

A Meta-Analysis Study on *Spodoptera exigua* and *Spodoptera litura* Control: Biopesticides vs. Synthetic Pesticides

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ABSTRACT

Polyphagous lepidopterans like *Spodoptera exigua* (Hübner) and *S. litura* (F.) (Lepidoptera: Noctuidae) are well-known economic importance insect pests that defoliate a variety of economically important crops. Currently, the most widely used control for both is the use of insecticides. Applying large amounts of synthetic pesticides to control these armyworms has resulted in resistance and resurgence of these insect pests. Developing an environmentally friendly method to control insect pest populations, such as investigating natural components from plants as natural insecticides, can help to reduce insecticides' harmful effects. The current effort is in line with this call and concern, but it focuses on biopesticides and synthetic pesticides with different targets, methods of exposure, and stages of exposure. The study used information extracted from 100 scientific papers. Descriptive statistics, agglomerative hierarchical clustering, and identifying the model, heterogeneity, and Log Response Ratio (LRR) of LC₅₀ were performed to identify the development of biopesticides and synthetic pesticides in controlling these armyworms. The studies with botanical insecticides started in the mid 1988, and it experienced exponential growth after 2001. The trend is currently maintained and peaked in 2012. The agglomerative hierarchical clustering analysis indicated that 3rd instars were assessed in paper frequently, while 1st, 2nd, 4th, and 5th instars were located in a separate cluster. The agglomerative hierarchical clustering analysis indicated that leaf dip bioassays were assessed in paper frequently, while artificial diet bioassay, leaf disc bioassay, and topical application instars were located in a separate cluster. Highly heterogeneous values for a fixed-effect model on the effect of pesticides on *S. exigua* and *S. litura* showed that other factors gave a stronger influence than the treatment. The LRR of LC₅₀ showed that *S. exigua* is more sensitive than the *S. litura*.

Keywords: Biopesticides, Insecticides, Meta-analysis, *Spodoptera exigua*, *S. litura*

1. INTRODUCTION

Polyphagous lepidopterans like *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) are well known as important insect pests that defoliate many economically important crops [1]. The beet armyworm (*S. exigua* Hübner) is a polyphagous pest that attacks various vegetables and ornamental plants [2]. The insect has been reported to be the main pests of welsh onions in Vietnam, onions in Indonesia, onions and tobacco in India, cotton

in Egypt, corn in Turkey, and onions in the Philippines [3]. *S. exigua* infects many shallot plants, turning it into a serious problem for shallot plantations in Indonesia [4,5].

Spodoptera litura is a polyphagous insect that damages various vegetables and field crops in Asian countries. *S. litura*, also known as the tobacco cutworm, is a significant threat to most cropping systems due to its generalist herbivorous behavior [6,7]. Cotton, tobacco, celery, tomato, chrysanthemum, groundnut, crucifers,

sunflower, soybean, kale, mung beans, and corn are among the 150 host types of *S. litura* [8,9,10,11]. In Indonesia, *S. litura* has a critical status and causes up to 80% damage to soybeans [12,13].

People began to employ synthetic insecticides to combat these armyworms and lessen the loss. Excessive usage of synthetic pesticides has resulted in insect pests developing resistance and resurgence, biological accumulation and biological magnification in the environment and non-target organisms [14]. However, according to the World Health Organization (WHO), synthetic pesticide poisoning kills over two hundred thousand people every year. According to the World Resource Institute, more than 500 insects are resistant to insecticides. Reducing synthetic pesticides' negative impact can be done by using biopesticides. On the other hand, biopesticides are less persistent, less toxic to non-target organisms, and eco-friendly [15].

In the present study, we conducted a bibliographical search and meta-analysis using information from 100 scientific papers mentioning biopesticides and synthetic pesticides for armyworms. The meta-analyses were performed for (i) recognizing the magnitude of the evolution and focus of interest within the subject of botanical insecticides and synthetic pesticides, (ii) identifying the model, heterogeneity, and Log Response Ratio (LRR) of LC₅₀ on such studies, and as well (iii) recognizing existing knowledge gaps that needed to be addressed. The results obtained can be used as a consideration in developing bio-insecticides based on plant extracts to control this severe lepidopteran pest.

2. MATERIALS AND METHODS

2.1. Strategy to Select Scientific Paper

The scientific papers for this study were obtained from online journals. Several bibliographic databases were accessed online through Google Scholar, PubMed, ScienceDirect, Scopus, and Journal Storage (JSTOR). The keywords used included "biopesticide", "synthetic pesticides", "*Spodoptera exigua*", "*Spodoptera litura*". A screening procedure was applied to select 100 papers following criteria study about biopesticides from botany or insecticides and the target is *S. exigua* or *S. litura*.

2.2. Construction of Bibliographical Database and Excel File for Meta-Data Analysis

The 100 selected papers were added to a Mendeley database. First, we tagged the papers with the discipline/study information: biopesticide or insecticide. Then, the papers were read thoroughly and information was extracted into an MS Excel table: authors, publication year, country, study, target, exposed stage, exposure method, control, and response variable (Supplementary data 1). Another MS Excel table was

prepared to identify the relationship between exposed instar stages assessed in each study from each paper (Supplementary data 2), to identify relationships between each exposure method in each study (Supplementary data 3), analysis of the targeted study and identify the effect of several synthetic pesticides on *S. exigua* larva (Supplementary data 4).

2.3. Data Analysis

MS Excel was used to summarize the data, the XLSTAT, RStudio, and Meta-Mar were used for statistical analysis. Supplementary data 1 was used for descriptive statistics. Supplementary data 2 and 3 were used for Agglomerative Hierarchical Clustering (AHC) analysis. Supplementary data 4 was used for models and the determination of heterogeneity and LRR of LC₅₀ analysis.

3. RESULTS

3.1. General Data Description and Qualitative Overview

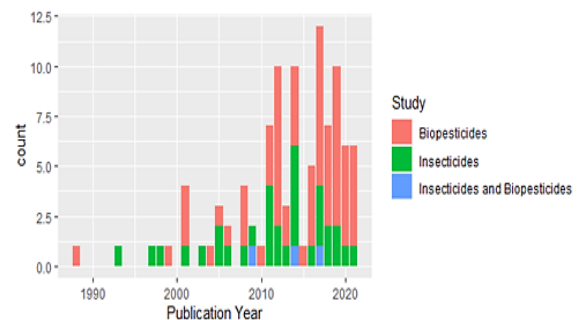


Figure 1. Number of literature by year published and type of study used (biopesticide, insecticide or both).

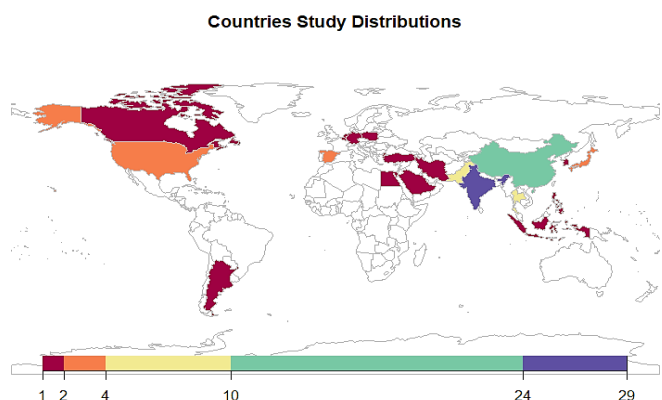


Figure 2. Countries distribution studied on *S. exigua* and *S. litura* concerning biopesticide and/or insecticide.

The studies with botanical insecticides started in the mid 1988, and it experienced exponential growth after the 2001. The trend is currently maintained and peaked in 2012 (Figure 1). Concerning the discipline, the largest number of papers was collected from studies in

Biopesticides (65 papers) compared to Insecticides (32) and Biopesticides and insecticides (3). The 100 papers reported studies conducted in 20 countries: India (29 papers), China (24), Thailand (10), Pakistan (8), Spain (4), USA (4), Japan (3), Korea (3), Germany (2), Netherlands (2), Turkey (2), Argentina (1), Belgium (1), Canada (1), Egypt (1), Iran (1), Indonesia (1), Philippines (1), Poland (1), Saudi Arabia (1) (Figure 2).

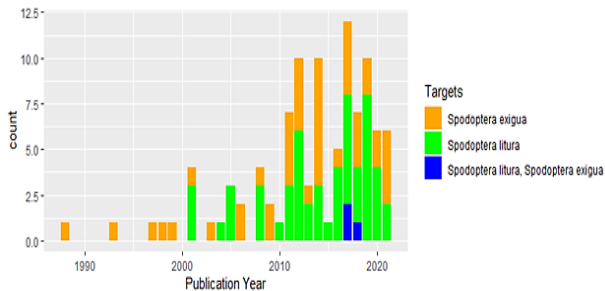


Figure 3. Target exposed species of study concerning biopesticide and/or insecticide (*S. exigua*, *S. litura*, or both).

Target exposed pest species received most of the attention among the identified studies, with *S. exigua* species receiving attention on only 44% of the studies. In comparison, 53% of the studies on *S. litura* species and the studies of *S. exigua* and *S. litura* is 3% (Figure 3). A large number of exposed methods were explored, but the topical application and leaf disc bioassay drew the bulk of the attention. These studies predominantly used different exposed stages, either eggs, instar 1st - 5th, pupa, and adult (Figure 4).

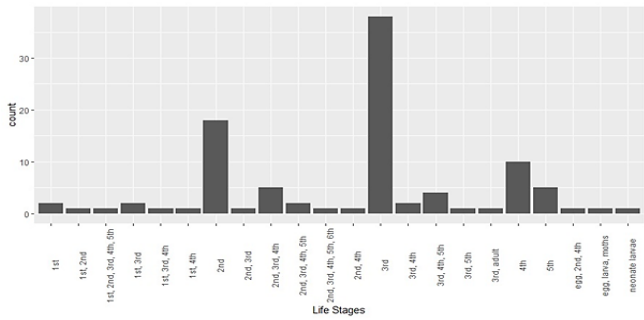


Figure 4. Exposed instar stage of studies on *S. exigua* and *S. litura* concerning biopesticide and/or insecticide.

3.2. Significance and Relationship between exposed stage Parameters

According to Supplementary data 2, early study of the exposed stage in biopesticides and insecticides for armyworms involved several stages, including eggs, 1st, 2nd, 3rd, 4th, 5th, and 6th instar, pupae, and imago. The exposed stage parameters were reduced to the fifth most frequent parameters used: 1st, 2nd, 3rd, 4th, and 5th instars. An AHC analysis was performed to identify the relationship between exposed instar stages assessed in each study. The AHC analysis was conducted in a binary manner: "1" indicates the assessed stage and "0" for the non-assessed stage. The result indicated that the assessed stages were grouped in 2 clusters: one for the 3rd instar stage and one for all other instar stages (Figure 5).

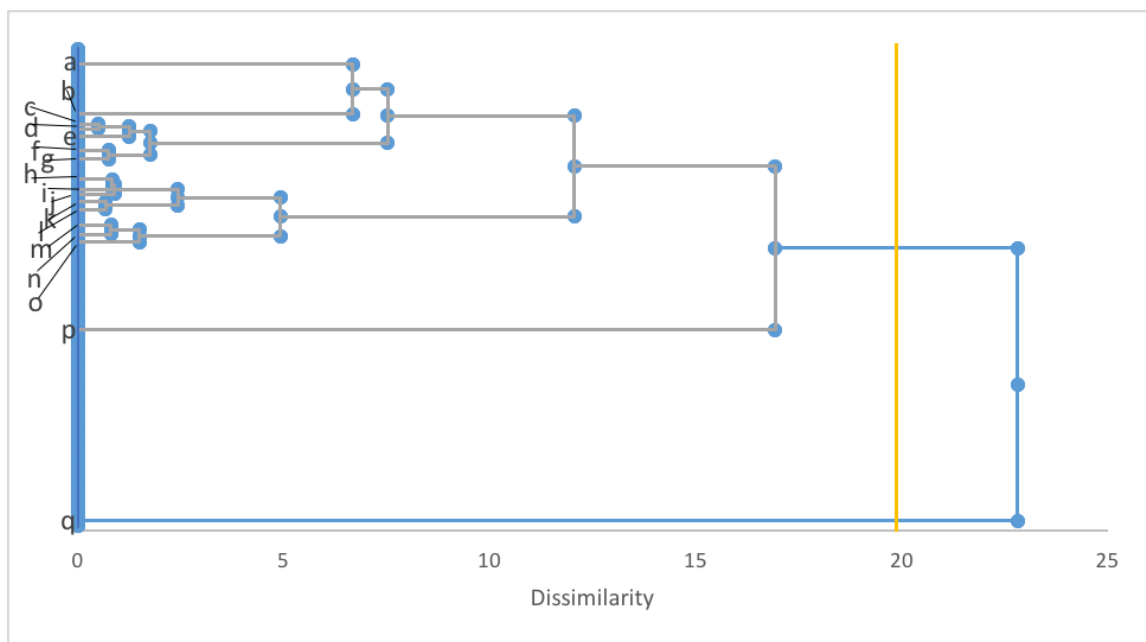


Figure 5. Hierarchical clustering of exposed stage parameters method in biopesticides and insecticides for *S. exigua* and *S. litura*. (a: 5th, b: 4th, c: 1st, 3rd, 4th, d: 1st, 4th, e: 1st, 3rd, f: 1st, g: 1st, 2nd, h: 2nd, 3rd, 4th, i: 2nd, 4th, j: 2nd, 3rd, k: 2nd, 3rd, 4th, 5th, l: 1st, 2nd, 3rd, 4th, 5th, m: 3rd, 4th, 5th, n: 3rd, 5th, o: 3rd, 4th, p: 2nd, q: 3rd)

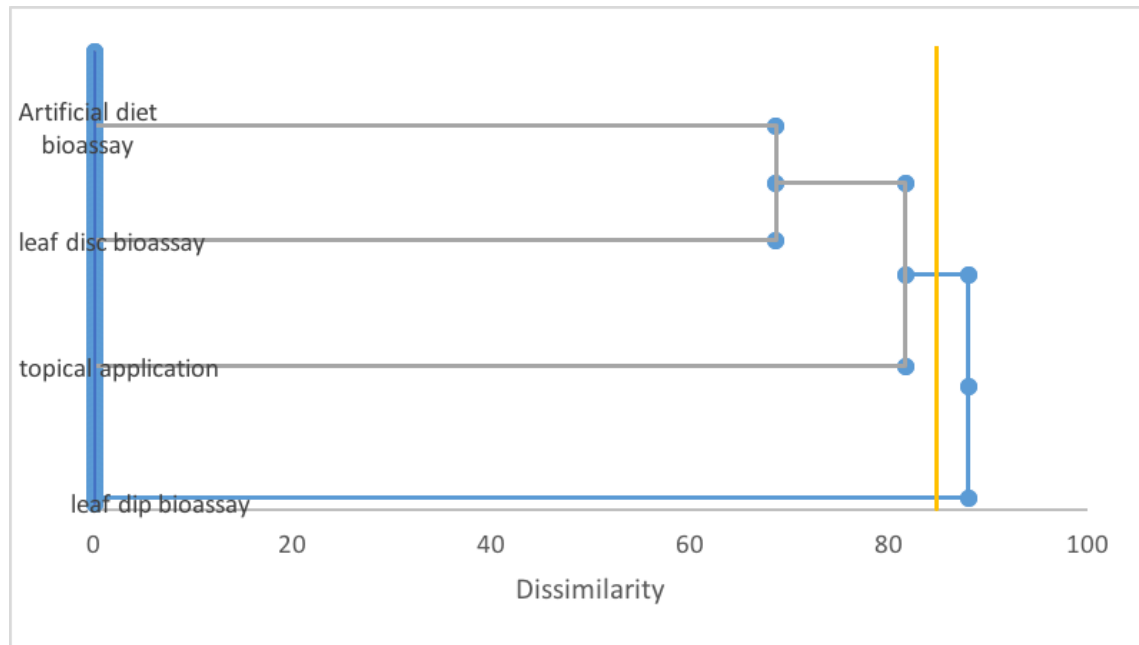


Figure 6. Hierarchical clustering of exposure method in biopesticides and insecticides for *S. exigua* and *S. litura*.

3.3. Significance and Relationship between Exposure Method Parameters

According to Supplementary data 3, early study of exposure methods in biopesticides and insecticides for armyworms involved several methods, including artificial diet bioassay, leaf disc bioassay, topical application, leaf dip bioassay, contact toxicity, injection bioassays, and oral exposure. The exposed stage parameters were reduced to the four most frequent parameters used: leaf dip bioassay, artificial diet bioassay, leaf disc bioassay, and topical application. The AHC analysis was also performed to identify relationships between each exposure method in each study. The analysis was also conducted in a binary manner: "1" indicates the used exposure method and "0" for the opposite. The result showed that the exposure methods were grouped in 2 clusters: one for the leaf dip bioassay and another for other methods (Artificial diet bioassay, leaf disc bioassay, topical application) (Figure 6).

3.4. Models and Determination of Heterogeneity

According to the dataset in Supplementary data 4, The heterogeneity (I^2) of the targeted studies was determined using Meta-Mar analysis website, referring to [16] fixed and random effect model. The analysis of the targeted study, in particular the effect of the synthetic pesticides on *S. exigua* larva, showed a highly heterogeneous value ($I^2 = 86.9%$) for a fixed-effect model.

3.5. Log Response Ratio of LC_{50}

Supplementary data 4 were transformed into a logarithmic to process Response Ratio (RR) of the LC_{50} ; therefore, the LRR of the LC_{50} was formed. The LRR was used to identify the effect of several synthetic pesticides on *S. exigua* and *S. litura* larva, whether relatively sensitive or not. The 'metafor' package of the RStudio was used and showed that the log ratios were lesser than zero (Figure 7).

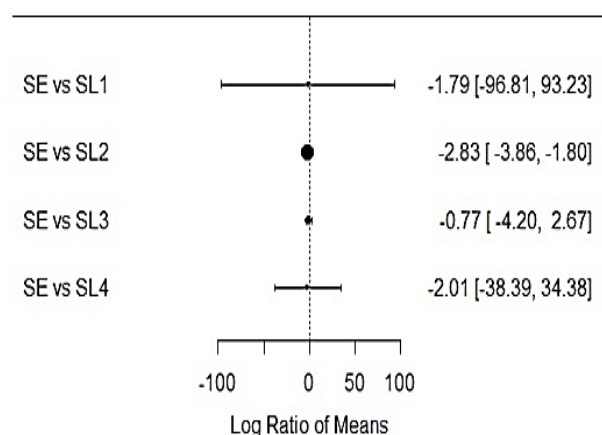


Figure 7. The LRR of LC_{50} of several synthetic pesticides on *S. exigua* (SE) and *S. litura* (SL).

4. DISCUSSION

4.1. Trend of Biopesticides and Synthetic Pesticides in *S. exigua* and *S. litura*

Studies have shown that the management of armyworms can be performed using synthetic pesticides. The pesticides used to control armyworms include spinosad, permethrin, cypermethrin, deltamethrin, cyhalothrin, emamectin benzoate, lufenuron, chlorpyrifos, chlorfenapyr, and tetra chlorantraniliprole [17-21]. However, widespread usage of insecticides has resulted in resistance to these armyworms. Several studies have reported the development of resistances in armyworms to different pesticides worldwide, including Pakistan [22-24], China [17], and Spain [25]. Insecticide resistance to armyworms has also been reported in Indonesia [26,27]. For example, the armyworm's resistance to methoxyfenozide was reported at the shallot plantation center in Java with LC_{50} between 0.53 - 127.61 ppm [5,28]. Armyworms in Indonesia have also been reported to be resistant to indoxacarb, spinosad, and emamectin benzoate [5,24]. As a result, much focus is required, especially on pest bio-ecology in the ecosystem. The scope of current Integrated Pest Management (IPM) methods used to manage other key pests, bio-control potential, and the identification of safer insecticides for successful management to avoid yield loss.

The present work showed that studies about biopesticides, particularly botanical pesticides, have increased due to public concern for the environment and safety of conventional insecticides in various parts of the world. The trend is currently maintained and peaked in 2012 with the target of study *S. exigua* [29,30] and *S. litura* [31-36]. Botanical pesticides, which are plant-derived products, are environmentally friendly, residue-free, biodegradable, and cost-effective. Therefore, the use of these products has caught the interest of scientists and policymakers in many countries [37,38]. The study of biopesticides in this work uses any kind of plant, for example, *Piper retrofractum* against *S. litura* [11], *Salvia veneris* Hedge. against *S. exigua* [39], and *Alpinia galanga* against *S. litura* [40]. Further studies on the biological activity of botanical insecticides typically include synthetic characterization of the crude plant extract, including essential oils, and the use of isolated compounds. Usually, it is necessary to include both negative and positive controls to ensure the quality of the results [33,41].

Most botanical insecticide research is still focused on detecting mortality-based activity on target pest species [11,42-44]. Mortality is a critical toxicological endpoint, but another response study such as repellents or sublethal responses may become of interest. The two last assays remain unexplored and represent another shortcoming for these compounds' commercial development [41].

Another response variable that is interesting to study include enzyme activity [45-47,40].

4.2. Different Exposed Stage and Exposure Methods for Assay Biopesticides and Insecticides

The papers used in this study showed that research in both bio- and/or synthetic insecticide used various insect exposed stages and exposure methods. Early study of the exposed stage in biopesticides and insecticides for armyworms involved several stages, including eggs, 1st, 2nd, 3rd, 4th, 5th, and 6th instar, pupae, and imago. Among these insect stages, the five most frequently used insect stages are 1st, 2nd, 3rd, 4th, 5th instars. Further analysis using the AHC analysis indicated that the 3rd instar was frequently assessed in many studies while 1st, 2nd, 4th, and 5th instars were located in a separate cluster, indicating lesser use. In addition, we can relate the selection of instar stages to the response variables that we will observe. For example, a study conducted by Lenora [48] used 1st instar larvae to observe the antifeedant activity of *Eichhornia crassipes* (Mart). An antifeedant is a synthetic that prevents insect pests from feeding while remaining near the treated foliage and getting killed from starvation [33]. The use of the 2nd instar is studied by Bullangpoti [42]. The reason for using this stage is the small body size of 1st instar larvae. They were unable to assess moribundity to the extract in the same way they did for the 2nd instar. This is because most smaller larvae are always more susceptible to insecticides than later instars, in addition to other reasons consistent with the manufacturer's recommendation to target application toward smaller larvae, and some toxicity research on *S. exigua* has started to compare insecticide toxicity with 2nd instar rather than other stages.

Early study of exposure methods in biopesticides and insecticides for armyworms involved several methods, including artificial diet bioassay, leaf disc bioassay, topical application, leaf dip bioassay, contact toxicity, injection bioassays, and oral exposure. The exposed stage parameters were reduced to the four most frequent parameters used: leaf dip bioassay, artificial diet bioassay, leaf disc bioassay, and topical application. The AHC analysis indicated that leaf dip bioassays were assessed in paper frequently, while artificial diet bioassay, leaf disc bioassay, and topical application instars were located in a separate cluster.

Several essential oils and plant extracts were tested against *Spodoptera* spp. using a topical method. Even at sub-lethal doses, *Pimenta racemosa*, *Origanum vulgare*, *Salvia sclarea*, and *Thymus vulgaris* were highly toxic to *S. littoralis* larvae and pupae (70 % mortality at LD_{30} dose) [47]. The leaf-dipping method was used to evaluate the activity of the test samples [44]. Furthermore, according to Duncan's New Multiple's Range Test, the dipping method was more toxic than the sprayer method,

with a significant difference ($P=0.0006$ and 0.0024 at $P<0.05$ for 24 and 48 hr after exposure) [42].

4.3. Models, The Determination of Heterogeneity and LRR of LC_{50}

According to our dataset, studies using *S. exigua* are fewer compared to *S. litura*. However, we could still compare the sensitivity to pesticides of multiple armyworm species using the corpus of studies included for this meta-analysis. We demonstrated that, aside from *S. litura* species, *S. exigua* species are the least susceptible, as reported by eight publications. Figure 5. showed a high variability of the log ratios of LC_{50} ; this ratio ranged from -2.83 to -0.77 . Log ratios were lesser than zero, indicating that *S. exigua* is generally more sensitive to pesticides than *S. litura* [49]. Thus, it may explain that research on biopesticides of *S. exigua* is lesser because people are still satisfied with the results of insecticides. However, it should be remembered that excessive use of insecticides can cause severe problems to the environment, such as pest resistance, environmental pollution, and human health effects. Thus, developing more friendly pest control for the environment is urgent to be done. Highly heterogeneous value ($I^2=86.9\%$) for a fixed-effect model on the effect of pesticides on *S. exigua* and *S. litura* showed that other factors gave more decisive influence than the treatment.

A limitation of the dataset used in our meta-analysis was that standard deviations of the LC_{50} value were frequently missing. Therefore, we decided to use the standard error and convert it into a standard deviation. Despite this constraint, we discovered significant differences between species. Therefore, in the future, we strongly advise scientists to include standard deviations and/or confidence intervals for LC_{50} in their publications.

Our study shows that biopesticide studies are still increasing and have become of interest to many researchers worldwide. The study started in the mid-1988 but experienced exponential growth after 2001, which is currently maintained and peaked in 2012. The analysis of the targeted study of the effect of the synthetic pesticides on *S. exigua* larvae showed a highly heterogeneous value ($I^2=86.9\%$) for a fixed-effect model on the effect of pesticides on *S. exigua* and *S. litura*. Showed that other factors gave stronger influence compared to the treatment. The LRR of LC_{50} indicates that *S. exigua* is more sensitive than *S. litura*. The information gathered from this study provides a better picture of how the future design and approach to developing biopesticides, especially in beet armyworms.

AUTHORS' CONTRIBUTIONS

All authors worked together to complete this project. Author TRN and NSSS designed the study, performed the statistical analysis, and wrote the first draft of the

manuscript. Authors HA managed the analyses of the study. Author LHN, SS, TRN, NSS, and HA wrote the manuscript. All authors read and approved the final manuscript.

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