7 Recent Advancements in Bio-botanical Pesticide Formulation Technology Development

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Abstract

The misuse and overuse of synthetic pesticides have led to the vast destruction of beneficial organisms along with detrimental effects on environment. The use of biopesticides including microbial agents, biochemical pesticides, and botanicals is considered as a more convincing strategy for the management and control of various insect pests. The biochemical pesticides and the botanicals possess pesticidal as well as repellent properties and have come up as better substitutes to synthetics as they are eco-friendly with target specificity and biodegradability and are economically feasible. The chapter provides information on the different types of the biological product-based pesticide formulations for the control of mosquitoes and other disease vectors.

7.1 Introduction

The escalating problems of pest resistance and pesticide residues along with the contamination of the ecosphere due to the irrational use of synthetic chemical pesticides have necessitated the replacement of the same with the pesticides ensuring biodegradability and target specificity. Also, their excessive use for controlling stored grain pests and agricultural pests makes the edibles completely unpalatable for human consumption. In the case of household pests, viz., mosquitoes and cockroaches, there is every possibility of insecticide coming in contact with the human beings. An awareness was circulated worldwide to develop newer class of pesticides (Rajashekhar et al. 2012). The new category of insecticides must have the characteristics, viz., non-phytotoxicity, target specificity, no or low mammalian toxicity, availability, cost-effectiveness, etc. (Hermawan et al.. 1997).

Biopesticides refer to all biologically derived pesticides which help in reducing the pest population (Koul and Walia 2009) such as plants (e.g., pyrethrum – *Chrysanthemum* sp., *Azadirachta* sp., etc.) microbes, nematodes, bac-

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teria (e.g., *Bacillus thuringiensis*), viruses (e.g., nucleopolyhedrosis virus), fungi (e.g., *Beauveria* sp., *Metarhizium* sp.), and the transgenic plants containing a pest-combating gene (e.g., Bt cotton). The biochemical pesticides include pheromones, plant extracts and oils, plant growth regulators, and insect growth regulators. Biopesticides work in harmony with the integrated pest management programs. A number of plant derivatives are known to function as insecticides, insect repellents, antifeedants, and insect growth and development regulators. The effects of plant secondary metabolites responsible for insecticidal activity are described as suppression of calling behavior, growth retardation, toxicity, oviposition deterrence, feeding inhibition, and reduction of fecundity and fertility.

7.2 Categorization of Biopesticides

7.2.1 Microbial Insecticides

7.2.1.1 Bacteria

The most potent and effective microbial insecticide is *Bacillus thuringiensis*. More than 90 % of the bacteria species with different strains that are manufactured and sold throughout the world are for the control of caterpillar pests, mosquitoes, and black flies. The specific orders against which these organisms are useful are Lepidoptera, Diptera, and Coleoptera. Basically the technique involves the utilization of pathogenic microorganisms isolated from diseased insects (Dubey et al. 2008).

7.2.1.2 Viruses

Baculoviruses are rod-shaped DNA viruses, which initiate their life cycle by reproducing inside the cells. Generally, caterpillar cells are reservoirs for baculoviruses, which multiply and get incorporated into protective polyhedron-shaped protein structures called occlusion bodies. The caterpillars infected with the baculoviruses die and contaminate the leaf surfaces with the occlusion bodies. Then the healthy caterpillars ingest the occlusion bodies and release the virus while feeding on contaminated leaves, thus continuing the life cycle of infection and replication. They eliminate some of the caterpillar populations like tobacco budworm, cotton bollworm, etc. (Ben 2002).

7.2.1.3 Fungi

Epizootic outburst in some areas with insect pest populations is a result of inoculating the populations with entomopathogenic fungi, especially with *Entomophthorales* that often successfully regulate the insect pest populations. It instigates an efficient biological control of pests, most notably against the gypsy moth. The most common method of employing fungi for insect control is through inundatory means. The conidia of most of the entomophthoralean fungi species are relatively difficult to produce, and their primary conidia are short lived, making the timing of the inundator applications difficult (Isman and Akhtar 2007). The utility of these fungi has been increased by developing effective methods for production of resting spores and competent mycelia of entomophthoralean species. A broad range of insect pests, including whiteflies, aphids, thrips, termites, grasshoppers and locusts, and beetles, were chosen to investigate the effect of entomopathogenic hyphomycetes.

7.2.2 Biochemical Pesticides

7.2.2.1 Essential Oils

Steam distillation of aromatic plants yields the volatile organic compounds, essential oils produced as secondary metabolites in plants. Approximately 3,000 essential oils are known, out of which 300 are commercially important for cosmetics, perfume, and pharmaceutical industries besides their pesticidal potential (Chang and Cheng 2002, Dubey et al. 2011). Various plant families, for example, Myrtaceae, Lauraceae, Rutaceae, Lamiaceae, Asteraceae, Apiaceae, Cupressaceae, Poaceae, Zingiberaceae, and Piperaceae, are known to produce essential oils. Since the Middle Ages, essential oils have been widely used for bactericidal, virucidal, fungicidal, parasiticidal, and insecticidal applications.

Also, the essential oils can be exploited as insect repellents and insect-feeding behavior alterants, along with those affecting ecdysis (moulting) and behavior during mating and oviposition.

7.2.2.2 Insect Growth Regulators

Insect growth regulators (IGRs) have a unique mode of action different from most chemical insecticides. Generally, these products avert insects from reaching a reproductive stage, thereby plummeting the spreading out of pest populations. The direct impact of IGRs on target pests combined with the preservation of beneficial insects and pollinators aids the growers in maximizing yield and product quality. IGRs can be divided into two broad categories, i.e., those that disrupt the hormonal regulation of insect metamorphosis and those that disrupt the synthesis of chitin, a principal component of insect exoskeletons. As far as the agricultural applications are concerned, the first category of compounds is mainly focused, known as "hormone mimics." The most widely used botanical insect growth regulators is azadirachtin, which interrupts moulting due to structural resemblance to the natural insect moulting hormone, ecdysone.

7.2.3 Botanicals

7.2.3.1 Pyrethrum

Pyrethrin is one of the most widely and heavily used botanical insecticide worldwide, used in household aerosols for fast knockdown of pests. 20–25 % of pyrethrins are present in the technical grade pyrethrum (resin), which is used in formulating commercial insecticides. Piperonyl butoxide (PBO), derived from sassafras or N-octyl bicycloheptene dicarboximide, is added to it to increase insect mortality and shelf life of the product.

7.2.3.2 Rotenone

Rotenone, an isoflavonoid, is a traditional botanical insecticide mainly used for organic food production. It is obtained from the roots or rhizomes of tropical legumes in the genera *Derris*, *Lonchocarpus*, and *Tephrosia*. The dust formulation of rotenone contains 1–5 % active ingredients and is most commonly marketed for household and garden use. The liquid formulations used in organic agriculture can contain as much as 8–15 % of total rotenoids (Isman et al. 2011).

7.2.3.3 Nicotine

The aqueous extracts of tobacco (*Nicotiana* spp.: Solanaceae) and *Anabasis aphylla* (Chenopodiaceae) yield the alkaloid, nicotine. Nicotine is a synaptic poison and therefore induces high level of insecticidal effects as it mimics the neurotransmitter, acetylcholine. The symptoms of poisoning caused by the neonicotinoids are similar to those seen with organophosphate and carbamate insecticides. Most commonly, nicotine is used as a fumigant in greenhouses against soft-bodied pests.

7.2.3.4 Neem

Azadirachta indica (neem) is widely grown in other Asian countries and in tropical and subtropical areas of Africa, America, and Australia and is considered a traditional botanical insecticide (Allan et al. 2002). Azadirachtin was isolated in 1968 and was used as a most potent locust antifeedant. In addition to that, neem possesses fungicidal, nematicidal, bactericidal, and molluscidial properties. Also it exhibits immunomodulatory, anti-inflammatory, antimalarial, antiviral, antioxidant, antimutagenic, and anticarcinogenic effects. Azadirachtin is a persistent insecticide against the crop pests as it has systemic effects (Al-Quraishy et al. 2012).

7.3 Active Ingredients in Botanicals Accountable for Biopesticidal Properties

7.3.1 Terpenes and Terpenoids

Terpenes are a group of molecules whose structure is based on a various but definite number of isoprene units (methylbuta-1,3-diene, named hemiterpene, with 5 carbon atoms). Terpenes are classified on the basis of the number of isoprene units integrated in the basic molecular skeleton as monoterpenes with two isoprene units, sesquiterpenes with three, diterpenes with four, sesterterpenes with five, and triterpenes with six isoprene units.

Modified terpenes, where methyl groups are moved or removed or oxygen atoms are added, are known as terpenoids. Terpenoids are represented as the most widespread group of natural products. Camphor, menthol, eucalyptus oil, lemongrass oil, orange oil, etc. are some of the commercially available terpenoids (Kokete et al. 2012). The essential oil of the basil shrub, *Ocimum kilimandscharicum*, contains camphor as a marker compound which was evaluated against the stored product beetles, *Sitophilus granarius*, *S. zeamais*, *Tribolium castaneum*, etc., in terms of toxicity, grain protectant potential, and repellency (Obeng-Ofori et al. 1998).

7.3.2 Flavonoids

Flavonoids are a group of polyphenolic phytochemicals that include flavones, isoflavones, (iso) flavonones, catechins, and chalcones. Fruits, vegetables, nuts and grains, and herbs and spices contain a high concentration of flavonoids. *P. trifoliata* yields flavonoids that can be employed in the development of commercial mosquitocidal products (Rajkumar and Jebanesan 2008).

7.3.3 Glycosides

Glycosides are colorless, crystalline, or amorphous solid substances generally poisonous in nature. They are optically active (L), usually soluble in water and alcohol, but insoluble in ether and chloroform. Glycosides are the condensation product of hydroxyl group or other groups of aglycon and hemiacetal hydroxyl group of sugar. The aglycon should possess at least one hydroxyl group to which glycosidal hydroxyl group of sugar joints. Glycosides are termed as O-, N-, S-, and C-glycosides on the basis of glycosidic linkage (Shah 2009). Cyanogenic glycosides, which liberate cyanide, are plant-based pesticides which block cytochrome c oxidase and NIS, hence toxic for a large group of parasites and herbivores (Kokete et al. 2012).

7.3.4 Alkaloids

Alkaloids refer to the nitrogen heterocycles extracted from plants with the help of organic solvent, e.g., methanol. They are found in plants as their salts of carboxylic acids as citric, lactic, oxalic, acetic, malic, and tartaric acids. Angiosperms are known to be the major source of alkaloids. The plant families identified with the highest alkaloid levels are Papaveraceae, Berberidaceae, Leguminosae, Liliaceae, etc.(Kokete et al. 2012; Shah 2009). Nicotine (*N. tabacum*, *N. glauca*) is considered as the most potent alkaloid derivative used for the control of piercing and sucking insects, e.g., aphids, thrips, etc. It mimics the neurotransmitter, acetylcholine, in the insect's central nervous system (Dayan et al. 2009).

7.4 Formulations of Bio-botanicals

Very few pesticidal materials, either synthetic or botanical, can be used as they exist in technical forms. Most of these, besides being highly hazardous, are sticky, oily, gummy, greasy, or solid materials which are insoluble in water and difficult to handle. For example, essential oils are highly volatile in nature due to the presence of oxygenated monoterpenes. These have, therefore, to be brought to forms in which they could be used as such (Koul et al. 2008). The process of mixing a pesticide with other materials like wetters, spreaders, stickers, deflocculators, stabilizers, etc. to give it certain desirable properties constitutes its "formulation." It also includes combining with inerts like silica and synergists like PBO to increase the effectiveness.

7.5 Advantages of Biological Pesticides over Synthetic Chemical Pesticides

1. As compared with the persistent and highly toxic synthetic chemical pesticides, the biological pesticides are biodegradable in nature and do not leave any harmful residue.

2. The biopesticides are eco-friendly in contrast to the synthetic ones, which are hazardous to the environment and users.
3. They are non-phytotoxic with greater selectivity toward the targeted pest unlike the synthetic pesticides having phytotoxicity and mammalian toxicity.
4. The biopesticides have an array of constituents responsible for the insecticidal action, so the chances of pests developing resistance are relatively less, whereas in case of synthetic chemical pesticides, the phenomenon of pest resurgence is quite common.
5. Availability of biological components (derived from naturally occurring plants) is trouble-free as compared to the synthetic ones which are always not easily procured.
6. The biopesticidal products have a great economic feasibility as low-cost infrastructure (equipments, raw materials, etc.) is used in their production in contrast to the synthetic-based products which use high-cost machines and other ingredients.

7.6 Types of Bio-botanical Formulations

7.6.1 Emulsions

7.6.1.1 Microemulsions

Microemulsions are isotropically clear dispersion of two immiscible liquids, having a droplet size less than 0.1 μm, and are thermodynamically stable and transparent. They form a single phase of three components, i.e., oily liquid or solid dissolved in organic solvent, water, and surfactant/co-surfactant containing micelles (Singla and Patanjali 2013) in which the nonaqueous phase of the active ingredient and solvent is dissolved by the surfactant aggregate system. The preparation of microemulsions involves the addition of two different types of surfactants, one water soluble (anionic with high HLB) and one oil soluble (ionic and low HLB), and a co-surfactant, like butanol, hexanol, etc., with low HLB value. As compared to the o/w emulsion, the microemulsion can have a total concentration of surfactants as high as 10–30 %, which is 5 % for o/w emulsions (Tadros 1995).

For example, microemulsion formulation of eucalyptus oil, citronella oil, etc. possesses certain desirable properties worth exploiting for pest management but has the drawback of being volatile in nature. However, this can be overcome by encapsulating the oils at the surfactant aggregate system, thereby reducing its volatility and increasing the effectiveness.

7.6.1.2 Nanoemulsions

Nanoemulsions are oil-in-water dispersions of an oil and water phase in combination with a surfactant with the droplet size ranging from 100 to 600 nm (Solans et al. 2003). They are thermodynamically and kinetically stable (Bouchernal et al. 2004). For example, nanoemulsions of citronella oil, hairy basil oil, and vetiver oil with mean droplet size ranging from 150 to 220 nm showed long-lasting mosquito repellent activity against *Aedes aegypti* (Nuchuchua et al. 2009).

Nanoemulsions utilizing eucalyptus oil (Fig. 7.1a) and aqueous extracts of botanicals (*Pongamia glabra* and *Jatropha curcas*) (Bar et al. 2009) have been developed at IPFT, Gurgaon, with long-term stability and increased efficacy against the stored grain pest, *Tribolium castaneum*. They also provide a better surface coverage property of the targeted pest due to nanoparticle size and therefore increased surface area.

Advantages

(a) Micro- and nanoemulsions are aqueous-based formulations.
(b) The use of solvents is eliminated and the pesticide dosage is also reduced to enhance their biological activity.
(c) They have an easy application.
(d) Very low particle size of nanoemulsions makes them a promising alternative for pest control.

Disadvantages

(a) The emulsifier level used for microemulsion is high.

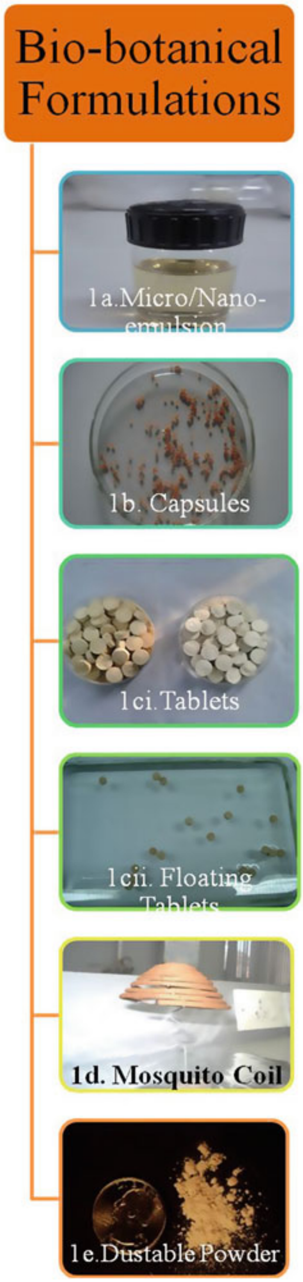

Fig. 7.1 Types of bio-botanical formulations developed at IPFT, Gurgaon

7.6.2 Controlled Release Formulations (CRF)

The technology refers to the triggered/controlled release of an active ingredient to the targeted site to achieve an intended effect on the target pest. Four major categories of controlled release formulations are as follows: (1) coated pesticide granules, (2) matrix systems containing physically trapped particles, (3) polymer systems containing covalently bound particles, and (4) polymer membrane-pesticide reservoir system, e.g., microencapsulation. More popularization of microencapsulation technique has come about in last some years (Beestman 2003). The technique uses the principle of interfacial polymerization (Knowles 2008). In this process the active ingredient, usually a liquid or low-melting waxy solid, is dissolved in an aqueous solution of monomer, e.g., sodium alginate along with slow addition of surfactant to get a homogeneous mixture of all the contents. This mixture is allowed to react with an aqueous solution of a cross-linking agent, e.g., calcium chloride. Interfacial polymerization occurs at the interface where the active component gets encapsulated. The rate of release of the pesticide is, therefore, a diffusion-controlled process (Fernández-Pérez 2007). Examples of CRF formulations are microencapsulations, capsulated suspensions, nanoencapsulated gels, etc. The efficacy of nanoparticle-encapsulated garlic essential oil as compared to free garlic oil was evaluated against *Tribolium castaneum*, the red flour beetle, where the nanoparticle loaded with essential oil gave good mortality over 5 months time indicating a slow and persistent release of active components (Yang et al. 2009). Microencapsulation (Fig. 7.1b) of neem seed oil and karanja oil has been successfully done at IPFT, Gurgaon, for the control of larvae of *Aedes aegypti* (Pant et al. 2012).

Advantages

(a) This technology allows the triggered release of pesticide.
(b) It doesn't affect the nontarget organisms and is safe for the humans.
(c) It also reduces the level of pesticides in the environment.

Disadvantages

The technology requires the use of expensive process/inert ingredients.

7.6.3 Botanical Tablet Formulations

Botanical active ingredients are compressed into a solid mass, i.e., tablet (Fig. 7.1ci), with the help of a tablet machine. The basic components of a pesticide tablet are the active material (e.g., powders of various active components derived from naturally occurring plants), diluents or filler, binder, lubricants, and wetting/dispersing agent (Patanjali 2012). Floating tablets are (Fig. 7.1cii) used for aquatic insects and pests like mosquito larvae. Botanical tablets have been successfully developed by IPFT, Gurgaon (patent filed, application No.:2705/DEL/2012), by using different botanical powders having insecticidal property and botanical wastes (deoiled cakes of *Karanja* and *Jatropha*) alone and in combination. These tablets were tested against the household pest, *Periplaneta americana* (American cockroach).

Advantages

(a) It is an eco-friendly innovation which is completely biodegradable and safe to the user.
(b) The formulation has no mammalian toxicity, phytotoxicity, and dispersion of insect populations like the synthetic sprays.
(c) All ingredients are available throughout India.
(d) Tablets are easy to formulate, store, and use as compared to commercially available synthetic products with an accurate dosage.

Disadvantages

Fungal infestation may occur when high concentration of botanicals is used.

7.6.4 Botanical Coil Formulations

Botanical coil formulations (Fig. 7.1d) comprise of botanical active ingredients, inerts, burning materials and binders (derived from naturally occurring plants), and preservatives (Patanjali 2010). The ingredients are properly dried, powdered, mixed well, and finally extruded through a coil machine to get the product, i.e., coil. Preservatives are used to preserve the repellant properties for a longer period and to increase their shelf life. Coil formulations have been successfully developed by IPFT, Gurgaon (patent filed, Application No.: 365/DEL/2010), by using different botanical powders having insecticidal property along with burning materials, binders, and preservatives in various combinations. The coils prepared were tested against adults of *Aedes aegypti*.

Advantages

(a) The coil formulations are non-hazardous to the environment, less toxic, and safe to the user.
(b) They are economical as they do not consume electricity.
(c) They possess mosquito repellant and knockdown properties.

Disadvantages

Burning of coil produces smoke, which may cause irritation when inhaled.

7.6.5 Dustable Powder

It consists of active ingredients (botanical powders) along with inerts and carriers like china clay. The active ingredient, either solid or liquid, is gradually added in china clay. After complete addition of the active ingredient, it is ground in a mixer to get a uniform composition. It is easy to formulate and use. Dustable powder formulations (Fig. 7.1e) have been developed by IPFT, Gurgaon, for the control of stored grain pests, and a patent has been filed.

Advantages

(a) It is a low-cost formulation and has a good stability aspect.

Disadvantages

(a) It has a low-technology image due to its dustiness and inconvenient application, which create toxic hazards on handling.
(b) It is not target specific, hence has poor bioefficacy.

7.7 Development and Testing of Botanical-Based Pesticide Formulations at IPFT

The bioefficacy of any formulation evaluated against a certain pest is calculated by determining the LC_{50} value using the probit analysis. The LC_{50} value measures the concentration of any pesticide which is able to kill 50 % of the population of the test organisms to which it is exposed. The lower the LC_{50} value, the higher the toxicity of pesticide and vice versa (Mikhaiel 2011).

To conclude the result of any bioefficacy trial, probit analysis is being performed. It relates with the transformation of the observed mortalities to log probit. The doses/concentrations are expressed as logarithmic values. The doses versus observed mortalities give a linear regression parameter. The LC_{50} value is calculated from the linear regression parameter.

Considering the intensive nature of calculations for the estimated LC_{50} and associated 95 % confidence interval using the probit method, the data analysis by a computer program is highly recommended (Rath et al. 2011). The bioefficacy results along with LC_{50} of two botanical pesticide formulations developed at IPFT, Gurgaon, are given below.

7.7.1 Microencapsulation

Encapsulation of neem and karanja oil was done using interfacial polymerization technique where calcium alginate beads were prepared incorporating the oils individually and in combination (Pant et al. 2012). The beads were oven-dried at 45 °C for 2 days and then used for the bioefficacy trials against the *Aedes aegypti* larvae.

Table 7.1 shows the synergistic larvicidal effect of the combination of neem and karanja oil as compared to individual oils. Better mortality was observed with the combination of neem and karanja oils. The LC_{50} value was also found to be less in this combination as compared to LC_{50} individual oils. FTIR analysis of the calcium alginate beads was done to confirm the stability of active ingredient. This formulation has shelf life of more than 6 months and can be exploited for commercial purpose.

7.7.2 Botanical-Based Mosquito Coils

Mosquito coils were developed using neem kernel powder (NKP) and keekar powder (KP) as active ingredients along with binders, preservatives, and burning materials. The efficacy of the coils was evaluated in terms of knockdown and repellency against the *Aedes aegypti* adults (Patent, Abstract published, Application No.: 365/DEL/2010).

The bioefficacy of the coils was evaluated against the mosquitoes, and the percent protection was calculated as % protection = [(No. of mosquitoes landing in control-No. of mosquitoes landing in tested)/(No. of mosquitoes landing in

Table 7.1 Bioefficacy of *Aedes aegypti* with encapsulated neem oil, karanja oil, and neem + karanja oil calcium alginate beads

Serial no.	Amount of calcium alginate beads (mg)	% Mortality in 48 h (Neem oil encapsulation) N	(Karanja oil encapsulation) K	(Neem + karanja oil encapsulation) N + K
1.	100	10.00	20.00	43.33
2.	150	13.33	43.33	53.33
3.	200	16.66	46.67	66.67
4.	250	20.00	53.33	73.33
5.	300	23.33	73.33	86.67
6.	Control	0	0	0

LC_{50} (N) = 5.1 mg/l, LC_{50} (K) = 4.0 mg/l, LC_{50} (N + K) = 3.1 mg/l control)] × 100. The percent protection was 83.49 % whereas the knockdown effect was 39 %.

The results clearly show that mosquito repellent and knockdown activity of the neem (*Azadirachta indica*) kernel powder was enhanced due to the presence of kabuli keekar (*Prosopis juliflora*) pods powder. It is also shown in the results that the maximum mosquito repellent activities and mosquito knockdown activities were shown by the composition, whereas the neem (*Azadirachta indica*) kernel powder and kabuli keekar (*Prosopis juliflora*) pods powder were taken in a specific ratio. The results indicate that kabuli keekar (*Prosopis juliflora*) pods powder has a synergistic effect over the neem (*Azadirachta indica*) kernel powder resulting in enhanced mosquito repellent properties and knockdown activities.

7.8 Conclusion

The current chapter elaborates the importance of botanical-based products/formulations with reference to the formulations developed by IPFT, Gurgaon. The work is in progress with screening and identification of more botanicals as a source of natural insecticides to compensate the loss/damage caused by the excessive use of synthetic chemical-based pesticides. Burning of mosquito coils is a traditional but significant means to repel or kill adult mosquitoes. Many synthetic chemicals such as octachlorodipropyl ether in coils are found to be one of the important genotoxic agents (Pauluhn and Mohr 2000). On the contrary, plant-derived coils are mostly safe and nontoxic (Singh et al. 2011). Increasing attention is being paid to develop safer, effective, and more eco-friendly pesticide formulations. Formulation technology improves operator safety and reduces dose rate and wastage of pesticides applied to crops along with reducing environmental impact and increasing food safety. This has led to the development of water-based liquid formulations regarded as a new technology. The current chapter elaborated the new possible technological developments in pesticide formulations and how the Institute of Pesticide Formulation Technology is progressing in the development of user and eco-friendly pesticide formulations.

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