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Ecosmart Biorational Insecticides: Alternative Insect Control Strategies

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1. Introduction

Pest insects can damage agricultural crops, consume and/or damage harvested food, or transmit diseases to humans and animals. The past 30 years has witnessed a dramatic re-emergence of epidemic vector-borne diseases throughout much of the world (Atkinson, 2010). Prior to the development and commercial success of synthetic insecticides in the mid-1930s to 1950s, botanical insecticides were the foremost weapons against insect pests. The synthetic insecticides (organochlorines, organophosphates, carbamates and later the pyrethroids and neonicotinoids) are characterized by efficacy, speed of action, ease of use, and low cost. Accordingly, they drove many natural control methods, such as using of botanicals, predators, and parasitoids to near obscurity. Twenty years after synthetic insecticides were overzealously entrenched in 'modern' agricultural production; they induce widespread environmental contamination, toxicity to non-target organisms, development of resistance against insecticides, and negative effects on animal and human health (Pretty, 2009). Consequently, there is an urgent need to explore and utilize naturally occurring products for combating pests.

The terms "biorational pesticide" and "biopesticides" are gaining popularity in the current climate of environmental awareness and public concern. Both terms are derived from two words, "biological" and "rational", referring to pesticides that have limited or no adverse effects on the environment, non-target organisms including humans. Biorational insecticides include: biochemicals insecticides (botanicals, insect growth regulators, insect pheromones, photoinsecticides, and inorganics); biological insecticides, using of natural enemies such as parasitoids, predators, nematodes, and pathogens (virus, bacteria, fungi, or protozoa); and transgenic insecticides (genetically modified plants or organisms). Natural enemies play an important role in limiting potential pest populations and they are more likely to survive in case of application of ecofriendly biopesticides. Approaches to the biological control of insects include: conservation of existing natural enemies; introducing new natural enemies and establishing a permanent population (called "classical biological control"); and mass rearing and periodic release, either as a seasonal introduction of a small population of natural enemies, or a massive, "inundative" release. In developing countries, biopesticides offer unique and challenging opportunities for exploration and development of their own biorational insecticides. Nanotechnology has become one of the most promising

new technologies in the recent decade for protection against insect pests. Such technology will revolutionize agriculture including pest management in the foreseeable future.

Integrated pest management (IPM) is the use of all available means to maintain pest populations below levels that would cause economic loss while minimally impacting the environment. Several tactics could be utilized in IPM programs as chemical, cultural, physical, and biological control (Vreysen et al. 2007). The introduction of more effective biorational products through IPM programs will reduced rates of chemical pesticides and prevent, or at least delay the development of resistance in target pests to both chemical pesticides and biopesticide toxins. Flourishing of organically produced food in the developed world facilitates greater farmer acceptance of biopesticides as the sales of organically produced food are increasing at a significantly faster rate than sales of any other food commodity. Consequently, biorational insecticides will dominate the market of pesticides in the near future. Here, I am concerned about control of insects and arachnids (ticks and mites) of agricultural, medical, and veterinary importance and referred to them as insects or insect pests. The words “biorational” and “biopesticide” as well as “pesticides” and “insecticides” are used interchangeably throughout this chapter. Finally, I review current biorational insecticides and their mode of actions, uses, commercial products, and safety concerns.

2. Biochemical control

2.1 Botanical insecticides

The practice of using plant derivatives or botanical insecticides in agriculture dates back at least two millennia in ancient Egypt, India, China, and Greece. In Europe and North America, the documented use of botanicals extends back more than 150 years, dramatically predating discoveries of the major classes of synthetic chemical insecticides beginning in the 1940s.

2.1.1 Traditional botanical insecticides

2.1.1.1 Pyrethrum

Pyrethrum is one of the oldest and safest insecticides. The ground, dried flowers of *Tanacetum cinerariaefolium* (Asteraceae) were used in the early 19th century to control body lice during the Napoleonic Wars. Pyrethrum contains three esters of chrysanthemic acid and three esters of pyrethric acid. Among the six esters, those incorporating the alcohol pyrethrolone, namely pyrethrins I and II, are the most abundant and account for most of the insecticidal activity. Technical grade pyrethrum, the resin used in formulating commercial insecticides, typically contains from 20% to 25% pyrethrins (Casida & Quistad, 1995). Recently, Australia produces almost one-half of the world supply and produces a technical grade material comprising 50% pyrethrins by weight. Pyrethrins affect the insect on contact, creating disturbances in the nervous system which eventually result in convulsions and death. Pyrethrin acts on insects with phenomenal speed causing immediate paralysis, notably in flying insects, some of which are immobilized within 1 s. It blocks voltage-gated sodium channels in nerve axons. The mechanism of action of pyrethrins is qualitatively similar to that of DDT and many synthetic organochlorine insecticides. Pyrethrums are mixed with a synergist such as piperonyl butoxide (PBO) to increase insect mortality and to extend their shelf life. In purity, pyrethrins are moderately toxic to mammals, but technical

grade pyrethrum is considerably less toxic (Casida & Quistad, 1995). Major uses of pyrethrum are for structural pest control, in public health, and for treatment of animal premises. Pyrethrins have limited use outdoors as they are especially labile in the presence of the UV component of sunlight (Ware & Whitacre, 2004). Pyrethrum products represent 80% of the total market of global botanical insecticides (Isman, 2005) and are favored by organic growers because of their low mammalian toxicity and environmental non-persistence making it among the safest insecticides in use. For more information about pyrethrum, see Taylor (2001), Collins (2006), and Gilbert & Gill (2010).

2.1.1.2 Other traditional botanicals

A handful of other plant materials have seen limited commercial use as insecticides and their uses are in decline, such as *sabadilla*, a powder based on the ground seeds of the South American plant *Schoenocaulon officinale*; Wood of the Caribbean tree *Ryania speciosa*; *Quassia amara*, a small tree from Brazil; woodchips and ground bark of this species have been used traditionally as an insecticide, as have plant parts from the related tree, *Ailanthus altissima*; rotenone, an isoflavonoid obtained from the roots or rhizomes of tropical legumes in the genera *Derris*, *Lonchocarpus*, and *Tephrosia*. Rotenone is used as insecticide and mainly a fish poison to paralyze fish, causing them to surface and be easily captured, but there is a growing concern about its safety and its relation to Parkinson's disease (Betarbet et al., 2000). For more niceties about traditional botanical insecticides, see Ware & whitecare (2004), Isman (2005, 2006, 2010), Isman & Akhtar (2007), Gilbert & Gill (2010), Kumar et al (2010), Dubey (2011), and Mehlorn (2011).

2.1.2 Newer botanical insecticide "Neem"

Neem (*Azadirachta indica* A. Juss: Meliaceae) is a large, evergreen, hardy tree, native to the Indian sub-continent and well known their as the ' Botanical Marvel', It is an old and new insecticide. The Indians used neem, from prehistoric times, primarily against household and storage pests, and to some extent against pests related to field crops. In addition, they traditionally burn neem leaves in the evening to repel mosquitoes. It is effective against more than 500 species of insects and arthropods. Neem has attracted global attention recently due to its potential as a source of natural drugs and as environment-friendly pesticides, see Schmutterer (1995), Kumar (2002), Isman et al. (2011), and Mehlhorn (2011) for more fine points.

2.1.2.1 Chemical composition

Neem seeds are a rich storehouse of over 100 tetranortriterpenoids and diverse non-isoprenoids. The neem tree contains more than 100 different limonoids in its different tissues (Isman et al., 1996). Many of them are insect feed deterrents. The highly oxygenated azadirachtin ($C_{35}H_{44}O_{16}$), a nortriterpenoid belonging to the lemonoids, is the most biologically active constituent of neem. Azadirachtin has shown bactericidal, fungicidal, and insecticidal properties, including insect growth regulating qualities (Ware & Whitacre, 2004). It is systemic in nature, absorbed into the plant and carried throughout the tissues, being ingested by insects when they feed on the plant. Thus, it is effective against certain foliage-feeders that cannot be reached with spray applications. In general, chewing insects are affected more than sucking insects and insects that undergo complete metamorphosis are also generally affected more than those that do not undergo metamorphosis (Dubey, 2011).

2.1.2.2 Mode of action

The effects of azadirachtin on insects include feeding and oviposition deterrence, growth inhibition, and fecundity and fitness reductions (Schmutterer, 1990). Azadirachtin is a common example of a natural plant defense chemical affecting feeding, through chemoreception (primary antifeedancy), that consists in the blockage of the input from receptors that normally respond to phagostimulants, or from stimulation of specific deterrent cells or both (Dethier, 1982) and through a reduction in food intake due to toxic effects if consumed (secondary antifeedancy), where food intake is reduced after application of azadirachtin in ways which bypass the mouth part chemoreceptors (Mordue & Blackwell, 1993). The antifeedant effect is highly variable among pest species, and even those species initially deterred are often capable of rapid desensitization to azadirachtin (Bomford & Isman, 1996). Azadirachtin is a tetranortriterpenoid, structurally similar to insect hormones “ecdysones”, its biological activity as ecdysone-blocker thus disturbing insect growth. This substance interferes with synthesis of the insect molting hormone, α -ecdysone, as well as other physiologically active neuropeptides in insects, producing a wide range of physiological and behavioral effects, such as anorexia. It also leads to sterility in female insects due to its adverse effects on ovarian development, fecundity, and fertility. For more information about the mode of action of neem, see Isman and Akhtar (2007) and Insect growth regulators below.

2.1.2.3 Safety

Azadirachtin is nontoxic to mammals. Different neem products were neither mutagenic nor carcinogenic, and they did not produce any skin irritations or organic alterations in mice and rats, even at high concentrations. The pure compound azadirachtin, the unprocessed materials, the aqueous extracts and the seed oil are the most safe to use as an insecticide to protect stored seeds for human consumption (Boeke et al., 2004). Ecologically, azadirachtin is non toxic to fish (Wan et al., 1996), natural enemies and pollinators (Naumann & Isman, 1996), birds, other wild life, and aquatic organisms as azadirachtin, breaks down in water within 50–100 h. It is harmless to non-target insects (bees, spiders, and butterflies). The effect of azadirachtin on natural enemies is highly variable (Hohmann et al., 2010, Kumar et al., 2010). Environmentally, azadirachtin induce no accumulations in the soil, no phytotoxicity and accumulation seen in plants, and no adverse effect on water or groundwater (Mehlhorn, 2011). Neem is sensitive to light and the half-life of azadirachtin is only one day (Kleeberg, 2006), leaving no residues on the crop and therefore are preferred over chemical pesticides. Azadirachtin is classified by the Environmental Protection Agency (EPA) in class IV.

2.1.2.4 Risk factors

The most critical adverse effects are reproduction disturbances, although these are often reversible. (Boeke et al., 2004). Neem pollen induces allergenic effect to some individuals (Karmakar & Chatterjee, 1994). Moreover, the oil can turn rancid (De Groot, 1991) and is easily contaminated with aflatoxins, so contaminated neem seeds with aflatoxin should not be picked from the ground but seeds that are greenish yellow in color should be picked from the trees or swept regularly under the tree (Gunaseena & Marambe, 1998). Ecto-endo parasitoids vulnerable to neem but soil application could reduce negative side effects compared to plant spraying and hence improve selectivity (Kumar et al., 2010). Treating the host with neem before parasitism was less deleterious to wasp emergence, especially for *Trichogrammatoidea annulata* (Hohmann et al., 2010). For more details about

safety of neem, see Boeke et al. (2004), Mehlhorn (2011), Kumar et al. (2010), and Homanni et al. (2010).

2.1.2.5 Production

In order to produce and use efficacious neem pesticides, Saloko et al. (2008) reviewed some points that should be notes: neem leaf extracts are less effective than seed extracts due to lower azadirachtin content; neem preparations should be kept away from sunlight to avoid photodegradation of active ingredients by UV light, and formulations are better applied at dusk when sun is weak; sun screens such as Para Amino Benzoic Acid (PABA) could be added to reduce the photo-oxidation of azadirachtin by UV light.

Neem seeds contain 0.2% to 0.6% azadirachtin by weight, so solvent partitions or other chemical processes are required to concentrate to be 10% to 50% as in the technical grade material used for commercial production. World wide, there are over 100 commercial neem formulations such as Margosan-O, Bio-neem, Azatin, , Neemies, Safer's ENI, Wellgro, RD-Repelin, Neemguard, Neemark, and Neemazal. Formulations include emulsifiable concentrates (ECs), suspension concentrates (SCs), ultra low volume (ULV) formulations and granular formulations. The chemistry of azadirachtin was reached in 2007 and its synthesis was completed, see Morgan (2009). Azadirachtin and botanical preparations based on neem seed extracts are environmentally friendly pesticide and virtually non-toxic to mammals and wildlife, making them among the safest of all insecticides that used for integrated pest management and organic farming, For more details about neem, see Collins (2006), Isman & Akhtar (2007), Saloko et al. (2008), Gilbert & Gill (2010), Dubey (2011), and Regnault-Roger (2011).

2.1.3 Essential oils

Aromatic oils obtained through steam distillation of many plant families, ex. Myrtaceae, Lamiaceae, Asteraceae, Apiaceae, and Rutaceae are highly targeted for anti-insect activities against several insect orders. Approximately 3000 essential oils are known, and 10% of them have commercial importance in the cosmetic, food, and pharmaceutical industries. They are generally recognized as safe, GRAS, by the US Food and Drug Administration. Complete essential oils are more effective than individual constituents or even a combination of constituents.

2.1.3.1 Essential oil chemistry

The volatile components of essential oils can be classified into four main groups: terpenes, benzene derivatives, hydrocarbons, and other miscellaneous compounds. The major constituent of some oils are 8-cineole from rosemary (*Rosmarinus officinale*) and eucalyptus from (*Eucalyptus globus*); eugenol from clove oil (*Syzygium aromaticum*); thymol from garden thyme (*Thymus vulgaris*); and menthol from various species of mint (*Mentha* species). More information about essential oil chemistry is given by Isman (2006) and Tripathi et al. (2009).

2.1.3.2 Mode of action

Aromatic plants produce many compounds that act as ovicidal, larvicides, adulticides, insect arrestants and repellents or act to alter insect feeding behavior, growth and development, ecdysis (molting) and behavior during mating and oviposition.

Essential oils are lipophilic in nature and interfere with basic metabolic, biochemical, physiological, and behavioral functions of insects. Commonly, essential oils can be inhaled,

ingested or skin absorbed by insects. The rapid action against some pests is indicative of a neurotoxic mode of action, and there is evidence for interference with the neuromodulator octopamine (Enan, 2005) or GABA-gated chloride channels (Priestley et al., 2003).

Several essential oil compounds have been demonstrated to act on octopaminergic system of insects. Octopamine is a neurotransmitter, neurohormone, and circulating neurohormone – neuromodulator (Hollingworth, et al., 1984) and its disruption results in total break down of nervous system in insects. The lack of octopamine receptors in vertebrates likely accounts for the profound mammalian selectivity of essential oils as insecticides. Eugenol mimicked octopamine in increasing intracellular calcium levels in cloned cells from the brain of *Periplaneta americana* and *Drosophila melanogaster* (Enan, 2005). Consequently, octopaminergic system of insects represents a biorational target for insect control. Plant volatile oils have long been known to affect the behavioural responses of pests, with the monoterpenoid components appearing most useful as insecticides or antifeedants (Palevitch & Craker, 1994). LMW terpenoids may be too lipophilic to be soluble in the haemolymph after crossing the cuticle, and proposed a route of entry through the tracheae (Veal, 1996). Most insecticides bind to receptor proteins in the insect and, in doing so; they interrupt normal neurotransmission, which lead to paralysis and subsequently death. Recent evidence suggests that low-molecular-weight (LMW) terpenoids may also bind to target sites on receptors that modulate nervous activity. Ionotropic, γ -aminobutyric acid, GABA receptors, the targets of organochlorine insecticides lindane and dieldrin, are modulated by LMW terpenoids with vastly different structures (Priestley et al., 2006). Valuable appraisals about the mode of action are those of Price & Berry (2006), Isman (2006, 2010); Tripathi et al. (2009); and Dubey (2011). Some essential oils have larvicidal effect and the capacity to delayed development and suppress adults emergences and induce abnormalities during development of insects of medical and veterinary importance, Fig (1-5) (Khater, 2003; Shalaby & Khater, 2005; Khater & Shalaby, 2008; Khater & Khater (2009); Khater et al., 2009, 2011).

2.1.3.3 Repellent effect

Repellents are substances that provide a vapor barrier deterring the arthropod from coming into contact with the surface or flying to, landing on or biting human or animal skin. The use of insect repellent compounds dates back to ancient times as plant oils, smokes, tars, etc. were used to displace or kill insects. The use of repellents by travelers may reduce infection with local diseases in temperate areas. DEET (N,N-diethyl-m-toluamide) is a broad spectrum repellent and the most effective and persistent on skin. Unfortunately, it may cause environmental and human health risks (Pitasawat et al., 2003). Therefore, there has been an increase in search efforts for natural and eco-friendly repellents.

2.1.3.3.1 Plant-based repellents

Some plant-based repellents are comparable to, or even better than synthetics; however, essential oil repellents tend to being short-lived in their effectiveness due to their volatility. Nerio (2010) review some splendid ideas for improvement of repellency of essential oils. Repellency assays with essential oils were done for Diptera species, especially mosquitoes and to a lesser extent to coleopteran insects related to losses in stored food. Plants with strong smell, such as French marigold and coriander act as repellents and can protect the corps nearby. Several essential oil- producing plants have been widely studies, such as *Cymbopogon* spp., *Eucalyptus* spp., *Ocimum* spp., the osage

orange (hedgeapple) (*Maclura pomifera*), and catnip (*Nepeta cataria*). Several plant oils or their constituents have been commercialized as insect repellents in the past decade, such as soybean, lemon grass, cinnamon, and citronella. Neem oil, from *A. indica*, when formulated as 2% in coconut oil, provided complete protection (i.e. no confirmed bites) for 12 hours from *Anopheles* mosquitoes (Sharma et al., 1993). Essential oils have pronounced *In vitro* and *In vivo* pediculicidal activity as the number of lice infesting water buffaloes in Egypt was significantly reduced 3, 6, 4, and 6 days after treatment with the essential oils of camphor (*Cinnamomum camphora*), peppermint (*Mentha piperita*), chamomile (*Matricaria chamomilla*), and onion (*Allium cepa*), respectively. Surprisingly, the same oils repelled flies (*Musca domestica*, *Stomoxys calcitrans*, *Haematobia irritans*, and *Hippobosca equine*) infecting buffaloes for almost 6 days post-treatment. No adverse effects were noted on either animals or pour-on operators after exposure to the applied oils (Khater et al., 2009).

2.1.3.3.2 Metabolites reliable for repellent activity

Nerio et al. (2010) reviewed the repellent activity of essential oils which contributed to some metabolites, such as monoterpenes (α -pinene, cineole, eugenol, limonene, terpinolene, citronellol, citronellal, camphor, and thymol) against Mosquitoes (Yang et al., 2004) sesquiterpenes, β -caryophyllene, repellent against *A. aegypti*; phytol, a linear diterpene alcohol, against *Anopheles gambiae*; and phenylethyl alcohol, β -citronellol, cinnamyl alcohol, geraniol, and α -pinene, isolated from the essential oil of *Dianthus caryophyllum*, against ticks (*Ixodes ricinus*). In addition, cineole, geraniol and piperidine found in bay leaves (*Laurus nobilis*, Lauraceae) possess repellent properties towards cockroaches. Repellents may have an increasingly important role in eliminating insects from certain environments and essential oils could play a major role in new repellent technology. Valuable review on the repellent activity of essential oils are those of Tripathi et al. (2009), Isman (2010), Kumar et al. (2010), Nerio et al. (2010), Dubey (2011), and Maia & Moore (2011).

2.1.3.4 Fumigant

Today, The used fumigants, for instance, phosphine, methyl bromide, and DDVP (2,2-dichlorovinyl dimethyl phosphate) do have adverse effects. Phosphine is the major cause of suicidal deaths in India. Methyl bromide has ozone-depleting potential (UNEP, 2000) and DDVP has a possible human carcinogen potential (Lu, 1995). Thus, there is an urgent need for development of safe alternative that have the potential to replace the toxic fumigants against pests attacking grains, dry stored food, and other agricultural products. The active principles are monoterpenes, sesquiterpenes and their biogenically related phenols. In addition to direct toxicity to insects, many of these substances are deterrents or repellents. Essential oils of *Artemisia* species, *Anethum sowa*, *Curcuma long*, and *Lippia alba*. Clove, rosemary, thyme, eucalyptus and various mint species have demonstrated contact and fumigant toxicity to a wide spectrum of insects, including human head lice (Tolosa et al., 2008). Isolates like d-limonene, carvones and 1,8-cineole have been well documented as fumigants. The exact mode of action of these oils as fumigant is unknown, but the oils mainly act in the vapour phase via respiratory system. Physical properties of essential oils such as high boiling point, high molecular weight and low vapor pressure are barriers for application in large scale fumigation. For more details about fumigants, see Tripathi et al., (2009), Isman (2010), and Dubey (2011).

2.1.3.5 Commercialization

Although essential oils are effective when freshly applied, their protective effects usually dissipate relatively quickly. In their review, Nerio et al. (2010) discussed methods to access repellency effects, the synergistic phenomena of such oils and some novel ideas to increase the repellent efficiency. Some fixative materials such as liquid paraffin, vanillin, salicylic acid, mustard, and coconut oils have been used. Formulations based on creams, polymer mixtures, or microcapsules for controlled release, resulted in an increase of repellency duration. Still, essential oils can be incorporated with polymers into sheets and attractant adhesive films with essential oils were prepared to control insects in agriculture and horticulture. Novel ideas are needed to be explored for better commercialization of essential oil-based pesticides. Several essential oil constituents are already in use as an alternative to conventional insecticides, such as Green Ban® (containing oils of citronella, cajuput, lavender, safrole free sassafras, peppermint, and bergapten free bergamot oil); Buzz Away® (containing oils of citronella, cedarwood, eucalyptus, and lemongrass); Valero™, a miticide/fungicide for use in grapes, berry crops, citrus, and nuts; and Cinnamite™, an aphidicide /miticide/fungicide for glasshouse and horticultural crops. The last two products are based on cinnamon oil, with cinnamaldehyde (30% in EC formulations) as the active ingredient. In addition, d-limonene is an active ingredient of commercially available flea shampoos, plus pulegone and citronellal are used as mosquito repellents.

2.1.3.6 Safety of essential oils

Currently, the US Environmental Protection Agency (US EPA) has registered citronella, lemon, and eucalyptus oils as insect repellent ingredients for application on the skin. Using essential oils or some of their products could cause dermatitis, they should be rubbed on a small portion of skin to determine if there will be an allergic reaction before treating your whole body. The most attractive aspect of using essential oils and/or their constituents for pest control is their favorable mammalian toxicity because many essential oils and their constituents are commonly used as culinary herbs and spices. Many of the commercial products including essential oils are included on the GRAS list fully approved by FDA and EPA in USA for food and beverage consumption (EPA, 1993). Some of the purified terpenoid constituents of essential oils are moderately toxic to mammals, but, with few exceptions, the oils themselves or products based on oils are mostly nontoxic to mammals, birds, and fish. Although natural enemies are susceptible via direct contact, predators and parasitoids reinvading a treated crop one or more days after treatment are unlikely to be poisoned by residue contact as often occurs with conventional insecticides. Owing to their volatility, the oils and their constituents are environmentally nonpersistent, with outdoor half lives of 24 h on surfaces, in soil and in water (Isman et al., 2011). There is no harvest restrictions or worker re-entry restrictions for treated crops; they are compatible with biological control agents and indigenous natural enemies of pests, and they bring about reduce risks to honeybees and other foraging pollinators. For additional information about safety of essential oils, see Isman (2006, 2010), Tripathi et al. (2009), Nerio et al. (2010), and Regnault-Roger (2011). Because many conventional pesticide products fall into disfavour with the public, botanical-based pesticides should become an increasingly popular choice for pest control.

2.2 Insect growth regulators

Insect growth regulators (IGRs) are chemical compounds that alter growth and development in insects. They don't directly kill insects, but interfere with the normal

mechanisms of development, resulting in insects dying before they reach adulthood. IGRs are classified into two general categories based on mode of action: chitin synthesis inhibitors and substances interfering with the action of insect hormones.

2.2.1 Chitin synthesis inhibitors

Chitin synthesis inhibitors (CSIs) affect the ability of insects to produce new exoskeletons when molting. They act on the larval stages by inhibiting or blocking the synthesis of chitin which represent 30-60% of the insect exoskeleton structure. They also increase egg mortality. CSIs include conventional benzoylureas, triazine/pyrimidine derivatives, and buprofezin.

2.2.1.1 Benzoylphenylurea

Typical effects benzoylureas or benzoylphenylurea (BPUs) on developing larvae are the rupture of malformed cuticle or death by starvation. BPUs act as ovicides, reducing the egg laying rate or hindering the hatching process by inhibiting embryonic development or failure of hatchability. Commercial products of BPUs include diflubenzuron (Dimilin®, Adept®, Micromite®); triflumuron (Alsystin®); teflubenzuron (Nomolt®, Dart®), hexaflumuron (Trueno®, Consult®); chlorfluazuron (Atabron®); flufenoxuron (Cascade®); and flucycloxuron (Andalin®). Among the newer benzoylureas only hexaflumuron (1993) and novaluron (2001) have been registered by EPA. Studies with diflubenzuron, the most investigated BPU, revealed that it alters cuticle composition, especially inhibition of chitin, resulting in abnormal endocuticular deposition that affects cuticular elasticity and firmness, and cause abortive molting. Diflubenzuron (Dimilin® EI -Delta Company, Egypt) is highly effective in controlling mosquitoes, *Culex pipiens* than house flies, *Musca domestica*. LC 50 values were 1.26 and 1000 ppm, respectively. All treated late 3rd and early 4th larvae of *C. pipiens* (concentrations: 0.04 - 40 ppm) were eventually died as Dimilin® prolonged the larval durations (11.9 days vs. 4 days in the control group) and increased larval abnormalities (46.7%). Such abnormalities were larvae with transparent cuticle, splitting of cuticle, and pharate pupae (Fig. 4). It induces pupal abnormalities as well (Fig. 5). Treatment of *M. domestica* with the same product (at 1ppm) induced larval and pupal malformations reached 23.3 and 56.5%, respectively, and reduce adult emergence (66.7%). Abnormalities of *M. domestica* include small, shrunken, macerated larvae and larvae with weak cuticle as well as distorted puparia and failure of adult eclosion (Khater, 2003) (Fig. 1-3).

2.2.1.2 Triazine/pyrimidine derivatives

2.2.1.2.1 Cyromazine

Cyromazine (Larvadex®, Trigard®), a triazine, is a potent CSI and it is selective toward dipterous species and fed to poultry or sprayed to control flies on animals, in manure of broiler and egg producing operations. It controls blowfly infesting sheep and persist for up to 13 weeks (O'Brien & Fahey, 1991) after a single pour-on application, or longer if applied by dip or shower. Moreover, it is used as a leafminers spray in vegetable crops and ornamentals. Cyromazine may inhibit growth or expansion of the body wall (or both) sufficiently to prevent normal internal growth, producing the observed symptoms and leading to abnormal development. The presence of three resistant house fly populations to cyromazine in Brazilian poultry farms strongly suggests that the operational aspects of larvicide use are important for the development of resistance. Cyromazine is applied as a feed-through, both in Brazil and in the USA, where resistance has already been documented. However, in Denmark, where it was approved only as a topical manure spray, no case of resistance has yet been detected (Pinto & do Prado, 2001).

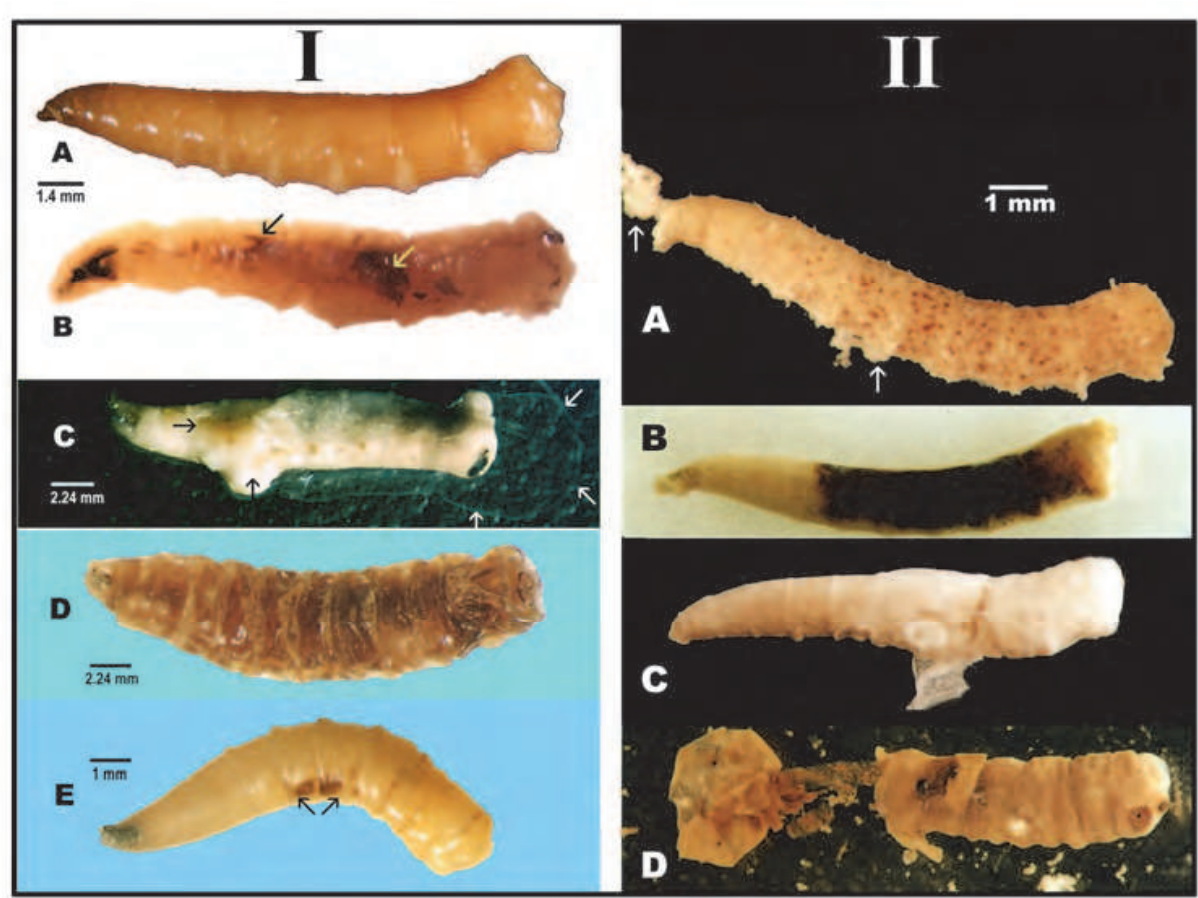


Fig. 1. Morphological malformations of larvae of house flies. I. A. Normal larva. B-E. Malformed larvae, treated with essential oils and insect growth regulators showing signs of pigmentation. C. Macerated larva with weak transparent cuticle. II. Larvae infected with fungi. A. Red Pin -point pigments all over the larval body with apparent fungal growth (arrow heads). B. Larva with diffuse blackish pigmentation. C. Larva with an ulcer in the middle. D. Ulcerated and macerated larva with white nodules and fungal growth.

2.2.1.2.2 *Dicyclanil*

Dicyclanil (ZR ®, ComWin ®), a pyrimidine derivative, is highly active against dipteran larvae and available as a pour-on formulation for blowfly control in sheep in Australia and New Zealand providing up to 20 weeks' protection (Bowen et al., 1999). On the whole,

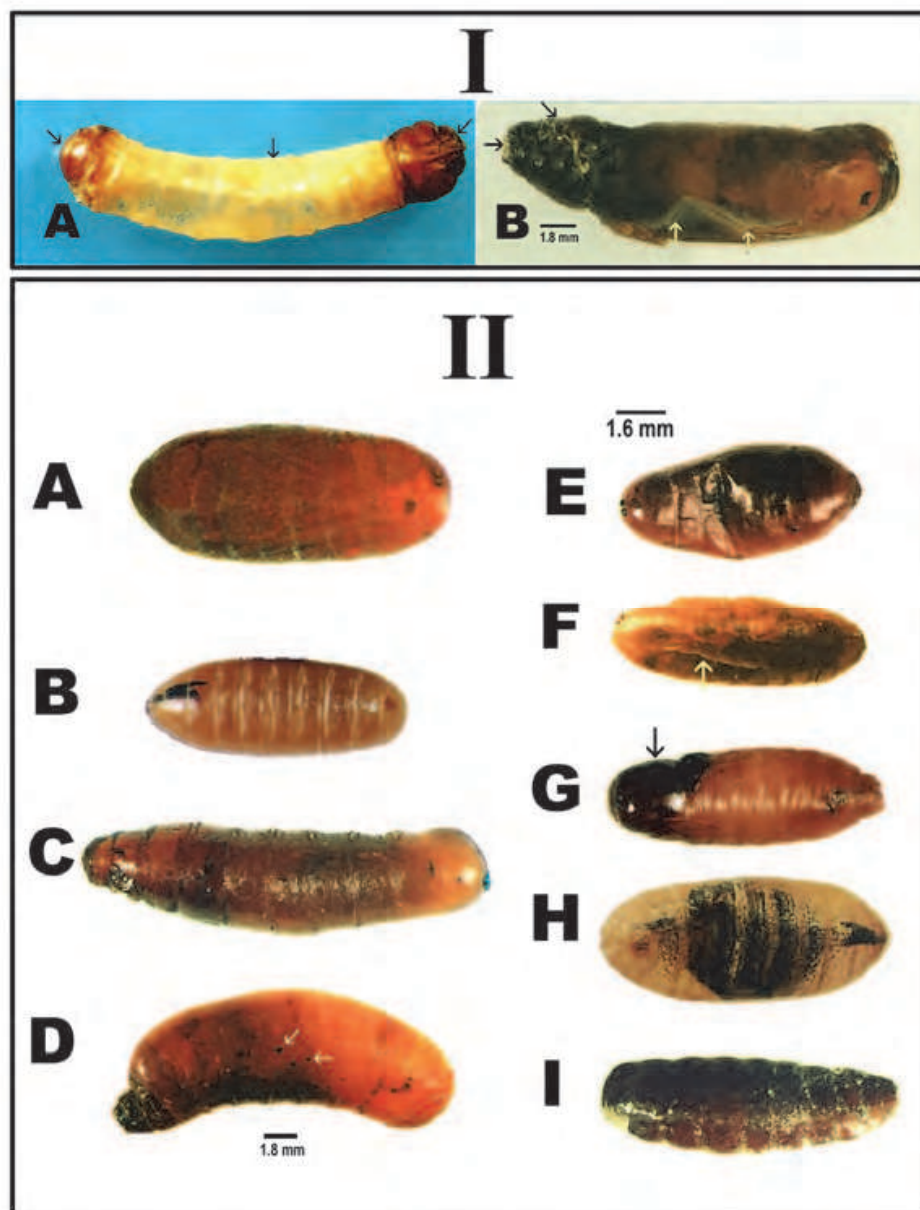


Fig. 2. Pupal abnormalities of house flies: I. Larval pupal- intermediates: A. The anterior and posterior ends as pupae (left and right arrows), while the rest of the body as larva (middle arrow). B. Fungal growth anteriorly (left arrow) and cracked pupal case at the middle (lower arrows). II. Pupae: A. Normal pupa. B. Small pupa with visible cephalopharyngeal skeleton. C. Larviform pupa, pigmented with small dark spots at the intersegmental regions. D. C-shaped pupa with anterior constriction and small patches of black pigments (arrow heads). E. Distorted puparia. F. Pupa with a groove (arrow head). G. Blackish posterior end (arrow head). H. Transparent puparia. I. Hyphal growth appears on a small puparia. Adapted from Khater (2003).

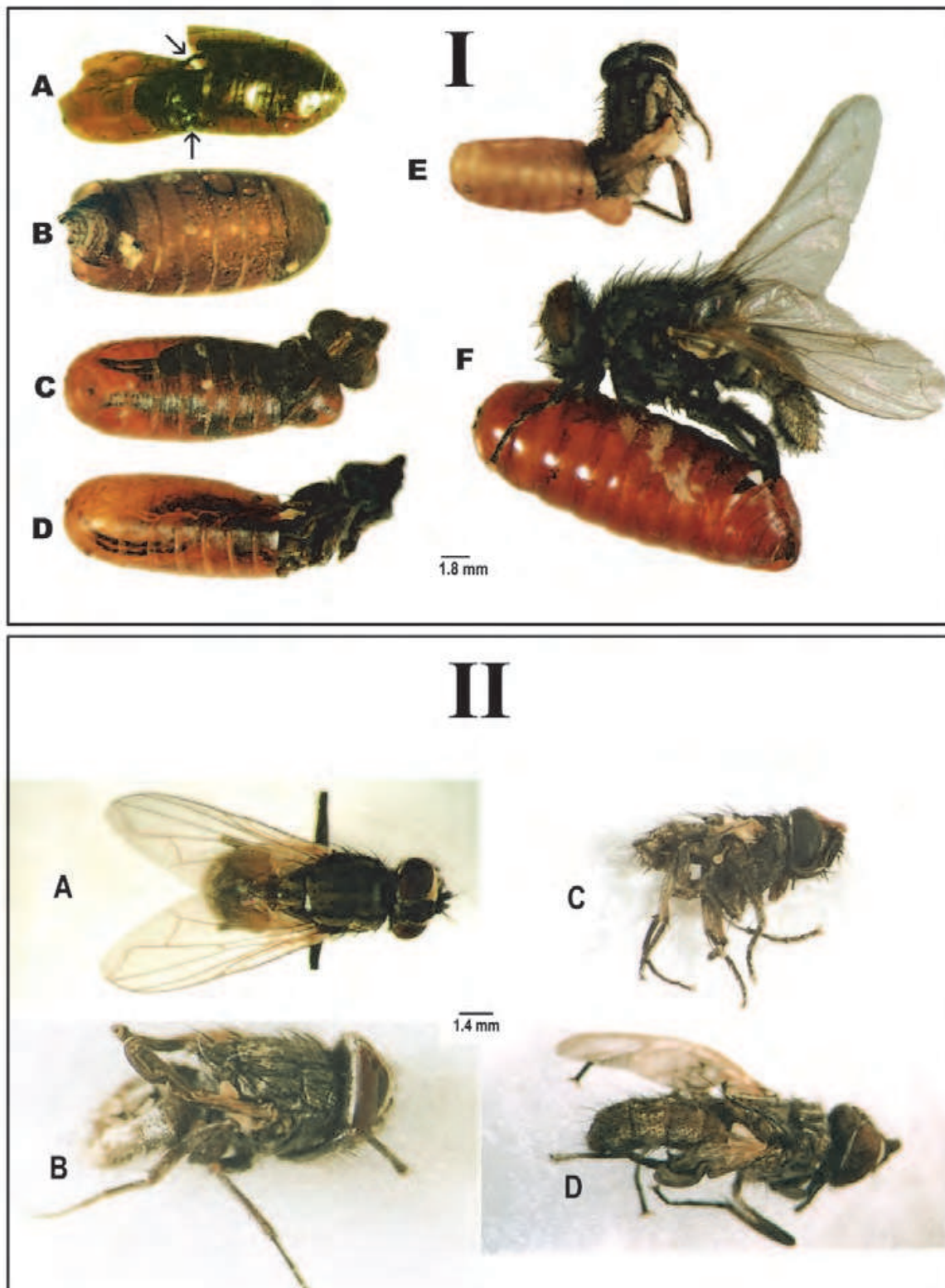


Fig. 3. Morphological abnormalities of house flies. I. (A-F) Failure of adult eclosion. II. Adults (A. Normal adult, B. Crumpled adult with poorly developed wing and legs, C. Small adult, D. Elongated adult with deformed wing, abdomen, and legs. Adapted from Khater (2003).

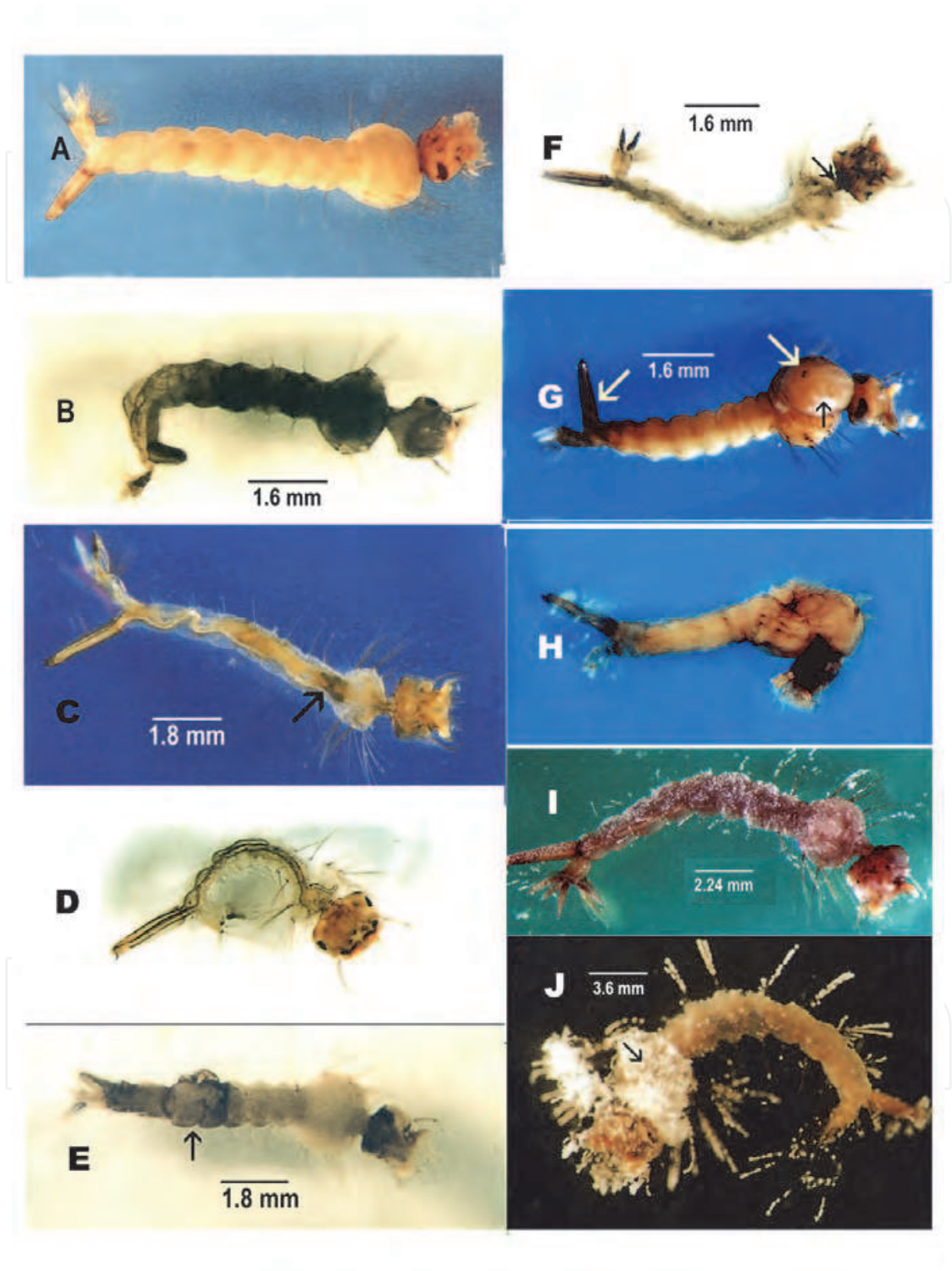


Fig. 4. Morphological abnormalities of mosquito larvae: A. Normal larva, B. Pigmented larva, C-F. Larvae with deformed cuticles, G. Larva with an opaque swelling on the thorax and black coloration at the posterior end, H. Pharate pupa (prepupa), I, J. larvae show symptoms of mycosis. Adapted from Khater (2003).

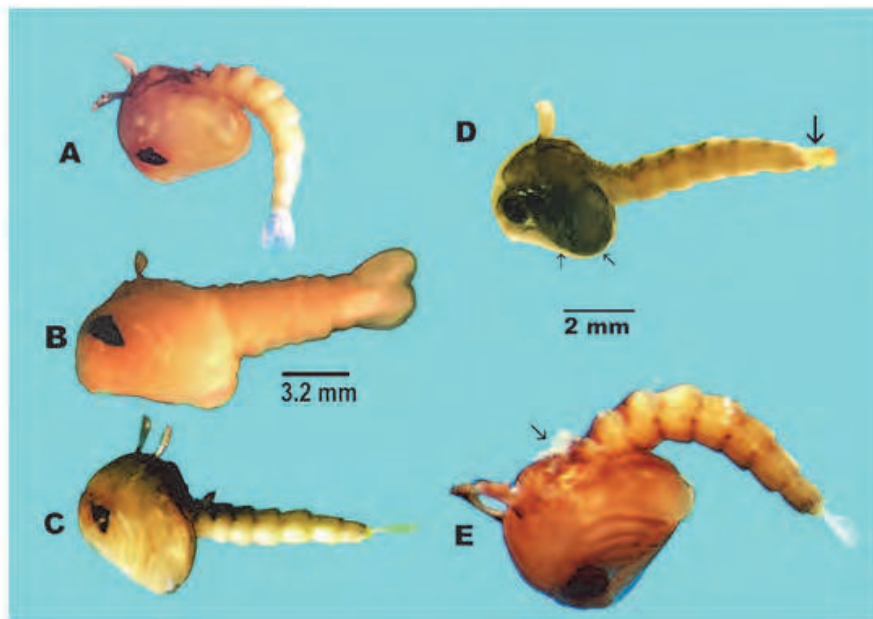


Fig. 5. Pupal abnormalities of mosquitoes: A. Normal pupa. B. Albino pupa. C. Elephantoid pupa, with enlarged cephalic region and extended abdomen. D. Black cephalothorax (lower arrows) and extended abdomen with transparent posterior end (upper arrow) and no anal gills. E. Pupa with apparent fungal growth (arrow head). Adapted from Khater (2003).

most CSI compounds are very potent against a variety of different pests, especially lepidopterous insects and whiteflies. They are harmless or exert little adverse effect on bees, predators, or parasitoids (Tomlin, 2000) which renders them acceptable for inclusion in IPM programs.

2.2.2 Substances interfering with the action of insect hormones

Growth and development of insects are regulated by hormones: prothoracicotropic hormones (PTTH) (brain hormone), ecdysteroids, and juvenile hormones (JH). PTTH controls the secretion of the molting hormone (ecdysone) from the prothoracic gland. Ecdysone is responsible for cellular programming and, together with JH, initiating for the molting process. High secretion of JH from the corpora allata allows the epidermis to be programmed for a larval molt, or else it is programmed for metamorphosis. JH is virtually absent in the pupae, but is present in adults to serve some functions in reproduction. JH suppresses pupation and induces vitellogenesis during the reproductive stage of the insect (Eto, 1990).

2.2.2.1 Ecdysteroid agonist

Bisacylhydrazine (BSH), a newer class of IGRs, is ecdysone agonists or disruptors with molting hormone activity. BSHs include chromafenozide (Virtu®), Tebufenozide (Mimic®, Confirm®), Halofenozide (Mach-2®), and Methoxyfenozide (Intrepid®). They have a greater metabolic stability than the insect steroid molting hormone 20-hydroxyecdysone (20E) *In vivo* (Retnakaran et al., 1995). They are toxic after ingestion or exposure to higher doses of topical application. Ingestion of BSH creates hyperecdysionism in susceptible insect, including molting events. The effect starts with feeding inhibition within 3-14 h (Retnakaran et al., 1997), which is very important for preventing further crop damage. In the mean while,

larvae become moribund, slip their head capsule, and the hind gut may be extruded in extreme cases. Synthesis of new cuticle begins and apolysis of the new cuticle from the old one takes place. The new cuticle is not sclerotized or tanned. Consequently, the food intake by larva is prevented as the mouthparts become soft and mushy. Larval death is due to incomplete molting, starvation, and desiccation due to hemorrhage. Both Tebufenozide and methoxyfenozide, selectively toxic to Lepidopteran pests, have been classified by US EPA as reduced risk pesticides. It worth to mention that azadirachtin in neem tree acts as ecdysone blocker and disrupt insect growth, see botanical insecticides above. In general, BSHs have insect selectivity, reduced risk ecotoxicology, and mammalian profile as they have new mode of action, therefore, they are ideally integrated in IPM programs.

2.2.2.2 Juvenile hormone analogues

The juvenile hormone analogues (JHA) mimic the activity of naturally occurring JHs and prevent metamorphosis to the adult stage. JH is one of the most pleiotropic hormones known and functions in various aspects such as embryogenesis, molting and metamorphosis, reproduction, diapause, communication, migration/ dispersal, caste differentiation, pigmentation, silk production, and phase transformation. The major function of JH is the maintenance of larval status or the so- called juvenilizing effect, See Tunaz (2004). JHAs are highly effective at the beginning stage of metamorphosis and embryogenesis, for instance, freshly ecdysed last larval instars, freshly ecdysed pupal instars, and deposited eggs. Thus embryogenesis is disrupted when young eggs are treated with JHAs. Application to early last instar larvae would result in the development of supernumerary instars, whereas treatment at the later stage would result in abnormal pupation and development of larval-pupal mosaics or intermediates (Khater, 2003). Treatment of *M. domestica* and *C. pipiens* with pyriproxyfen (Sumilarv® Somitomo Co.) interferes with normal metamorphosis and results in various developmental abnormalities including larval pupal intermediates that do not survive (Fig. 1-5); Lower concentrations significantly prolonged larval and pupal durations, 10.5 and 11 days (at conc. 0.1 ppm) and 9.9 and 15.1 days (at conc. 4 ppm), respectively, than those of the control (3, 5.7 days and 3.9, 1.1 days, respectively) (Khater, 2003). Methoprene (Altosid®, Apex®, Phororid®, Precor®), the first compound introduced into the market, is a terpenoid compound with very low mammalian toxicity and is regularly used for flea control. It is sensitive to light. Thus, It has been used extensively and successfully in indoor environments and on pets in the form of collars, shampoos, sprays, and dips and also as a feed through larvicide for hornfly, *Haematobia*, control on cattle (Graf, 1993). Other IGRs available for use against household and agricultural pests are pyriproxyfen and fenoxycarb. Fenoxycarb (Logic®, Award®, Comply®, Torus®) is a carbamate stomach insecticide that has also JH-type effects when contacted or ingested by various arthropod pests, e.g., ants, roaches, ticks, chiggers and many others. Some JHAs are of plant origin, such as Juvenoids, isolated from plants, the “paper factor” from the balsam fir (*Abies balsamea*) and Juvocimenes from the sweet basil plant (*Ocimum basilicum*) (Bowers & Nishida, 1980). Morphogenetic control is effective for controlling insects that are pests as adults, like most insects of medical and veterinary importance, ex. mosquitoes, nuisance flies, and fire ants. They are not effective against lepidopteran agricultural pests because the larval stage is responsible for plant destruction. Many JH analogs (or mimics) (JHAs) are attractive candidates for pest control because of the ease of their synthesization and their pest selectivity than those of other peptide and steroid hormones (Eto, 1990).

2.2.2.3 Antijvenile hormones

Antijvenile hormones, anti- JHs, are very effective pest control agents as they prevent JH production, facilitate JH degradation, or destroy corpus allatum. Intoxicated newly emerged larvae with anti- JH would create miniature pupae, thus abbreviating the destructive part of insect life cycle. Some of these plant-derived substances actually serve to inhibit the development of insects feeding and protecting the host plant. These are referred to broadly as antijvenile hormones, more accurately, antiallatotropins, or precocenes. Although the mode of action of the precocenes is still unclear, it is known that they depress the level of juvenile hormone below that normally found in immature insects. As anti-JHs is competing with JH in binding to the JH receptors or to the JH carrier proteins, injuring the corpora allata cells, or interfering with JH biosynthesis (Leighton et al., 1981), other JHAs may also function as anti-JHs, such as ETB [ethyl 4-(2- pivaloyloxybutyloxy)-benzoate], which showed JH agonist and antagonist activities in *Manduca sexta* larvae as it compete with JH at the receptor site and become feedback inhibitors of JH biosynthesis (Staal, 1986).

2.2.3 Safety

Several IGRs are registered by the EPA, such as methoprene (Altosid ®) which is used as a grain protectant, as mosquito growth regulator; Precor ® for indoor control of dog and cat fleas; Hydroprene (Gentrol ® , Mator ®) for use against cockroaches, and stored grain pests; and kinoprene (Enstar II ®), which is effective against aphids, whiteflies, mealybugs, and scales (both soft and armored) on ornamental plants and vegetable seed crops grown in greenhouses and shadehouses. Although some insects acquired resistance against some IGR- based products, IGRs are typically “safer” to use around humans, pets, and natural enemies than conventional insecticides and acaricides. IGRs are effective when applied in very minute quantities and generally have few or no effects on humans and wildlife. They are, however, nonspecific, since they affect not only the target species, but other arthropods as well. For more information, see Taylor (2001), Tunaz (2004), Ware & Whitacre (2004), Collins (2006), and Gilbert & Gill (2010).

2.3 Pheromones

Pheromones are a class of semiochemicals that insects and other animals release to communicate with other individuals of the same species. Such behavioral chemicals, range from small hydrophobic molecules to water-soluble peptides, leave the body of the first organism, pass through the air (or water) and reach the second organism, where they are detected by the receiver. Signalling chemicals play an essential role in arthropod life cycles. They provide the means whereby mates, host and oviposition sites are located and recognized (Mordue Luntz, 2003). Pheromones may signal various information. Long-lasting pheromones allow marking of food sources or territorial boundaries. Other signals are very short-lived and provide an immediate message, such as a brief period of reproductive readiness or short-term warning of danger.

2.3.1 Uses of pheromones

There are five principal uses for sex pheromones: population monitoring, mass trapping of insects, movement studies, detection of exotic pests and, mating disruption. Such disturbance is very important in reducing the population density of pests as synthetic

pheromone is dispersed into the field and the false odor plumes attract males away from females that are waiting to mate, thus reduce the population density of the pests. In contrast to the previous benefits, the high degree of selectivity may be a barrier to large-scale implementation where secondary pests become a problem as insecticides are eliminated (Walker & Welter, 2001). Other obstacles include the following: the lack of an identified pheromone for some pest species, high development and production costs, requirements for specialized application techniques or equipment, and the need to supplement the pheromone program in high pest-pressure situations (Welter et al., 2005). Large-scale implementation projects have yielded significant reductions in pesticide use while maintaining acceptably low crop-damage levels. Pheromones manipulate the behavior of insect pests. With these non-toxic and biodegradable chemicals, insects can be lured into traps or foiled into wasting energy that they normally need for locating food and mates. Pheromones are species-specific chemicals that affect insect behavior and they are not toxic to insects or other non-target organisms, creating opportunities for the biological control of other pest species. They are attractive in extremely low doses and used to bait traps or confuse a mating population of insects. Other advantages include negligible health risks, no accumulation in wildlife or groundwater, limited impacts on other management practices; manage insecticide resistance, and a more rapid registration process. Pheromones can play an important role in integrated pest management for medical or veterinary, structural, agricultural, landscape, and forest pest problems. Pheromone programs are most effective with low to moderate population densities. Because of some difficulties with high populations of pests, these programs should not be viewed as stand-alone strategies but rather as one tactic within a suite of integrated pest management options (Welter et al., 2005). For more information about pheromones, see; Cooping & Menn (2000), Witzgall (2001), Leal et al. (2003), Bray et al. (2009), and van Emden & Service (2011).

2.4 Photosensitizers

Development of new, ecologically safe technologies to control insect pest populations is of great importance. Photosensitizers are activated by illumination with sunlight or artificial light sources, have been shown to be accumulated in significant amounts by a variety of insects when they are administered in association with suitable baits. The subsequent exposure of such insects to UV/visible light leads induces lethal photochemical reactions and death. The most famous photosensitizers are xanthenes (e.g. phloxin B) and porphyrins (e.g. haematoporphyrin) which appear to be endowed with the highest photoinsecticidal activity. In particular, porphyrins absorb essentially all the UV/visible light wavelengths in the emission spectrum of the sun; hence they are active at very low doses. Photoactive compounds usually used for photosensitization might be effective as pesticide agents, with low impact on the environment, being non-toxic and not mutagenic, see Ben Amor & Jori (2000), Mangan & Moreno (2001), Ragaei & Khater (2004), Lukšienė et al. (2007), and Awad et al. (2008) for more fine points.

2.5 Inorganics and organic acid

Several inorganic substances are well known with their insecticidal effect, such as potassium silicate, diatomaceous earth (DE, diatomite or kieselgur), mineral oils, sulfur, boric acid, sodium borate, silica gels, kaolin clay, and soap spray. For more details about inorganic and their uses, see Ware & Whitacre (2004), and Collins (2006). It is worth to mention that peracetic

acid, an organic acid, $C_2H_4O_3$, has strong acaricidal effect which was discovered for the first time (Khater & Ramadan, 2007). PPA had a great potential as acaricide against the cattle tick, *Boophilus annulatus*, and the fowl tick, *Argas persicus*, *In vitro*. Two minutes after treatment with 0.5%, PAA induced 100% mortality of both tick species and LC50 values for cattle and fowl ticks, after treatment for 30 min, were 0.06 and 0.05%, respectively. Following treatment with 0.25%, the LT50 values were 0.02 and 3.12 min, respectively. Furthermore, the detrimental effect of PAA against cattle tick extended beyond the adult stage, it significantly prolonged the preoviposition period, shortened the oviposition period, and decreased the mean number of the laid eggs, and such parameters were 14.75 and 6.57 days, and 457.50, respectively, after treatment with 0.25% of PAA. Therefore, PAA is highly effective when used at lower doses and short exposure time. The high speed of killing ticks is very important for avoidance of the hazard ensued by pathogen transmission in the course of delayed mortality caused by the currently used acaricides (Khater & Ramadan, 2007). PAA is highly effective against lice, *In vitro* and soft tick (*In vivo*), (Khater, H.F., Unpublished data).

3. Biological control

Biological control is the reduction or protection of pest populations by natural enemies which include four categories: microbes or pathogens (such as viruses, bacteria, protozoa, and fungi); entomopathogenic nematodes; predators (such as lady beetles and lacewings); and parasitoids (wasps and some flies). Protozoa, predators, and parasitoids are out of the scope of this chapter. Natural enemies are responsible for natural suppression of pest population. The first successful large scale microbial control application using coidiospores of the fungus *Metarhizium anisopliae* was carried out in the Russian Ukraine against the beet weevil, *Bothynoderes punctiventris* (Metchnikoff, 1879).

3.1 Virus

Entomopathogenic viruses are obligate disease-causing organisms that can only reproduce within a host insect. Among the fifteen or more families of viruses of invertebrates, it is mainly those having virus particles (virions) occluded within a proteinaceous matrix, an occlusion body (OB), have been used successfully in controlling pest populations. Such families are Entomopoxviridae (Entomopoxviruses, EPVs), Reoviridae (cypoviruses, CPVs), and Baculoviridae (Baculoviruses, BVs) (Lacy & Kaya, 2007). Only BVs have been used as pesticides (Szewczyk et al., 2009, 2011). In the past, the classification of the family Baculoviridae was based on virus morphology. It was divided into two genera: the Nucleopolyhedrovirus (NPVs) and the Granulovirus (GVs). A new division on the basis of comparison of genomic sequences indicate that virus phylogeny followed more closely the classification of the hosts than the virion morphological traits. Accordingly, family Baculoviridae contains four genera: Alphabaculovirus (lepidopteran-specific NPVs), Betabaculovirus (lepidopteran-specific GV), Gammabaculovirus (hymenopteran-specific NPVs), and Deltabaculovirus (dipteran-specific NPVs) (Jehle et al., 2006). Although wider use of BVs as commercial insecticides was restricted because of their slow killing action and difficulties in large scale production, a very successful project was carried out in Brazil (Moscardi, 1999); over 2.0 million hectare of soybean had been already controlled annually by velvetbean caterpillar BV. Consequently, many countries have increased the area of fields and forests protected by BV pesticides.

3.1.1 Life cycle and viral stability

Virul infection begins in the insect's digestive system after consumption of plant material with viral particles. The virus spread though out the body but the digestive system is among the last part to be destroyed, so the insects usually continue to feed until they die leading to economic loss. See Lacey & Kaya (2007) and McNiel (2010) for more information about biology and ecology of viruses. The virus manipulates the behavior of infected larvae for its own dissemination. For example, larvae of the cabbage moth, *Mamestra brassicae* infected with NPV moved up to five times more than their healthy counterparts during the middle stages of infection (Vasconcelos et al., 1996b). Adult insects can also disperse viruses via vertical and horizontal transmission. Understanding the biology and ecology of viruses is crucial for optimizing pest control strategies of exotic and genetically modified organisms, see Lacy & Kaya (2007) and Szewczyk et al. (2009, 2011) for more details. UV protectants are very important for stability of the viral product, stilbene fluorescent brighteners (e.g. Phorwite AR ®, Blankophor ®, and others) induce the best results. Plant metabolites as peroxidases generate free radicals (Hoover et al., 1998) inactivate BVs. As a result, addition of free radical scavengers such as mannitol or enzyme superoxide dismutase to BV preparations can reduce such inactivation (Zhou et al., 2004). Tillage buries virus particles in the soil, thus good agricultural practices can reduce viral persistence between seasons.

3.1.4 Products

Some BVs are produced as commercial products, mainly for caterpillars, such as Gemstar LC (NPV of *Heliothis/Helicoverpa* spp. e.g., corn earworm, tobacco budworm, cotton bollworm); Spod-X LC (NPV of *Spodoptera* spp. e.g., beet armyworm); CYD-X and Virosoft CP4 (GV of *Cydia pomonella*, the codling moth); and CLV LC (NPV of *Anagrapha falcipera*, the celery looper). Successful infections can perpetuate the disease outbreak making repeat applications within a season unnecessary.

3.1.5 Safety

Members of BVs are regarded as safe to vertebrates. Their specificity is usually very narrow, often limited to single insect species. Regarding beneficial arthropods, immature larvae of parasitoids in infected hosts may die not due to virus infection, but relatively to premature loss of the host or to variation in quality of the host. Virus can provide safe, effective and sustainable control of a variety of insect pests as a part of a varied IPM programs, see El-Husseini (2006) and Szewczyk et al. (2009). Entomopathogenic viral agents are ideal for pest management purposes due to their specificity, dispersal capacity, and self propagation. Viruses are the most environmentally acceptable components of direct and integrated management regimes. Accordingly, there is global interest in use of viral agents. Valuable reviews on entomopathogenic viruses are those of Szewczyk et al. (2009, 2011), Lacey & Kaya (2007), McNiel (2010), Ahemed et al. (2011), and Singh et al. (2011).

3.2 Bacteria

The insecticidal bacterium, *Bacillus thuringiensis* (Bt) is a widely occurring gram-positive, spore-forming soil bacterium that produces parasporal, proteinaceous, crystal inclusion-bodies during sporulation. Bt has been the most successful commercial microbial insecticide, and also has been the subject of the overwhelming majority of genetic engineering studies to improve efficacy (Federici, 2010; Lacey & Kaya, 2010; Mehlhorn, 2011). Bt is actually a

complex of bacterial subspecies that occur in soil, leaf litter, leaf surfaces, insect feces, and as a part of the flora in the midguts of many insect species. There are several insecticides based on various sub-species of *Bacillus thuringiensis* Berliner (Bt), such as *B. thuringiensis israelensis* (Bti), with activity against mosquito larvae, black fly (simuliid), fungus gnats, and related dipterans species; *B. thuringiensis kurstaki* (Btk) and *B. thuringiensis aizawai* (Bta) with activity against lepidopteran larval species; *B. thuringiensis tenebrionis* (Btt), with activity against coleopteran adults and larvae; and *B. thuringiensis japonensis* (Btj) strain *buibui*, with activity against soil-inhabiting beetles.

3.2.1 Mode of action

The insecticidal properties of Bt are largely a function of the presence of extra-chromosomal plasmids in the cell. These carry genes such as *cry* genes that encode a diverse array of these protein crystalline inclusion bodies which are toxic to insects. Upon ingestion by an insect, the crystal proteins (Cry) are solubilised and the insect gut proteases convert the original protoxin into smaller toxins. These hydrolysed toxins bind to the insect's midgut cells at high-affinity and specific receptor binding sites where they interfere with the potassium ion dependent, active amino acid symport mechanism decreasing absorption of minerals and nutrition from midgut and finally death of the columnar cells. This disruption causes the formation of large cation-selective pores that increase the water permeability of the cell membrane. A large uptake of water causes cell swelling and eventual rupture, disintegrating the midgut lining. Different toxins bind to different receptors with different intensities and this explains the selectivity of different Bt strains in different insect species (Baum et al., 1999; Cooping & Menn, 2000; Lacey & Kaya, 2007; Federici, 2010). Delayed larval mortalities (2–48 h) is caused by the crystal inclusions which have to be ingested and then processed within the insect's gut. Biological control products may contain the endotoxins plus live bacterial cells. The toxin stops feeding, this action hinders further damage caused by the feeding larva, and do not directly kill insects, but young larvae may starve to death and may die from bacterial infection over a longer period. Some commercial products contain Bti crystal proteins and spores, such as Bactimos and VectoBac (Valent BioSciences). VectoBac, Bti, (12 AS, Wady El-Niel for agricultural development Co. Egypt) is highly effective against *C. pipiens* than *M. domestica*, LC₅₀ values were 1×10^{-5} and 3.86×10^3 spores/ml, respectively, and LC₉₀ values were 0.04 and 37.28×10^3 spores/ml, respectively. Survived mosquito larvae died as pupae (Khater, 2003). Bti is a practical substitute to organophosphate insecticides, but it is unsuitable for application to environmentally sensitive water bodies. *Bacillus sphaericus* control mosquito larvae, particularly *Culex* and anopheline spp., especially those breeding in polluted water. It controls also black fly, *Simulium* sp., the vector of river blindness disease. Bs is effectively controlled *C. pipiens* in Egypt (Ragaei et al., 2004). It is widely used in Europe and Africa. Registered *B. sphaericus* product is Vectolex CG (Valent BioSciences). Recent studies in Kenya have shown that at least in some areas, biting rates by *Anopheles gambiae* can be reduced by more than 90% by using a combination of existing commercial formulations of Bti and Bs (Filingier & Lindsay, 2000).

3.2.2 Bacterial toxins

The use of insect-specific toxins from Bt and Bs is forming an increasingly component of biological control strategies. The protein crystals (protoxins) contain several toxins which

are classified according to their insecticidal activity and molecular relationship into four major groups (Höfte and Whiteley, 1989): Cry I toxins, active against larvae of lepidopterans; Cry II toxins, active against larvae of lepidopterans and dipterans; Cry III toxins, active against larvae of coleopterans (Chrysomelidae); and Cry IV toxins, active against larvae of nematoceran flies. Cry toxins bind to glycoprotein or glycolipid receptors. The toxins can also be further categorized into subclasses, ex. CryIA etc. The genes of the toxins are named as the toxins but without capital letters and in italic (e.g., *cryIV*). Cytotoxins (Cyt) found in strains toxic to larvae of nematoceran flies and coleopterans. The CytA protein binds to lipids and does not exhibit the specific binding mechanism which the Cry proteins do (Höfte and Whiteley, 1989). Cyt proteins are thought to have a similar mode of action to that of Cry proteins, with the exception that they directly bind to the microvillar lipid bilayer. Similar to Bti, the toxicity of Bs is due to protein endotoxins produced during sporulation and assembled into a parasporal body. The main toxin of Bs is a binary toxin (BinA and BinB) which are proteolytically activated in the mosquito midgut to release peptides (43 and 39 kDa, respectively) to form the binary toxin. The toxins bind to microvilli of the midgut epithelium, trigger hypertrophy and lysis of cells, leading to destruction of midgut and death of mosquito larva.

3.2.3 Resistance

Despite decades of use, there is no reported resistance among mosquito populations probably due to biochemical properties of Cyt protein. Bti produces a parasporal body that contains four major endotoxins, Cry4Aa, Cry4Ba, Cry11Aa, and Cyt1Aa. Both Cry and Cyt are must be ingested to yield active toxins in the midgut of insect. Cyt protein synergizes the toxicity of the Bti Cry proteins resulting in delayed the phenotypic expression of the evolution of resistance. Such synergistic interactions are also known to occur between the Bti Cry proteins. When Cyt protein combined with the Bs Bin toxin, it can also overcome resistance to Bti. For detailed information, see Wirth et al. (2005) and Federici (2010). As a rule, bacterial control agents are less likely to provoke resistance because their mode of action is more complex (Wirth et al., 2005; Federici, 2010). However, resistance had developed against Btk in some pests, e.x., the stored grain pest, *Plodia interpunctella*, and the diamondback moth, *Plutella xylostella*. In most cases, resistance has been associated with a recessive or partially recessive trait(s) and appears to be linked to a single gene. The combination of protoxins in Bti is very important in reducing the likelihood of resistance. In contrary, when the gene encoding a single toxin protein was cloned into a microorganism and then fed to larval mosquitoes, resistance was induced within a few generations; see transgenic organisms below and Mehlhorn (2011) for more details. For avoidance of resistance, products with different arrays of endotoxins should be alternated (Schuster & Stansly, 2006).

3.2.4 Safety

The U.S. Environmental Protection Agency (USEPA) categorizes the risk posed by Bt strains to nontarget organisms as minimal to nonexistent. After using of Bt products for more than 50 years, it can be concluded that Bt belong to the most environmentally safe products as they kill target organisms and usually do not harm nontarget organisms, such as beneficial insects, plants, or humans. The safety record for occupational exposure to Bt based biopesticides with regard to human health is considered good (Barford, 2010). Some Bt

subspecies produce other types of toxins besides Cry proteins as β -exotoxin and α -toxin. Both toxins are toxic to vertebrates and they are banned in Bt products, see Lacey & Kaya (2007) and Makonde et al. (2010) for more particulars about bacterial toxins. Bt and BS are the most used biocontrol agents against nuisance, pest or vector species. They are strongly incorporated in IPM programs because they are highly efficient, easily be mass-produced, easy to handle, stable when stored, cost-effective, pest specific, and safe to people and the environment. For more information about bacteria as a biological control agent, see Lacey & Kaya (2007), Gilbert & Gill (2010), Ahmed et al. (2011), Mehlhorn (2011), Singh et al. (2011), and van Emden & Service (2011).

3.3 Entomopathogenic fungi

Entomopathogenic fungi are important natural control agents that limit insect populations. Survival of fungi requires a delicate balance of interactions between fungi, host, and the environment. Most fungi cause insect diseases spread by means of asexual spores called conidia which vary greatly in their ability to survive adverse environmental conditions, desiccation, and ultraviolet radiation.

3.3.1 Life cycle

In general, insects get infected when they come into contact with spores on the bodies of dead insects, on the surface of plants, in the soil, or in the air as windborne particles. High humidity is usually required for germination of conidia on the insect cuticle and production of germ tube that allow the fungus to penetrate the cuticle often at joints or creases where the insect's protective covering is thinner. In contrast to virus and bacteria, fungi do not have to be ingested to cause infections. The fungus multiplies within the host insect and kill it. Many fungi produce toxins to increase the speed of kill or prevent competition from other microbes. Death is due to toxin produced by the fungus or fungal multiplication. Under favorable conditions, the fungus grows out of the cadavers, usually at thinner areas and form conidiophores or analogous structures and sporulates. Some species go into a resting stage which survive periods of adverse conditions before forming or releasing spores. Soil incorporating fungi usually avoid the adverse effects of ultraviolet radiation and desiccation, but other microorganisms that act as competitors or antagonists often alter pathogen effectiveness. Fungal pathogens differ in the range of hosts. Many important fungi attack eggs, immature, and adults of a variety of insect species. Others are more specific to immature stages or to a narrow range of insect species. Several fungi are used to control insects such as *Leptolegnia* spp., *Coelomomyces* spp., *Hirsutella thompsonii*, *Nomuraea rileyi*, and *Vericillium lecanii*. The most famous entomopathogenic fungi will be discussed below.

3.3.2 Oomycetes (water molds)

Lagenidium giganteum was first described by Couch (1935) from a combined collection of copepods and mosquito larvae, *Culex* and *Anopheles*, in North Carolina, USA. It has a wide geographical distribution: North America, Europe, Africa, Asia, Antarctica (Federici, 1981). The only species of the genus *Lagenidium* is known to be pathogenic to mosquito larvae is *L. giganteum* (formerly: *L. culicidum*). As a facultative parasite, *L. giganteum* can grow as pathogen on mosquito larvae, or as a saprophyte in aquatic environments (Sur et al., 2001). This aquatic fungus is highly infectious to larvae of several mosquito genera. It cycles effectively in the aquatic environment even when mosquito density is low, but its

effectiveness is limited by high temperatures. The fungus is not effective for mosquitoes in brackish or organically rich aquatic habitats (Merriam and Axtell, 1982). *L. giganteum* has a wide geographical distribution: North America, Europe, Africa, Asia, Antarctica (Federici, 1981). Fungal reproduction is both asexual (zoospores) and sexual (oospores) (Federici, 1981). In order to infect mosquito larvae, biflagellate zoospores must be formed. Motile zoospores are the asexual stage of the fungus. Oospores, the sexual stage of *L. giganteum*, can also be used as inoculum. They are dormant propagules, resistant to desiccation and mechanical abrasion and stable for at least seven years, which allows multivoltine persistence of the fungus in some habitats (Kerwin et al., 1994).

Lagenidium spp. was isolated from Egypt for the first time from *Culex pipiens* larvae infesting a polluted creek in Miet El- Attar, Benha, Egypt, by Khater (2003). Such fungus was propagated on SDA medium and Peptone yeast glucose (PYG) for sporulation. Five concentrations (5×10^5 - 1.6×10^7) were used to infect *C. pipiens* late 3rd larval stage *In vitro* and the biological parameters had been followed up till emergence of adults. LC50 and LC90 values were 2.79×10^6 and 3.94×10^8 spores/ ml, respectively. At the lowest concentration, larval duration reached 8.3 days (3.6 days in the control group) and morphological changes (symptoms of mycosis) was 66.7%, (Fig. 4.5). All survived larvae died as pupae; consequently, the isolated fungus inhibited adult emergence. As this fungus has the ability to be self- propagated, it could be an effective control agent for the vector of Bancroftian filariasis and Rift valley fever virus in Egypt (Khater, 2003). The fungus has caused high mortalities in mosquito populations, see Schotle et al. (2004) for a review. Results from a small scale field trial in North Carolina indicated that *L. giganteum* recycled for an entire season despite periodic scarcity of hosts and short-term drought with infections ranging from 0-100% (Jaronski & Axtell, 1983). A large-scale field trial in Californian rice fields, using mycelium from either 20 or 30 liters of fermentation beer per hectare resulted in 40%-90% infection of *Culex tarsalis* and *Anopheles freeborni* sentinel larvae (Kerwin & Washino, 1987).

3.3.2.1 The relative potency

The relative potency indicated that Vectobac (Bti), and *Lagenidium* spp. were 12×10^{11} and 4.3 times, respectively, more effective than the fungal product Biosect®, *Beauveria bassiana*, on the basis of LC50 values (Khater, 2003). *L. giganteum* is compatible with the bacterial agents Bti and Bs Meyer and Neide when used against *Culex quinquefasciatus* (Orduz & Axtell, 1991), with the fungus having the distinct advantage over Bti in that it is able to recycle in stagnant water, infecting multiple and overlapping generations of mosquitoes (Legner, 1995). In field trials in which Lagenex 25 was compared with Vectobac-12AS (Bti), Lagenex reduced *C. quinquefasciatus* larvae by 100% for 22 days whereas Vectobac-12AS required retreatment by the 10th day (Hallmon et al., 2000).

3.3.2.2 Production

Unfortunately, mass production yields of oospores remain orders of magnitude below that of the less stable mycelial (asexual, presporangial) stage, and continued problems with spore activation have prevented large-scale field tests (Kerwin and Petersen, 1997). For solving this problem, improving oospore yields would be much more useful than zoospores in large-scale operational mosquito control programs. In contrast to the previous obstacles, Lagenex® is a *L. giganteum*-based product by AgraQuest (California, USA) until 1999. It is effective against larvae of most pest mosquito species; remains infective in the environment through dry periods but it is unable to survive high summertime temperatures; the kind of

spore used was not mentioned. Shathele (2009) made an excellent potential for investigations on the use of newly developed isolate (Saudi-1 and Saudi-2) media for an isolated strain of *L. giganteum* from Louisiana for its maintenance and zoospore release.

3.3.2.3. Safety

Zoospores of the fungus appear harmless to vertebrates (Kerwin et al., 1990), most aquatic invertebrates (Nestrud & Anderson 1994) except *Daphnia* spp. and copepods (Couch, 1935), three cladoceran species and a chironomid species (Nestrud & Anderson, 1994).

3.3.3 Hyphomycetes

Most soil fungi used for the control of insect pests belong to the group hyphomycetes, such as *Beauveria bassiana* and *Metarhizium anisopliae*, formerly known as *Entomophthora anisopliae*. Some species have been developed as commercial products because of their ability to be mass produced. Most fungi in this group can cause natural outbreaks, to a wide range of insect hosts, on their own when environmental conditions are favorable. Soil provides protection against UV along with optimal conditions of temperature and moisture. Furthermore, the fungi may survive in the soil through recycling in insects or roots (Leger, 2008). Thus, they provide a long-term strategy for larvae and puparia control (Quesada-Moraga et al., 2006). *B. bassiana* infects both larvae and adults of a broad host range. Understanding the interactions between *B. bassiana* and other soil microorganisms is very important for the success of using of this fungus. Commercially available products based on *B. bassiana* are Mycotrol O (Emerald BioAgriculture), Naturalis Home and Garden (H&G), Naturalis L (Troy BioSciences, Inc.), and Biosect® (Kafr El Zayat - KZ Chemicals, Egypt). Khater (2003) used Biosect® to control larvae of both *M. domestica* and *C. pepiens* *In vitro*. LC₅₀ and LC₉₀ values were 29.2×10^7 and 9.97×10^8 spores/ ml for house flies and 1.2×10^7 and 4.17×10^9 spores/ ml, for mosquitoes, respectively. The total larval mortalities of mosquitoes were almost 100%, the few survived larvae died as pupae. At the lowest concentrations, Larval and pupal malformations were 30% and 45%, See fig (1,2,4,5) for symptoms of mycosis.

3.3.4 Entomophthorales

Entomophthorales is a group of fungi that tend to be much more host specific and can cause natural outbreaks in insect populations. Several different *Entomophthora muscae sensu stricto* genotypes were documented and each type was restricted to a single host species, indicating a very high degree of host specificity at or below the level of the subfamily (Jensen et al., 2001). Currently, no commercially-based product is available because of difficulties in mass production. All available literature deal with *E. muscae* as a pathogenic fungi of adult *M. domestica*, but Khater (2003) isolated it for the first time in Egypt, from Moshtohor, Toukh, Qlubia governorate, such strain has a unique ability to infect larvae of house flies for the first time also. The isolated fungus was replicated on Sabouraud Dextrose Agar (SDA) medium for hyphenation and on liquid medium for laboratory - scale Entomophthora' hyphal production for enhancement of zoosporogenesis. Khater (2003) also made a bioassay using early 3rd larval stages of house flies which were infected *In vitro* by adding different concentrations of *E. muscae* (from 25 to 16×10^2 spores/ml) to the breeding medium of larvae and followed up till adult emergence. LC₅₀ and LC₉₀ values for larval mortalities were 2.19×10^2 and 20×10^2 spores/ ml, respectively. At lower concentrations, larval durations were elongated (7.2 days) than that of the control group (2.6 days). Larval abnormalities were larval pigmentation, black, red- pin point pigmentation, ulceration, maceration and larval

pupal intermediates. Mortality and morphological changes of pupae (symptoms of mycosis) reached 100% at 8×10^2 spores /ml. Adult mortalities, deformity, and emergence reduction rates increased as the concentrations increased (79.3%, 60%, 98.2%, respectively, at 4×10^2 spores/ ml). Adult abnormalities were failure of adult eclosion, small adults, and deformed wings and legs (Fig. 1-3). In addition, this pathogen manipulates the behavior of infected host for his own dissemination as the apparently normal adults exhibited the characteristic position of *Entomophthora* spp. (Fig. 6) (Krasnoff et al., 1995; and Khater, 2003) as follows:

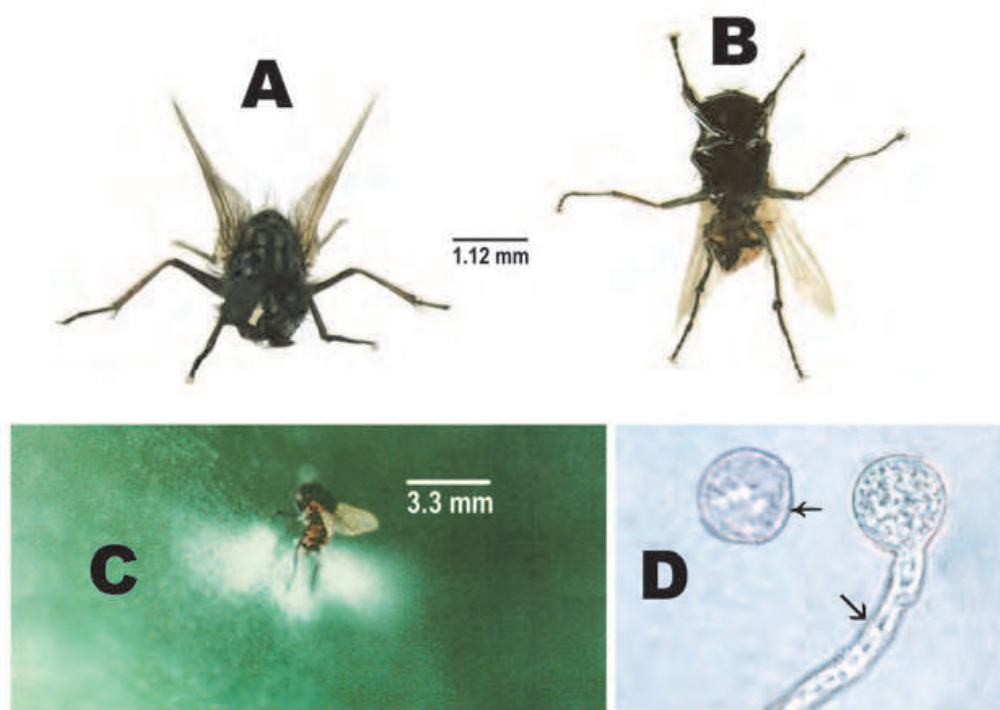


Fig. 6. Characters of adult house fly infected with *Entomophthora* spp.: A. Adult exhibited elevated abdomen, wings, and extended legs whereas the mouth parts were stoked downwards (anterior view), B. Adult with extended legs (ventral view), C. Conidial shower (white color), D. Hyphal body (zoosporangia) right arrow, spore (left arrow) of *Entomophthora* spp. Adapted from Khater (2003).

First, adults died in elevated positions, with the proboscis extended and attached to the substrate, legs spread, the abdomen angled away from the substrate, and the wings rose above the thorax. The conidial discharge reached 3-9 cm, depending on the size of the cadavers (Fig. 6). Conidia are forcibly ejected from conidiophores that emerge most profusely from the intersegmental membranes on the insect's abdomen, such posture enhance dispersal of conidia (Krasnoff et al., 1995; Khater, 2003). Similarly, an onion maggot fly, *Delia antiqua* (Meigen), infected by *E.muscae* Cohn (Fresenius) and dying at the top of a grass stem with its proboscis fastened to the substrate and its abdomen well raised by an atypical disposition of the legs appears to be an ideal launch pad for maximizing the effective area covered by the conidial shower (see Krasnoff et al., 1995 for more details). Second, the pathogen also benefits indirectly from the sexual attractiveness of female cadavers. Males attempting necrophilous copulations invariably become infected with the

fungus (Moller, 1993). Third, both field observations (Roffey, 1968) and controlled experiments (Bellini et al., 1992; Khater 2003, unpublished data) indicate that host mortality usually occurs in the late afternoon. This may be a temporal manipulation serving to enhance the likelihood that conidia produced from the dead host will be released at a time when the possibility for germination is highest, after dark when dew has set and surface moisture has had a chance to accumulate. The presumed adaptive payoff of elevation and posture for the fungus enhances dispersal of propagules.

3.3.5 Safety

Concerning the safety of entomopathogenic fungi developed for commercial use in microbial control of insect pests. Most fungi showed no infectivity to man or other vertebrates. Safety tests with *Nomuraea rileyi*, *Hirsutella thompsonii*, *Verticillium lecnii*, and *L. giganteum* assured negative findings to different mammals and birds. On the other hand, *B. bassiana* has been reported to cause allergies in humans and is at least an opportunistic pathogen to man and other mammals. *B. bassiana* and *M. anisopliae* affect non-target invertebrates with various degrees; see El-Husseini (2006) for more details. Soil treatment with *M. anisopliae* and *B. bassiana* on *Tapinoma nigerrimum* colonies indicated that there were no significant differences in ant, as a non-target host, activity before and after fungal treatment (Garrido-Jurado et al., 2011). For more information about entomopathogenic fungi, see Hakjek et al. (2007), Lacey & Kaya (2007), Gilbert & Gill (2010), Singh et al. (2011), and van Emden & Service (2001). For using of fungi for controlling arthropods of medical and veterinary importance, see Steenberg et al. (2001), Samuels, et al. (2002), Khater (2003), Kirkland et al. (2004), Schotle et al. (2004), Hartelt et al. (2008), Zabalgozteaga et al. (2008), Mochi et al. (2010), and Stephen & Kurtböke (2011).

3.4 Nematodes

Entomopathogenic nematodes cause damage to soilborne insect pests. Nematodes from the families Steinernematidae and Heterorhabditidae have proven to be the most effective as biological control organisms (Lacey & Kaya, 2007) to control a wide range of insect pests including filth flies, German cockroaches, cat fleas, armyworms, carpenter worms, crown borers, cutworms, flea beetles, leaf miners, mole crickets, phorid flies, plume moths, root weevils, sciarid flies, stem borers, webworms, and white grubs (Smart, 1995).

3.4.1 Life cycle

Generally speaking, the life cycle of most nematodes includes an egg stage, four juvenile stages, and an adult stage. The third juvenile stage, dauer, is the only infective and free-living stage which is capable of surviving in the soil; its function is to locate, attack, and infect an insect host through its breathing holes, mouth, or anus, but some species are capable of penetrating thin areas of the insect's cuticle. After that, the nematodes release special bacteria into the insect. The toxins produced by the bacteria kill the insect after a few days. The bacteria multiply inside the body of the insect and the nematodes eat the bacteria. The nematodes mature, mate, and multiply inside the insect. Eventually, the insect's body becomes filled with nematodes. Infective stage nematodes then exit the insect body searching for other insects to infect. Once inside the body cavity of the host, the infective

juveniles release bacteria that live symbiotically within the nematode's gut. The nematode-bacterium relationship is highly specific: only *Xenorhabdus* spp. bacteria co-exist with steinernematids, and only *Photorhabdus* bacteria co-exist with heterorhabditids. Under optimal conditions, it takes 3–7 days for steinernematids and heterorhabditids to complete one life cycle inside a host from egg to egg. Emergence of infective juveniles from the host requires about 6–11 days for steinernematids and 12–14 days for heterorhabditids (Kaya & Koppenhöfer, 1999). The ability of any biological control nematode to infect a particular insect can be affected by nematode and insect behavior, physical barriers, and immune responses.

3.4.2 Using beneficial nematodes

Over 30 species of beneficial nematodes have been identified. Seven species have been commercialized worldwide and seven are currently available in the United States: *Steinernema carpocapsae*, *S. feltiae*, *S. glaseri*, *S. riobravus*, *Heterorhabditis bacteriophora*, *H. megidis*, and *H. marelatus*. (Kaya & Koppenhöfer, 1999). There were two commercial nematode products available for termite control, Spear® and Saf T-Shield®.

The use of the entomopathogenic nematodes *S. feltiae* and *H. megidis* in high numbers was shown to be effective against housefly larvae (Renn, 1995) and effective after formulation into a housefly bait, as effective as the spraying of a carbamate pesticide in pig units (Renn, 1998). Also the nematode *Paraionchium muscadomesticae* could infect housefly larvae, but mortality was low except at high nematode concentrations, but *P. muscadomesticae* infect housefly larvae and descendants of the nematodes invade and damage the ovaries of adult female flies and are deposited in the larval habitat when the flies attempt to oviposit. Infected adults lived about half as long as uninfected flies (Geden, 1997; Khater, unpublished data); consequently, *P. muscadomesticae* reduce fly population indirectly. Nematodes were effective in the laboratory but persisted in manure only for 3–7 days. Moreover, mermithid nematodes which parasitize grasshoppers, cockroaches, and mosquitoes can play a part in natural suppression of pest insects. Nematodes must be raised in live insects and this is not a very efficient means of production.

3.4.3 Safety

Entomopathogenic nematodes are not harmful to humans, animals, plants, or earthworms. They can therefore be used as biological control organisms. Such beneficial nematodes have been released extensively in the field with negligible effects on nontarget insects and are regarded as remarkably safe to the environment.

Finally, microbial control agents provide a more environmentally acceptable and sustainable form of insect pest management than chemical insecticides, but are most effective when underpinned by a detailed knowledge of specific host-pathogen interactions. For more information about successful application of entomopathogenic nematodes, see Mill et al. (2000), Gaugler (2002), Crow (2006), Hajek et al. (2007), Lacey & Kaya (2007), and Gilbert & Gill (2010).

4. Transgenic pesticides

Genetically modified organisms (GMO) and crops (GMC) could be engineered to augment pest control. Transgenic organisms are genetically altered by artificial introduction of DNA

from another organism and the artificial gene sequence is referred to as a transgene. Plants with such transgenes are called GMC, plant pesticides or plant incorporated protectants.

4.1 Genetically modified crops

By the mid 1980s, one company, Monsanto had committed to a research program designed to create crop protection products through the application of biotechnology (Glover, 2008). Charles (2001) has produced a very readable history of pesticide-related transgenic crops. Different endotoxins have different biological spectra, thus different genes are used in GMC, for protection from attack by various insects. For example, Monsanto has used *cryIA(c)* genes from Btk in cotton and tomatoes and *cryIIIA* genes from Bt in potatoes. Moreover, Novartis and Mycogen used *cryIA(b)* genes from Btk and Monsanto used *cryIIA* and *cryIA(b)* genes, both from Btk in corn. AgrEvo (Plant Genetic Systems) is using *cry9C* toxin genes from *B. thuringiensis* subsp. *tolworthi* in transgenic corn. GMC have been enhanced sometimes by use of stacked genes. i.e. more than one transgene is introduced into the same crop to achieve multiple desired characteristics and avoidance of resistance. Many experimental studies conducted to date indicate that transgenic plants have no adverse effects on non-target organisms. In addition, there is no scientific evidence as yet that the commercial cultivation of GM crops has caused environmental impacts beyond the impacts that have been caused by conventional agricultural management practices. Nonetheless, studies are still on-going to assess the potential environmental impacts of GM crops (see Makonde et al., 2010). The effect of GM crops on natural enemies remains a controversial topic (Andow et al., 2006; Marvier et al., 2007; Lövei et al., 2009).

4.2 Genetically modified organisms

Molecular biology impact searching for new products through understanding the chemistry and mode of action of a natural product and the discovery of a new protein (and its gene) that may be used to transform a target crop or organism. For more information about proteases as insecticidal agents; see Harrison & Bonning (2010).

4.2.1 Recombinant bacteria

It has been shown that plasmids with a molecular weight of 60–94 MDa play an essential role in the crystal toxin production. Cloning various toxin genes into host organism's increase the persistence or the insecticidal properties. The development of recombinant DNA techniques improves the efficacy of Bti through combining the most potent insecticidal proteins from Bti, Btj, and Bs into new bacterial strains that are ten-fold more toxic than wild type species of Bti and Bs used in current commercial formulations. New bacterial larvicides offer environmentally compatible options for use as components in integrated vector control programs aimed at reducing malaria, filariasis, and many important viral diseases of humans (Federici, 2010). Recombinant (new) bacterial larvicides are used as components in IPM programs because they are much more highly efficient than the wild type strains from which they were originally derived. Their costs are similar to that of new chemical insecticides, and they are much more environmentally compatible than most chemical insecticides. Therefore, recombinant bacterial larvicides will play an important role in controlling pests and vectors in the near future. For more information, see Berón & Salerno (2007), Lacey & Kaya (2007), Gilbert & Gill (2010), Federici (2010), and Singh et al. (2011).

4.2.2 Recombinant baculoviruses

Most insect viruses take several days to kill their host insect. In the same time, the pest is still causing damage. Very high doses are often necessary for adequate control and early larval life stages are highly most susceptible to virus infection. For solving the previous problems, there has been steady interest in the potential to produce recombinant BVs to increase their speed of kill or reduce insect feeding (Inceoglu et al., 2001). The activity of BVs against their natural hosts may be enhanced by introduction of insect-specific toxins, such as toxin genes isolated from the scorpion or spiders, or by interference with insect physiology (Bonning & Hammock, 1996). Arthropod toxins usually attack insect sodium channels producing final effect similar to the chemical insecticides of the pyrethroid group. Though, the specific target in sodium channels is different, so there is a potential possibility to produce synergistic effect by biopesticide/chemical pesticide application (McCutchen et al., 1997). BV recombinants that produced occlusion bodies incorporating Bt toxin were constructed by making a fusion protein consisting of polyhedron and Bt toxin (Chang et al., 2003). This new biopesticide is highly pathogenic than wild type virus as it combines the advantages of the virus and the bacterial toxin.

The changes to host physiology were done by introducing genes coding for some insect hormones or hormone-modifying enzymes into BV genome, or by deletion of the BV-encoded ecdysteroid glucosyltransferase (*egt*) gene. Cloning juvenile hormone esterase gene into BV genome which over expressed decreases the concentration of the juvenile hormone which is a signal for a caterpillar to stop feeding and pupate (Inceoglu et al., 2001). The product of the *egt* gene interacts with larval moulting and indirectly affect the time of feeding of infected caterpillars. Furthermore, recombinant juvenile hormone esterase act as a biochemical anti- JH hormone agent and it affects ovarian development in the house cricket *Acheta domesticu* (Bonning et al., 1997). The deletion of the baculovirus encoded *egt* gene was used first by O'Reilly and Miller (1991). Such deletion from BV genome resulted in 30% faster killing of caterpillars. The *egt* gene is not essential for viral replication and can be replaced with an exogenous gene; the product of which may enhance the insecticidal activity of the recombinant virus (Sun et al., 2004). Genetically modified BVs are safe as they infect only their hosts. They are not pathogenic to bees and vertebrate species (Sun et al., 2004) as well as to the natural enemies of larvae such as parasitoids and predators (Boughton et al., 2003). Consequently, recombinant BVs lead to the expansion of BV use worldwide. For more details about recombinant BVs, see Szewczyk et al. (2009, 2011), Gilbert & Gill (2010), Ahmed et al. (2011), and Singh et al. (2011).

4.2.3 Transgenic insects

Genetic modification of mosquitoes (which renders them genetically modified organisms, GMOs) offers opportunities for controlling malaria. Transgenic strains of mosquitoes have been developed to replace or suppress wild vector populations and reduce transmission and deliver public health gains. The transition of this approach from confined laboratory settings to open field trials in disease endemic countries (DECs) is a staged process that aims to maximize the likelihood of epidemiologic benefits while minimizing potential pitfalls during implementation. Unlike conventional approaches to vector control, application of GM mosquitoes will face contrasting expectations of multiple stakeholders, the management of which will prove critical to safeguard support and avoid antagonism, so that potential public health benefits can be fully evaluated. Inclusion of key stakeholders in decision-

making processes, transfer of problem-ownership to DEC, and increased support from the wider malaria research community are important prerequisites for this. It is argued that the many developments in this field require coordination by an international entity to serve as a guiding coalition to stimulate collaborative research and facilitate stakeholder involvement. Contemporary developments in the field of modern biotechnology, and in particular GM, requires competencies beyond the field of biology, and the future of transgenic mosquitoes will hinge on the ability to govern the process of their introduction in societies in which perceived risks may outweigh rational and responsible involvement (Knols et al., 2007). For more information about transgenic insects and improving their ecological safety for field release, see Vreysen et al. (2007).

5. Future trends, nanoparticles

The potential uses and benefits of nanotechnology are enormous. These include enhancement involving nanocapsules for vector and pest management and nanosensors for pest detection. Nanoparticles are 1-100 nm in diameter, whereas the size of a virus is roughly 100 nm. Such particles are agglomerated atom by atom. Nanotechnology deals with the targeted nanoparticles which exhibit different physical strength, chemical reactivity, electrical conductance, and magnetic properties. Nanoparticles are present in insect entire body parts. Insects are incredible nanotechnologists. The surfaces of many insect wings have evolved properties materials scientists only dream of for their creations (Watson et al., 2010). Nanoparticles help to produce new pesticides, insecticides and insect repellants (Owolade et al., 2008). Nanoencapsulation is a process through which a chemical (ex. an insecticide) is slowly but efficiently released to a particular host for insect pest control. Release mechanisms include diffusion, dissolution, biodegradation and osmotic pressure with specific pH (Vidhyalakshmi et al., 2009). Such process can also deliver DNA and other desired chemicals into plant tissues for protection of host plants against insect pests (Torney, 2009). Nanoparticles loaded with garlic essential oil are efficacious against *Tribolium castaneum* Herbst (Yang et al., 2009). Aluminosilicate filled nanotube can stick to plant surfaces while nano ingredients of nanotube have the ability to stick to the surface hair of insect pests and ultimately enters the body and influences certain physiological functions (Patil, 2009).

Nanotechnology is used widely in agriculture and food (Joseph & Morrison, 2006). One of the world's largest agrochemical corporations, Syngenta, is using nanoemulsions in its pesticide products. Encapsulated product from Syngenta delivers a broad control spectrum on primary and secondary insect pests of cotton, rice, peanuts, and soybeans. Marketed under the name Karate® ZEON, a quick release microencapsulated product containing the active compound lambda-cyhalothrin (a synthetic insecticide based on the structure of natural pyrethrins) which breaks open on contact with leaves. The encapsulated product "gutbuster" only breaks open to release its contents when it comes into contact with alkaline environments, such as the stomach of certain insects. Furthermore, the new technology improve pesticide and fertilizer delivery systems which can take action to environmental changes, ex. they will release their cargo in a controlled manner (slowly or quickly) in response to different signals e.g. heat, moisture, ultrasound, magnetic fields, etc. Recently, nanotechnology is widely acceptable publicly because it is not yet linked to any toxicological and ecotoxicological risks. Research on nanoparticles and insect control should be directed toward production of faster and ecofriendly pesticides to deliver into the target host tissue

through nanoencapsulation. This will control pests efficiently and accelerate the green revolution. For more in rank about usages of nanoparticles, see Joseph & Morrison (2006), Torney (2009), Yang et al. (2009), Bhattacharyya et al. (2008, 2010), Ahmed et al. (2011), and Hashim (2011).

6. Conclusion

IPM programmes have demonstrated that current levels of pesticide use in many circumstances are not necessary and, frequently, are even counter-productive. Excessive and otherwise inappropriate pesticide use is an unnecessary burden on farmers' health and income, on public health, and on the environment (Pretty, 2009). Biorational insecticides have emerged as an alternative or as supplemental forms of pest control. The use of biopesticides will help in preventing the discarding of thousands of tons of pesticides on the earth and provide the residue free food and a safe environment to live. In spite of intensive research on plant natural products and insect-plant chemical interactions over the past three decades, only two new types of botanical insecticides have been commercialized in the past 15 years, those based on neem seed extracts (azadirachtin), and those based on plant essential oils. There are some obstacles toward commercialization of new botanical products, such as the availability of sufficient quantities of plant material to produce the pesticides, standardization, refinement, and quality control of the products; regulatory approval, patents, difficulties in registration; as well as problems related to their volatility, poor water solubility, and aptitude for oxidation. These challenges should be overcome for botanicals to be of particular use for human and high value animals and crops. For more information about standardization, regulatory approval, and commercialization of botanical insecticides, see Tripathi et al. (2009), Isman (2006, 2010), Dubey (2011), and Isman et al. (2011). The insect growth regulator, nonsteroidal ecdysone agonist bisacylhydrazine (BSH) insecticides are generally faster acting than the JHA and CSI insecticides, thus, preventing crop damage by inhibition of feeding within 3–12 h after application. Although both JHA and CSI were discovered long before the BSH, the mode of action of BSH is the best understood at the molecular level. This allows cloning and expression of cDNAs encoding the ecdysone receptor complex, a heterodimer of two proteins: ecdysone receptor (EcR) and ultraspiracle (USP), from several insects, and the availability of stable and easy to synthesize BSH. Moreover, the molecular targets for BSH and reasons for the selective insect toxicity of bisacylhydrazine insecticides are also well known and understood. More investigations are required to explore several aspects about other IGRs, for instance, the molecular targets and basis of actions of about JHAs and CSI and if the JHAs use the same molecular target/site as the natural JHs do. Development of more efficient high-throughput assays for discovery of new and novel CSIs is allowed through understanding of the biosynthetic pathway for chitin synthesis and cuticle deposition, molecular characterization of the various enzymes involved, and the precise mode of action of CSIs, see Gilbert & Gill (2010) for more details. For designing better applications and protocols for pheromone application, understanding the mechanisms of mating disruption systems for different target species and dispensers is very curtail. It is better to suppress highly mobile insects at regional rather than local scales and use newer formulations, such as puffers, attract-and-kill, and sprayable formulations, that offer opportunities to reduce costs, increase program flexibility, and mix strategies. More reliable, economic, and widespread applications of pheromone can be achieved if a joint effort is made by people from different disciplines and organizations. Because of some

difficulties with high populations of pests, pheromones should not be viewed as stand-alone strategies but integrated with other IPM options.

Simply, biological control could be defined as using biota to reduce biota safely and economically. In the last 50 years, microbial insecticides illustrate remarkable developments associated with distinct good results under optimized laboratory conditions, followed many times by unsatisfactory results in the field applications. It is very important to produce reliable, effective, and safe entomo-pathogens for microbial control through the integration of research efforts about physiology, pathology, genetics, mass production, formulation, and application strategies, see Ravensberg (2011), for more information about a roadmap to the successful development and commercialization of microbial pest control products for control of arthropods. Production of biopesticides sharpens the action of genetic engineering for amalgamation of two or more effective lethal processes to finally tailor them into one agent/organism. As a result, the probability of development of resistant strains will decrease due to little morbidity and effective mortality. In spite of concerns about the potential environmental hazards of their long-term use, such opportunities will continue to persuade companies to seek new products and producers. GM microbes and transgenic crops are the new comer in IPM strategies and are gaining popularity because of their efficacy in eradicating pests. Nanotechnology is a promising field of research launches in the present decade a wide array of prospects and is anticipated to give major force in pest control. It is accepted by the public and not yet linked to any great concerns about health and the environment.

Limited shelf-life, high specificity and variable field performance could be considered as advantages and disadvantages of biorational insecticides. As a result, proper identification of a target insect pest is essential and biorational products must be applied when the pest is in its most vulnerable life stage. Otherwise, applications may be ineffective, and applications of a conventional product may be necessary. Insect could also be controlled through environmental/ cultural controls as well as genetic control, see Vreysen et al. (2007), Gurr & Kvedaras (2010), Atkinson (2010), and van Emden & Service (2011) for more details. For more information about area-wide control of insect pests from research to field implementation, see Vreysen et al. (2007). Achieving a zero pesticide strategy in tropical agroecosystems may be easier than in temperate zones, as in many instances farmers have not yet begun the generalized use of pesticides. This gives special opportunities for scientists and farmers to work on a systems approach to minimize pest impact before agroecosystems have been disturbed. For those areas already heavily impacted by the use of pesticides, such as cash crops, horticultural crops and livestock, adjustment to pesticide-free systems management will take some adaptation. Time is needed to establish or re-establish conditions in the system that are conducive to increased natural control such as habitat management and agronomic practices, as well as to introduce farmers to new biological and physical control concepts and methods (Pretty, 2009). In general, biorational insecticides are much slower acting than those acting on neural target sites. The end user has, of course, been used to seeing insects die within a very short time following application of neuroactive insecticides. The change to the new insecticides has necessitated educating the distributors and the users on the mode of action and safety of the new products. There is a great potential to clean up agriculture from conventional insecticides. The road will be open to detoxify agriculture in case of endorsement of strong political will, consumer awareness, and market responses. Indisputably, the number and quality of biorational products will

increase and the costs will fall. These progresses will assure an increasing place in the market for biopesticides for the foreseeable future.

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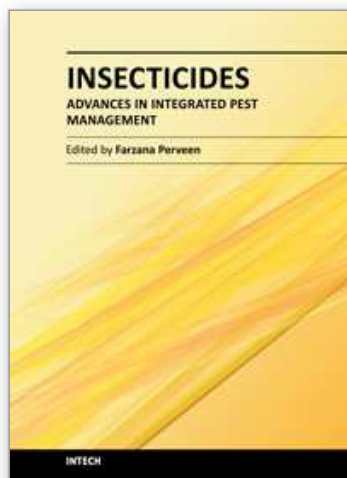
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This book contains 30 Chapters divided into 5 Sections. Section A covers integrated pest management, alternative insect control strategies, ecological impact of insecticides as well as pesticides and drugs of forensic interest. Section B is dedicated to chemical control and health risks, applications for insecticides, metabolism of pesticides by human cytochrome p450, etc. Section C provides biochemical analyses of action of chlorfluazuron, pest control effects on seed yield, chemical ecology, quality control, development of ideal insecticide, insecticide resistance, etc. Section D reviews current analytical methods, electroanalysis of insecticides, insecticide activity and secondary metabolites. Section E provides data contributing to better understanding of biological control through *Bacillus sphaericus* and *B. thuringiensis*, entomopathogenic nematodes insecticides, vector-borne disease, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

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