia 1992).

Studies have documented that AZ does not affect *T. cruzi* growth in axenic media, AZ-A-treated infected blood remains infectious, the compound does not interfere with *T. cruzi* infection in mammalian hosts, and ecdysone and juvenile hormone in vitro do not interfere directly with the development of *T. cruzi.* Thus, the direct effect of these compounds on *T. cruzi* could be excluded. Therefore, it is suggested that the extensive physiologic and biochemical changes in the neuroendocrine system and gut have been suggested to create a microenvironment that was no longer suitable for the survival and development of trypanosomes, and resulted in failure of infection in vector (Garcia 1988, Garcia et al. 1992). Further studies are needed as to how neem or other products might be used in blocking trypanosome growth and reproduction in triatomine vectors in a practical manner.

## Cockroaches

Antifeedancy, IGR type of activity, and reduced biological fitness have been shown in several species of cockroaches through oral or topical administration of AZ or commercial formulations. Qadri and Narsaiah (1978) studied the effects of AZ on the molting processes of last-stage nymphs of *Periplaneta americana* L. The 24-h $LD_{50}$ of AZ for last-stage male and female nymphs was 1.5 mg/g body weight. Azadirachtin at 0.75 mg/g body weight delayed molting from the 23rd to the 38th day without a toxic effect. A positive correlation existed between the concentration of Az and histopathologic changes in hemocytes and levels of hemolymph proteins, cholesterol, and nucleic acids. Disruptive and vacuolated changes were characterized both in cytoplasmic and nuclear regions of hemocytes. Reductions as much as 20–25% were observed in the hemocyte population 24 h after injection; a 25–40% reduction in albumin and globulin levels occurred 1–24 days after injection; a 15–45% reduction was found in cholesterol levels; and a 7–10% reduction in RNA and DNA levels in hemolymph occurred 1–6 days after injection. Margosan-O, a commercial preparation of 40% neem seed extract in 95% ethanol (340 μg AZ/ml), was tested for its effects as a repellent, toxicant, or growth inhibitor against 6 species of cockroach: *Blatta orientalis* L., *Blattella germanica* L., *Byrsotria fumigata* Guerin-Meneville, *Gromphadorhina protentosa* Schaum, *P. americana,* and *Supella longipalpa* F. Last-stage nymphs of these species fed on Lab-Chow pellets impregnated with neem extract at a rate of 0.5 ml/pellet had increased mortality and retarded development. All 1st-stage nymphs of *B. orientalis, B. germanica,* and *S. longipalpa* died after continu-

ously consuming treated Lab-Chow pellets during a 24-wk observation period. Topical application of Margason-O to the abdomen of last-stage nymphs of *B. orientalis* at 2 μl/nymph, or injection of this material at 0.5 μl/nymph, resulted in reduction of growth and increased mortality. However, placing 1st-stage *Blatta orientalis* nymphs on a surface treated with the neem extract had no notable effect (Adler and Uebel 1985). The $LD_{50}$ of a neutral fraction of winter neem leaves (mixture of triterpenoids) against 4th-stage nymphs of *B. germanica* was 0.5 μg/individual. This treatment caused inhibition of cholinesterase (39%), of acid phosphatase (62%), and of alkaline phosphotase (74%) (Naqvi 1987).

Recently, Prabhakaran and Kamble (1996) studied the toxicity of technical grade AZ (11.46% AI) against insecticide-resistant and -susceptible strains of *B. germanica* by injection and diet incorporation. The effect of AZ was dose-dependent. The $LD_{50}$ and $LD_{90}$ for 3 strains ranged between 2.49 and 2.56 μg per insect and 6.49 and 6.92 μg per insect, respectively. Incorporation of AZ in food pellets resulted in median lethal times ($LT_{50}$s) of 22–23 days for different strains. No significant differences were observed in toxicity of AZ to either insecticide-resistant or -susceptible strains. The body weight of test animals varied with time depending upon the dosage administered. The cockroaches injected with 2 and 3 μg of AZ showed a continuous weight reduction. The toxicity of AZ to the cockroach may be associated with altered feeding behavior and disruption of hormonal events.

## Fleas

Fairly good results were achieved in flea control by neem products. Fourth-stage larvae of laboratory-reared *Ctenocephalides felis* Bouche and *Xenopsylla brasiliensis* Baker were exposed to 50% NSKEs incorporated into the culture medium for periods varying from 30 min to 24 h. Mortality of larvae was observed for exposure periods of 2 h and longer in both species. At 24 h of exposure, 95.3 and 94.7% of *C. felis* and *X. brasiliensis,* respectively, were killed. The $LT_{50}$s were approximately 6 h and 10.5 h for *C. felis* and *X. brasiliensis,* respectively. It was generally concluded that NSKEs were potentially larvicidal against the 2 flea species and that NSKEs could be effectively used for controlling the insects by properly dusting the breeding sites with appropriate concentrations of the extract (Kilonzo 1991). Chamberlain et al. (1988) tested 31 different compounds against larvae of the oriental rat flea, *Xenopsylla cheopis* Rothschild. These 31 compounds represented 6 chemical groups: organophosphates, carbamates, pyrethroids, diphenylureas, dodecadienoates, and sulfonates, plus a miscellaneous group including AZ. The inhibitory effect $IE_{50}$ dose of AZ was as low as 0.31 ppm.

## Lice

Azadirachtin was also tested to control the sheep biting louse *Bovicola ovis* Schrank. A variety of insecticidal substances (considered acceptable by Organic Production Standards) (New Zealand) such as AZ, pyrethrum, and soap, were applied to louse-infested sheep and their efficacies compared with that of a commercial formulation of cypermethrin. The sheep treated with AZ and pyrethrum had significantly fewer lice than either the control or soap-treated sheep over the 48-day period of the trial. Both AZ and pyrethrum were as effective as cypermethrin. Control (reduction in louse score) of 85.0–100% was achieved over the period of the trial (Heath et al. 1995).

## Sand flies

The landing and bloodfeeding repellent action of neem oil applied to human skin was evaluated against sand flies under laboratory and field conditions. Concentrations of 2% neem oil mixed in coconut or mustard oil provided 100% protection against *Phlebotomus argentipes* Ann and Brun throughout the night under field conditions. Under laboratory conditions, 2 and 5% neem oil provided 100% protection from the bites of *Phlebotomus papatasi* Scopoli up to a mean of 7 h 20 min ± 34 min and 7 h 54 min ± 25 min, respectively (Sharma and Dhiman 1993).

## Ticks

Little work has been done on the activity of neem products against hard and soft ticks. In a few studies, the relationship between salivary gland degeneration and levels of ecdysteroids as well as effects of AZ on this relationship were elucidated in ixodid ticks. Injection of the ecdysteroid inhibitor AZ (up to 100 μg/g body weight) into engorged female *Amblyomma americanum* L. inhibited the onset of oviposition by a few days and the rate of oviposition during the next 6 days, but did not significantly inhibit vitellogenesis or reduce total egg production. The salivary gland degeneration probably caused by ecdysteroid could not be prevented by the application of AZ, which implied that ticks possess at least one AZ-insensitive ecdysteriod system (Kaufman 1988, Lindsay and Kaufman 1988).

## CONCLUSIONS

Considerable information has been gathered during recent years on the insecticidal properties of botanical pesticides, especially neem products. The "village pharmacy" philosophy of use of neem products in India and elsewhere where they have been used for its antiseptic, medicinal, and insecticidal properties for many years, has now developed into the "neem technology" approach where-

by the neem tree is being cultured and promoted in tropical and subtropical areas of the world in reforestation programs as source of wood, fertilizer, cattle feed, oil, shade, medicinals, and a source of environmentally friendly insecticides for worldwide use (Koul et al. 1990, Mordue and Blackwell 1993, Schmutterer 1995). Azadirachtin and other bioactive compounds from neem extracts can exert multiple actions affecting feeding, growth and development, reproduction, or even the development of vector-borne pathogens in their vectors. Neem products are degradable in the environment and are relatively safe to nontarget organisms. No cases of resistance to neem products have been reported. Lack of resistance is due to the complexity of the contained components and multiple modes of action in insects. Equally important, the neem tree is adapted to vast areas in tropical and subtropical countries. This tree and its products provide an abundant resource for the manufacturing of a variety of products having industrial, medicinal, and agricultural applications. The costs of planting and managing neem trees and producing neem insecticides are relatively low.

The production and application of neem insecticides have several drawbacks. First, even though considerable effort has been made to synthesize the predominant insecticidal component in neem products, we are still a long way from achieving economic synthesis, because the chemical structure of AZ is too complicated. To date, the manufacturing of neem products is still dependent on the natural resources that are renewable, thus providing for a sustainable source of the bioactive agents at a relatively modest cost. Fortunately, the research by Allan et al. (1994) and Wewetzer (1998) has shown that callus from leaves of the neem tree produce natural AZ when grown in defined media. Second, neem products, like many other natural products, show a limited spectrum of persistence under field conditions because of UV light, temperature, pH, and microbial degradation. The UV absorber, 2,4-dihydroxybenzophenone added to AZ-A at a concentration of 1:1 (w/w) could increase the half-life of AZ-A (Sundaram 1996). Finally, the delayed effects and relatively low degree of efficacy (if not applied in high doses) of neem products may irritate users accustomed to synthetic insecticides with a broad-spectrum of activity and strong contact efficacy.

Compared with the amount of effort and application technology of neem products in agricultural pest control, the developmental effort in medical and veterinary entomology is meager. Regarding the activity and application of neem products, especially of AZ against mosquitoes, flies, and other insects of medical and veterinary importance, further research is needed on the practical use of AZ products in disease vector control programs. Studies are necessary to determine effects on feeding behavior; the mechanism of growth regulation, that

is, the interference of AZ and related products with ecdysone and juvenile hormone levels; effects on reproduction including spermatogenesis, mating, vitellogenesis, oogenesis, oviposition, and the hatching ability of the eggs; effects on metabolism; effects on aspects of biological fitness; effects on the development of vector-borne pathogens (which only has been studied in triatomine bugs); and the possibility of resistance development. Detailed laboratory and field studies on a variety of neem products, especially those that are commercially available and developed for agricultural pest control, will facilitate the practical use of AZ applications for the control of arthropods of medical and veterinary importance.

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