Brassica nigra and Curcuma longa Compounds Affecting Interactions Between Spodoptera exigua and Its Natural Enemies Cotesia flavipes and Podisus maculiventris

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Abstract
The interaction Spodoptera exigua Hübner (Lepidoptera: Noctuidae) × its natural enemies Cotesia flavipes Cameron (Hymenoptera: Braconidae) and Podisus maculiventris Say (Heteroptera: Pentatomidae) × botanical compounds with and without synergist is unknown; therefore, it was studied under controlled conditions. The objective of this study was to evaluate the direct mortality of P. maculiventris nymphs and adults and indirect by this predator feeding on S. exigua larvae treated after being exposed to parasitism by C. flavipes. Brassica nigra L. (Brassicales: Brassicaceae) and Curcuma longa L. (Zingiberales: Zingiberaceae) compounds, with and without lead (II) oxide (PbO), were tested as insecticides. The mortality of first and second instars P. maculiventris was high with turmeric essential oil by topical application. The PbO increased the predator mortality in combination with turmeric powder, crude essential oil, and ar-turmerone. This last derivative caused also the highest mortality of P. maculiventris nymphs when ingested through treated S. exigua larvae that were previously subjected to parasitism. Turmeric powder and its derivatives, with and without PbO, should not be used in areas with P. maculiventris due to the high mortality caused to this predator.

Keywords
ar-turmerone, black mustard, curcuminoid pigments, lead (II) oxide, mortality, turmeric

Introduction
Some synthetic insecticides can kill nontarget organisms, such as aquatic species, parasitoid, pollinator, and predator insects, besides soil decomposers.¹⁻⁵ Some natural insecticides are as effective as synthetics in pest control and more selective in terms of mortality to nontargets.⁶⁻⁹ Commercial insecticides, based on plant compounds, generally have natural or synthetic synergistics in their formulation, such as lead (II) oxide (or lead monoxide). This is an inorganic with the molecular formula PbO¹⁰ and component of some botanicals, such as Schultz Insecticide, Houseplant & Indoor Garden Insect Spray.¹¹ This product, with a broad-spectrum action, is recommended to control pests on plants in indoor and outdoor environments, including edible vegetables.¹² The mortality of the cabbage looper, Trichoplusia ni Hübner (Lepidoptera: Noctuidae) caterpillars treated by topical application was 4 times higher with PbO at 0.20% + pyrethrin at 0.02% than with pyrethrin at 0.02%

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alone. The high mortality of cabbage looper caterpillars by insecticides with PbO is attributed to the inhibition activity of this synergist on the detoxifying enzymes of this pest, including cytochrome P450 monoxygenases (CYP).

Turmeric, Curcuma longa L. (Zingiberales: Zingiberaceae), native to southwestern India, is a spice utilized to dye and color the condiments and medicine sources. The properties of turmeric against stored product and by-product insect pests and as a mosquitos repellent are well known. Most maize weevil Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae) adults were killed six days after contact with ar-turmerone (sesquiterpene extracted from turmeric rhizomes essential oil) at 1% (m/m; n = 24) and mortality of fall armyworm Spodoptera frugiperda J.E. Smith (Lepidoptera: Noctuidae) caterpillars was 58% (n = 150) after ingestion of this compound at 1% (m/v) through an artificial diet.

Black mustard Brassica nigra L. (Brassicaceae) is an annual herbaceous cultivated plant and its seeds are utilized as a spice. This plant is likely native to the Mediterranean Asia and Europe. The black mustard seed essential oil repellent 89% (n = 100) faba bean beetle Bruchidius incarnatus Boheman (Coleoptera: Bruchidae) adults seven days after treatment of infested stored beans Vicia faba L. (Fabales: Fabaceae) and presented the highest mortality (76%) of this pest (n = 100) 168 h after the treatment. The black mustard seed powder repellent most cowpea bruchid, Calllosobruchus chinensis L. (Coleoptera: Chrysomelidae) adults in a choice test using an olfactometer. The mortality of the cotton aphid Aphis gossypii Glover (Hemiptera: Aphididae) adults was higher with the black mustard seed ethanolic extract as synergist + cyano phos (cholinesterase inhibitor) or KZ oil (an aficide).

The parasitoid Cotesia flavipes Cameron (Hymenoptera: Braconidae) and the predatory spined soldier bug Podisus maculiventris Say (Heteroptera: Pentatomidae) are natural enemies of the beet armyworm Spodoptera exigua Huëbner (Lepidoptera: Noctuidae). They can coexist in a same crop of parasites and natural enemies. Spodoptera exigua Say (Heteroptera: Pentatomidae) are natural enemies of the beet armyworm Spodoptera exigua Huëbner (Lepidoptera: Noctuidae) adults were killed six days after contact with ar-turmerone (sesquiterpene extracted from turmeric rhizomes essential oil) at 1% (m/m; n = 24) and mortality of fall armyworm Spodoptera frugiperda J.E. Smith (Lepidoptera: Noctuidae) caterpillars was 58% (n = 150) after ingestion of this compound at 1% (m/v) through an artificial diet.

Materials and Methods

Insects

Predatory spined soldier bug was obtained from the US Department of Agriculture (USDA), Agricultural Research Service (ARS), Center for Medical, Agricultural and Veterinary Entomology (CMAVE), in Tallahassee, FL. This predator was reared in metal cages (30 × 30 × 30 cm) and fed yellow mealworm larvae Tenebrio molitor L. (Coleoptera: Tenebrionidae) and provided distilled water in 2-mL plastic vials. Yellow mealworm larvae and adults were reared on plastic trays (40 × 40 × 20 cm) containing at pleasure wheat flour (Triticum aestivum L. Poales: Poaceae; 95%) mixed with yeast (5%), vegetable slices (carrot, Daucus carota subspecies sativus (Hoefn) Schübl. & G. Mar tens (Apiaceae: Apiaceae) and sweet potato, Ipomoea batatas (L.) Lam. (Solanales: Convolvulaceae)] as food and moisture supplied once a week. A beet armyworm colony and C. flavipes cocoons were obtained from the USDA-ARS, CMAVE, in Gainesville, FL. Beet armyworm larvae were reared on an artificial diet and parasitoid adults on a distilled water–honey solution (90:10%). All insects were reared under controlled environmental conditions at 25 ± 2°C, 70 ± 5% RH, and a 12:12-h (light:dark) photoperiod. Predatory spined soldier bug first, second, third, fourth, and fifth instars, and 1-day-old adults, three days old beet armyworm larvae, and parasitoid adults were obtained after daily observations on the colonies of these insects.

Plant Materials

Turmeric rhizomes were obtained from a commercial crop grown on the Macaúba Farm in Catalão, Goiás State, Brazil (18º08’ S, 47º57’ W, 515 m above sea level). Five large plants, lacking diseases and pests, were aleatorily selected within the crop. The soil was dug with a hoe until rhizomes became visible. Entire plants were harvested and the rhizomes cut from the plants with a scalpel. The rhizomes were placed in a polystyrene box lined with ice cubes and brought to a laboratory, where they were washed in running water, dried, and stored at 2°C. This farm uses no synthetic pesticides for their produce.

Chemicals

Black mustard seed organic virgin oil, extracted through the cold pressed unrefined method and its purity from 98 to 100%, was purchased from Organic & Beauty (Germany).
There were both a positive and a negative control. Positive control was the natural insecticide causing the highest mortality on beet armyworm larvae according to the literature. Negative control was the solvent used to extract the natural compounds tested in the bioassays. Schultz Insecticide, Houseplant & Indoor Garden Insect Spray (Premier Tech Home & Garden Inc., Brantford, Canada) was used as positive control. This product contains 0.02% pyrethrins [organic compound derived from dried flower heads of Chrysanthemum cinerariaefolium (Trevir.) Vis. (Asterales: Asteraceae)] as active ingredient and 0.20% PbO as synergist. Schultz Insecticide, Houseplant & Indoor Garden Insect Spray was purchased from Sigma-Aldrich Corporation (Canada) with a 98 to 100% purity.

**Experimental Procedures**

$^{1}$H, $^{13}$C, Heteronuclear Single Quantum Coherence, and heteronuclear multiple bond correlation-nuclear magnetic resonance measurements were carried out on a Bruker Avance III 500 instrument (Bruker Corporation, Germany) (operating at 500.13 MHz for $^{1}$H) equipped with a 5 mm triple resonance broadband inverse probe head with Z-gradient. Deuterated chloroform was used as solvent and tetramethylsilane as the internal standard. Mass spectra were obtained by gas chromatography coupled to a mass spectrometry (GC-MS). The GC-MS analyses were performed using a gas chromatograph (GC-17A Shimadzu, GC-MS/QP5,000 Shimadzu, DB-5 column; 30 × 0.32 mm), with ionization by electronic impact, under the following conditions: 60°C for three min; 5 to 240°C for eight min, with an injector of 180°C, a detector of 260°C, and an injection of 1 L. Mass spectra were compared with the National Institute of Standards and Technology Database 62.

**Extraction and Structural Characterization of $^{1}$ turmerone**

Turmeric rhizomes were air-dried at 40°C for three days and ground into a fine reddish yellow powder (turmeric powder). The major chemical turmeric powder constituents are curcuminooids, including bisdemethoxycurcumin, curcumin (3.14%), and demethoxycurcumin, besides general constituents including proteins, resins, and sugars. Some volatile components could be lost after drying the rhizomes at 40°C. However, the objective was to test the nonvolatile components. Moreover, $^{1}$ turmer one has high molecular mass and it is not volatile at 40°C.

An aliquot of the turmeric powder was reserved for bioassays and part of the remainder extracted by steeping in hexane freshly distilled at 25 ± 3°C with occasional stirring for a 6-hour period. Five hundred grams of rhizome powder were extracted with 1-L hexane. The solution obtained was filtered and the solvent removed in a rotary evaporator under low pressure, yielding a light yellow oil (= crude essential oil). Some volatile compounds in the turmeric crude essential oil include atlantone, turmerone, and zingingiberene.

An aliquot of the crude essential oil was reserved for bioassays and the remainder separated by column chromatography on silica gel (Vetec, 60-270 mesh), eluted with hexane:ethyl acetate (9:1). The fractions of interest, containing $^{1}$ turmerone, were analyzed by thin-layer chromatography (0.20 mm thickness, 60-mesh silica gel; Macherey-Nagel, Duren, Germany) visualized with iodine vapor (sublimation) and compared with a previously isolated and identified standard.

Topical Application of Turmeric Powder, Its Derivatives, and Black Mustard Seed Essential Oil Solutions With and Without PbO as Synergist on the Predatory Spined Soldier Bug Nymph and Adult Dorsum

Ten nymphs each of first, second, third, fourth, or fifth instar, or 10 1-day-old predatory spined soldier bug adults were placed per Petri dish (90 × 15 mm). The insect dorsum was treated each with 2 μL ethanolic solutions of turmeric powder, its derivatives (crude essential oil, $^{1}$ turmerone, or curcuminooid pigments), or black mustard seed essential oil at 1, 0.5, 0.25, or 0.125% (m/m; 1:1 ratio) plus or minus PbO using a microsyringe equipped with a dispenser (Trajan Scientific and Medical, Austin, Texas, USA). Two microliters absolute ethanol (Sigma-Aldrich Corporation) or Schultz Insecticide, Houseplant & Indoor Garden Insect Spray applied on the insect dorsum was used as negative and positive controls, respectively. Treatments had three replicates each with a Petri dish with 10 insect individuals. The number of dead individuals per Petri dish 24 h after treatment was evaluated.

Beet Armyworm Larvae Subjected to Parasitism by C. flavipes and Treated With Turmeric Powder, Its Derivatives, or Black Mustard Seed Essential Oil Solutions, as Prey for the Predatory Spined Soldier Bug Nymphs and Adults

Three days old beet armyworm larvae were subjected to parasitism for 48 h by C. flavipes females, which, in turn, had been exposed to mating for 2 days. The larvae (five days old) that were subjected to parasitism were then dipped for one second into each ethanolic solution of turmeric powder, its derivatives (crude essential oil, $^{1}$ turmerone, or curcuminooid pigments), or black mustard seed essential oil at 1% (m/m) using a forcep. The 1-second period was used because it was sufficient to wet uniformly without causing mortality of larvae according to a preliminary test. The preliminary test evaluated the wet uniformity and mortality of three day old beet armyworm larvae after being dipped into pure ethanol for one, two, and three seconds. Treated larvae were introduced in Petri dishes each with 10 second, third, fourth, or fifth instar, or 10 1-day-old predatory spined soldier bug adults. A 5 g cube S. exigua artificial diet was placed per Petri dish and replaced...
after dried being or consumed. Cannibalism and natural dead or consumed larvae were replaced by fresh ones for feeding from the same treatment. Absolute ethanol and Schultz Insecticide, Houseplant & Indoor Garden Insect Spray were used as negative and positive controls, respectively. The predatory spined soldier bug mortality was assessed per Petri dish every 24 h for five consecutive days.

### Statistical Analyses

All bioassays were repeated three times and results presented are means of the repetitions. The survival in the insecticide treatments was corrected according to the mortality in the negative control using Abbott formula when necessary. The data were subjected to a 1-way analysis of variance and of insecticide treatments without synergist and parasitism compared by the Tukey range test at 5% probability. The mortality data by insecticide treatments with and without synergist and no parasitism were subjected to a 1-way analysis of variance and of insecticide treatments without synergist and parasitism compared by the paired t test at 5% probability and of parasitism analyzed using regression equations with the 95% confidence limits calculated by PROBIT analysis. The median lethal time (LT\(_{50}\)) of the insects was obtained with PROC PROBIT. All data were analyzed using SAS version 9.1 software.

### Results

#### ar-Turmerone Yield

The turmeric essential oil yield extracted with hexane was 0.39% (dwt; 1.93 g) and of ar-turmerone obtained from this essential oil was 82% (dwt) after the chromatographic separations of the initial material (1.58 g turmeric powder). A total of 3.2 g of ar-turmerone was present per kilogram of rhizomes of this plant grown in Catalão, Goiás State, Brazil.

#### Toxicity of Insecticides Without PbO by Topical Application on the Predatory Spined Soldier Bug Nymph and Adult Dorsum

The mortality of first (\(F = 45.08\) and \(P < .0001\)) and second (\(F = 51.05\) and \(P < .0001\)) instar predatory spined soldier bug was higher with Schultz Insecticide, Houseplant & Indoor Garden Insect Spray (0.02% pyrethrins + 0.20% PbO) and turmeric essential oil at the highest tested concentration (1%, [m/m]) than with turmeric powder, its derivatives (ar-turmerone or curcuminoid pigments), or black mustard seed essential oil applied on the insect dorsum regardless of the concentration (Table 1). The fourth (\(F = 127.70\) and \(P < .0001\)) and fifth (\(F = 196.0\) and \(P < .0001\)) instar and adult (\(F = 12.0\) and \(P < .0001\)) mortality of the predatory spined soldier bug was minimum or

### Table 1. Mortality caused by Topical Application of 2 μL Ethanolic Solutions of Turmeric Powder, Curcuma longa (Zingiberaceae), Its Derivatives (Crude Essential oil, ar-Turmerone, or Curcuminoid Pigments), or Black Mustard Seed Essential Oil, Brassica nigra (Brassicaceae) at 1, 0.5, 0.25, or 0.125% (m/m) to Podusis maculiventris (Heteroptera: Pentatomidae) Nymphs and Adults With 3 Replications Each With 10 Nymphs of Each Instar or 10 Adults, per Petri Dish 24 hours After Treatment.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>% First Instar</th>
<th>Second Instar</th>
<th>Third Instar</th>
<th>Fourth Instar</th>
<th>Fifth Instar</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turmeric powder</td>
<td>63.3 ± 3.3</td>
<td>33.3 ± 3.3</td>
<td>6.6 ± 3.3</td>
<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>0.5</td>
<td>46.6 ± 3.3</td>
<td>23.3 ± 3.3</td>
<td>3.3 ± 3.3</td>
<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>0.25</td>
<td>16.6 ± 6.6</td>
<td>3.3 ± 3.3</td>
<td>0 c</td>
<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>0.125</td>
<td>13.3 ± 3.3</td>
<td>0 g</td>
<td>0 c</td>
<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>Turmeric oil</td>
<td>96.6 ± 3.3 a</td>
<td>36.6 ± 3.3 bc</td>
<td>23.3 ± 3.3 b</td>
<td>3.3 ± 3.3 b</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>0.5</td>
<td>33.3 ± 3.3 cdef</td>
<td>26.0 ± 3.3 bcde</td>
<td>6.6 ± 6.6 c</td>
<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>0.25</td>
<td>26.6 ± 3.3 defg</td>
<td>10 ± 5.7 efg</td>
<td>0 c</td>
<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>0.125</td>
<td>23.3 ± 3.3 efgh</td>
<td>3.3 ± 3.3 g</td>
<td>0 c</td>
<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>ar-Turmerone</td>
<td>46.6 ± 3.3 bcd</td>
<td>23.3 ± 3.3 cdef</td>
<td>3.3 ± 3.3 c</td>
<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>0.5</td>
<td>50 ± 0 bc</td>
<td>43.3 ± 3.3 b</td>
<td>13.3 ± 3.3 b</td>
<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>0.25</td>
<td>43.3 ± 3.3 bcde</td>
<td>23.3 ± 3.3 cdef</td>
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<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>0.125</td>
<td>33 ± 3.3 cdef</td>
<td>10.0 ± 5.7 efg</td>
<td>0 c</td>
<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
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<tr>
<td>Curcuminoids</td>
<td>23.3 ± 3.3 efgh</td>
<td>6.6 ± 3.3 fg</td>
<td>0 c</td>
<td>0 b</td>
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<td>0 b</td>
</tr>
<tr>
<td>0.5</td>
<td>10.0 ± 5.7 gh</td>
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<tr>
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<td>0 c</td>
<td>0 b</td>
<td>0 b</td>
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<tr>
<td>Mustard oil</td>
<td>33 ± 3.3 cdef</td>
<td>16.6 ± 3.3 defg</td>
<td>0 c</td>
<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
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<td>20.0 ± 5.7 gh</td>
<td>10.0 ± 5.7 efg</td>
<td>3.3 ± 3.3 c</td>
<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>0.25</td>
<td>10.0 ± 5.7 gh</td>
<td>0 g</td>
<td>0 c</td>
<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>0.125</td>
<td>3.3 ± 3.3 h</td>
<td>0 g</td>
<td>0 c</td>
<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>Schultz</td>
<td>100.0 ± 0 a</td>
<td>100.0 ± 0 a</td>
<td>86.6 ± 3.3 a</td>
<td>53.3 ± 3.3 ab</td>
<td>46.6 ± 3.3 a</td>
<td>20.0 ± 5.7 a</td>
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<tr>
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<td>57.45</td>
<td>127.70</td>
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</tbody>
</table>

*Abbott effectiveness. The data were subjected to a 1-way analysis of variance (ANOVA) after correction. Means followed by the same lower letter per column, do not differ by the Tukey range test (\(p = .05\)). Absolute ethanol and Schultz Insecticide, Houseplant & Indoor Garden Insect Spray were used as negative and positive controls, respectively.

\(\text{LET}_{50}\) of the insects was obtained with PROC PROBIT. All data were analyzed using SAS version 9.1 software.
Synergistic Effects of PbO to Turmeric Powder, Its Derivatives, and Black Mustard Seed Essential Oil

The addition of PbO to turmeric powder at 1 and 0.5% (m/m), crude essential oil, and ar-turmerone at 1% (m/m) caused synergism by increasing the predatory spined soldier bug nymph and adult mortality. Minimum or zero synergism on mortality was observed for all insecticides tested in combination with PbO on the fourth and fifth instars and adult predatory spined soldier bug (Figure 1).

Mortality of Predatory Spined Soldier Bug Nymphs and Adults Fed on Beet Armyworm Larvae Subjected to Parasitism by C. flavipes and Treatment With Turmeric Powder, Its Derivatives, and Black Mustard Seed Essential Oil

Beet armyworm larvae that were subjected to parasitism and later treated with the insecticides were consumed by the predatory spined soldier bug. The time spent to kill 50% individuals of second, third, and fourth instar predatory spined soldier bug was lower with ar-turmerone (LT50 = 95.72, 121.19, and 138.42 h, respectively) than turmeric powder (LT50 = 130.23, 169.94, and 182.42 h), its derivatives (crude essential oil LT50 = 142.48, 146.63, and 182.61 h, and curcuminoid pigments LT50 = 130.42, 161.70, and 161.84 h), or black mustard seed essential oil (LT50 = 105.37, 138.02, and 147.44 h), respectively. However, the mortality of second (\( \chi^2 = 3.00 \) and \( P = 1.00 \)), third (\( \chi^2 = 12.77 \) and \( P = .47 \)), fourth (\( \chi^2 = 7.53 \) and \( P = .87 \)), and fifth (\( \chi^2 = 3.06 \) and \( P = 1.00 \)) instars and adult (\( \chi^2 = 14.33 \) and \( P = .35 \)) predatory spined soldier bug was fastest with Schultz Insecticide, Houseplant & Indoor Garden Insect Spray (Table 2).

Discussion

The finding of high concentration of ar-turmerone in Catalão, Goiás State, Brazil, confirms reports of this compound extracted from nonpolar extracts and essential oils of turmeric from China, India, Pakistan (Asia), Nigeria, and São Tomé and Príncipe (Africa).48-51 The quantitative and qualitative compositions of plant extracts and essential oils depend on genetic factors and on the environmental conditions where the plant is grown, with turmeric essential oil concentration variations occurring at different places.52-54 Turmeric can be cultivated at low cost and sustainable in Brazil using minimal labor in different growing seasons and spacing with organic fertilization using 50 tons of cattle manure/ha.55

The mortality of predatory spined soldier bug nymphs and adults treated with turmeric powder, its derivatives (ar-turmerone, essential oil, and curcuminoid pigments), and black mustard seed essential oil solutions with or without PbO as a synergist was assessed. The mortality of nymphs and adults of this predator fed on treated beet armyworm larvae after being exposed to parasitism by C. flavipes was also evaluated. There is little information about the mortality caused by turmeric powder, its derivatives, and black mustard seed essential oil to nontarget organisms such as generalist predators including the predatory spined soldier bug. There is almost no information on the mortality of the predatory spined soldier bug fed on beet armyworm larvae subjected to parasitism by C. flavipes and postrally treated with solutions of these botanicals.

The higher mortality of first, second, and third instar predatory spined soldier bug according to concentration increases, by insecticide solutions without synergist, is similar to reports for turmeric powder and its derivatives solutions to the cabbage looper12 and fall armyworm stages and maize weevil adults.21 The toxicity of turmeric rhizomes essential oil indicates the presence of insecticide active compounds in its composition such as curcuminoid pigments as reported for the turmeric pigments inhibiting the growth of the desert locust Schistocerca gregaria Forsskål (Orthoptera: Acrididae) nymphs by 60%. A total of 20 μg applied per nymph controlled 45% of those of the red cotton stainer Dysdercus koenigii F. (Hemiptera: Pyrrhocoridae) and 50 μg per nymph controlled both species by 50 to 60%.56 The turmeric essential oil, extracted from the leaves of this plant and applied by contact and fumigation, controlled and reduced the progeny of the lesser grain borer Rhyzopertha dominica F. (Coleoptera: Bostrichidae; LD50 = 36.71 μg/mg insect weight) and the rice weevil Sitophilus oryzae L. (Coleoptera: Curculionidae; LC50 = 11.36 mg L/ar) adults, and reduced the number of eggs and the emergence percentage of the red flour beetle larvae by 72 to 80% at 5.2 mg/cm².57 Turmeric extracts obtained from rhizomes of this plant caused the highest mortality to the red flour beetle Tribolium castaneum Herbst (Coleoptera: Tenebrionidae) adults (LD50 = 0.337 mg/cm²; n = 30) compared to those of aerial parts (LD50 = 0.695 mg/cm²; n = 30) 24 h after exposure.58

The high synergism of PbO in turmeric powder, crude essential oil, and ar-turmerone solutions through topical application on predatory spined soldier bug nymphs and adults suggests blockade of the metabolic system of detoxifying enzymes of this insect.59 The PbO increased the toxicity of turmeric powder and its derivatives (90-97% mortality) in most binary combinations (5 μg of turmeric powder or its derivatives + 5 μg PbO). The ar-turmerone compound alone or in combination with PbO reduced the weight of cabbage looper larvae on treated Brassica oleracea L. (Brassicaceae) plants under laboratory and greenhouse conditions compared to the negative control (pure acetone).12 The combination of permethrin (pyrethroid insecticide) and PbO inhibited CYP activity by 81% and reduced the longevity period of the itch mite Sarcoptes scabiei L. (Sarcoptiformes: Sarcoptidae).60

The consumption of treated larvae, subjected to parasitism by C. flavipes, by the predatory spined soldier bug suggests
Figure 1. Mortality (%) of the first (A), second (B), third (C), fourth (D), and fifth (E) instars and (F) Podisus maculiventris (Heteroptera: Pentatomidae) adults with three replications each with 10 nymphs of each instar or 10 adults, per Petri dish, 24 h after topical application of 2 μL ethanolic solutions of turmeric, Curcuma longa (Zingiberaceae) powder (T1), its derivatives (crude essential oil [T2], ar-turmerone [T3], or curcuminoid pigments [T4]), or black mustard seed essential oil, Brassica nigra (Brassicaceae; T5) at 1, 0.5, 0.25, or 0.125% (m/m) with or without the synergist lead (II) oxide (PbO; 1:1 ratio), respectively. Absolute ethanol and Schultz Insecticide, Houseplant & Indoor Garden Insect Spray were used as negative and positive controls, respectively. * denotes significant values; ns nonsignificant values, at 5% probability by the paired t test. The left side is based on Abbott effectiveness44 of the tested botanicals without PbO and the right side with PbO.
mortality of the parasitoid by the insecticides, because heteropteran predators avoid preying on parasitized larvae. In addition, dead parasitoids in host larvae body are unable to produce chemical signals responsible to prevent predation. The faster dying of the predatory spined soldier bug with the positive control, \(-\)turmerone, and black mustard seed essential oil in the current study shows knockdown effect of these products to the predator. However, this mortality could be reduced because host larvae parasitized by *Cotesia* species increase the CYP activity. The higher mortality of the confused flour beetle *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) adults by ingestion of a mustard synthetic oil compared to a topical application was due to the great percentage (90\%) of allyl isothiocyanate in this oil. Allyl isothiocyanate vapor alone controlled 100\% of the book louse *Liposcelis entomophila* Enderlein (Psocoptera: Liposcelididae), lesser grain borer, maize weevil, and *Tribolium ferrugineum* F. (Coleoptera: Tenebrionidae) adults 72 h after exposure to a three mg/mL atmospheric concentration.

### Conclusions

The mortality of the second and third instar predatory spined soldier bug by topical application of turmeric essential oil without synergist was high. It suggests that this compound should not be used in integrated pest management programs with these instars. However, the fourth and fifth instars and adults of this predator survived after being treated through topical application of turmeric powder and its derivatives without synergist regardless of the concentration. The fourth and fifth instars and

<table>
<thead>
<tr>
<th>Treatments</th>
<th>n</th>
<th>df</th>
<th>LT50 (hours)</th>
<th>Slope ± SE</th>
<th>(\chi^2)</th>
<th>P Value</th>
</tr>
</thead>
</table>
| **Second instar**  
Turmeric powder | 30 | 13 | 130.23 (103.61-212.24) | 2.85 ± 0.66 | 6.49 | .93 |
| Turmeric oil | 30 | 13 | 142.48 (110.82-257.46) | 2.83 ± 0.69 | 7.76 | .86 |
| \(-\)Turmerone | 30 | 13 | 95.72 (79.85-125.50) | 2.89 ± 0.56 | 3.89 | .99 |
| Curcuminoids | 30 | 13 | 130.42 (100.32-233.21) | 2.37 ± 0.57 | 4.70 | .98 |
| Mustard oil | 30 | 13 | 105.37 (89.38-137.18) | 3.44 ± 0.68 | 10.08 | .69 |
| Schultz | 30 | 13 | 37.71 (32.25-42.93) | 5.44 ± 0.77 | 3.00 | 1.00 |
| **Third instar**  
Turmeric powder | 30 | 13 | 169.94 (127.47-500.64) | 3.42 ± 1.08 | 3.27 | .99 |
| Turmeric oil | 30 | 13 | 146.63 (123.68-289.47) | 6.39 ± 2.12 | 3.10 | .99 |
| \(-\)Turmerone | 30 | 13 | 121.19 (94.97-200.33) | 2.41 ± 0.56 | 10.71 | .63 |
| Curcuminoids | 30 | 13 | 161.70 (119.27-370.91) | 2.58 ± 0.68 | 13.85 | .38 |
| Mustard oil | 30 | 13 | 138.02 (109.32-232.88) | 3.01 ± 0.71 | 11.22 | .59 |
| Schultz | 30 | 13 | 61.67 (53.34-70.29) | 4.20 ± 0.61 | 12.77 | .47 |
| **Fourth instar**  
Turmeric powder | 30 | 13 | 182.42 (133.87-857.52) | 3.80 ± 1.35 | 2.10 | .99 |
| Turmeric oil | 30 | 13 | 182.61 (134.31-988.25) | 3.97 ± 1.45 | 3.89 | .99 |
| \(-\)Turmerone | 30 | 13 | 138.42 (116.32-218.01) | 4.84 ± 1.28 | 9.34 | .75 |
| Curcuminoids | 30 | 13 | 161.94 (128.79-517.85) | 5.25 ± 1.89 | 3.32 | .99 |
| Mustard oil | 30 | 13 | 147.44 (117.97-276.60) | 3.76 ± 1.03 | 5.31 | .97 |
| Schultz | 30 | 13 | 78.05 (67.80-90.93) | 3.86 ± 0.64 | 7.53 | .87 |
| **Fifth instar**  
Turmeric powder | 30 | 13 | – | – | – | – |
| Turmeric oil | 30 | 13 | – | – | – | – |
| \(-\)Turmerone | 30 | 13 | – | – | – | – |
| Curcuminoids | 30 | 13 | – | – | – | – |
| Mustard oil | 30 | 13 | – | – | – | – |
| Schultz | 30 | 13 | 113.89 (97.72-150.65) | 4.02 ± 0.88 | 3.06 | 1.00 |
| **Adult**  
Turmeric powder | 30 | 13 | – | – | – | – |
| Turmeric oil | 30 | 13 | – | – | – | – |
| \(-\)Turmerone | 30 | 13 | – | – | – | – |
| Curcuminoids | 30 | 13 | – | – | – | – |
| Mustard oil | 30 | 13 | – | – | – | – |
| Schultz | 30 | 13 | 169.38 (128.20-457.64) | 3.59 ± 1.11 | 14.33 | .35 |

Abbreviation: df, degree of freedom.  
* Abbott effectiveness44. – indicates mortality lower than 20\% after 120 hours. LT50 was calculated using PROC PROBIT analysis. Absolute ethanol or Schultz Insecticide, Houseplant & Indoor Garden Insect Spray were used as negative and positive controls, respectively.
adults of this predator should be selected for releasing activities in areas treated with turmeric-based insecticides. As expected, the synergist PbO enhanced the toxic effect (eg, mortality) of turmeric powder, crude essential oil, and ar-turmerone to the predatory spined soldier bug nymphs and adults. ar-Turmerone showed knockdown effect to the predator by its ingestion through treated beet armyworm larvae that were previously exposed to parasitism.

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