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Botanical insecticide research: many publications, limited useful data

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Our analysis of >20 000 papers on botanical insecticides from 1980 to 2012, indicates major growth in the number of papers published annually (61 in 1980 to 1207 in 2012), and their proportion among all papers on insecticides (1.43% in 1980 to 21.38% in 2012). However, only one-third of 197 random articles among the 1086 papers on botanical insecticides published in 2011 included any chemical data or characterization; and only a quarter of them included positive controls. Therefore, a substantial portion of recently published studies has design flaws that limit reproducibility and comparisons with other and/or future studies. In our opinion, much of the scientific literature on this subject is of limited use in the progress toward commercialization or advancement of knowledge, given the resources expended.

The allure of plant natural products

Consumers in many regions of the world continue to be drawn to products based on plant (or other) natural products as alternatives to mainstream medicines, cosmetics, and cleaning products. The allure of natural products is based on the often invalid assumption that 'natural is safe – synthetic is hazardous' (e.g., [1,2]), likewise with products intended to kill or otherwise mitigate insect pests (i.e., insecticides). The negative public perception of conventional (synthetic) insecticides is well founded, given the demonstrated negative environmental and health impacts of many synthetic insecticides. However, that perception, deeply ingrained in the public mindset particularly in wealthy countries, is largely based on insecticides used through the latter half of the 20th century. In fact, many of these insecticides were eliminated from the agrochemical arsenal as early as 20 years ago, and replaced by synthetic insecticides with reduced health and environmental impacts.

This negative perception, together with a greater scientific understanding of potential negative impacts of pesticides on human health and the environment, is driving their increasingly stringent regulation in highly developed countries. These considerations provide an impetus for the discovery and development of more environmentally benign and less hazardous insecticides. Among these are the botanicals, either crude extracts or naturally occurring chemicals derived from plants. Plants have evolved complex, and often effective, chemical arsenals to limit the damage inflicted on them by herbivorous insects [3]; and the use of botanicals is one of many strategies to protect plants from microbial pathogens [4] and nematodes [5]. Many plant defensive metabolites have clearly demonstrated mechanisms of insecticidal [6] or repellent [7,8] actions. Examination of the scientific literature in the field of botanical insecticides covering the past 30 years demonstrates a strong and growing academic interest in this area. However, is this surge in research activity on botanicals driving progress toward the discovery and/or commercialization of effective plant-based insect control methods? Unfortunately, for a large proportion of recently published studies, we think not. Here, we document the growth of botanical insecticide research, the recent surge in research on essential oils as insecticides, and the widespread occurrence of two methodological flaws that undermine the reproducibility and interpretation of the results from this fast-growing body of literature.

The renaissance of botanical insecticides research

Botanical insecticides, such as nicotine and pyrethrum, once dominated crop protection and domestic pest control, before the discovery of the insecticidal properties of DDT and methyl parathion during the late 1930s [9]. With the discovery of additional inexpensive and highly efficacious synthetic insecticides (organochlorines, organophosphates, and carbamates), botanicals were quickly trivialized in the pest control marketplace from the 1970s onward [10], and today occupy a small market share (e.g., <0.05% of all pesticides used in California in 2011), even within the category of biopesticides, which is dominated by products derived from the bacterium *Bacillus thuringiensis*.

Our analysis indicates that only 1.43% of papers published on insecticides in 1980 dealt with botanical insecticides. However, a scientific 'renaissance' in interest in botanical insecticides was spurred by the Western 'discovery' of the profound anti-insect bioactivity of the triterpenoid azadirachtin, isolated from the seeds of the Indian neem tree (*Azadirachta indica*, Meliaceae) during the 1960s [9]. Neem and neem-based insecticides became the subject of numerous studies, and several international conferences focusing on neem were held during the 1980s

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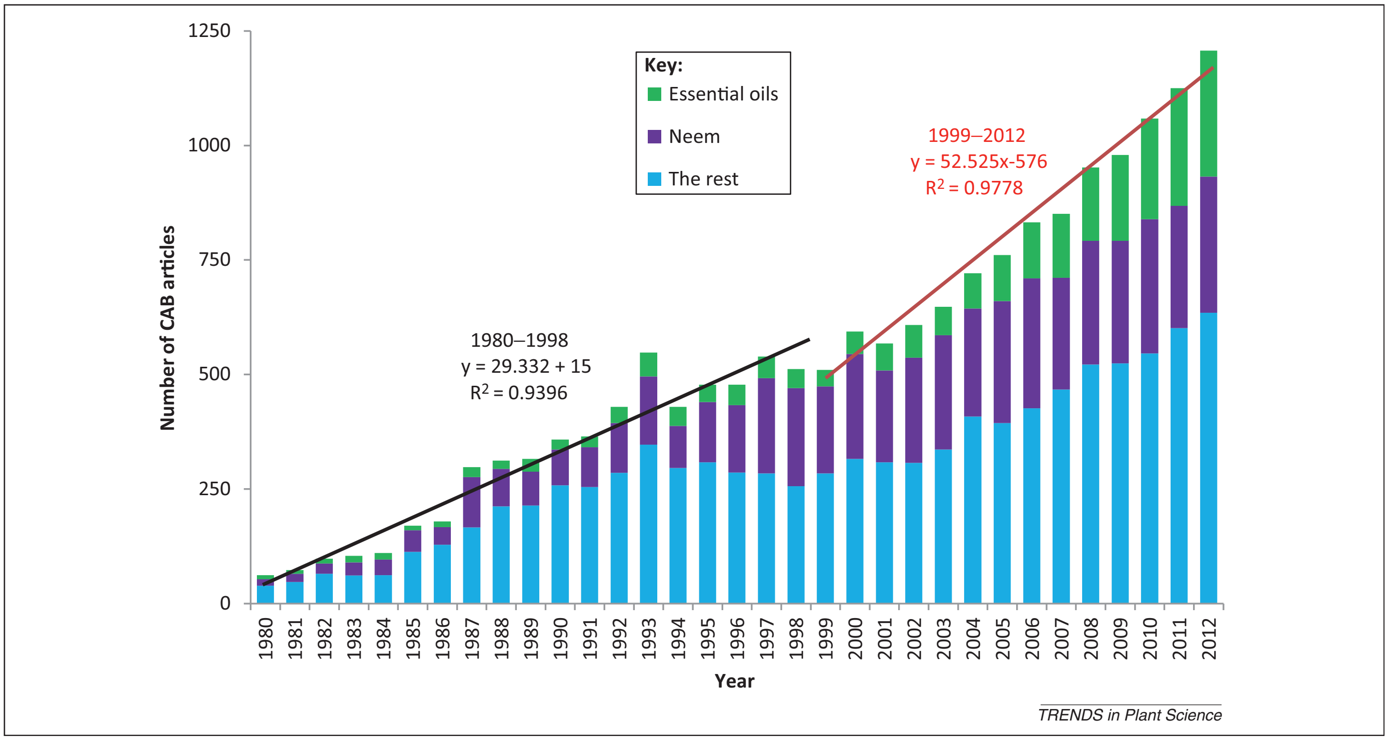

Figure 1. The botanical insecticide literature retrieved from CAB using Query B (‘*botanical insecticide literature*’): (antifeed* or deterr* or repell* or acaricid* or insecticid* or larvicid*) and (precocene* or neem* or azadiracht* or margosan* or (plant and extract*) or ‘essential oil*’ or ‘botanical insecticid*’ or ‘plant oil*’ or ‘vegetable oil*’ or derris or ‘insecticid* plant*’ or ‘leaf extract*’ or limonoid* or triterpen* or diterpen* or sesquiterpen* or saponin* or terpenoid* or flavonoid*) on 24 May 2013. The regression equations indicate this literature grew by approximately 30 articles per year from 1980 to 1998, and by over 50 papers per year from 1999 to 2012. In 2011 and 2012, the numbers of papers on essential oils and neem and its products were very similar. Over half of the papers on essential oils (1,111) were published from 2007 to 2012.

and 1990s. A seminal volume on neem was first published in 1995, with a second edition published in 2002 (http://neemfoundation.org). Regulatory approval of neem insecticides in the USA and Germany made neem the first new botanical insecticide for commercial use in almost 50 years. Our analysis suggests that the subject of neem insecticides became a dominant part of the scientific literature on botanicals over the past 20 years (Figure 1).

A second, and even greater, growth spurt in publications on botanical insecticides began with the commercialization of insecticides based on plant essential oils around the beginning of this century [11,12]. Plant essential oils have several attributes that lend themselves to the production of natural insecticides, including their pre-existing availability through worldwide commerce as flavorings and fragrances [13]. Another important factor in their consumer acceptance and regulatory approval as pesticides is their long history of human use in the aforementioned products. As subjects of scientific investigation, plant essential oils are attractive due to their relative ease of preparation (principally through steam distillation), ease of analysis (gas chromatography–mass spectrometry coupled with computer-based spectral libraries of common compounds), and the wide diversity of plants that produce them [13].

Dramatic growth in publications on botanical insecticides

Our scientometric analysis of publications on botanical insecticides is based on documents retrieved from the CAB Abstracts database (see supplementary material online for methods). A comparative search using the Thomson Reuters Web of Science, which is more commonly used in scientometric analyses, produced fewer than half as many records.

Growth in the field of botanical insecticides research has been explosive, from only 61 papers in 1980 to 1207 in 2012 (Figure 1). This growth can be divided into two somewhat distinct periods: 1980–1998, and from 1998 to date. Much of the growth in the earlier period can be attributed to publications on neem insecticides, whereas the latter period has seen rapid growth in publications on essential oils as insecticides. Over the entire period, almost 5000 papers were published on neem insecticides, more than double the number published on essential oils.

Overall, the proportion of papers focusing on botanical insecticides among all papers on insecticides has increased from 1.43% in 1980 to 21.38% in 2012, and approximately 2.6-fold between 1992 and 2012 (Figure 2). At the same time, the proportion of all papers on botanical insecticides that focus on essential oil insecticides has increased from 8.42% in 2000 to 22.78% in 2012 (Figure 2). Thus, within the realm of insecticide research in general, botanicals, particularly essential oils, are becoming increasingly important. Köhler and Triebskorn [14] noted a similar increase in articles on effects of pesticides on wildlife since the mid-1980s, although proportionally, articles on this subject grew only 1.5-fold between 1992 and 2012.

Papers on the effects of botanical insecticides on wildlife [15,16] and on human health [17] are not common. This may be a consequence of the relatively low acute vertebrate

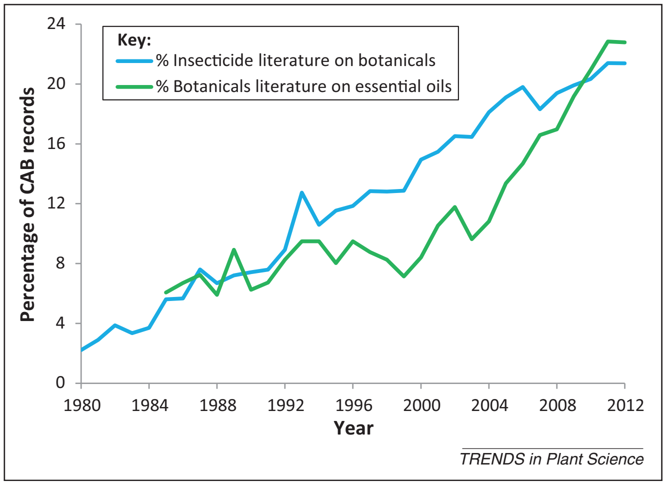

Figure 2. Proportions of botanicals in the overall insecticide literature (100*Query B/Query A), and of essential oils in the botanical insecticides literature (100*Query C/Query B). *Query A* ('*all insecticide literature*'): antifeed* or deterr* or repell* or acaricid* or insecticid* or larvicid*; *Query B* ('*botanical insecticide literature*'): Query A and (precocene* or neem* or azadiracht* or margosan* or (plant and extract*) or 'essential oil*' or 'botanical insecticid*' or 'plant oil*' or 'vegetable oil*' or derris or 'insecticid* plant*' or 'leaf extract*' or limonoid* or triterpen* or diterpen* or sesquiterpen* or saponin* or terpenoid* or flavonoid*); *Query C* ('*essential oil insecticide literature*'): Query A and ('essential oil' or 'essential oils').

toxicity of most botanicals, and the fact that many, including neem and plant essential oils, have long histories of human medicinal uses.

Geographic patterns: the dominance of India, China, and Brazil

Examination of the country of origin for these studies indicates some clear trends (Figure 3). India dominates the category: from 2000 to 2010, numbers of published papers on botanical insecticides increased from approximately 125 per year to just over 300 per year. Over the same period, papers from China increased from approximately 30 per year to approximately 100 per year, and in Brazil the numbers increased from approximately 20 per year to approximately 80 per year. In fact, India, China, and Brazil (the 'big 3') accounted for 494 (40.9%) of the 1207 botanical insecticide articles published in 2012. Recent growth in this category has been more modest in some African (Egypt and Nigeria) and Middle Eastern countries (Iran and Turkey), whereas the numbers of articles have remained essentially constant in most developed countries (UK, USA, Germany, and Japan).

Veracity of studies and contributions to knowledge

Isman and Paluch [18] previously commented on the wide disconnect between the large volume of scientific investigations and publications on botanical insecticides, and the sparse commercialization of new botanicals. However, it must be noted that such research undertaken by major multinational agrochemical companies is unlikely to find representation in the scientific literature, owing to intellectual property concerns. To further understand this issue, we carried out a more detailed examination and review of a random subset ($n = 197$) of papers on botanical insecticides published in 2011 (of a total of 1086). We specifically determined whether each of the 197 papers examined: (i) included any chemical characterization of active fractions or compositional analysis; and (ii) whether bioassay results reported included any positive controls.

Our rationale for the relevance of chemical characterization or composition should be obvious: for a given plant species, plant defensive chemistry can be highly variable in time and space, and can also be affected by environmental conditions, such as soil type, history of predator and/or pathogen attack, and others [2,19]. Investigations based on a single collection of plant material do not address the question of natural chemical variation; neither do they indicate whether the material collected is truly representative of the species. Variation in plant defensive chemistry can be important if extracts of a given plant species are to be used for pest management. Plant populations can comprise chemotypes that either have varying amounts of the bioactive principles of interest, or even lack them entirely. For example, chemical variation and chemotypes of the legume *Tephrosia vogellii*, a plant often used in sub-Saharan Africa for insect control, is particularly instructive in this regard [20,21]. What this means to the researcher is that the biological activity of a given mass of plant material cannot necessarily be predicted based on species alone.

The use of an appropriate positive control (which in this context could be another botanical insecticide although a conventional synthetic insecticide would suffice) greatly enhances the value of any bioassay, because it provides a yardstick against which bioactivity (i.e., pest control efficacy) of new substances or extracts can be measured. It also provides an opportunity, although sometimes tenuous at best, for comparing results from different studies where the same positive control was used. In the absence of a positive control, reports of potency lack credibility.

Our analysis based on these two criteria was not encouraging. Among the 197 papers from 2011 we examined, only 65 (33.0%) included any chemical characterization or compositional information, and only 53 (27.0%) included any positive control (Table S1 in the supplementary material online). Assuming our sample of papers (approximately one-fifth of those published in 2011) is representative, most papers on botanical insecticides published in 2011 are wanting in terms of veracity and utility. Another shortcoming of many papers (62.6% of those examined) is that they report bioactivity of extracts from just a single plant species; and only 21.6% of the studies included more than two plant species (Table S1 in the supplementary material online). When the focus is on a single plant species, plant taxonomy becomes especially important, yet few of the studies we examined reported that voucher specimens of subject plants were retained for future taxonomic validation. Comparable studies of Chinese herbal medicines found a disturbingly high rate of taxonomic misidentifications (M.S.J. Simmonds, personal communication) [22]. An additional potential problem in reporting bioassay results from chemically uncharacterized plant material is the risk of contamination with residues of conventional pesticides or heavy metals [23–25]. Even when plant material is collected in 'nonagricultural' areas, pesticide contamination may occur from pesticides used in residential, structural, golf course, or municipal mosquito control contexts.

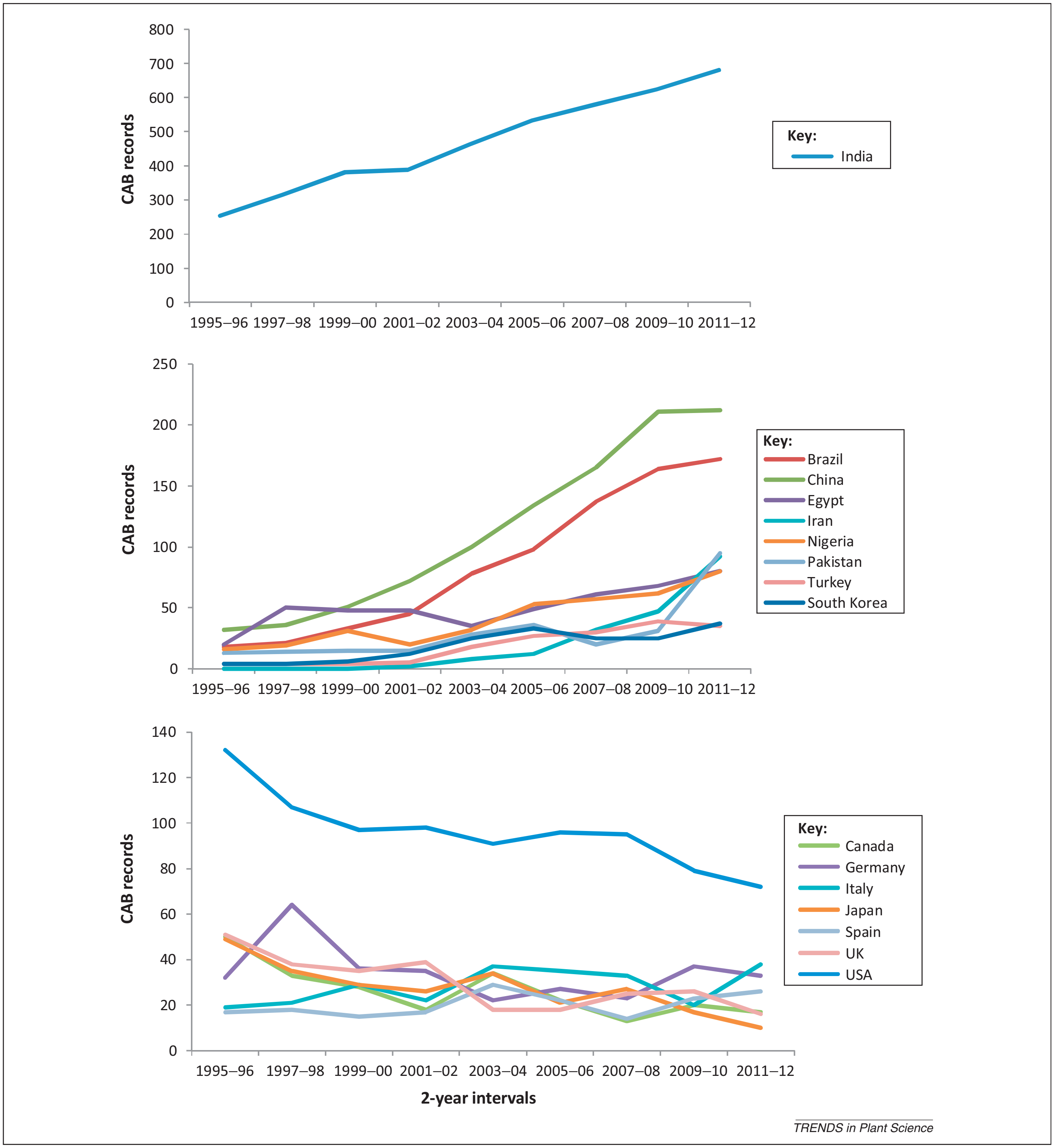

Figure 3. Country affiliations of botanicals literature authors retrieved from CAB by Query B ('*botanical insecticide literature*'): (antifeed* or deterr* or repell* or acaricid* or insecticid* or larvicid*) and (precocene* or neem* or azadiracht* or margosan* or (plant and extract*) or 'essential oil*' or 'botanical insecticid*' or 'plant oil*' or 'vegetable oil*' or derris or 'insecticid* plant*' or 'leaf extract*' or limonoid* or triterpen* or diterpen* or sesquiterpen* or saponin* or terpenoid* or flavonoid*). Note that CAB includes only the affiliation of the author of correspondence. In 2012, authors from India, China, and Brazil together accounted for over 40% of the botanical insecticide literature.

In fact, many studies have shown environmental pesticide-residue levels from residential, industrial, and other non-agricultural uses to exceed those from agricultural uses (e.g., [26,27]); and agricultural pesticide residues have been detected in pristine wilderness areas over 100 km from the nearest agricultural sources [28].

Study quality versus 'journal quality'

The fact that so many studies are published which lack positive controls, chemical characterization, or both, reflects the stringency of editors and manuscript reviewers of the journals, in addition to the decisions made by the authors of these studies. We wondered whether the presence of chemical composition data or positive controls were related to 'journal quality.' ISI® Impact Factors (IF) are a widely used, although far-from-perfect, indicator of 'journal quality' [29]. IF values are based on rates at which all articles in a given journal are cited within 2 years of publication, so they only exist for journals that have been covered for at least 3 years in the Web of Science database. In addition, the *Journal Citation Reports®* of ISI list 'aggregate IF' values, which represent the IF calculation of all articles in a given field of study, such as entomology.

Among the 197 articles in our survey, 40–65% of articles in each 'Yes' or 'No' category of Table S1 in the supplementary material online appeared in journals without IF values. Of those in journals with IF values, the average IF value was markedly higher for articles with chemical composition data (Table S1 in the supplementary material online). Given that such articles are more likely to be published in chemistry journals, this may simply reflect the higher 2012 aggregate IF of fields in chemistry (e.g., 2.957 for chemistry, organic) versus fields where articles without chemical composition data are more likely to be published (e.g., 1.384 for entomology or 1.468 for agriculture, multidisciplinary). The average IFs of articles with and without positive controls were nearly identical, and similar to the aggregate IFs of entomology and agriculture, multidisciplinary (Table S1 in the supplementary material online). Among studies that lack chemical composition or positive controls, 8% and 15%, respectively, appeared in journals with IF values above 2.000. In fact, our survey found some studies lacking these elements that were nonetheless accepted by journals with IFs over 3.000.

Concluding remarks

Our analysis suggests that, although there is a rapidly growing literature on botanical insecticides, much of this literature is limited in its' reproducibility and does not provide a basis for comparison with existing or future studies. These issues likely apply to other categories of botanical pesticides, and other areas of biologically active natural product research. Investigators would be well advised to define the goal of their research, whether it is the use of crude or semirefined plant extracts for resource-poor farmers in developing countries, or simply the first step in phytochemical prospecting for new lead chemistries for industrialized pesticide development. We observed that too many of the studies we examined are little more than first reports on the screening of (chemically uncharacterized) crude plant extracts from a single plant species to one insect pest in a laboratory bioassay. In our opinion, these are examples of preliminary research that may not have sufficient novelty or reproducibility to merit publication. By contrast, we clearly recognize the desire, if not necessity, for scientists in all countries to publish their results. Where research facilities and funding are especially limiting, such studies may be the best that can be accomplished in those settings. Unfortunately, these studies do little to advance knowledge, except to add another species to the list of potentially useful plants. However, even in the absence of chemical analysis, crude plant extracts can have valuable utility for resource-poor farmers, as has been demonstrated recently [30,31], provided that the plant materials used do indeed contain the active substances at biologically meaningful levels. We also encourage scientists in developing countries to make greater efforts to investigate the utility of plant extracts for crop protection in field trials, in collaboration with local farmers, because such studies should prove more valuable than laboratory-only studies.

Disclosure

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 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 EU-funded project on the utilization of plant pesticides in sub-Saharan Africa.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tplants.2013.11.005.

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