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BIOLOGICAL CONTROL

Effects of Botanical Insecticides on Hymenopteran Parasitoids: a Meta-analysis Approach

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Abstract
Botanical insecticides (BIs) are considered a valuable alternative for plant protection in sustainable agriculture. The use of both BIs and parasitoids are presumed to be mutually compatible pest management practices. However, there is controversy on this subject, as various studies have reported lethal and sublethal effects of BIs on hymenopteran parasitoids. To shed new light on this controversy, a meta-analytic approach of the effects of BIs on adult mortality, parasitism, and parasitoid emergence under laboratory conditions was performed. We show that BIs increased mortality, decreased parasitism, and decreased parasitoid emergence. Botanical insecticides derived from *Nicotiana tabacum* and *Caceolaria andina* were particulary lethal. Most of the parasitoid groups showed susceptibility to BIs, but the families Scelionidae and Ichneumonidae were not significantly affected. The negative effects of BIs were seen regardless of the type of exposure (topical, ingestion, or residual). In conclusion, this meta-analysis showed that under laboratory conditions, exposure of hymenopteran parasitoids to BIs had significant negative effects on adult mortality, parasitism, and parasitoid emergence.

Introduction

Hymenopteran parasiotids have been one of the most prominent groups for biological control programs in crop production systems (Cluasen 1978; Stiling & Cornelissen 2005). They have been essential in suppressing phytophagous insect populations (Dolphin & Quicke 2001). The use of parasitoids on pest management can be accompained by another method as long as both are compatible. In this sense, botanical insecticides (BIs) have been considered as attractive alternative for pest management due to their low impact on non-target organisms. In addition, the advantages of using BIs relative to those of their chemical-synthetic counterparts include low residual environmental effects, rapid degradation under field conditions, and low toxicity for humans (Regnault-Roger & Philogène 2008; Montes-Molina *et al* 2008).

Interest in the development of BIs has increased recently, especially in countries like India, China, and Brazil, and other emerging economies (Isman & Grieneisen 2014). Various BIs have been successfully marketed for agriculture, including products derived from *Azadirachta indica* that contain mainly triterpenes with effects on the endocrine system and odor/-taste signal reception (Isman 2006). Products that contain alkaloids have been related to alteration of the insect nervous system (Isman 2006). Likewise, monoterpene-rich essential oils are also common products evaluated on pest management that have demonstrated neurotoxicity (Kostyukovsky *et al* 2002). Apart from mortality, the effects of BIs on pest insects include feeding reduction, developmental alteration, reproductive adnormalities, and behavioral changes (Montes-Molina *et al* 2008; Regnault-Roger & Philogène 2008).

Few studies have examined the effects of BIs on parasitoids to date. With variable outcomes reported, there is a need for a systematic study to draw clear conclusion. For example, some studies have reported that *A. indica*-derived BIs have very low or no negative effects on parasitoids (Tang

et al 2002; Chiasson *et al* 2004; Haseeb *et al* 2004; Almeida *et al* 2010), whereas other studies have found that these BIs have significant lethal and sublethal effects (Perera *et al* 2000; Kumar *et al* 2008; Tunca *et al* 2014). The variability of these results might be related to factors, such as type of exposure, parasitoid species, or developmental period of treated insects. When *Melia azedarach*-derived BIs were assayed on *Cotesia ayerza* (Brèthes) (Hymenoptera: Braconidae), the effect of oral exposure caused high mortality, but topical application had no effect (Defagó *et al* 2011). Conversely, the effects of a *Chenopodium*-based oil on parasitoids was much higher when applied topically compared to residual applications (Hall & Nguyen 2010; Bostanian *et al* 2005).

The present work integrates a meta-analysis of independent studies on the effects of BIs on hyemenopteran parasitoids to determine the effect size and trends about factors that contribute to the effects. We evaluated the effects of BIs on adult mortality, percentage parasitism, and parasitoid emergence. The analysis was broken down in three factors: plant source of BI, taxonomic group of parasitoid, and type of exposure to BIs.

Materials and Methods

Data selection

The studies were obtained from eight electronic databases, Wiley Online Library, BioOne, Elsevier, Cambridge Univ Press, Taylor & Francis, Cabdirect.org, SciELO, and Ingentaconnect. To find primary literature on the effects of BIs on hymenopteran parasitoids, the search terms used were botanical insecticides, plant extracts, parasitoid, benefical insects, natural enemies, toxicological effects, toxicity, and side-effects. The citations from the papers resulting from the abovementioned searches were also included. Data were included if the studies met the following criteria: (1) the studies were carried out after the year 2000; (2) the studies evaluated the toxicity of plant-derived products (experimental or commercial) on hymenopteran parasitoids under laboratory conditions; (3) the studies reported data on adult mortality, percentage parasitism, or adult emergence; (4) the studies provided clear methods for the evaluation of BIs at relevant concentrations and rates used under field conditions; and (5) the studies provided means, sample sizes, and measures of variance (standard deviation or standard error) for both a control group (non-BI exposed) and an experimental group (BI exposed).

Data from a single paper reporting more than one parasitoid family, BIs from diffrent plant sources, different concentrations of BI, or time of exposure were considered independent studies. Plant source of BI refers to the plant species from which the product was obtained. The type of exposure refers to the method used for the toxicological studies, which includes topical, oral, and residual application. Parasitoid group refers to the family of parasitoid where the species evaluated belonged.

Effect sizes

The effects of BIs on adult mortality, percentage parasitism, and parasitoid emergence were evaluated using a ratio (response ratio) of the effect compared to that of a control group where parasitoids were not exposed to BI. As a result, negative values in the response ratio indicate a negative effect or a reduction in parasitism and parasitoid emergence, whereas for adult mortality, a positive value indicates an increase in mortality. To calculate the effect size for each variable, the mean, sample size, and standard deviation were obtained for both control and experimental groups. If the studies did not provide these data, univariate statistical data (F, t y p) were used to calculate the effect size (Rosenberg *et al* 2002). All standard error values were transformed into standard deviation according to the equation: $SD = SE \sqrt{n}$, where *SD* is the standard deviation, *SE* is the standard error and *n* is the sample size.

Data analysis

Separate analysis was performed to evaluate the influence of plant source of BI, taxonomic group of parasitoid, and type of exposure to BI. The variables analyzed were adult mortality, percentage parasitism, and parasitoid emergence.

The effect size was calculated by Hedges: $d = [(X_O\text{-}X_Y)/s]\,J$, where X_O is the mean response to the control group, X_Y is the mean response to experimental group, s is the combined SD, and J is the correction factor for bias due to small sampling size (Gurevitch & Hedgez 2001).

The analysis was performed in Meta Win 2.1 statistical program using fixed effects models (Rosenberg *et al* 2002). Confidence intervals were generated to a 95% confidence level (Bias-corrected bootstrap) for all the effect sizes from 999 iterations. The effects were considered significant if the confidence intervals did not overlap with zero.

To evaluate if the categorical groups (plant source of BI, taxonomic group of parasitoid and type of exposure to BIs) were homogeneous with respect to the effect size, the heterogeneity within each group (Q_W) and among groups (Q_B) were calculated and the significance was evaluated by X^2.

The risk of bias existing in the dataset was assessed by the failsafe number (n_{fs}), using the Rosenberg method (Rosenthal 1979; Rosenberg 2005). The n_{fs} indicates the number of nonsignificant, unpublished or missing studies that would need to be added to the meta-analysis to change the results from significant to nonsignificant. The results are

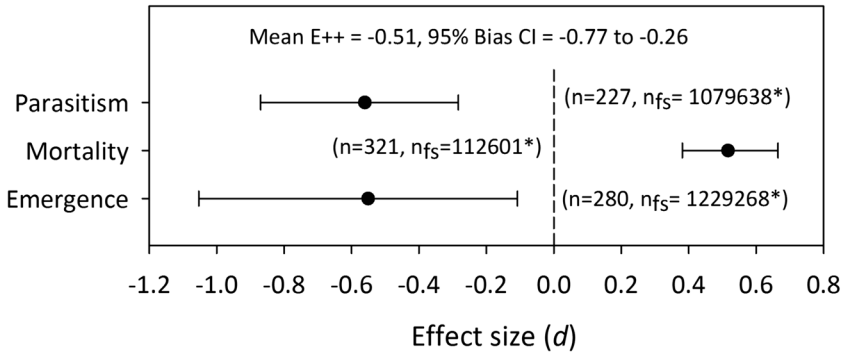

Fig 1 Effect size (mean and 95% confidence interval) of BIs on adult mortality, parasitism, and parasitoid emergence. The number of point samples used to calculate each mean is shown for each analysis. Means with confidence intervals that did not overlap with zero were considered significant. *n*: sample size; n_{fs}: failsafe number; *: indicate statistical robustness of n_{fs}. Mean effect size (E++) and 95% Bias CI are presented for all variables of responses.

considered robust when the *n*fs is larger than 5*n* + 10, where *n* is the number of studies (Rosenthal 1979).

Results

Heterogeneity and general effect

Among 104 studies collected, only 57 met the selection criteria. Not all selected studies provided data for all response variables. We synthesized 24 studies for adult mortality, 24 studies for parasitism, and 39 studies for parasitoid emergence. For the metanalysis, the negative effect of BIs is indicated by the positive values in the variable adult mortality, and negative values in the variables parasitism and parasitoid emergence.

Overall, BIs had significant effects on adult mortality, percentage parasitism, and parasitoid emergence (Fig 1). For adult mortality, exposure to BI resulted in an effect size of 0.5168 (Bias % CI = 0.3810 to 0.6649), which represents a significant negative effect compared to control. For parasitism and parasitoid emergence, exposure to BIs resulted in effect sizes of − 0.5604 (Bias % CI = − 0.8697 to − 0.2834) and − 0.5508 (Bias %CI = − 1.0529 to − 0.1080), respectively, which represents a significant negative effect relative to controls (Fig 1; Table 1).

Mortality

Across all studies, BIs caused significant mortality of adult parasitoids (Table 1; Fig 1). The analysis by plant source of BI, parasitoid group and type of exposure, showed that most of the BIs produced significant mortalities on adult parasitoids, except those derived from *Origanum vulgare* and *Tephrosia vogelii*. The highest effect on adult mortality was observed for BIs derived from *Nicotiana tabacum* (Fig 2). The analysis by parasitoid group showed that the effects of BIs were significant in all parasitoid families, except Eulophidae, Ichneumonidae, and Scelionidae (Fig 2). Results also showed that all types of exposures had negative effects on mortality (Fig 2).

Parasitism

Overall, exposure to BIs reduced significantly parasitism (Table 1). The analysis performed by plant source of BI showed that, with the exception of those derived from *Blumea aurita, Chenopodium* sp., and *Leptospermum petersonii*, all others had significant negative effects on parasitism (Fig 3). Analysis by parasitoid group showed an overall negative effect in all parasitoid families, but the effect was significant only in Aphelinidae, Ichneumonidae, and Trichogrammatidae (Fig 3). All types of exposure had negative effects, but these effects were significant only when parasitoids were exposed by ingestion and residual contact (Fig 3).

Table 1 Heterogeneity statistics for each model in the adult mortality, parasitism, and parasitoid emergence frequency analysis.

	Mortality			Parasitism			Emergence		
	df	Q_B	*P*	df	Q_B	*P*	df	Q_B	*P*
Full model	320	5245	< 0.0001	226	56,149	< 0.0001	279	169,063	< 0.0001
Plant source of BI	9	453	< 0.0001	10	1089	< 0.0001	10	4581	< 0.0001
Parasitoid group	7	242.5	< 0.0001	5	521	< 0.0001	8	1222	< 0.0001
Type of exposure	2	30.6	< 0.0001	2	69.8	< 0.0001	2	70	< 0.0001

df, degrees of freedom; Q_B, variation in effect size explained by the model.

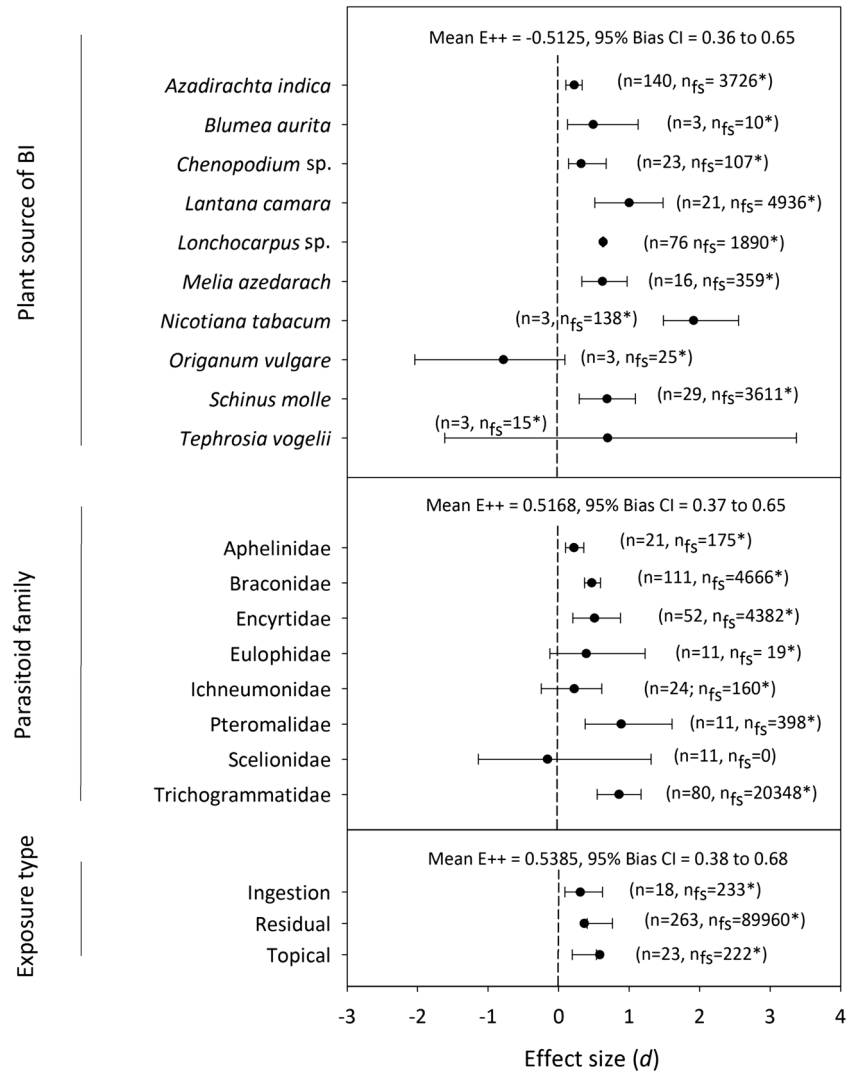

Fig 2 Effect size (mean and 95% confidence interval) of BIs on adult mortality for the categories considered for the a priori defined groups (type of BI, parasitoid family and type of exposure). Effect sizes were considered significant if 95% confidence intervals did not overlap with zero. Effect sizes within analyses were considered different from one another if their 95% confidence interval did not overlap. *n*: sample size; n_{fs}: failsafe number; *: indicate statistical robustness of n_{fs}. We showed mean effect size (E++) and 95% Bias CI for all response variables.

Parasitoid emergence

Parasitoid emergence significantly decreased when hosts were exposed to BIs (Table 1). When the effects were categorized by plant source, BIs derived from *Azadirachta indica*, *Calceolaria andina*, *Cymbopogon schoenanthus*, *Lantana camara*, and *Schinus molle* caused significant negative effects on parasitoid emergence (Fig 4). Analysis by parasitoid group indicated significant negative effects on Aphelinidae, Braconidae, Encyrtidae, Pteromalidae, and Trichogrammatidae. Significant negative effects were also observed when parasitoids were exposed by ingestion and residual contact.

Discussion

This work presents a systematic analysis of the effects of BIs on hymenopteran parasitoids under laboratory conditions. Overall, we found that BIs had negative effects on adult mortality, parasitism, and parasitoid emergence. The extent of the BI effects on parasitoids was dependent upon the plant source, parasitoid group, and type of exposure to BIs. It is important to mention that BIs included in this analysis come from widespread commercial-use products, some are used regionally as crude products and some are used on a very limited basis.

Botanical insecticides have been developed from a wide range of plant species, including those that contain highly toxic metabolites. Various studies have reported that BIs are harmless for non-target organisms. However, in the present meta-analysis, we observed that all BIs caused adult mortality, decreased parasitism and decreased parasitoid emergence. Mortality was particularly high when parasitoids were exposed to *C. andina*- and *N. tabacum*-based BIs, which suggest that these BIs may contain high concentrations of non-selective metabolites. Phytochemical studies have shown that *C. andina* contain naftoquinones, metabolites that inhibit mitochondrial respiration and cause DNA fragmentation

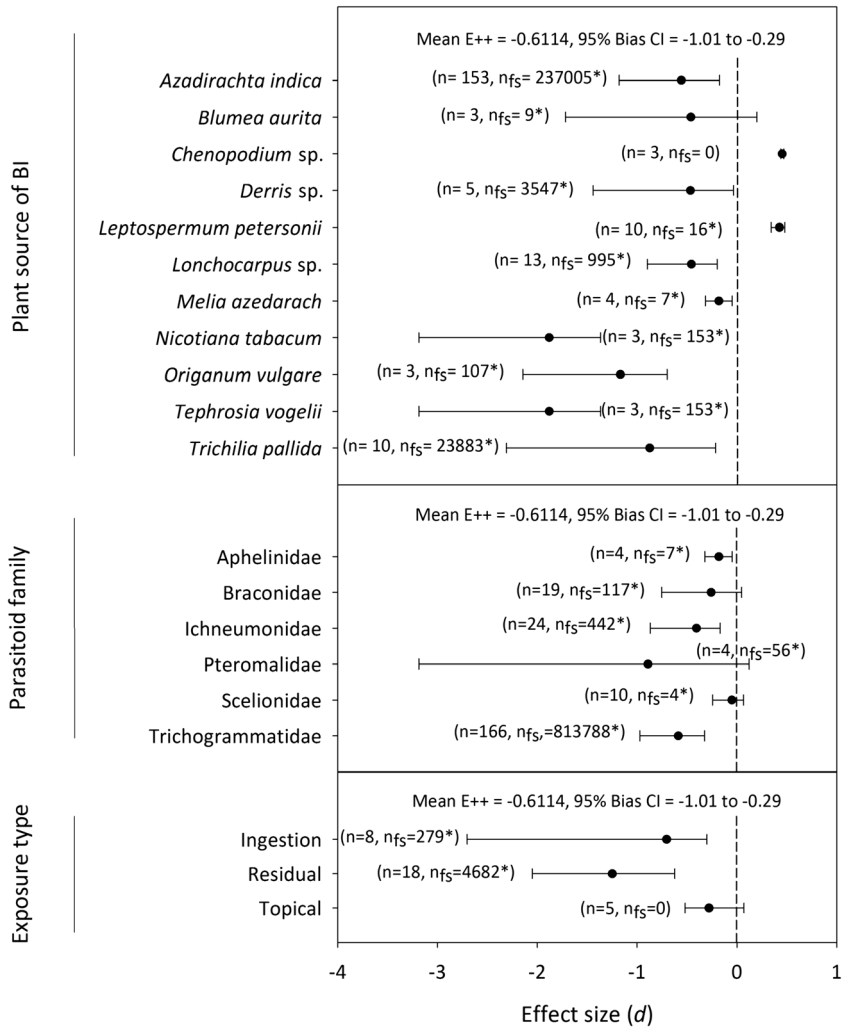

Fig 3 Effect size (mean and 95% confidence interval) of BI on parasitism for the categories considered for the priority defined groups (type of BI, parasitoid family and type of exposure). Effect sizes were considered significant if 95% confidence intervals did not overlap with zero. Effect sizes within analysis were considered different from one another if their 95% confidence.

(López *et al* 2002; Simmonds *et al* 2002; Babu *et al* 2008; Akhtar *et al* 2012). Similarly, *N. tabacum*-based BIs contain high concentrations of alkaloids, like nicotine, nornicotine, and anabasine, which are metabolites with general neurotoxic activity (Boeke *et al* 2003; Isman 2006). Other BIs, especially those that have essential oils in their constituents, may target to some extent the insect nervous system (Iannacone & Lamas 2003; Abdel-Sattar *et al* 2010; Dua *et al* 2010; Huerta *et al* 2010). Other metabolites commonly present in BIs, like isoflavonoids, can also have lethal effects by blocking electron transport and in turn inhibiting mitochondrial ATP production (Hollingworth *et al* 1994). Therefore, direct mortality should be expected when BIs containing the aforementioned metabolites are applied. Our analysis also showed that overall, exposure to BIs reduced parasitism and reduced parasitoid emergence. Surprisingly, *Chenopodium* sp.-based BI had positive effects on both variables. This should be taken with care as the results were not robust based on our failsafe number. It is worth noting that studies dealing with the effects of *A. indica*-based BIs have shown highly variable outcomes, with some reporting negative effects, while others found no effects (Haseeb *et al* 2004; Kumar *et al* 2010). Our results clearly indicate that *A. indica*-based BIs have lethal effects on adult parasitoids and decrease parasitism as well as parasitoid emergence.

We did not analyze other effects of BIs at sublethal concentrations such as host localization and host acceptance. These processes are critical when a parasitoid must localize and choose an adequate host to ensure its reproductive success (Desneux *et al* 2007). These effects may occur specifically with BIs that cause deterrence/repellence. In this regard, Luckmann *et al* (2014) documented that repellent BIs provide a barrier to parasitism (Desneux *et al* 2007; Luckmann *et al* 2014). Other studies have also documented the negative effects of botanical insecticides on repellence of other beneficial insects, like honey bees, resulting in a change of foraging behavior of these pollinators (Xavier *et al* 2015).

Susceptibility of the parasitoid group to BIs was also analyzed. Direct adult mortality was observed in all analyzed

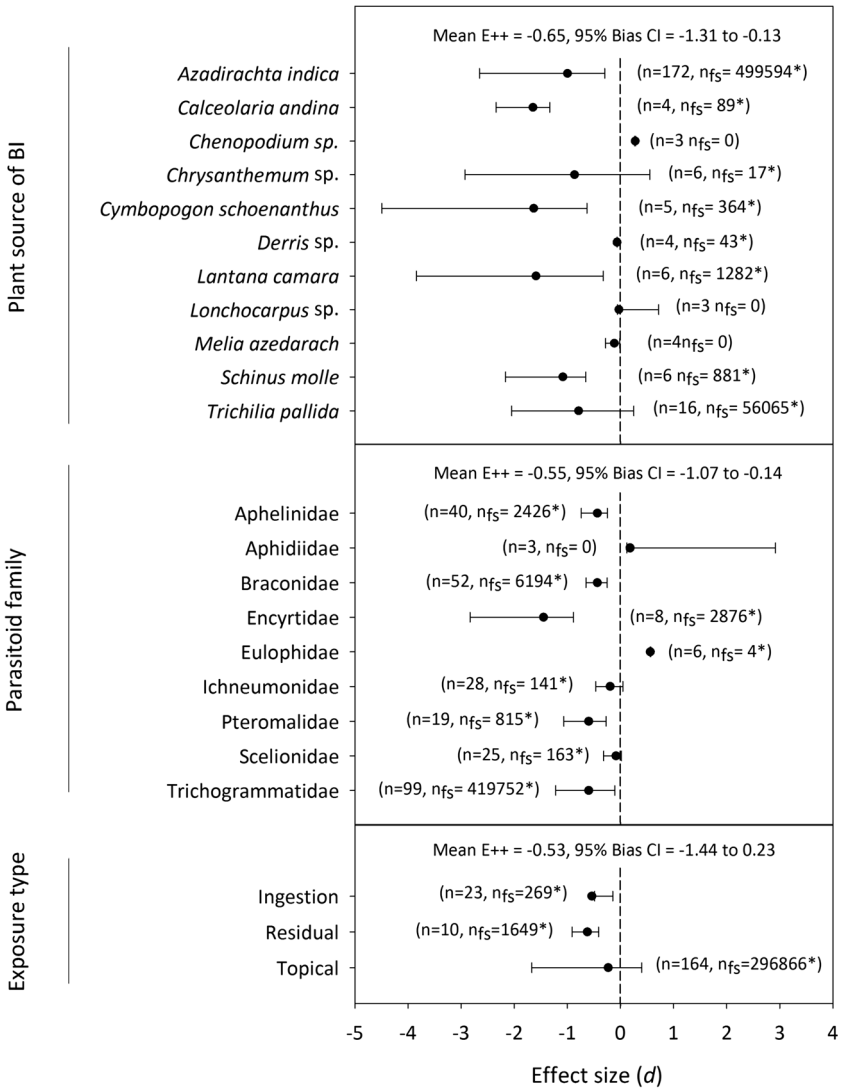

Fig 4 Effect size (mean and 95% confidence interval) of BI on adult emergence when applied to parasitized hosts for the categories considered for the appropriate groups (type of BI, parasitoid family and type of exposure). Effect sizes were considered significant if 95% confidence intervals did not overlap with zero. Effect sizes within analysis were considered different from one another if their 95% confidence interval did not overlap. *n*: sample size; n_{fs}: failsafe number; *: indicate statistical robustness of n_{fs}. We showed mean effect size (E++) and 95% Bias CI for all response variables.

families, except Ichneuminodae and Scelionidae. We observed a significant decrease in parasitism in Ichneumonidae and Trichogrammatidae, and in parasitoid emergence in Aphelinidae, Braconidae, Encyrtidae and Pteromalidae. A plausible explanation for this response may be related to the low capacity of these parasitoid groups to excrete and/or detoxify the chemical components of BIs. The effects of BIs then should not be generalized for all parasitoid families. We also observed that two families, Ichneumonidae and Scelionidae, were not significantly affected in at least two of the studied variables, adult mortality and parasitoid emergence. As for the type of exposure to BIs, at first sight one may think that residual exposure could be less harmful that topical exposure or exposure by ingestion. Surprisingly, residual exposure was one of those causing the largest negative effects.

It important to mention that the negative effects of BIs on parasitoids found in this meta-analysis should be interpreted with caution as all data used were obtained from reports of experiments carried out under laboratory conditions, and it is well known that the evaluation of toxicants under these conditions overestimate realistic effects in the field (Blümel *et al* 1993). To obtain more reliable information and a better characterization of the effects of BIs on parasitoids, it is necessary to include data from a combination of laboratory and field toxicity testing. Other studies on the side-effects of insecticides on non-target organisms, like aquatic arthropods and predatory mites, have pointed out the differential effects of insecticides when evaluated under both laboratory and field conditions. Overexposure of the organisms in laboratory essays results in an increase in toxicity (Baughman *et al* 1989; Blümel *et al* 1993). Other factors influencing the toxicity of the BIs on parasitoids in field experiments include rapid degradation due to weather conditions, formulation of the BIs, and time of spraying (El-Wakeil 2013). It is common that highly toxic insecticides in the laboratory have moderate to low toxicity in the field and greenhouse (Studebaker & Kring

2003). Thus, multiple testing methods should be included when evaluating the effects of pesticides on beneficial arthropods.

Conclusion

Our study showed that under laboratory conditions, BIs cause adult mortality, decrease parasitism, and decrease parasitoid emergence. Variation in the effect size was observed among plant source of BI, parasitoid family, and type of exposure.

Most of the analyzed parasitoid families were susceptible to BIs, except Scelionidae and Ichneumonidae. Particularly, residual and ingestion treatments caused significant negative effects on parasitoids.

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