Botanical insecticides inspired by plant–herbivore chemical interactions

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Plants have evolved a plethora of secondary chemicals to protect themselves against herbivores and pathogens, some of which have been used historically for pest management. The extraction methods used by industry render many phytochemicals ineffective as insecticides despite their bioactivity in the natural context. In this review, we examine how plants use their secondary chemicals in nature and compare this with how they are used as insecticides to understand why the efficacy of botanical insecticides can be so variable. If the commercial production of botanical insecticides is to become a viable pest management option, factors such as production cost, resource availability, and extraction and formulation techniques need be considered alongside innovative application technologies to ensure consistent efficacy of botanical insecticides.

Phytochemicals

Although plants are sessile organisms and cannot escape danger in the way that animals do, they are not completely defenseless. Plants have different forms of defense, ranging from structural traits [1] and barriers [2] to physiological [3] and chemical defensive mechanisms [4]. For decades, researchers have been studying the defensive mechanisms that plants use against different enemies, the variety of defensive responses, and the evolution and ecological impact of those responses [5–9]. Although the evolutionary raison d'être of those traits is to protect plants from herbivores and pathogens in nature, humans have also found many uses for them. Plant secondary chemicals are of particular interest because they can be used as medicines [10], food- and beverage-flavoring agents, fragrances, textile dyes, hygiene products [11], and pest and disease management tools [12]. Plants produce a wide spectrum of chemicals in various tissues above and below ground that are used not only to defend themselves against biotic or abiotic stressors [13,14], but also to communicate with other plants [15] and organisms [16] (Box 1).

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In this review, we focus on botanical insecticides that are inspired by plant–insect chemical interactions. We briefly look at the phytochemicals that have been used for pest management and compare these with conventional (synthetic) pesticides and examine the different ways that plants use their secondary chemicals in nature in contrast to how we use them for pest management. We also discuss the practical challenges of producing commercial botanical insecticides and examine certain assumptions behind commercial botanical insecticides that are available on the market.

Botanical insecticides

All living organisms share certain chemicals and biochemical reactions that constitute their basic metabolism: for example, nucleic acids, proteins, and particular carbohydrates. In addition to the substances that participate in this primary metabolism, plants have also evolved diverse secondary metabolic pathways that produce a plethora of novel substances. Most secondary metabolites are produced from universally present precursors and, therefore, they are often classified based on their biosynthetic pathways [17]. Using a simplified classification, they can be classified as nitrogen-containing compounds, phenolics, polyacetates, and terpenoids (Box 1).

Pesticidal compounds exist within almost all classes of secondary metabolite. For example, the alkaloids nicotine [which is found in the nightshade (Solanaceae) family of plants] and strychnine (which is found in the seeds of *Strychnos* spp.) have been historically used as pesticides [18]. However, the only new botanical pesticides that have come on the North American market over the past 20 years are those based on the terpenoid azadirachtin [a limonoid found in seeds of the Indian neem tree (*Azadirachta indica; Meliaceae*)], which has been used traditionally to control pests and diseases [19], and those based on plant essential oils [20], which are used as contact toxicants, fumigants, attractants, and repellents to control agricultural pests (i.e., two-spotted spider mite, green peach aphid, and greenhouse whitefly), urban pests (i.e., housefly, bedbug, cockroaches, and ants), medical pests (i.e., mosquitoes, ticks, and lice) and veterinary pests (i.e., fleas and horseflies).

Production of botanical insecticides versus synthetic pesticides

Botanical insecticides are generally complex mixtures of several, often closely related secondary metabolites that may or may not have an important role in the toxicity of the

Box 1. Major classes of secondary metabolite used in commercial botanical pest management products

Plants have evolved diverse groups of chemicals that act as major barriers to herbivory. Some chemicals are constitutive [39], meaning that they are always present, whereas others are induced after attack [40]. Many compounds directly affect the herbivore, whereas others attract organisms from other trophic levels [44]. These chemicals can be found in, and are emitted from, all plant tissues above and below ground: toxic terpenes and volatile infochemicals are emitted from the foliage [73,74]; flowers have behavior-modifying floral scents [75]; phytotoxic root exudates are exuded from roots [76,77]; and toxic latex is exuded from the stem [78].

Plants have the capacity to convey certain information about herbivores to their natural enemies via the emission of specific chemical signals [45,47,79]. They can even respond chemically to herbivore oviposition before feeding damage occurs [80,81].

The most important botanical pesticides on the market in commercial terms are pyrethrum, neem, and essential oil-based products (Figure I). Essential oil-based products are the most diverse among the three different types. They are complex mixtures of low-molecular-weight, highly volatile secondary metabolites. Owing to their versatile nature, they have been used in variety of products, from contact toxicants to fumigants and even in behavior-modifier products, such as attractants and repellents.

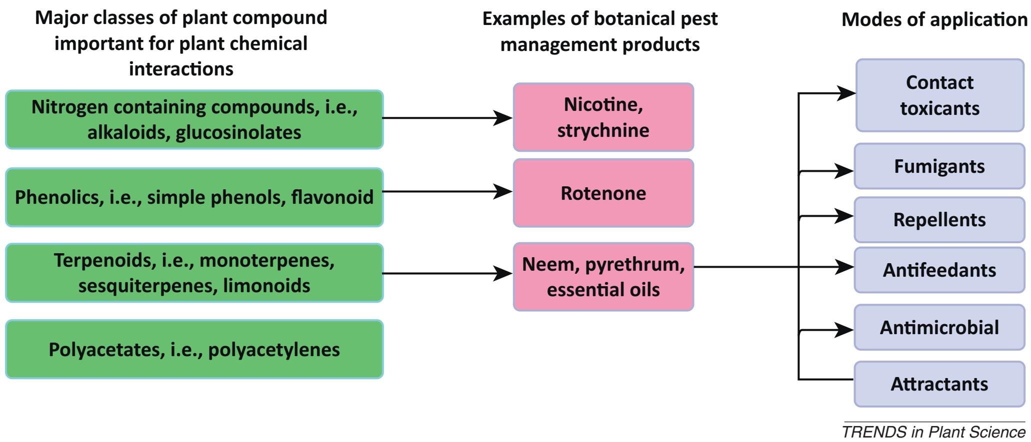

Figure I. Examples of botanical pest management products based on major classes of plant compound.

mixture. They can exhibit internal interactions in the form of synergy or antagonism, which can affect the overall toxicity of the mixture [21]. By contrast, synthetic pesticides are generally based on a single active ingredient (Table 1).

The most important difference between the production of botanical insecticides and the manufacture of synthetic pesticides is the difficulties associated with standardizing the active ingredients found in botanical pesticides: there can be great variability in the quality and composition of these toxic plant extracts. The source of this variability might be natural [22–25] or might occur as a result of using different harvest or extraction methods [26,27]. The initial biomass resource is generally outsourced and minimally monitored, in contrast to quality-control protocols that exist for synthetic pesticides. The extracts are usually specified based on the level of one or two marker compounds (putatively the active principles) even though the presence and level of other constituents in the mixture can significantly influence the overall toxicity and efficacy of the extract [21]. As a result of limited chemical standardization, the efficacy of botanical products may not be consistent [28]. However, synthetic pesticides do not have these problems owing to their simpler compositional structure compared with that of botanical insecticides, and the degree of control and standards relating to their manufacture.

Scalability limitation can also be an issue for manufacturers of botanical insecticides and depends on natural resource availability. Formulations may have to be changed to compensate for scarce and/or expensive ingredients that are not readily available to maintain the competitiveness of the product. Thus, the availability of the ingredients in the market dictates the scalability of botanical products. This is not the case for synthetic pesticides.

Botanical extracts and essential oils often comprise lipophilic and highly volatile constituents and are known to be susceptible to conversion and degradation reactions, such as oxidative and polymerization processes, which can result in loss of quality and of certain properties [29]. The stability of these substances is affected when exposed to elements such as air, light, and elevated temperatures [30]. For this reason, the residual effects of botanical insecticides can be limited and, in some cases, lacking entirely.

Despite these limitations, the use of botanical insecticides in California between 2006 and 2011 grew by almost 50% (http://www.cdpr.ca.gov/docs/pur/pur06rep/chmrpt06.pdf and http://www.cdpr.ca.gov/docs/pur/pur11rep/chmrpt11.pdf), in part because the public perceives natural products to be safer than synthetic chemicals, despite evidence to the contrary [31]. To put this in context, botanical insecticide use represents only 5.2% of biopesticides, and only 0.04% of all pesticide use in California [32]. Biopesticides represent approximately 2% of the US$60 billion global pesticide market (2012 estimate), but the segment is dominated by microbial insecticides led by products based on *Bacillus thuringiensis* [33]. The biopesticide segment is currently growing at 16% per year, compared with conventional agrochemicals that are growing at a rate of 5.5% per year [34].

Table 1. Commercial botanical pesticides versus conventional synthetic pesticides

Differentiators	Botanical pesticides	Synthetic pesticides	Refs
Active ingredients	Mixture of several secondary metabolites with various modes of actions. Concentration of active ingredients in the final product must be at certain level (usually higher than synthetic pesticides) to be effective	Usually one or two active ingredients with specific mode of action, for example neurotoxins. Usually small amount of active ingredient is needed in the final product for effective control	[21,82]
Manufacturing	Simple extraction methods and blending; enzymatic alterations of some secondary products by enzymes such as peroxidases and polyphenol oxidase may occur during extraction; materials are usually outsourced; various formulations	Multistep synthesis of active ingredients; various formulations; in-house production	[83,84]
Scalability	Limited, depending on availability of biomass; limited chemical standardization	Scalable for mass production; rigorous standards in place	
Shelf life	Limited, can breakdown and/or change over time	Relatively stable and/or long shelf life	[30]
Production cost	Variable, depending on biomass availability and/or market price	Generally lower than commercial botanical pesticides, especially off-patent	
Application	Limited applications in urban, medical, stored products, forestry, and large-scale agriculture	Various applications in almost all pest management sectors	[28]
Regulatory hurdles	Exemptions in some jurisdictions, certain products still require full registration	Require full registration	
Social hurdles	Generally considered safe	Generally considered harmful	
Marketing channels	Mostly retail and limited agriculture	Retail and large-scale agriculture, aviation, and military	

Regulatory exemptions such as that provided by List 25(b) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) of the US Environmental Protection Agency (EPA), which allows certain essential oils and inert materials to be used as pesticide active ingredients without regulatory review, has facilitated the commercialization of some essential oil-based pesticides in the USA over the past decade. Many of the essential oils and their products are included in the Generally Recognized as Safe (GRAS) list, which have been approved by the US Food and Drug Administration (FDA) and the US Environmental Protection Agency (EPA) for food and beverage consumption. However, to determine whether we are benefiting from the full potential of these natural substances, we believe that we need to look at their source and see how they are used in their natural context.

Secondary metabolites: use in pesticides versus use by plants

Millions of years before humans became the dominant species on the planet, plants started to evolve sophisticated defensive mechanisms. The diversification of flowering plants during the Cretaceous period (circa 145 million–66 million years ago) is associated with a sudden burst of speciation and adaptive radiation of insects that acted as a major selective force in plant evolution, and led to selection of plants that had defensive adaptations [35]. Plant–insect coevolution has been the subject of several studies [1,5,6,36–38] and several defensive traits have been identified that plants coevolved as a result of interacting with coevolving insect herbivores [2,4]. For the purpose of this review, we only explore traits and features that plants use specifically for chemical defense (Figure 1).

Chemical defenses in plants can be constitutive, meaning that the toxic chemicals are always present [39], or specifically induced after herbivore attack [40]. Some plants have evolved specific ways of storing toxic chemicals to protect themselves from their harmful effects by following two different strategies. Plants either (i) store less-toxic precursors, which are transformed into active toxins only when needed [for example, dhurrin, a cyanogenic glycoside, in sorghum (*Sorghum bicolor*) can produce hydrogen cyanide upon herbivore damage as a result of enzymatic degradation] or, (ii) store the toxic chemicals in specific protected cell compartments, such as vacuoles to prevent self-toxicity [for example, in white melilot (*Melilotus alba*), the tonoplast and the plasmalemma separate glucosinolates from enzymes that can produce toxic mustard oil] [41]. Plants have also evolved special anatomical features to release toxic chemicals. For example, some plants have evolved glandular trichomes that contain highly specialized secretory cells that synthesize and accumulate a variety of secondary metabolites. The glandular trichomes of some plants can continuously exude secretions, for example, capitate trichomes, such as those of tobacco (*Nicotiana tabacum.*), whereas others are touch sensitive and release the toxic materials, when ruptured, to trap and kill small arthropods, for example, peltate trichomes of the Lamiaceae (mint family) [42,43].

In the case of induced chemical responses, plants exhibit a strong degree of specification and control. Plants can respond differentially to different types of herbivore [44] and through those responses, change the behavior of predatory and parasitic arthropods that use those chemicals as cues to find their prey [45]. Plants can also use their volatile chemicals to signal pest density, location of pests in the canopy, and the duration of damage [46–48]. Furthermore, plants can control the composition and emission rates of their volatile chemicals [49–51].

Destructive extraction of these chemicals from plant tissues negates most of these traits. Simply put, we render millions of years of plant evolution, chemical specification, compartmentalization, and structural development useless by crudely combining all the extractable phytochemicals together. To take better advantage of the attributes of these secondary metabolites in the production of bio-inspired botanical insecticides, we must first closely examine the underlying assumptions that are made when developing these products and then address the practical challenges and limitations.

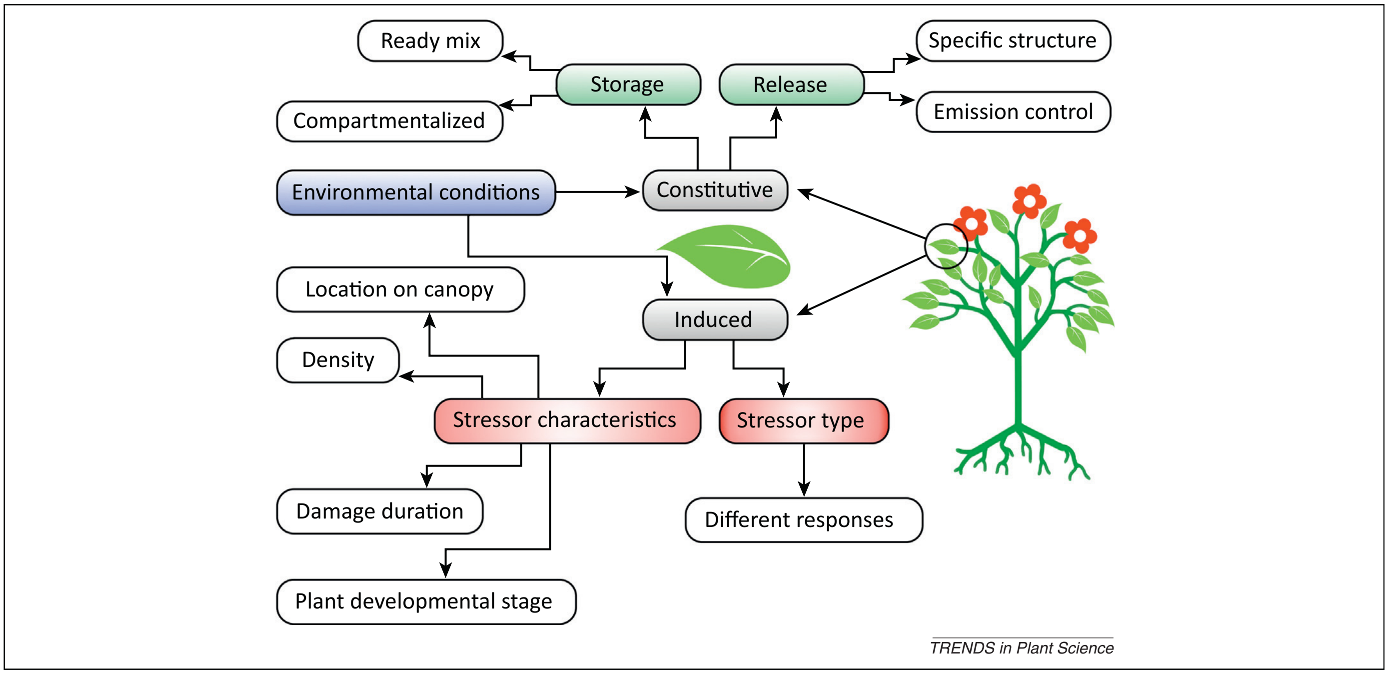

Figure 1. Factors affecting the elicitation of different secondary metabolites in plants. Plants have evolved specific traits that enable them to control and use their chemical arsenal robustly. They have perfected these traits over millions of years of evolution. However, most of these traits are destroyed by crude extraction techniques.

Practical challenges and opportunities

Developing standards, addressing chemical variation, blending and fortification: what is natural?

Botanical extracts and plant essential oils comprise several potentially bioactive constituents. Rosemary (*Rosmarinus officinalis*) oil for instance, comprises more than 50 different constituents, of which approximately ten are considered major compounds that determine specific characteristics of the oil. In addition to these major compounds, minute amounts of other compounds may also be present in the mixture and these can have an active role in the bioactivity of the total mixture [21]. The composition of plant essential oils is influenced by, for example, season, geography, harvest time, species chemotype, and extraction methods [22–27]. Plants can also actively change the composition of their volatile organic compounds in response to minute changes in their microclimate [49]. Most botanical insecticide manufacturers outsource their essential oils and usually screen for one or two marker compounds to determine the quality. To compensate for variability in the composition of essential oils and to create a uniform mixture with a known level of key compounds, essential oil traders and botanical pesticides manufacturers resort to blending essential oils of different origin to obtain the desired mixture. A greater understanding of the role of each constituent in the overall bioactivity of the mixture could enable manufacturers to create more effective blends and mixtures with relatively consistent efficacy [52].

It is also possible to fortify essential oils and botanical extracts with certain compounds that naturally occur in these mixtures to maintain a desired overall composition. However, can fortified mixtures be considered natural? This might be a concern for consumers that perceive 'natural products' to be 'pure, unadulterated substances' even though large-scale essential oil and botanical product traders significantly process these products by, for example, blending and filtration. This issue might also be a concern for regulatory agencies. At this point, there is no official definition or criteria as to what constitutes a natural essential oil despite the variability in their composition. By creating standard criteria for botanical product composition, extraction methods, blending, or fortification, it might be possible to achieve greater uniformity in pesticidal efficacy.

Stability and formulation methods

Compared with synthetic pesticides, botanical insecticides are relatively unstable and breakdown significantly faster when exposed to the elements, such as light, temperature, and air [30]. Constituents of botanical extracts originate from different biosynthetic pathways. Aromatic phenylpropanoids are formed via the shikimic acid pathway resulting in phenylalanine, whereas terpenoids are derived from the C_5 building blocks isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) [53–55]. Once plant chemicals have been removed from their protective compartments as a result of destructive extraction methods, their constituents are prone to oxidative damage, chemical transformations, or polymerization reactions. Furthermore, as plant extracts age, their quality declines further. Over time, they might lose some of their attributes, such as odor, flavor, color, and consistency [56,57]. The compositional diversity of the botanical extracts and the instability of their constituents can make botanical insecticides unsuitable for applications where residual effects over long periods of time are desirable.

To overcome the instability of botanical extracts and essential oils when used as pesticides, several formulation techniques and methods have been developed and deployed in recent years. Microencapsulation, for example, is a method that is used to protect sensitive materials that can easily suffer degradation [58]. Encapsulation techniques can be divided into three classes: (i) chemical processes, such as molecular inclusion or interfacial polymerization [59]; (ii) physicochemical techniques, such as coacervation and liposome encapsulation [60]; and (iii) physical processes, such as spray drying, spray chilling or cooling, co-crystallization, extrusion, or fluidized bed coating [61,62]. Microencapsulation techniques are generally used to prepare pesticide nanoemulsions that provide some level of controlled release of the botanical active ingredient [63].

These microencapsulation techniques generally slow down the release or decay of the entire mixture that is obtained by the destructive extraction of plant tissues; however, no specific attention is paid to the behavior of individual constituents of the mixture. By contrast, plants rely on specific structural features, cellular compartments, and chemical pathways to control proactively the production, storage, and release of individual compounds within their defensive chemical arsenal [50,51,54,55]. Novel technologies that consider the behavior and control level of individual constituents of botanical insecticides are paving the way for a new generation of botanical insecticides that are applied in a manner that is closer to the natural defense methods used by plants against herbivores [64].

Botanical insecticides: stand-alone solutions or complementary supplements?

The defensive arsenal of plants extends beyond chemical barriers [2,3,5]. Phytochemicals often work in harmony with other means of defense to protect plants from herbivores. However, does maximizing the efficacy of botanical insecticides by producing more potent versions of phytochemical mixtures via blending and fortification, despite the higher cost, provide a better option than integrating botanical products with other methods of pest management? Botanical insecticides have been successfully used in combination or rotation with synthetic pesticides [20] and biological control agents [65,66]. Owing to their instability and lack of residual toxicity, botanical pesticides can be easily incorporated into integrated pest management programs along with biological control agents. A closer look at the role of phytochemicals in plant–insect interactions across different trophic layers could inspire further development of effective integrated solutions. Considering the relatively higher cost of botanical insecticides and their scalability limitations, the integrated use of botanical insecticides with other control measures could be a more economically viable option both for consumers and pest management solution providers.

Concluding remarks and outlook

Most botanical insecticides are based on toxic chemicals that plants generate as part of their constitutive defensive arsenal. Many phytochemicals are induced by herbivore attack on demand when needed; however, our strategies for using commercial pesticides do not emulate this particular type of defensive behavior.

We have identified three important areas for future research to improve the efficacy of botanical insecticides. The first is improved extraction methods with specific attention to preserving the integrity of phytochemical mixtures. Sophisticated extraction methods based on physical [67], biological [68], and chemical [69] techniques allow greater control over the composition of plant extracts and provide opportunities for selective extraction of specific bioactive compounds [70]. However, because of their complexity and cost, these methods have not yet been adopted for the mass production of plant extracts by most botanical product producers. For example, traditional steam distillation is still a preferred method for obtaining essential oils in many countries that mass-produce essential oils [12]. Further research is needed to develop advanced extraction methods that are simple and economically viable yet provide adequate levels of control of the composition of botanical extracts.

The second area relates to novel formulation methods that mimic the chemical compartmentalization and storage capacity of plants. Compartmentalization of drug substrates to prevent unwanted reactions is a common practice in the development of pharmaceuticals [71]. The compartments can be formed in various sizes from a visible to a nano-scale [63,72]. The same techniques that have been successfully used in pharmaceuticals can be used for enhancing pesticide formulations. The main challenge for incorporating these techniques for industrial botanical insecticide production is again, cost and complexity. Although the effectiveness and innovative aspects of a product are well regarded in certain societies, cost and economic viability are still the primary factors that determine the commercial success of a product; therefore, more research is needed to find economical solutions and novel formulation methods that address the compartmentalization issue yet maintain commercial competitiveness of botanical insecticides.

The third area is the development of advanced technologies and delivery methods that provide qualitative and quantitative release control at the level of individual constituents. In recent years, micro- and nanoencapsulation techniques have been investigated as means of providing controlled release of botanical insecticides [58–62]. These technologies can extend the efficacy of botanical insecticides over longer periods of time. Despite these formulation advances, the controlled release remained at the whole formula mixture level without addressing differences in volatilization and biological characteristics of individual constituents of botanical materials used in production of botanical insecticides. A better understanding of the behavior and bioactivity of individual components of botanical insecticides coupled with more advanced methods of compartmentalization and formulation will allow greater degrees of control over the availability and activity of individual components of complex botanical mixtures and, consequently, should enhance the efficacy of botanical insecticides.

Disclaimer statement

S.M. was formerly the executive science officer and is a shareholder at Sumatics, LLC based in New York, NY, USA. Through Sumatics, Saber Miresmailli filed a patent on "Apparatus and method for controlled release of botanical fumigant pesticides" which is still under revision at the US patent office. Prior to that, S.M. was a member of the Scientific Advisory Panel for EcoSMART Technologies Inc., based in Roswell, GA, USA, providing consultation and scientific advise on the development of novel botanical pesticides. In collaboration with Scientific Animations Without

Boarders (SAWBO- University of Illinois at Urbana-Champaign), using funds from EcoSMART Technologies, S.M. produced educational animations promoting safe botanical pesticides and the correct way of using, handling, and storing insecticides. M.B.I. formerly served as a member of the Scientific Advisory Panel for EcoSMART Technologies Inc. He has conducted research on botanical insecticides for over 30 years, supported by EcoSMART Technologies, BC Chemicals Ltd., Safer Ltd., Arbokem Inc., and PheroTech Inc. in addition to several government-funding agencies. He currently receives support from SemiosBIO Technologies Inc. and is a consultant to DE Laboratories Inc. He is currently consulting on the development of essential oil-based insecticides for a university in China and a Government institute in Cuba and serves as an international advisor to an European Union-funded project on the utilization of plant pesticides for several countries in sub-Saharan Africa.

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