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

Wagner de Souza Tavares¹,2,3, Jesusa Crisostomo Legaspi², Ancidérton Antonio de Castro²,3,4, Hany Ahmed Fouad4,5, Muhammad Haseeb³, Robert L. Meagher, Jr⁶, Lambert H.B. Kanga³, and José Cola Zanuncio⁴

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 α-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
α-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%
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 mosquitoes 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. (Brassicales: 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 repelled 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 repelled most cowpea bruchid, Callosobruchus 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- panthos (cholinesterase inhibitor) or KZ oil (an acicide).

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 Huibner (Lepidoptera: Noctuidae). They can coexist in a same crop of celery 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* (Trevis.) Vis. (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 $\times$ 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 ar-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 curcuminoids, 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, ar-turmerone 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 $\pm$ 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 zingiberene.

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 ar-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.

The other portion of the turmeric powder was separated by column chromatography on silica gel (Vetec, 60-270 mesh), eluted with hexane:ethyl acetate (1:1), to obtain curcuminoid pigments. The curcuminoid pigments consisted of a mixture of bisdemethoxycurcumin, curcumin, and demethoxycurcumin.

**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 $\times$ 15 mm). The insect dorsum was treated each with 2 μL ethanolic solutions of turmeric powder, its derivatives (crude essential oil, ar-turmerone, or curcuminoid 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, ar-turmerone, or curcuminoid pigments), or black mustard seed essential oil at 1% (m/m) using a forceps. 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 of *S. exigua* artificial diet was placed per Petri dish and replaced...
Table 1. Mortality caused by Topical Application of 2 μL Ethanolic Solutions of Turmeric Powder, *Curcuma longa* (Zingiberaceae), Its Derivatives (Crude Essential oil, -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 *Podisus 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.*

| Treatments          | %    | First Instar | Second Instar | Third Instar | Fourth Instar | Fifth Instar | Adult |
|---------------------|------|--------------|---------------|--------------|---------------|--------------|-------|
| Turmeric powder     | 1    | 63.3 ± 3.3 b | 33.3 ± 3.3 c | 6.6 ± 3.3 c | 0 b           | 0 b          | 0 b   |
|                     | 0.5  | 46.6 ± 3.3 b | 23.3 ± 3.3 c | 3.3 ± 3.3 c | 0 b           | 0 b          | 0 b   |
|                     | 0.25 | 16.6 ± 6.6 g | 3.3 ± 3.3 g  | 0 c          | 0 b           | 0 b          | 0 b   |
|                     | 0.125| 13.3 ± 3.3 g | 0 g           | 0 c          | 0 b           | 0 b          | 0 b   |
| Turmeric oil        | 1    | 96.6 ± 3.3 a | 36.6 ± 3.3 b | 23.3 ± 3.3 b | 3.3 ± 3.3 b   | 0 b          | 0 b   |
|                     | 0.5  | 33.3 ± 3.3 cdef | 26.0 ± 3.3 bcde | 6.6 ± 6.6 c | 0 b           | 0 b          | 0 b   |
|                     | 0.25 | 26.6 ± 3.3 defg | 10 ± 5.7 efg | 0 c          | 0 b           | 0 b          | 0 b   |
|                     | 0.125| 23.3 ± 3.3 efgh | 3.3 ± 3.3 g  | 0 c          | 0 b           | 0 b          | 0 b   |
| *α*-Turmerone       | 1    | 46.6 ± 3.3 bcd | 23.3 ± 3.3 cdef | 3.3 ± 3.3 c | 0 b           | 0 b          | 0 b   |
|                     | 0.5  | 50 ± 0 bc     | 43.3 ± 3.3 b  | 13.3 ± 3.3 b | 0 b           | 0 b          | 0 b   |
|                     | 0.25 | 43.3 ± 3.3 cdef | 23.3 ± 3.3 cdef | 3.3 ± 3.3 c | 0 b           | 0 b          | 0 b   |
|                     | 0.125| 33 ± 3.3 cdef  | 10.0 ± 5.7 efg | 0 c          | 0 b           | 0 b          | 0 b   |
| Curcuminoids        | 1    | 23.3 ± 3.3 efgh | 6.6 ± 3.3 fg  | 0 c          | 0 b           | 0 b          | 0 b   |
|                     | 0.5  | 10.0 ± 5.7 gh  | 0 g           | 0 c          | 0 b           | 0 b          | 0 b   |
|                     | 0.25 | 6.6 ± 6.6 gh   | 0 g           | 0 c          | 0 b           | 0 b          | 0 b   |
|                     | 0.125| 3.3 ± 3.3 h    | 0 g           | 0 c          | 0 b           | 0 b          | 0 b   |
| Mustard oil         | 1    | 33.3 ± 3.3 cdef | 16.6 ± 3.3 defg | 0 c          | 0 b           | 0 b          | 0 b   |
|                     | 0.5  | 20.0 ± 5.7 gh  | 10.0 ± 5.7 efg | 3.3 ± 3.3 c | 0 b           | 0 b          | 0 b   |
|                     | 0.25 | 10.0 ± 5.7 gh  | 0 g           | 0 c          | 0 b           | 0 b          | 0 b   |
|                     | 0.125| 3.3 ± 3.3 h    | 0 g           | 0 c          | 0 b           | 0 b          | 0 b   |
| Schultz             | 100.0| ± 0 a         | 100.0 ± 0 a   | 86.6 ± 3.3 a | 53.3 ± 3.3 ab | 46.6 ± 3.3 a | 20.0 ± 5.7 a |
| *F*                | 45.08| 51.05         | 47.45         | 127.70       | 196.0         | 12.0         |
| *P* value           | <.0001<.0001 | <.0001        | <.0001        | <.0001       | <.0001        | <.0001       |

*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.

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.

**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.

**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 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 (LT50) of the insects was obtained with PROC PROBIT. All data were analyzed using SAS version 9.1 software.

**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
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 (LT$_{50}$ = 95.72, 121.19, and 138.42 h, respectively) than turmeric powder (LT$_{50}$ = 130.23, 169.94, and 182.42 h), its derivatives (crude essential oil LT$_{50}$ = 142.48, 146.63, and 182.61 h, and curcuminoid pigments LT$_{50}$ = 130.42, 161.70, and 161.84 h), or black mustard seed essential oil (LT$_{50}$ = 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). 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. 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.

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 pestically 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 looper and fall armyworm stages and maize weevil adults. 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 (Hemiptera: Pyrrhocoridae) and 50 µg per nymph controlled both species by 50 to 60%. 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; LD$_{50}$ = 36.71 µg/mg insect weight) and the rice weevil Sitophilus oryzae L. (Coleoptera: Curculionidae; LC$_{50}$ = 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/cm$^2$. Turmeric extracts obtained from rhizomes of this plant caused the highest mortality to the red flour beetle Tribolium castaneum Herbst (Coleoptera: Tenebrionidae) adults (LD$_{50}$ = 0.337 mg/cm$^2$; n = 30) compared to those of aerial parts (LD$_{50}$ = 0.695 mg/cm$^2$; n = 30) 24 h after exposure.

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. 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). 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).

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 effectiveness* 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.\textsuperscript{61,62} In addition, dead parasitoids in host larvae body are unable to produce chemical signals responsible to prevent predation.\textsuperscript{63} The faster dying of the predatory spined soldier bug with the positive control, \textit{ar}-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 \textit{Cotesia} species increase the CYP activity.\textsuperscript{64} The higher mortality of the confused flour beetle \textit{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.\textsuperscript{65} Allyl isothiocyanate vapor alone controlled 100\% of the book louse \textit{Liposcelis entomophila} Enderlein (Psocoptera: Liposcelididae), lesser grain borer, maize weevil, and \textit{Tribolium ferrugineum} F. (Coleoptera: Tenebrionidae) adults 72 h after exposure to a three \textit{mg}/mL atmospheric concentration.\textsuperscript{66}

### 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 adults of the parasitoid by the insecticides, because heteropteran predators avoid preying on parasitized larvae.\textsuperscript{61,62} In addition, dead parasitoids in host larvae body are unable to produce chemical signals responsible to prevent predation.\textsuperscript{63}

### Table 2.

| Treatments                          | n  | df  | \textit{LT}_{50} (hours) | Slope ± SE | \(\chi^2\) | \(P\) Value |
|-------------------------------------|----|-----|--------------------------|-----------|-----------|------------|
| **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        |
| \textit{ar}-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        |
| \textit{ar}-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        |
| \textit{ar}-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  | –                         | –         | –         | –          |
| \textit{ar}-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  | –                         | –         | –         | –          |
| \textit{ar}-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 effectiveness\textsuperscript{44} – indicates mortality lower than 20\% after 120 hours. \textit{LT}_{50} 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.

Acknowledgments
We thank Amy Rowley (USDA-ARS, CMAVE, in Gainesville, FL, USA) for providing the beet armyworm, its artificial diet, and C. flavipes cocoons. This article presents the results of research only. The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the US Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship and/or publication of this article: To “Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq),” “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES),” “Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG),” and “Programa Cooperativo sobre Proteção Florestal (PROTEF)” of the “Instituto de Pesquisas e Estudos Florestais (IPEF)” for scholarship and financial support.

References
1. Tavares WS, Costa MA, Cruz I, Silveira RD, Serrão JE, Zanuncio JC. Selective effects of natural and synthetic insecticides on mortality of Spodoptera frugiperda (Lepidoptera: Noctuidae) and its predator Eriopis connexa (Coleoptera: Coccinellidae). J Environ Sci Health B. 2010a;45(6):557-561.
2. Tavares WS, Grael CFF, Menezes CGW, Crux I, Serrão JE, Zanuncio JC. Residual effect of extracts of native plants from Brazil and a synthetic insecticide, chlorpyrifos, on Sitophilus zea-mais (Lepidoptera: Pyralidae). J Stored Prod Res. 2012;48(3):115-120.
3. Tavares WS, Freitas SS, Grael CFF, et al. Tenebrio molitor (Coleoptera: Tenebrionidae) as a guinea pig for the analysis of the toxicity of natural products. Vie Milieu. 2013c;63(3-4):193-204.
4. Fouad HA, Faroni LRD, Tavares WS, Ribeiro RC, Freitas SS, Zanuncio JC. Botanical extracts of plants from the Brazilian Cerrado for the integrated management of Sitotroga cerealella (Lepidoptera: Gelechiidae) in stored grain. J Stored Prod Res. 2014;57(1):6-11.
5. de Castro AA, Poderoso JCM, Ribeiro RC, Legaspi JC, Serrão JC, Zanuncio JC. Demographic parameters of the insecticide-exposed predator Podisus nigrispinus: implications for IPM. BioControl. 2015;60(2):231-239.
6. Tavares WS, Cruz I, Petacci F, et al. Potential use of Asteraceae extracts to control Spodoptera frugiperda (Lepidoptera: Noctuidae) and selectivity to their parasitoids Trichogramma pretiosum (Hymenoptera: Trichogrammatidae) and Telenomus remus (Hymenoptera: Scelionidae). Ind Crop Prod. 2009;30(3):384-388.
7. Tavares WS, Cruz I, Fonseca FG, Gouveia NL, Serrão JE, Zanuncio JC. Deleterious activity of natural products on postures of Spodoptera frugiperda (Lepidoptera: Noctuidae) and Diatraea saccharalis (Lepidoptera: Pyralidae). Z Naturforsch C. 2010;65(5-6):412-418.
8. Tavares WS, Cruz I, Petacci F, Freitas SS, Serrão JE, Zanuncio JC. Insecticide activity of pipeline: toxicity to eggs of Spodoptera frugiperda (Lepidoptera: Noctuidae) and Diatraea saccharalis (Lepidoptera: Pyralidae) and phytotoxicity on several vegetables. J Med Plants Res. 2011;5(21):5301-5306.
9. Mourão SA, Zanuncio JC, Tavares WS, Wilcken CF, Leite GLD, Serrão JE. Mortality of Anticarsia gemmatalis (Lepidoptera: Noctuidae) caterpillars post exposure to a commercial neem (Azadirachta indica, Meliaceae) oil formulation. Fla Entomol. 2014;97(2):555-561.
10. Sun YP, Johnson ER. Synergistic and antagonistic actions of insecticide-synergist combinations and their mode of action. J Agric Food Chem. 1960;8(4):261-266.
11. Afshar FH, Maggi F, Iannarelli R, Cianfaglione K, Isman MB. Comparative toxicity of Helosciadium nodiflorum essential oils and combinations of their main constituents against the cabbage looper, Trichoplusia ni (Lepidoptera). Ind Crop Prod. 2017;98(1):46-52.
12. Tavares WS, Akhatar Y, Gonçalves GLP, Zanuncio JC, Isman MB. Turmeric powder and its derivatives from Curcuma longa rhizomes: insecticidal effects on cabbage looper and the role of synergists. Sci Rep. 2016;6:34093.
13. Tak JH, Isman MB. Enhanced cuticular penetration as a mechanism for synergy of insecticidal constituents of rosemary essential oil in Trichoplusia ni. Sci Rep. 2015;5:12690.
14. Cecílio Filho AB, Souza RJ, Braz LT, Tavares M. Curcuma: medicinal, spice and of other potential use plant. Ciência Rural. 2000;30(1):171-175.
15. Araújo CAC, Leon LL. Biological activities of Curcuma longa L. Mem Inst Oswaldo Cruz. 2001;96(5):723-728.
16. Iqbal J, Jilani G, Asham M. Growth inhibiting effects of plant extracts against the grain moth, Sitotroga cerealella (Lepidoptera: Pyralidae). Pak J Zool. 2010;42(5):597-601.
17. Tavares WS, Grazziotti GH, Souza Juñior AA, et al. Screening of insecticide-synergist combinations and their mode of action. J Med Plants Res. 2013b;76(11):1892-1901.
18. Tavares WS, Faroni LRD, Ribeiro RC, Fouad HA, Freitas SS, Zanuncio JC. Effects of astilbin from Kaempferia galanga (Zingiberaceae) on the quality of turmeric rhizomes: implications for IPM. BioControl. 2015;60(2):231-239.
19. Sukari MA, Rashid NY, Neoh BK, Abu Bakar NH, Riyanto S, Ee GCL. Larvicidal activity of some Curcuma and Kaempferia
rhizome extracts against dengue fever mosquito Aedes aegypti Linnaeus (Diptera: Culicidae). *Asian J Chem*. 2010;22(10):7915-7919.

20. Damalas CA. Potential uses of turmeric (*Curcuma longa*) products as alternative means of pest management in crop production. *Plant Omics*. 2011;4(3):136-141.

21. Tavares WS, Freitas SS, Grazziotti GH, Parente LML, Liao LM, Zanuncio JC. Ar-turmerone from *Curcuma longa* (Zingiberaceae) rhizomes and effects on *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Ind Crop Prod*. 2013a;46(1):158-164.

22. Platel K, Sririvasan K. Influence of dietary spices or their active principles on digestive enzymes of small intestinal mucosa in rats. *Int J Food Sci Nutr*. 1996;47(1):55-59.

23. Odouor AMO, Lankau RA, Strauss SY, Gómez JM. Introduced *Brassica nigra* populations exhibit greater growth and herbivore resistance but less tolerance than native populations in the native range. *New Phytol*. 2011;191(2):536-544.

24. Sabbour M, E-Abd-El-Aziz S. Efficacy of certain weed extracts and their combinations as insecticides against *Bruchudis incarnatus* (Boh.) (Coleoptera: Bruchidae) infestation during storage. *J Plant Prot Res*. 2010;50(1):28-34.

25. Li WZ, An JJ, Fu GX, et al. Repellent effects of 12 spices and the combinations with aphicides against *Aphis gossypii* Glov. *J Henan Univ Technol (Nat Sci Ed)*. 2008;29(1):45-47.

26. Ali IHH, Rodina AH. Toxicity of certain weed extracts and their combinations with aphicides against *Aphis gossypii* Glov. *Arab Univ J Agric Sci*. 2002;10(3):1105-1113.

27. Ruberson JR, Herzog GA, Lambert WR, Lewis WJ. Management of the beet armyworm (*Lepidoptera: Noctuidae*) in cotton: role of natural enemies. *Fla Entomol*. 1994;77(4):440-453.

28. Sertkaya E, Bayram A, Kornosor S. Egg and larval parasitoids of the beet armyworm *Spodoptera exigua* on maize in Turkey. *Phytoparasitica*. 2004;32(1):305-312.

29. Beckage NE, Tan FF, Schleifer KW, Lane RD, Cherubin LL. Characterization and biological effects of *Cotesia congregata* polydnavirus on host larvae of the tobacco hornworm, *Manduca sexta*. *Arch Insect Biochem Physiol*. 1994;26(2-3):165-195.

30. de Buron I, Beckage NE. Characterization of a polydnavirus (PDV) and virus-like filamentous particle (VLPF) in the braconid wasp *Cotesia congregata* (Hymenoptera: Braconidae). *J Invertebr Pathol*. 1992;59(3):315-327.

31. McPherson JE. A list of the prey species of *Podisus maculiventris* (Hemiptera: Pentatomidae). *Great Lakes Entomol*. 1980;13(1):17-24.

32. Legaspi JC, Legaspi BC Jr. Does a polyphagous predator prefer prey species that confer reproductive advantage?: case study of *Podisus maculiventris*. *Environ Entomol*. 2004;33(5):1401-1409.

33. Goh HG, Park JD, Choi YM, Choi KM, Park IS. The host plants of *beet armyworm, Spodoptera exigua* Hübner, (*Lepidoptera: Noctuidae*) and its occurrence. *Korean J Appl Entomol*. 1991;30(2):111-116.

34. Zheng XL, Cong XP, Wang XP, Lei CL. A review of geographic distribution, overwintering and migration in *Spodoptera exigua* Hübner (*Lepidoptera: Noctuidae*). *J Entomol Res Soc*. 2011; 13(3):39-48.

35. Idris AB, Emelia O. Development and feeding behaviour of *Spodoptera exigua* L. (*Lepidoptera: Noctuidae*) on different food plants. *Online J Biol Sci*. 2001;1(12):1161-1164.

36. Azidah AA, Sofian-Azirun M. Life history of *Spodoptera exigua* (Lepidoptera: Noctuidae) on various host plants. *Bull Entomol Res*. 2006;96(6):613-618.

37. Herrick NJ, Reitz SR, Carpenter JE, O’Brien CW. Predation by *Podisus maculiventris* (Hemiptera: Pentatomidae) on *Plutella xylostella* (Lepidoptera: Plutellidae) larvae parasitized by *Cotesia platellae* (Hymenoptera: Braconidae) and its impact on cabbage. *Biol Control*. 2008;45(3):386-395.

38. Zanuncio JC, Tavares WS, Fernandes BV, Wilcken CF, Zanuncio TV. Production and use of Heteroptera predators for the biological control of *Eucalyptus* pests in Brazil. *Ekolooji*. 2014;23(91):98-104.

39. Ali A, Gaylor MJ. Effects of temperature and larval diet on development of the beet armyworm (*Lepidoptera: Noctuidae*). *Environ Entomol*. 1992;21(4):780-786.

40. Overholt WA, Ochieng JO, Lammers P, Ogedah K. Rearing and field release methods for *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), a parasitoid of tropical gramineous stem borers. *Int J Trop Insect Sci*. 1994;15(3):253-259.

41. Tayyem RF, Health DD, Al-Delaimy WK, Rock CL. Curcumin content of turmeric and curry powders. *Nutr Cancer*. 2006;55(2):126-131.

42. Nagpal M, Sood S. Role of curcumin in systemic and oral health: an overview. *J Nat Sci Biol Med*. 2013;4(1):3-7.

43. Père-Almeida L, Cherubino APF, Alves RJ, Dufossé L, Glória MBA. Separation and determination of the physico-chemical characteristics of curcumin, demethoxycurcumin and bisdemethoxycurcumin. *Food Res Int*. 2005;38(8-9):1039-1044.

44. Abbott WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol*. 1925;18(2):265-267.

45. Tukey J. Comparing individual means in the analysis of variance. *Biometrics*. 1949;5(2):99-114.

46. Finney DJ. *PROBIT Analysis*. 3rd ed. Cambridge, England: Cambridge University Press; 1971.

47. *SAS User’s Guide: Statistics*. Cary, NC: SAS Institute; 1999.

48. *Martins AP, Salgueiro L, Gonçalves MJ, et al. Essential oil composition and antimicrobial activity of three Zingiberaceae from S. Tome e Principe. *Planta Med*. 2001;67(6):580-584.

49. Raina VK, Srivastava SK, Syamsundar KV. Rhizome and leaf oil composition of *Curcuma longa* from the lower Himalayan region of northern India. *J Essent Oil Res*. 2005;17(5):556-559.

50. Qin NY, Yang FQ, Wang YT, Li SP. Quantitative determination of *Curcuma longa* leaf and rhizome oil using pressurized liquid extraction and gas chromatography–mass spectrometry. *J Pharmaceut Biomed*. 2007;43(1):486-492.

51. Ajayeco EO, Sama W, Essien EE, et al. Larvicidal activity of gumbo–rich essential oils of *Curcuma longa* leaf and rhizome from Nigeria on *Anopheles gambiae*. *Pharm Biol*. 2008;46(4):279-282.

52. Bansal RP, Bahl JR, Garg SN, Naqvi AA, Kumar S. Differential chemical compositions of the essential oils of the shoot organs,
rhizomes and rhizoids in the turmeric Curcuma longa grown in indograngetic plains. Pharm Biol. 2002;40(5):384-389.

53. Chane-Ming J, Vera R, Chalchat JC, Cabassu P. Chemical composition of essential oils from rhizomes, leaves and flowers of Curcuma longa L. from Reunion Island. J Essent Oil Res. 2002;14(4):249-251.

54. Naz S, Ilyas S, Jabeen S, Parveen Z. Composition and antibacterial activity of the essential oil from the rhizome of turmeric (Curcuma longa L.). Asian J Chem. 2011;23(1):1639-1642.

55. Sigrist MS, Pinheiro JB, Azevedo Filho JA, Zucchi MI. Genetic divergence among Brazilian turmeric germplasm using morpho-agronomical descriptors. Crop Breed Appl Biotechnol. 2011;11(1):70-76.

56. Chowdhury H, Walia S, Saxena VS. Isolation, characterization and insect growth inhibitory activity of major turmeric constituents and their derivatives against Schistocerca gregaria (Forsk) and Dysdercus koenigii (Walk). Pest Manag Sci. 2000;56(12):1086-1092.

57. Tripathi AK, Prajapati V, Verma N, et al. Bioactivities of the leaf essential oil of Curcuma longa (var. Ch-66) on three species of stored-product beetles (Coleoptera). J Econ Entomol. 2002;95(1):183-189.

58. Abida Y, Tabassum F, Zaman S, Chhabi SB, Islam N. Biological screening of Curcuma longa L. for insecticidal and repellent potentials against Tribolium castaneum (Herbst) adults. Univ J Zool Rajshahi Univ. 2010;28(1):69-71.

59. Bernard CB, Philogene BJ. Insecticide synergists: role, importance, and perspectives. J Toxicol Environ Health. 1993;38(2):199-223.

60. Pasay C, Arlian L, Morgan M, et al. The effect of insecticide synergists on the response of scabies mites to pyrethroid acaricides. PLoS Negl Trop Dis. 2009;3(1):e354.

61. Oliveira HN, De Clercq P, Zanuncio JC, Pratissoli D, Peduzzi EP. Nymphal development and feeding preference of Podisus maculiventris (Heteroptera: Pentatomidae) on eggs of Ephesia kuehniella (Lepidoptera: Pyralidae) parasitized or not by Trichogramma brassicae (Hymenoptera: Trichogrammatidae). Braz J Biol. 2004;64(3):459-463.

62. Varshney R, Ballal CR. Intraguild predation on Trichogramma chilonis Ishii (Hymenoptera: Trichogrammatidae) by the generalist predator Geocoris ochropterus Fieber (Hemiptera: Geocoridae). Egypt J Biol Pest Control. 2018;28(1):1-6.

63. Pehlivan S, Kurtuluş A, Alinec T, Atakan E. Intraguild predation of Orius niger (Hemiptera: Anthocoridae) on Trichogramma evanescens (Hymenoptera: Trichogrammatidae). Eur J Entomol. 2017;114(1):609-613.

64. Takeda T, Nakamatsu Y, Tanaka T. Parasitization by Cotesia plutellae enhances detoxifying enzyme activity in Plutella xylostella. Pestic Biochem Physiol. 2006;86(1):15-22.

65. Demirel N, Kurt S, Gunes U, Uluc FT, Cabuk F. Toxicological responses of confused flour beetle, Tribolium confusum du Val (Coleoptera: Tenebrionoidea) to various isothiocyanate compounds. Asian J Chem. 2009;21(1):6411-6414.

66. Wu H, Zhang GA, Zeng S, Lin KC. Extraction of allyl isothiocyanate from horseradish (Armoracia rusticana) and its fumigant insecticidal activity on four stored-product pests of paddy. Pest Manage Sci. 2009;65(9):1003-1008.