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Potential Insecticidal Effect of a Wasp (*Polistes dominulus*) and a Bee (*Apis mellifera*) Venoms in Controlling *Spodoptera littoralis*

Reham Z. Sadek*, Shereen M. El Banna, Fayez M. Semida, Samy M. Zalat

Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt
Email: *dr.reham_science@hotmail.com

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Abstract

Cotton leaf worm, *Spodoptera littoralis*, is considered one of the most common arthropod pests that contribute to crop devastation of cotton. Previously, agricultural pests were controlled through the application of chemical insecticides. However, insecticide-resistant insect populations emergence, as well as increasing concerns about the environmental and human health risks. Venomous animals introduce valuable sources of bioactive compounds which are employed for defence. Some of these toxins have high phyletic specificity, making them appropriate for use in IPM programs. This study aims to test the insecticidal effects of *Polistes dominulus* and *Apis mellifera* venoms. Crude venoms were examined for their insecticidal effect against cotton leaf worms by four different application methods including: leaf dipping, integument dropping, spraying, and injection. The data demonstrated a strong response to purified *(Polistes dominulus*) venom at an initial time than that of honey bee *(Apis mellifera*) which increases response with increasing the dose and the time interval. A dosage of 0.015 - 0.16 ml of both venoms had notably varied in LD_{50} values on *Spodoptera littoralis* that showed higher wasp venom toxicity. Cotton leaf worms showed more susceptibility and mortality to the *Polistes* sp. venom than that of honey bees.

Keywords

Social Insects, Honey Bee, Yellow Jacket, Enzymes, Pest Control, Bio-Insecticides, Biological Control

1. Introduction

Pests cause great damage to crop plants in Egypt, larvae of these pests can feed

on ≈90 economically important plant species belonging to 40 families. *Spodoptera littoralis* (Boisd.) is a severely destructive insect pest of cotton. The widespread use of pesticides to control *S. littoralis* larvae has resulted in environmental and health issues and risks, including resistance development and residual effects [1].

Chemical pesticides are primarily used to control agricultural insect pests as well as insect vectors of major human diseases. However, the usage of chemical pesticides has resulted in a number of issues, including degradation of the environment and a rise in human health impacts. Secondary pest outbreaks focused on using bio-insecticides as chemical alternatives.

Efforts were focused on finding new control agents with new modes of action. These substances are pesticides that are selective and particular to the pest in concern. [2] elicits their primary action on insect mortality, and metabolism and ultimately affects the development and growth of the target insect. They induce morphological abnormalities as well as the death of treated insects. These characteristics allow the most promising new control agents for controlling *S. littoralis* larvae.

For decades, scientists have been curious by the secretions of venomous creatures. The modern era of molecular toxicology was fueled by a desire to purify and understand the mechanism of action of lethal components from medically important animals like marine cone snails [3], stonefish [4], insects, and snakes [5]. In the late 1980s and early 1990s, pioneering work by [6] [7] [8], and others led to an understanding that most animal venoms are made up of a complex cocktail of peptide and protein components, the fatal toxin being only one of them, represents only a minor proportion. Furthermore, it became clear that many of the non-lethal venom components have useful bioactivities that allow them to be used as research tools, such as in the characterization of ion channels [7] [9] [10] [11], or as leads for the development of pharmaceutical agents [12] and insecticides [13] [14].

The majority of research has been carried out on isolating pesticide toxins with bio-control action. Hymenoptera venoms were selected because of their relevance as a model for social and solitary insects, respectively, as well as their taxonomic relationship to economically important pest insects.

Various chemical cocktails are produced by venomous animals for defence, prey acquisition, competitor deterrence, and/or extra-oral digesting [15]. With some venoms containing over 1000 distinct peptides, these venoms have shown to be a valuable source of pharmacologically active chemicals [16]. While some of these peptides have been shown to be invertebrate-specific [10] [11] [14] [17] [18] [19], many venom toxins exhibit activity in a wide range of phyla, therefore, the careful selection of peptides tracked for commercial pesticide manufacture is required [20]. However, some of these toxins may be ideal for use in IPM programmes due to their high phyletic specificity.

The venom characteristics and components of solitary and social bees and wasps are believed to differ depending on their social activity. Although it has

been assumed to have more diversified bioactive components with pest inactivation and physiological manipulation capabilities, there are just too little data on the venom compositions of social and solitary wasps and bees to make this assumption. However, several neurotoxic peptides and proteins appear to be unique to the venoms of solitary bees and wasps in addition to several other bioactive proteins.

Venom components target the main critical systems of an organism, such as biological, neuromuscular, and hemostatic systems, to achieve the most efficient and rapid immobilization or death of the victim. Since venomous animals prey on many different species, as well as have a defense system against unspecified intruders, they produce various effects and mechanisms both with specific molecular targets and those that are active across a wide range of animal species. Bee venom is made up of a complex mixture of proteins, peptides, and low-molecular-weight components. Its constituents have been identified and classified in recent years. Phospholipase, Hyaluronidase, Phosphatase, and Glucosidase are some of the most important components [21]. Wasp venoms, on the other hand, are made up of a complex mix of proteinacious and non-proteinacious components that could be used as agrichemicals or pharmaceuticals to help with pest control.

Similarly, preliminary research indicates that infected cotton worm pests with venom might have a reduced propensity to feed prior to death [12] [18] and there is evidence that bee and wasp venoms can affect not only the pest mortality, but also the survivorship [18]. The mechanisms that underlie this effect are unknown but might include alterations in host nutritional balance, which lead to resource competition, upregulation of immune responses, or production of secondary metabolites in the haemolymph.

The purpose of this research was to create a bioassay method that would allow new active bio-insecticides to be discovered using the venom of bees and wasps. When venom amounts are treated on cotton worms, which are considerably more sensitive to insect venoms, provides a better bioassay for the development of new insecticidal toxins.

Therefore, this study aims to: investigate the possible insecticidal activity of crude venom of bees and wasps against cotton leaf worm pest and investigate some of the biological mortality, toxicological and morphological effects of the venom components on cotton leaf worm.

2. Materials and Methods

2.1. Biological Studies

Polistes dominulus and *Apis mellifera* species were collected. The individuals were caught and subjected to a low-temperature, ice-controlled environment right away. To euthanize wasps and bees, freeze them for 5 hours at −20˚C.

Venom was obtained from poison glands of both social bees and wasps, and poisonous secretions were separated from venom glands using dissection instruments, a light microscope, were used to extract venoms from 208 wasp venom sacs, which

were meticulously dissected from the wasps, macerated in a 1:1 (v:v) acetonitrile/water solution, and centrifuged at 5000 g for 5 minutes at room temperature and vacuum drying. The supernatant was collected, vacuum dried, weighed in a precision balance, and stored at 20˚C. Lipholization was used to obtain venom powder, vacuum drying the venom in silica gel.

2.2. Rearing Technique of Cotton Leaf Worm, *Spodoptera littoralis*

A laboratory strain of *S. littoralis* was reared in the laboratory away from any insecticidal contamination at the Department of Cotton Leaf Worm, Branch of Plant Protection Research Institute at Zagazig, Sharqia Governorate under constant conditions 25˚C ± 2˚C and 60% ± 5% R.H. to provide insects used in the present investigation. Egg-masses were placed on leaves of castor bean oil, *Ricinus communis* in cylindrical glass jars (El-Defrawi *et al.*, 1964).

After egg hatching, the newly hatched larvae were transferred into large rearing jars and provided with filter paper at the bottom of the jars to absorb excess moisture. Larvae were reared on fresh castor bean leaves until the end of larval stage. The formed pupae were collected and placed in clean jars until adult emergence. Each jar was provided with 10% honey solution soaked in cotton wool, which was renewed daily to avoid fermentation and growth of microorganisms. Fresh green castor leaves were introduced daily into clean jars.

2.3. Venom Application Experiments

Preliminary tests for each of the separate treatments were conducted using a variety of concentrations (in distilled water) for each of the venom concentrations (0.01, 0.02, 0.04, 0.08, 0.16 µl). Cotton worms were treated in glass jars with various concentrations of bee and wasp venoms then provided to early 4th instar larvae to pupate on. According to [22], the offered treated larvae were in a wettable state, and the leaf-dipping strategy was used. Castor bean leaves (*R. communities*) were dipped in each concentration and dried at room temperature before being fed to newly moulted 4th instar larvae. The larvae were allowed to feed for 24 hours before being fed new, untreated castor bean leaves until pupation. Controls for bee and wasp treatments were larvae that ate untreated castor bean leaves. For each concentration, eight replicates were carried out, with each replicate containing five larvae. The percentages of larval mortality and morphological deformity were calculated. The data were then subjected to probit analysis [23] to obtain the LC_{50} values of both social bee and wasp venom as well as the concentration which causes 50% adult malformation (MC_{50}). Measuring the mortality and morphological changes through cotton worm pest treated with different concentrations of social wasp and bee venom through topical food, integument dropping, injection and spraying applications.

Injection: Cotton worms between 3rd and 4th instar (90 - 110 mg/individual) were used for injections. 0.1 mL of venom diluted in insect saline was injected into the metathoracic pleurite, and for all five concentration till 1.6 mL were in-

jected. Injections were performed using a 29.5 gauge insulin syringe (B-D Ultra-Fine, Terumo Medical Corporation, Elkton, MD, USA). A cohort of ten insects was injected at each venom concentration, and a similar number of control insects were injected with insect saline.

Integument dropping: Lyophilized crude bee and wasp venoms were tested for biological activity by topically dropping µl of aqueous samples into newly eclosed fourth instar *S. littoralis* larvae (40 - 70 mg). For each concentration tested, 80 larvae were topically dropped on integument and toxic effects were monitored over 4 - 5 days.

Spraying: treatment doses are then harvested from the bees and wasps venom and formulated in acetone solvent for application using hand-held (panel d) or aerial-mounted sprayers.

2.4. Morphological Malformations of Cotton Leaf Worm, *Spodoptera littoralis*

Honey bee venom as well as yellow jacket the concentration which causes 50% larval malformations (MC_{50}) for cotton worm *Spodoptera littoralis* was measured. The sequential cumulative effect among treatments of cotton worm with either venoms were carried out by treatment of the 4th instar larvae with the considered concentrations in order to obtain MC_{50} effects of *Apis mellifera* and *Polistes dominulus* then, at the end of larval stage, the late 6th instar larvae were allowed to pupate on castor bean treated with both venoms at which pupal MC_{50} were estimated.

2.5. Toxic Effects of Tested Venoms against Cotton Leaf Worm, *Spodoptera littoralis* under Laboratory Conditions

The effectiveness of various venom concentrations was tested on 4th instar larvae by application, topical on food, spraying, topical on integument, and injection. Using distilled water, serial successive concentrations of each venom were created, starting with the recommended concentration. Castor bean leaf discs (9 cm in diameter) were dipped in the tested concentrations for 10 seconds, dried, and fed to larvae that had been starved for 4 - 6 hours before treatment [24]. The larvae were placed in 5 pound glass jars, and each treatment was repeated eight times (5 larvae per each). Only distilled water was used to dip the control discs. The larvae were given 48 hours to feed on the treated discs before being moved to the untreated ones. For everyone, mortality percentages were obtained after 24, 48, 72, and 96 hours. Probit analysis was used to statistically examine the dose toxicity regression lines [23]. Sun formulae were used to compute the Toxicity Index and Relative Potency [21]. At the end of each testing period, total mortalities were determined and corrected using Abbott's formula [25].

2.6. Mortality of Treated Cotton Leaf Worm, *Spodoptera littoralis*

Samples of cotton leaves were picked up at random for each treatment at zero time directly after applying with four different treatment methods for cotton

worm insect pest *S. littoralis* through spraying, topical on food application, dropping on integument and direct injection. The prepared samples concentrations were sealed in small flasks and transferred to the laboratory where they were offered to cotton leaf worm larvae (Aly, 1999). Five larvae were placed in each glass jar and allowed to feed on the treated leaves for 1 to 5 days then the survived larvae were transferred to other clean jars and supplied with fresh clean castor bean leaves for another 3 days. Eight replicates were used for each treatment. Cumulative mortalities were calculated at the end of each testing time [26].

The mortalities at the zero time were considered as initial kill; while the mean of the cumulative mortalities of the remaining tested times were considered as cumulative final effect.

2.7. Statistical Analysis

Data were subjected to statistical analyses using a software package CoStat® Statistical Software (2005) a product of Cohort Software. The significance of the main effects was determined by analysis of variance (ANOVA) and chi-square tests. The significance of various treatments was evaluated by Duncan's multiple range tests ($P < 0.05$) [27].

3. Results

The current study showed advances in the development of bio-pesticides based on some Hymenoptera venom which involved successful development of bio pesticides for the control of cotton leaf worms to examine their potential on pest mortality, morphology, feeding and development.

3.1. Biological Studies

Morphological Malformation against *S. littoralis*

Two bio pesticides from social bee and wasp venom [Hymenoptera] (used at MC_{50}, concentration caused more than 50% morphological malformation) were used for treatment of larvae of cotton leaf worm, *Spodoptera littoralis*. The obtained results revealed that wasp is a potent toxin ($LC_{50} \leq 0.001$ ppm) at initial time compared to bee venom ($LC_{50} = 0.49$ ppm) when applied topically on food (**Table 1** and **Table 2**).

On the other hand, the mode of action through morphological malformation, the malformation concentration fifty (MC_{50} for cotton leaf worms treated by yellow jacket venom which was significantly higher at integument dropping and injection applications. All treatments caused higher significant morphological differences within integument, pores and dark bands when treated with Yellow jackets venom at final time interval.

The topical food application results revealed that wasp venom is a potent toxin causing morphological malformations that showed the highest effect on cotton leaf worm larvae while integument drooping highest effect was shown on the

Table 1. LC_{50} values for social bee venom on 4th instar larvae of cotton leaf worm *S. littoralis* LC_{50} (µl/g) showing different relative susceptibility evaluation of the tested insecticides.

Conc.	Conc. χ 100	Log (Conc. χ 100)	Treated	Observed response%	Linear response%	Linear probit
0.03	3	0.4771	40	17.949	12.7925	3.8635
0.04	4	0.6021	40	17.949	31.5304	4.5191
0.05	5	0.6990	40	58.974	51.0852	5.0272
0.01	1	0.0000	40	32.500	29.0434	4.4479
0.02	2	0.3010	40	40.000	46.5250	4.9128
0.03	3	0.4771	40	57.500	57.3286	5.1848
0.04	4	0.6021	40	65.000	64.7223	5.3779
0.05	5	0.6990	40	72.500	70.1079	5.5275
0.03	3	0.4771	40	20.000	20.9798	4.1929
0.04	4	0.6021	40	27.500	25.2413	4.3330
0.05	5	0.6990	40	27.500	28.8326	4.4417
0.01	1	0.0000	40	37.500	27.0683	4.3893
0.02	2	0.3010	40	37.500	41.9800	4.7976
0.03	3	0.4771	40	40.000	51.4533	5.0365
0.04	4	0.6021	40	50.000	58.1625	5.2061
0.05	5	0.6990	40	87.500	63.2109	5.3375

Table 2. LC_{50} values for both social bee and wasp venoms on 4th instar larvae of cotton leafworm *S. littoralis* LC_{50} (µl/g) showing different relative susceptibility evaluation of the tested insecticides.

Conc.	Conc. χ 100	Log (Conc. χ 100)	Treated	Observed response%	Linear response%	Linear probit
0.01	1	0.0000	60	48.33	44.4905	4.8614
0.02	2	0.3010	60	51.667	56.7334	5.1696
0.03	3	0.4771	60	60.000	63.6789	5.3500
0.04	4	0.6021	60	70.000	68.3638	5.4780
0.05	5	0.6990	60	75.000	71.8088	5.5772
0.01	1	0.0000	60	83.333	82.2030	5.9233
0.02	2	0.3010	60	83.333	86.1911	6.0891
0.03	3	0.4771	60	90.000	88.2161	6.1861
0.01	1	0.0000	60	33.333	37.4306	4.6795
0.02	2	0.3010	60	51.667	52.5709	5.0645
0.03	3	0.6021	60	76.667	67.3532	5.4497

Continued

0.04	4	0.9031	60	80.000	79.8062	5.8348
0.05	5	1.2041	600	85.000	88.8706	6.2198
0.01	1	0.0000	60	48.333	44.2541	4.8554
0.02	2	0.3010	60	61.667	66.8273	5.4352
0.03	3	0.4771	60	71.667	78.0625	5.7743
0.04	5	0.6021	60	91.667	84.4914	6.0151

second level at morphological deformations. MC_{50} = 0.0013 ppm compared to bee venom MC_{50} = 0.0157 ppm, while integument dropping application showed MC_{50} = 0.0114 ppm) compared to bee venom MC_{50} = 0.0303 ppm). The spraying application of wasp venom showed MC_{50} = 0.014 ppm on treated cotton leaf worms compared to bee venom MC_{50} = 0.139 ppm. Finally the injection application was MC_{50} = 0.0412 ppm compared to bee venom (MC_{50} = 0.792 ppm) (Figure 1 and Figure 2).

It was observed that venom applied by spraying has significant effect rather than by topical application. Spraying application of venom affect by black spots than topical application. Topical affect more by shrinkage of insect body while venom application by injection was observed to have the least morphological effect on cotton worm larvae.

3.2. Toxicity of Bee and Wasp Venoms against *S. littoralis* through LC_{50} Value

According to LC_{50} and LC_{90} values, topical application of bee venom was the most effective method where the LC_{50} and LC_{90} values recorded 0.1, 0.809 ppm for 4th instar treated with bee venom and 0.472 and 6.838 ppm for the 4th instars larvae treated with wasp venom, respectively.

Meanwhile, injection application appeared to be the least effective against pest tested instars, where the LC_{50} and LC_{90} values against 4th instar were 9.901 and 36.447 ppm for bee venom and the values against the 4th one were 65.736 and 1000.775 ppm for wasp venom, respectively.

The rest venom application methods gave moderate effects against instars that manifested, the LC_{50} and LC_{90} levels were 0.204 and 2.311 ppm for bee venom, 0.255 and 5.484 ppm for wasp venom. 1.001, 12.34 ppm for topical on integument application with bee venom and 9.901, 36.447 ppm for topical on integument application with wasp venom, respectively for 4th instar larvae (Table 1 and Table 2).

The social honey bee venoms killed cotton worms with LD_{50} values in the range 69 - 126 mg/g, while the social *Polistes* sp. Wasp venom were slightly more potent, with LD_{50} values of 46 - 48 mg/g (Figure 3). These values are comparable to the LD_{50} value of 105 mg/g reported for injection of crude venom into 3rd - 4th instar *Spodoptera littoralis*. Thus, while there might be statistically significant

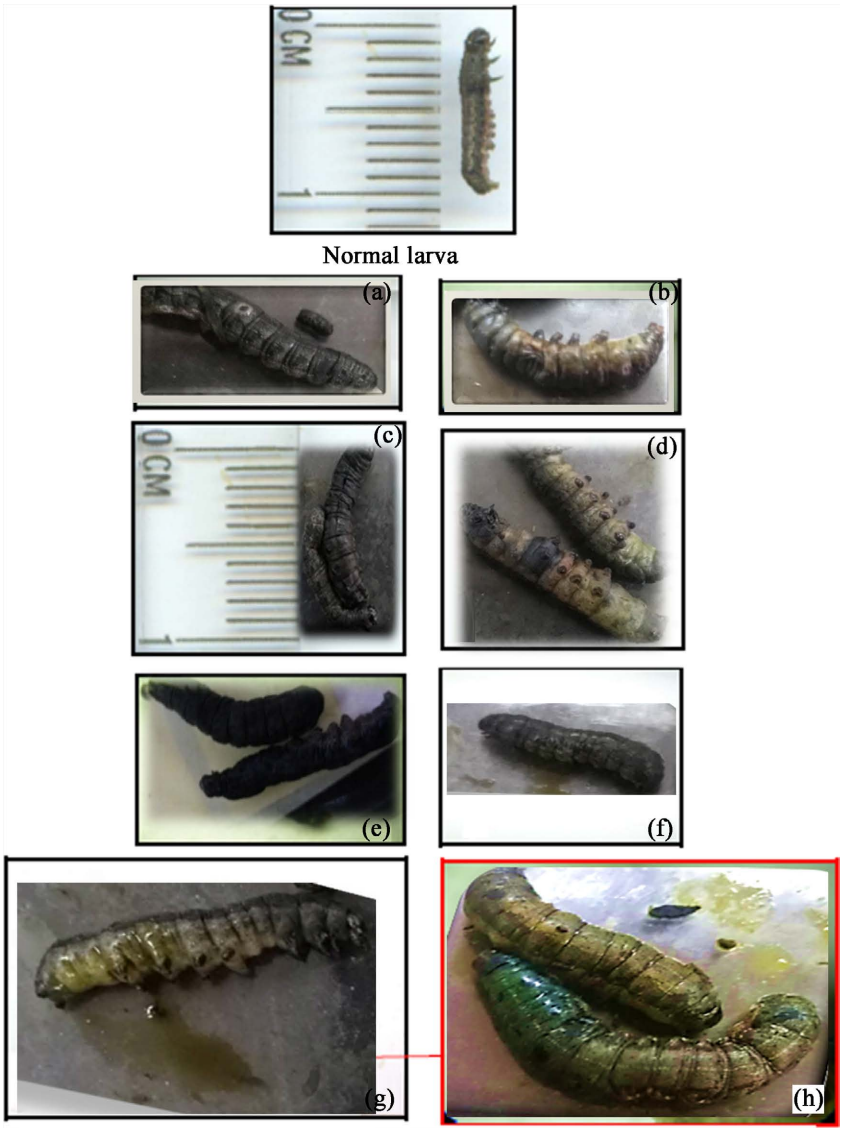

Figure 1. Morphological abnormalities resulted from treating *S. littoralis* larvae with bee and wasp venom concentrations causing presence of dark pores and white pores upon pest cuticle (a) & (b); formation of dark bands on thoracic segments (c) & (d); shrinkage and excess liqud elimination (f) & (g); constrictions within insect larva integument (h); and formation of liquid sacs projections (e) & (h).

differences in potency between social bee and wasp venoms, it is remarkable that the LD_{50} values of all of the venoms tested to date against cotton worm cluster in the range 69 - 242 mg/g.

3.3. Mortality of Bee and Wasp Venoms against *S. littoralis*

Results indicated the important role of feeding period on treated leaves for 48 h followed by untreated leaves for 3 days. So, the evaluation was assessed using the cumulative mortalities. In this study mortality calculated after 72 hrs after each application of either social bee or wasp venom. The initial effect that (calculated as the cumulative mortalities at zero time) recorded 100%, 100%, 92%, 88% and

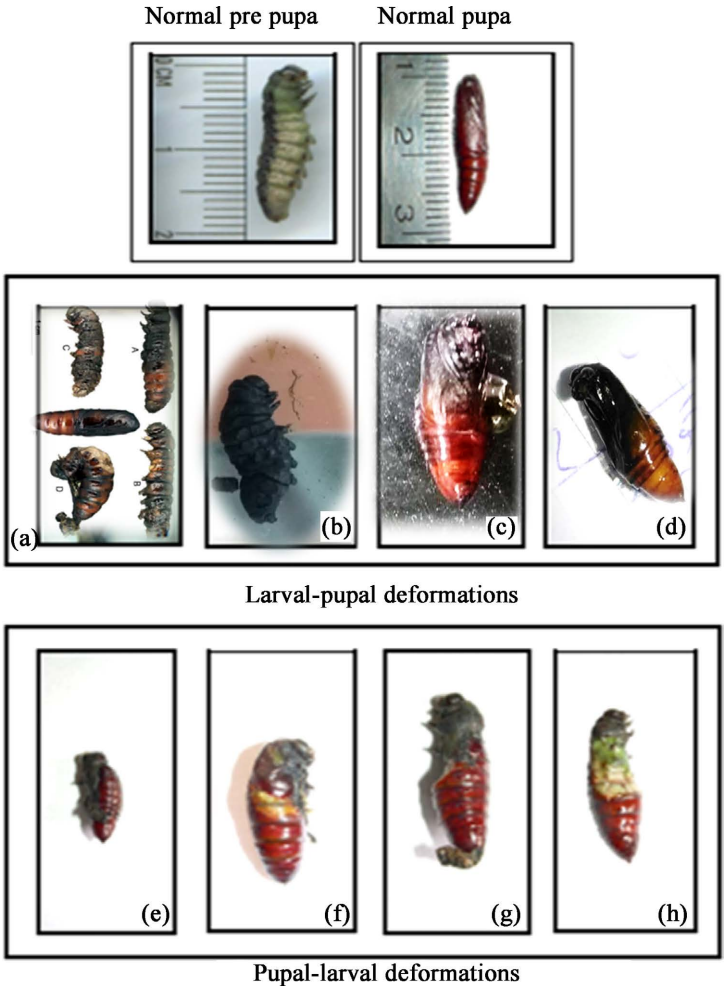

Figure 2. Illustrates the effects of feeding 4th instar larvae of cotton leaf worms *Spodoptera littoralis* with castor leaves treated with different bee and wasp venom concentrations on pupal development. There are various morphological abnormalities observed including abnormal pupa formation (a)-(d) and pre pupal deformations (e)-(h).

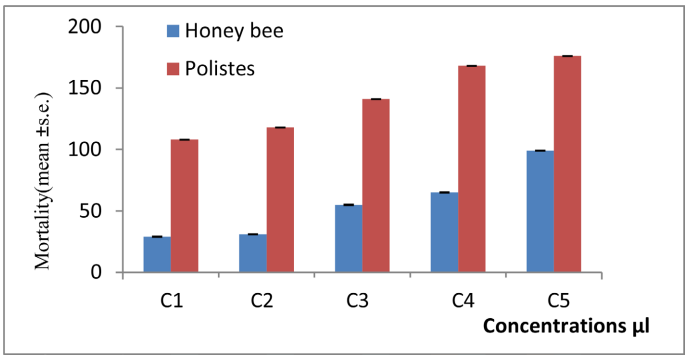

Figure 3. Mean difference in mortality with different concentrations between honey bee and *Polistes* sp. Venom affecting *S. littoralis.*

26% for spraying, topical on food, injection and on integument, respectively. Control recorded 1% mortality. Both topical application methods gave the highest significant mortalities compared to the control that manifested 0.76313 ± 0.43 and

0.921 ± 0.115 respectively for honey bee and 1.0169 ± 0.053 for wasp venoms ($F_{(4,389)} = 6.56$; $P < 0.0001$) ($F_{(4,389)} = 35.65$; $P < 0.0001$) (Table 3 and Table 4).

Cotton worm treatment with bee venom has accordingly reduced survival times at concentrations (0.04, 0.08, 0.16 ppm). Reduced survival times have reached 87% and 72% for *A. mellifera* while it was higher reached 98 and 100% for *Polistes* sp. (Table 4). For topical application on food and integument drop when compared with control. Caterpillars infected by spraying bee venom demonstrated significant survival reduction effects ($F_{(4,389)} = 25.59$; $P < 0.00001$) ($F_{(4,389)} = 5.12$; $P < 0.0001$) (Figure 3). It was low for both bee and wasp venoms effect on cotton worm mortality compared to infection by venom topical applications.

Topical application on food of bee venom indicated the mortality of *S. littoralis* was significantly different ($\chi^2 = 68.13$; d.f. = 2; $P < 0.0001$) among applications with highest number on *Polistes* sp. than *A. mellifera* but lowest mortality on injection of both bee and wasp venoms respectively (Figures 4(a)-(d), Table 3 and Table 4).

Table 3. Effect of five different concentrations of bee venom on cotton worm *Spodoptera littoralis* mortality with different application methods of venom respectively.

Conc. (µl)	Application			
	Spray (H.B)	*food* (H.B)	*Integument* (H.B)	*injection* (H.B)
C1	0.00 ± 0.00	0.34 ± 0.19	0.39 ± 0.20	0.00 ± 0.00
C2	0.00 ± 0.00	0.42 ± 0.20	0.42± 0.20	0.00 ± 0.00
C3	0.21 ± 0.16	0.68 ± 0.20	0.49 ± 0.20	0.21 ± 0.16
C4	0.25± 0.18	0.78 ± 0.19	0.52 ± 0.20	0.28 ± 0.18
C5	0.63 ± 0.19	0.86 ± 0.17	0.92 ± 0.11	0.39 ± 0.19

Data represents the mean value ± S.E. from 25 cotton leaf worm/group with significance difference between the five different concentrations, using Chisquare test ($\chi^2 = 52.11$, d.f. = 28, $P \leq 0.0037$).

Table 4. Effect of five different concentrations of bee venom on cotton worm *Spodoptera littoralis* mortality with different application methods of venom respectively.

Conc (µl)	Application			
	sray (P.)	*Food* (P.)	*integument* (P.)	*injection* (P.)
C1	0.5 ± 0.20	0.83 ± 0.15	0.49 ± 0.20	0.33 ± 0.19
C2	0.52 ± 0.20	0.86 ± 0.15	0.64 ± 0.19	0.52 ± 0.20
C3	0.61 ± 0.20	0.90 ± 0.12	0.72 ± 0.18	0.77 ± 0.17
C4	0.71 ± 0.18	1.00 ± 0.00	0.93 ± 0.10	0.81 ± 0.16
C5	0.76 ± 0.17	1.00 ± 0.00	1.00 ± 0.00	0.86 ± 0.14

Data represents the mean value ± S.E. from 25 cotton leaf worm/group with significance difference between the five different concentrations, using Chisquare test ($\chi^2 = 47.43$, d.f. = 28, $P \leq 0.01$).

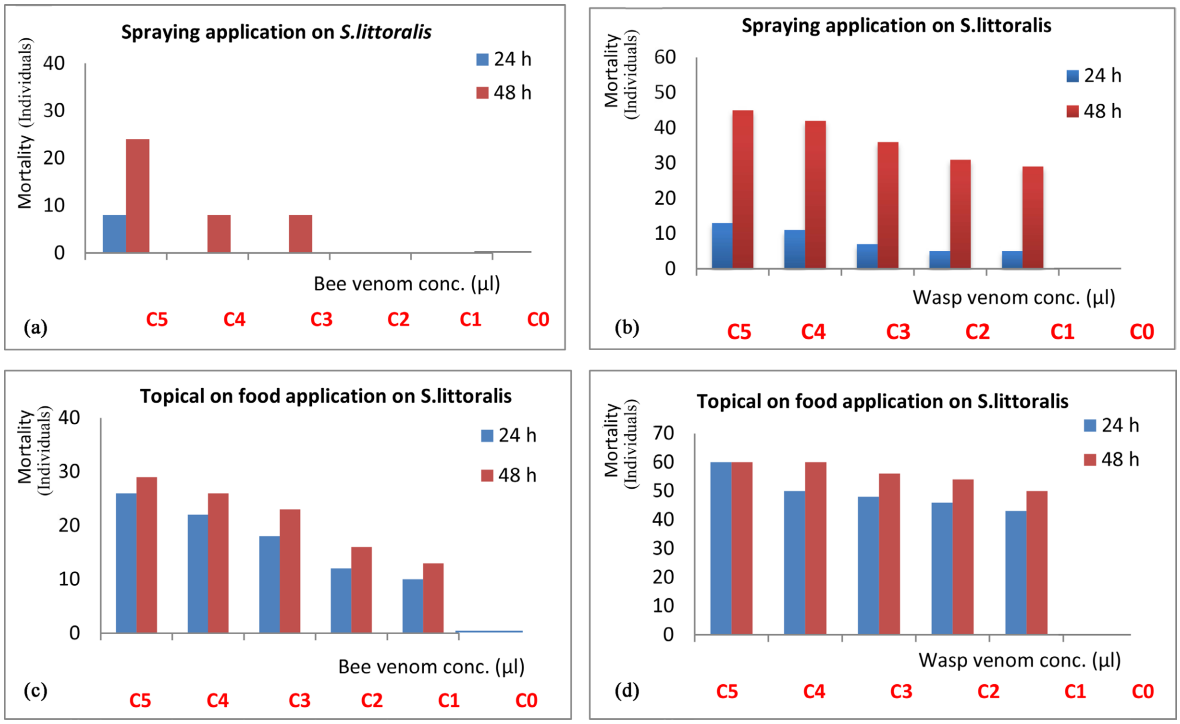

Figure 4. Difference in mortality % of cotton worm *Spodoptera littoralis* on different concentrations application methods of honey bee *Apis mellifera* and *P. dominulus* venom (mean value ± S.E.; $P \leq 0.05$).

Different application methods of crude venom toxins on integument or into the hemolymph had not compromise the same mortality effect derived from species selectivity. Spraying and injection application of a high concentration (1 - 1.16 ml^{-1}) of bee venom to cotton leaf worm caused significant morphological differences. However, by passing the cuticle by topical dropping and topical food applications of either social bee or wasp venom into the hemolymph (1 - 1.16 ml^{-1} per insect) caused 100% mortality in both species within 72 h. expressing different application effects to insect pests could be remedied by applying the venom in ways that targeted the pest.

Wasp venom applied on *S. littoralis* has the largest effect with ($\chi^2 = 53.10$; d.f. = 2; $P < 0.0001$ for topical on food application and $\chi^2 = 12.18$; d.f. = 2; $P < 0.002$ for integument application) which is higher than bee venom mortality and toxicological effects (**Figures 5(a)-(d)**).

Reduced cumulative survival by bee venom spraying caused 60% for bee and 75% for wasp ($F_{(4,389)} = 9.98$, $P \leq 0.004$) mortality for the highest concentration 0.16 while reached 75% by spraying *Polistes* sp. Venom compared with infection by injection that has the lowest effect for bee causing 25% mortality and 83% for wasp venoms on *Spodoptera littoralis* (**Figure 6** and **Figure 8**).

The mean of residual effect that calculated as the mean of cumulative mortalities from day 1 until day 5 after applying venom topically, injection and by spraying were manifested in (**Table 1** and **Table 2**). Topical application on food and integument were detected the highest significant mortalities effect, whereas injection recorded the least significant mortality effect (18.40%) (18.40%) ($F_{(4,389)} = 18.78$; $P < 0.001$)

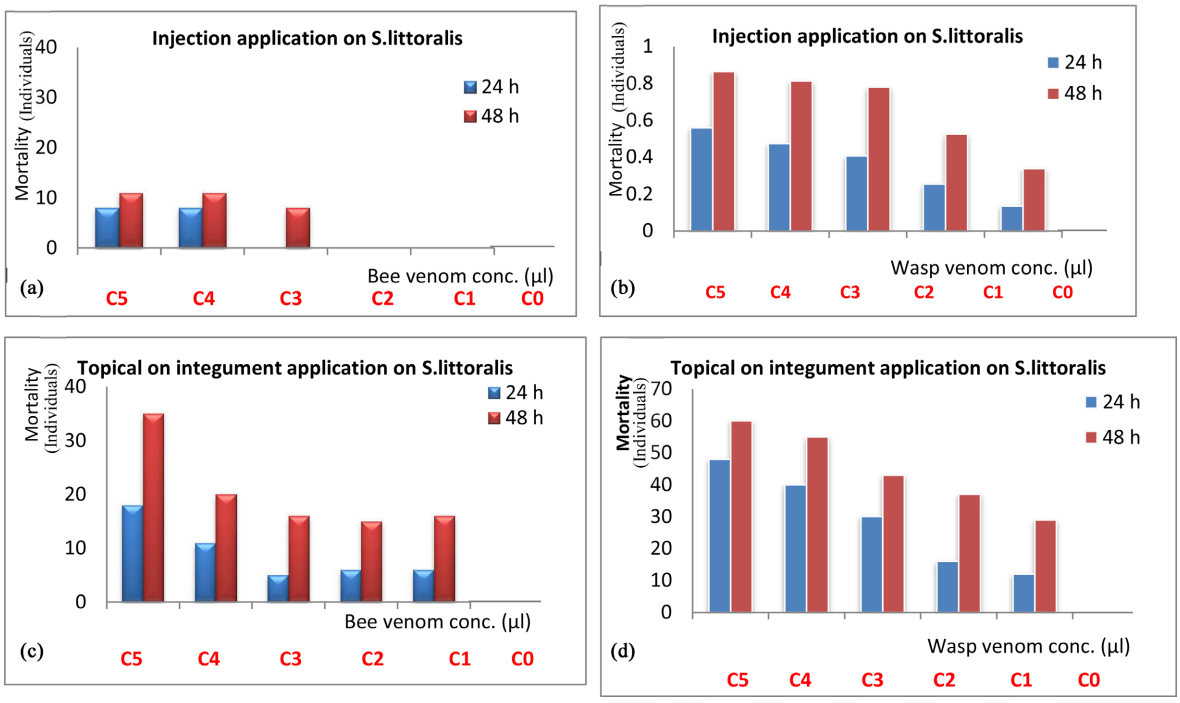

Figure 5. Difference in mortality % of cotton worm *Spodoptera littoralis* on different concentrations application methods of wasp venom *Polistes* sp. and *A. mellifera* (mean value ± S.E.; $P \leq 0.05$).

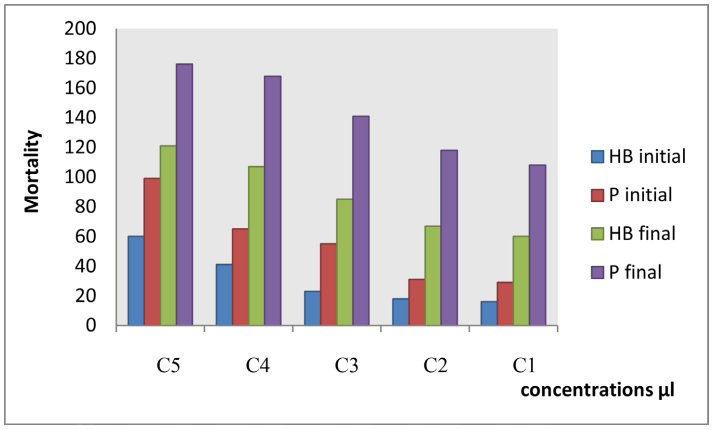

Figure 6. Total number of mortality of *S. littoralis* treated by HB and *P. dominulus* venoms with different concentrations of different application methods at initial and final time interval.

($F_{(4,389)} = 12.46$; $P < 0.0001$) as compared to other venom application (Figure 7).

All treatments caused significant increases in total mortality and morphological changes during the larval until pupal stage and the sequential combined effect treatments had more decreasing effect than the individual treatments. According to the obtained result, bee and wasp venom could be considered as a biopesticide, and become more effective when used in sequential treatment (Table 2 and Figure 6).

Furthermore, we demonstrate that, despite significant differences in their venom most venom have strikingly similar insecticidal effectiveness. The fact that similar social bee and wasp venoms are not acquiring the same mechanism of

action on lepidopteran insects suggests from results that bee venom toxins have a conserved mode of action. However, the precise role of their toxins identified as social insects still remains to be determined within our study.

3.4. Susceptibility of the Fourth Instar Larvae of *Spodoptera littoralis* to Different Tested Insecticides

Wasp venom applied on *S. littoralis* has the largest effect with (χ^2 = 53.10; d.f. = 2; P < 0.0001 for topical on food application and χ^2 = 12.18; d.f. = 2; P < 0.002 for integument application) which is higher than bee venom mortality and toxicological effects (Figure 7 and Figure 8).

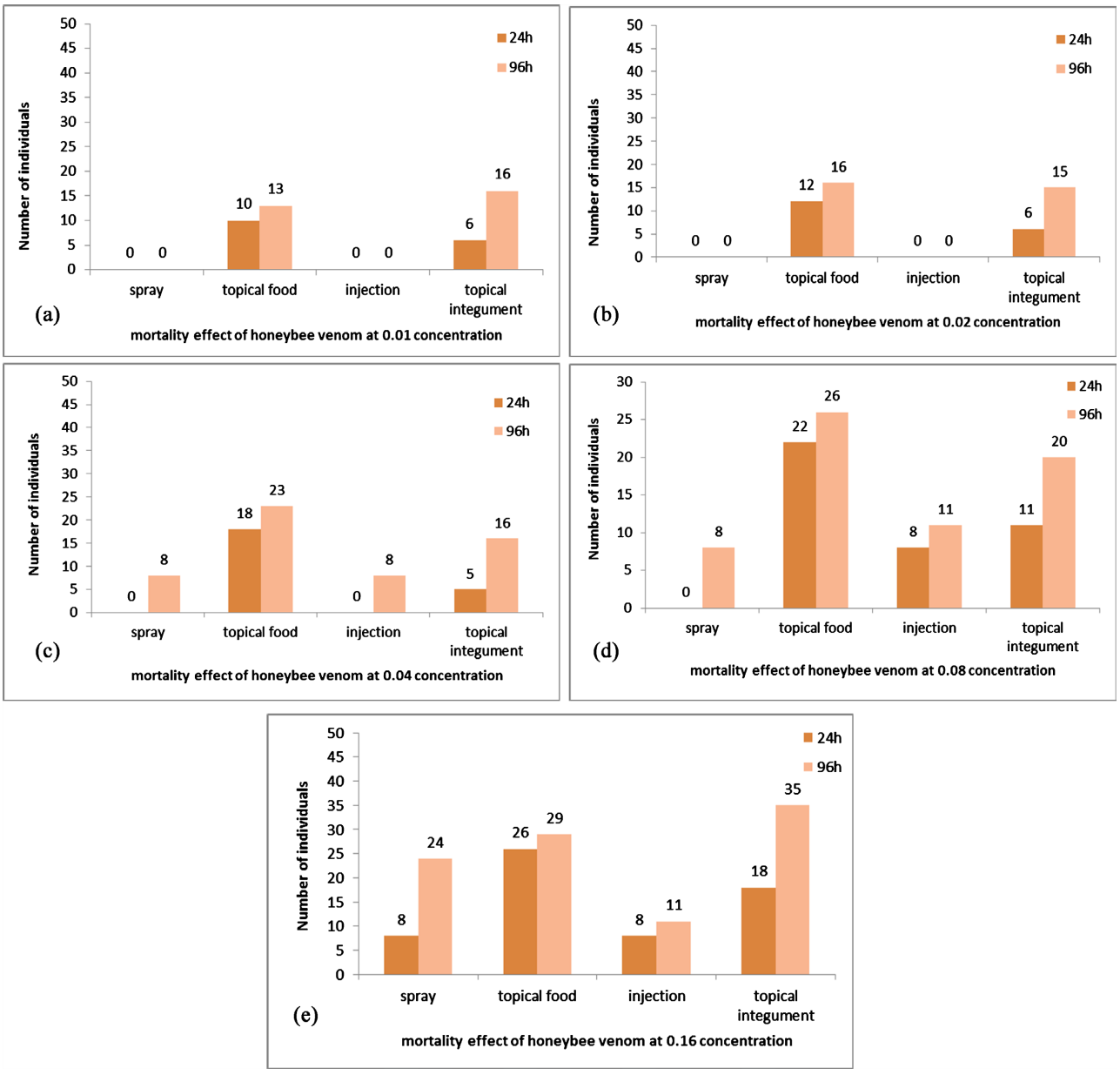

Figure 7. Difference between toxicity % of cotton worm *Spodoptera littoralis* on different concentrations application methods of honey bee venom *A. mellifera* (mean value ± S.E.; P ≤ 0.05).

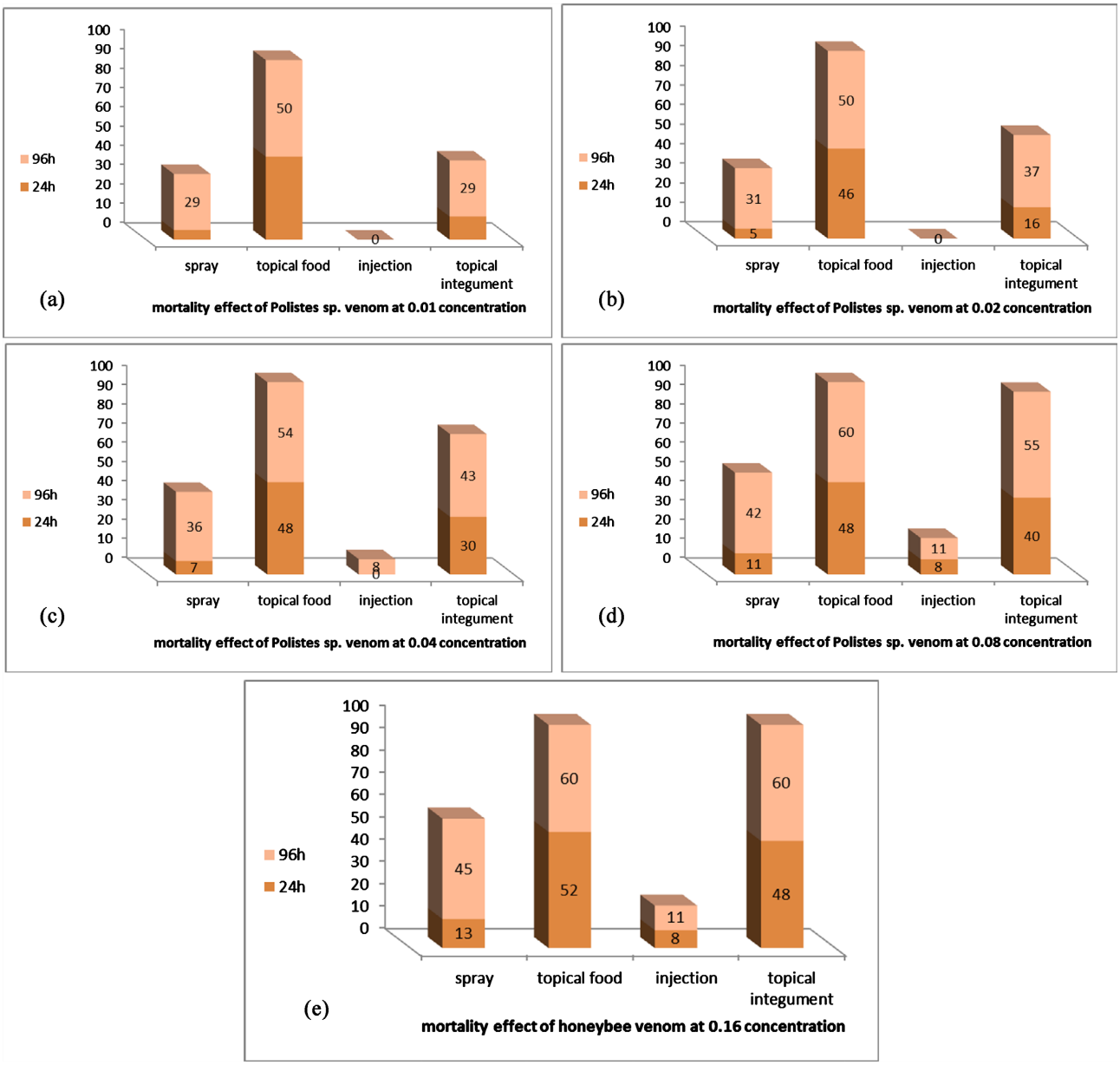

Figure 8. Difference between toxicity % of cotton worm *Spodoptera littoralis* on different concentrations application methods of wasp venom *Polistes* sp. (mean value ± S.E.; $P \leq 0.05$).

All treatments caused significant increases in total mortality and morphological changes. According to the obtained result, bee and wasp venom could be considered as a biopesticide, and become more effective when used in sequential treatment.

4. Discussion

The 4th instar larvae of *Spodoptera littoralis* were used in the current study for investigating the significant variations within the toxic biological effects of social bee and wasp venoms. The 4th instar larvae are considered as the most susceptible stage for toxic affection and when the host is at the active stage, e.g. larval/nymphal

stage [28].

Based on LC_{50} values, both venom compounds caused considerable toxic effects against the 4th instar larvae of *Spodoptera littoralis*. Wasp venom particularly had shown higher drastic toxic effects compared to bee venom toxicity. This finding agrees with [29] which demonstrated that honeybee venom-reactive proteins had weak cross toxic reactions compared with yellow jacket venom due to strong IgE binding significantly. This significant variation may be due to the presence of Melittin and apamin which are found in the genus *Apis*. However, is found in more genera, such as *Vespa*, *Parapolybia*, *Protonectarina*, and *Polistes* [30].

Great toxic biological effects were found to be on the development, mortality, and morphology of the larvae treated with a combination of the median lethal concentrations. Few numbers of larvae remained alive and reached the 4th larval were small size with slow motion cleared symptoms of death, while the control untreated larvae reached 6th larval stage. This may be due to the fact that venoms of both bees and wasps are being used as defensive agents against predators, competitors, and pathogens. These venoms are particularly rich in neurotoxic, cytolytic and antimicrobial peptides that fulfill key roles in capture and conservation of praise, defense against competitors [31] that agree with [32] that indicates the insecticidal activity of ectoparasitoid wasp on the cotton boll worm.

Aiming at underlying the potential of venom approaches for pharmaceutical discovery different application types for social bee and wasp venom were significantly ($\chi^2 = 239.47$, d.f. = 3, $P \leq 0.0001$) compared within the study. Venom application by injection had no toxic effects on larvae. However, it has the ability to cause a high malformation percentagein *Spodoptera littoralis*.

The concentration which causes 50% of adult mortality up to 70% resulted from the toxicity of honey bee venom components to *S. littoralis* larvae is due to the production of crystalline endotoxic protein. On digestion by susceptible larvae, the active toxin generated from the protoxin binds to the receptors on the gut epithelium. This leads to paralysis of gut and mouth parts causing the death of larvae [33]. These effects comprise destruction of epithelial cells; microvilli and the peritrophic membrane were curled and ruptured than those of control treatment. The current studies cleared the presence of liquid swelling within insect integument that can be resulted from the mixing of the gut contents with the haemolymph caused the larval mortality.

On the other hand, morphological malformations occurred to cotton worm larvae during their development may be resulted from both bee and wasp venom may be due to a potent chitin synthesis inhibitor classified as an insect growth regulator, inhibits the synthesis of chitin in larvae that have ingested it, causing the integument to become fragile, and leading to mortality during the moulting [34]. This finding agrees with [35] study on the cytotoxicity [36] of venom from *P. hypochondriaca*. Different cells of the mid-gut exhibited a swelling, appearance and microvilli showed complete disorders in many areas, increasing in goblet cells secretion with rupture of basement membrane, many vacuolations occurred in the cell cytoplasm. This can be expressed by pest response to venom

injection that is often accompanied by melanization, a cascade of proteolytic reactions leading to the deposition of melanin and production of phenolic intermediates [37] that agree with current study results. This may occur due to the enhanced rate of absorption [38], the swollen and elongated protruded villi into its lumen as a bulbous version was a result of enzymatic activity of the epithelial cells [39]. These results are in agreement with findings of [40] [41] and [42] studies on venom proteins from *A. ervi* [17] cause castration by causing apoptosis and subsequent tissue degradation [43] [44].

Thus social bee and wasp venom may find applications as biocontrol agents against insect pests especially cotton worms that agree with [45]. This might explain the high malformation and mortality percentage in *S. littoralis* treated with social bee and wasp venoms obtained in the present work that cope with [46] where the venom of *A. ervi* induces the castration of the pea aphid *Acyrthosiphon pisum*. Our study findings have important implications for future applications that would aim at using crude venom or other venom bioactive proteins to efficiently control cotton worm populations.

A correlation was observed between changes in morphology and mortality of cotton worms treated with both social bee and wasp venoms. From these data, it seems that the wasp venom interferes with the activity of chitin synthesis in the cuticle in such a way that the daily rate of chitin deposition and its growth are reduced or retarded. Vinson *et al.* [47] recorded that chitin deposition was inhibited in locusts that fed on Dimilin which resulted in wrecking the cuticle and thus its rigidity was reduced to half of that of the normal cuticle. While honey bee venom interferes more with a high toxic effect on larval mortality which agree with their defence mechanism of action as bees depend on killing their insect pest than depending on their paralysis as wasps [11] [48].

Thus, strains promise to fill an important gap in current insect-management programs. This should open the way for cost-effective bio-control of a range of insect pests of agricultural and medical importance. Produce a cheap, safe, and green tool for the control of insect pests including cotton worms, which, in contrast to most chemical insecticides, will not eventually be rendered useless by the evolution of resistance.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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