

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.¹² 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 monooxygenases (CYP).¹³

Turmeric, *Curcuma longa* L. (Zingiberales: Zingiberaceae), native to southwestern India, is a spice utilized to dye and color the condiments and medicine sources.^{14,15} The properties of turmeric against stored product and by-product insect pests¹⁶⁻¹⁸ and as a mosquitoes repellent^{19,20} 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.²¹

Black mustard *Brassica nigra* L. (Brassicales: Brassicaceae) is an annual herbaceous cultivated plant and its seeds are utilized as a spice.²² This plant is likely native to the Mediterranean Asia and Europe.²³ 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.²⁴ The black mustard seed powder repelled most cowpea bruchid, *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae) adults in a choice test using an olfactometer.²⁵ The mortality of the cotton aphid *Aphis gossypii* Glover (Hemiptera: Aphididae) adults was higher with the black mustard seed ethanolic extract as synergist + cyanophos (cholinesterase inhibitor) or KZ oil (an aficide).²⁶

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* Hübner (Lepidoptera: Noctuidae).²⁷ They can coexist in a same crop of *Apium* (Apiales: Apiaceae), *Asparagus* (Asparagales: Asparagaceae), *Beta* (Caryophyllales: Chenopodiaceae), *Gossypium* (Malvales: Malvaceae), *Lactuca* (Asterales: Asteraceae), *Nicotiana* and *Solanum* (Solanales: Solanaceae), and *Phaseolus* and *Pisum* (Fabales: Fabaceae), besides certain cereals, flowers, oilseeds, and weeds.²⁸ *Cotesia flavipes* adults lay eggs in the second or third instar beet armyworm²⁹ and larvae of this wasp pass through two instars in the caterpillar hemocoel for 12 to 16 days after oviposition depending on the environment conditions. Third instar of this parasitoid emerges from the caterpillar, pupate in cocoons, and its adults emerge in about four to eight days.³⁰ Predatory spined soldier bug feeds on about 90 insect species, including commercial crop pests.^{31,32} Beet armyworm is native to Asia, but it was introduced into different parts of the world where its host plants are grown.^{33,34} Smaller beet armyworm larvae feed on the leaf parenchyma, leaving the epidermis and veins intact.^{35,36}

The tritrophic interaction between beet armyworm and its natural enemies *C. flavipes* and predatory spined soldier bug is unknown.³⁷ Consequently, the impact of turmeric powder, its

derivatives, and black mustard seed essential oil solutions in this interaction is also unknown. The objective of this study was to evaluate the mortality caused by 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 to the predatory spined soldier bug nymphs and adults. 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.

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* (Hoffm) Schübl. & G. Martens (Apiales: Apiaceae) and sweet potato, *Ipomoea batatas* (L.) Lam. (Solanales: Convolvulaceae)] as food and moisture supplied once a week.³⁸ 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 diet³⁹ and parasitoid adults on a distilled water-honey solution (90:10%).⁴⁰ 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 (18°08' S, 47°57' 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.¹² 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

¹H, ¹³C, 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 ¹H) 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 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,⁴¹ besides general constituents including proteins, resins, and sugars.⁴² 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 ± 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.⁴³

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, 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.^{29,30} 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 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

Table 1. Mortality caused by Topical Application of 2 μ L 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 *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.^a

Treatments	%	First Instar	Second Instar	Third Instar	Fourth Instar	Fifth Instar	Adult
Turmeric powder	1	63.3 $\pm$ 3.3 b	33.3 $\pm$ 3.3 bcd	6.6 $\pm$ 3.3 c	0 b	0 b	0 b
	0.5	46.6 $\pm$ 3.3 bcd	23.3 $\pm$ 3.3 cdef	3.3 $\pm$ 3.3 c	0 b	0 b	0 b
	0.25	16.6 $\pm$ 6.6 fgh	3.3 $\pm$ 3.3 g	0 c	0 b	0 b	0 b
	0.125	13.3 $\pm$ 3.3 fgh	0 g	0 c	0 b	0 b	0 b
Turmeric oil	1	96.6 $\pm$ 3.3 a	36.6 $\pm$ 3.3 bc	23.3 $\pm$ 3.3 b	3.3 $\pm$ 3.3 b	0 b	0 b
	0.5	33.3 $\pm$ 3.3 cdef	26.0 $\pm$ 3.3 bcde	6.6 $\pm$ 6.6 c	0 b	0 b	0 b
	0.25	26.6 $\pm$ 3.3 defg	10 $\pm$ 5.7 efg	0 c	0 b	0 b	0 b
	0.125	23.3 $\pm$ 3.3 efgh	3.3 $\pm$ 3.3 g	0 c	0 b	0 b	0 b
<i>ar</i> -Turmerone	1	46.6 $\pm$ 3.3 bcd	23.3 $\pm$ 3.3 cdef	3.3 $\pm$ 3.3 c	0 b	0 b	0 b
	0.5	50 $\pm$ 0 bc	43.3 $\pm$ 3.3 b	13.3 $\pm$ 3.3 b	0 b	0 b	0 b
	0.25	43.3 $\pm$ 3.3 bcde	23.3 $\pm$ 3.3 cdef	3.3 $\pm$ 3.3 c	0 b	0 b	0 b
	0.125	33 $\pm$ 3.3 cdef	10.0 $\pm$ 5.7 efg	0 c	0 b	0 b	0 b
Curcuminoids	1	23.3 $\pm$ 3.3 efgh	6.6 $\pm$ 3.3 fg	0 c	0 b	0 b	0 b
	0.5	10.0 $\pm$ 5.7 gh	0 g	0 c	0 b	0 b	0 b
	0.25	6.6 $\pm$ 6.6 gh	0 g	0 c	0 b	0 b	0 b
	0.125	3.3 $\pm$ 3.3 h	0 g	0 c	0 b	0 b	0 b
Mustard oil	1	33.3 $\pm$ 3.3 cdef	16.6 $\pm$ 3.3 defg	0 c	0 b	0 b	0 b
	0.5	20.0 $\pm$ 5.7 fgh	10.0 $\pm$ 5.7 efg	3.3 $\pm$ 3.3 c	0 b	0 b	0 b
	0.25	10.0 $\pm$ 5.7 gh	0 g	0 c	0 b	0 b	0 b
	0.125	3.3 $\pm$ 3.3 h	0 g	0 c	0 b	0 b	0 b
Schultz		100.0 $\pm$ 0 a	100.0 $\pm$ 0 a	86.6 $\pm$ 3.3 a	53.3 $\pm$ 3.3 ab	46.6 $\pm$ 3.3 a	20.0 $\pm$ 5.7 a
F		45.08	51.05	57.45	127.70	196.0	12.0
P value		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

^a Abbott effectiveness⁴⁴. 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.

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 formula⁴⁴ 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 test⁴⁵ 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.⁴⁶ The median lethal time (LT₅₀) of the insects was obtained with PROC PROBIT.⁴⁷ All data were analyzed using SAS version 9.1 software.⁴⁷

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

zero by topical application of 2 μ L ethanolic solutions of turmeric powder, its derivatives (crude essential oil, *ar*-turmerone, or curcuminoid pigments), or black mustard seed essential oil at any tested concentration (Table 1).

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 (χ^2 = 3.00 and P = 1.00), third (χ^2 = 12.77 and P = .47), fourth (χ^2 = 7.53 and P = .87), and fifth (χ^2 = 3.06 and P = 1.00) instars and adult (χ^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 posttreated 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* Forsskal (Orthoptera: Acrididae) nymphs by 60%. A total of 20 μ g applied per nymph controlled 45% of those of the red cotton stainer *Dysdercus koenigii* F. (Hemiptera: Pyrrhocoridae) and 50 μ g 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; LD_{50} = 36.71 μ g/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/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 (LD_{50} = 0.337 mg/cm²; n = 30) compared to those of aerial parts (LD_{50} = 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 μ g of turmeric powder or its derivatives + 5 μ g 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

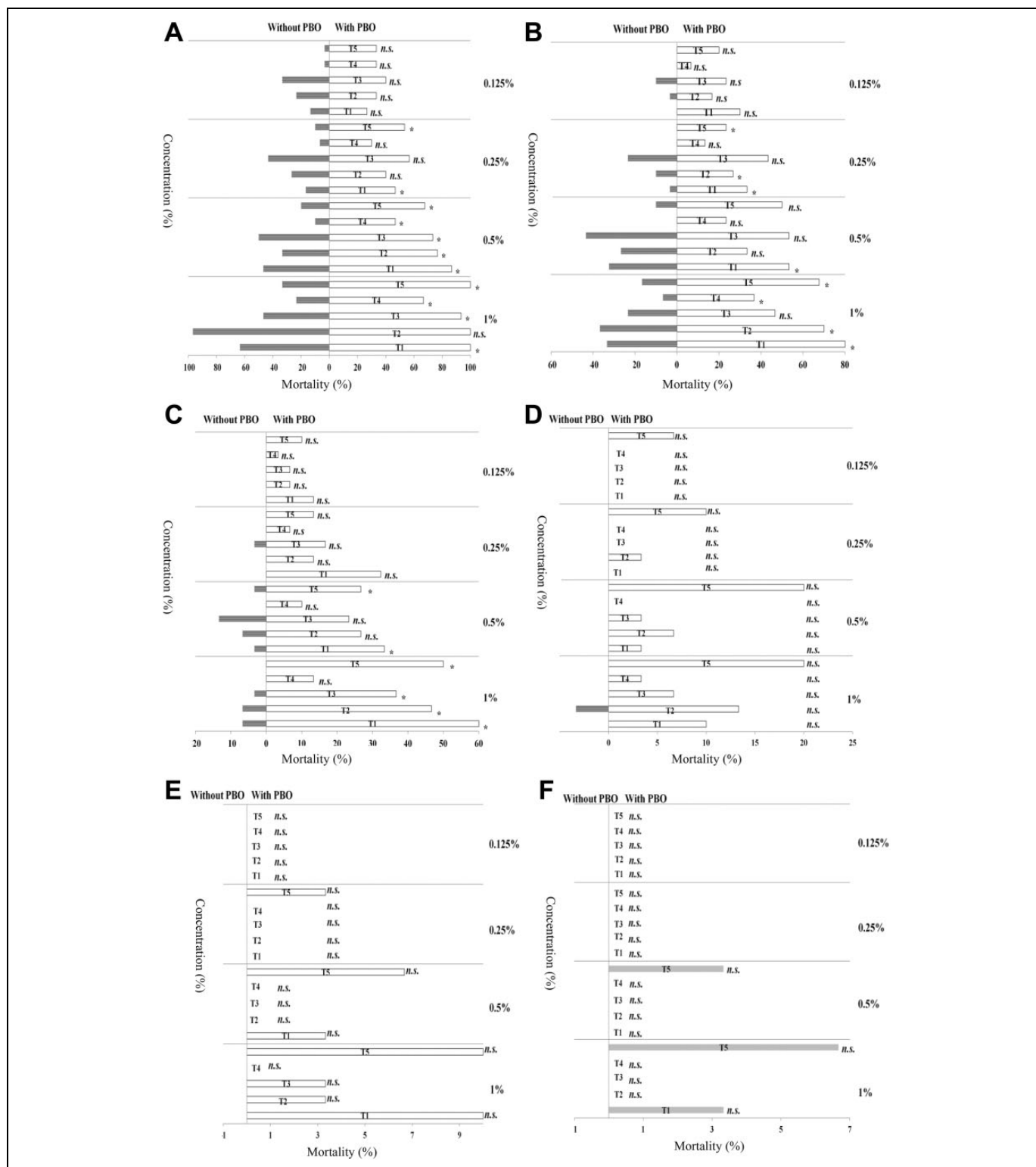


Figure I. Mortality (%) of the first (A), second (B), third (C), fourth (D), and fifth (E) instars and (F) *Podisus maculiventris* (Heteroptera: Pentatomidae) adults with three replications each with 10 nymphs of each instar or 10 adults, per Petri dish, 24 h after topical application of 2 μ L ethanolic 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.

Table 2. LT₅₀ in hours (Variation Interval), Slope $\pm$ Standard Error (SE), χ^2 , and P Value for Mortality Caused by 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% (m/m) to *Podisus maculiventris* (Heteroptera: Pentatomidae) Nymphs and Adults Feeding on *Spodoptera exigua* (Lepidoptera: Noctuidae) Larvae Subjected to Parasitism by *Cotesia flavipes* (Hymenoptera: Braconidae).

Treatments	n	df	LT ₅₀ (hours)	Slope $\pm$ SE	χ^2	P Value
Second instar						
Turmeric powder	30	13	130.23 (103.61-212.24)	2.85 $\pm$ 0.66	6.49	.93
Turmeric oil	30	13	142.48 (110.82-257.46)	2.83 $\pm$ 0.69	7.76	.86
<i>ar</i> -Turmerone	30	13	95.72 (79.85-125.50)	2.89 $\pm$ 0.56	3.89	.99
Curcuminoids	30	13	130.42 (100.32-233.21)	2.37 $\pm$ 0.57	4.70	.98
Mustard oil	30	13	105.37 (89.38-137.18)	3.44 $\pm$ 0.68	10.08	.69
Schultz	30	13	37.71 (32.25-42.93)	5.44 $\pm$ 0.77	3.00	1.00
Third instar						
Turmeric powder	30	13	169.94 (127.47-500.64)	3.42 $\pm$ 1.08	3.27	.99
Turmeric oil	30	13	146.63 (123.68-289.47)	6.39 $\pm$ 2.12	3.10	.99
<i>ar</i> -Turmerone	30	13	121.19 (94.97-200.33)	2.41 $\pm$ 0.56	10.71	.63
Curcuminoids	30	13	161.70 (119.27-370.91)	2.58 $\pm$ 0.68	13.85	.38
Mustard oil	30	13	138.02 (109.32-232.88)	3.01 $\pm$ 0.71	11.22	.59
Schultz	30	13	61.67 (53.34-70.29)	4.20 $\pm$ 0.61	12.77	.47
Fourth instar						
Turmeric powder	30	13	182.42 (133.87-857.52)	3.80 $\pm$ 1.35	2.10	.99
Turmeric oil	30	13	182.61 (134.31-988.25)	3.97 $\pm$ 1.45	3.89	.99
<i>ar</i> -Turmerone	30	13	138.42 (116.32-218.01)	4.84 $\pm$ 1.28	9.34	.75
Curcuminoids	30	13	161.84 (128.79-517.85)	5.25 $\pm$ 1.89	3.32	.99
Mustard oil	30	13	147.44 (117.97-276.60)	3.76 $\pm$ 1.03	5.31	.97
Schultz	30	13	78.05 (67.80-90.93)	3.86 $\pm$ 0.64	7.53	.87
Fifth instar						
Turmeric powder	30	13	—	—	—	—
Turmeric oil	30	13	—	—	—	—
<i>ar</i> -Turmerone	30	13	—	—	—	—
Curcuminoids	30	13	—	—	—	—
Mustard oil	30	13	—	—	—	—
Schultz	30	13	113.89 (97.72-150.65)	4.02 $\pm$ 0.88	3.06	1.00
Adult						
Turmeric powder	30	13	—	—	—	—
Turmeric oil	30	13	—	—	—	—
<i>ar</i> -Turmerone	30	13	—	—	—	—
Curcuminoids	30	13	—	—	—	—
Mustard oil	30	13	—	—	—	—
Schultz	30	13	169.38 (128.20-457.64)	3.59 $\pm$ 1.11	14.33	.35

Abbreviation: df, degree of freedom.

^a 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.

mortality of the parasitoid by the insecticides, because heteropteran predators avoid preying on parasitized larvae.^{61,62} In addition, dead parasitoids in host larvae body are unable to produce chemical signals responsible to prevent predation.⁶³ The faster dying of the predatory spined soldier bug with the positive control, *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 *Cotesia* species increase the CYP activity.⁶⁴ 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.⁶⁵ Allyl isothiocyanate vapor alone controlled 100% of the book

louse *Liposcelis entomophila* Enderlein (Psocoptera: Liposcelidae), lesser grain borer, maize weevil, and *Tribolium ferrugineum* F. (Coleoptera: Tenebrionidae) adults 72 h after exposure to a three μ g/mL atmospheric concentration.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 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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