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Evaluation of *Ipomoea carnea* (jacq.) extracts and chlorpyrifos insecticide against the cotton leafworms, *Spodoptera littoralis* (Boisd.)

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Abstract

The cotton leafworm, *Spodoptera littoralis* (Boisd.), is an insect that causes serious damages to more than 112 plant species belonging to 44 different families. Botanical extracts of *Ipomoea carnea* were very efficient against fourth instar larvae of *Spodoptera littoralis*. Based on LC_{50} and LC_{90} values, *Ipomoea carnea* acetone extracts were recorded 24.622 and 164.947 ppm, respectively. While hexane extracts were 232.677 and 15,377.590 ppm, respectively. Also, the treatment of fourth instar larva of *S. littoralis* with chlorpyrifos insecticide caused 9.497 and 91.126 ppm, respectively. The chemical constituents of acetone extract of *I. carnea* by using GC–MS analysis resulted in the most active compounds that were palmatic (iso propyl-hexadecanoate), silane, [[(3.alpha.,5.alpha.,20R)-pregnane-3,20-diyl]bis(oxy)] bis (trimethyl-(cas)5. and pederone that recorded (44.025%, 11.455% and 9.325%, respectively). *Ipomoea carnea* extracts were produced with different deformation abnormalities of all *S. littoralis* stages.

Keywords: *Schistocerca gregaria, Ipomoea carnea*, botanical extracts, bioassay.

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1. Introduction

The cotton leafworm, *Spodoptera littoralis*, causes serious damages to the cotton crop and the decreasing cultivation of cotton, the pest damage is more likely to be seen on vegetable crops. Recently, this pest attack a wild variety of vegetables, field crops, fruit orchards and ornamental plants (El-Massry, Ghamry, Hegab & Hassan, 1998; El-Okda, 1980; Ghamry, El-Deeb & Kokab,1993; Miller, Swails, Olsan & Staten, 1988; Salama, Nassar, Taha & Hala, 2014).

Mankind has used plant parts or extracts to control insects since ancient times. Higher plants are a rich source of novel natural substances that can be used to develop environmental safe methods for insect control (Jbilou, Ennabili, Abdeslam & Sayah, 2006). Insecticidal activity of many plants against several insect pests has been demonstrated (Carlini & Grossi, 2002; El-Shazly, Nassar & El-Sherief, 1996; Isman, 2000; Jilani & Su, 1983; Nassar, Hafez, Nagaty & Khalaf, 1999). The deleterious effects of plant extracts or pure compounds on insects can be manifested in several manners including toxicity, repellent, anti-feedant growth inhibitor, suppression of reproductive behaviour, reduction of fecundity and fertility (Euturk, 2004; Isman, 2000; Koul, 2004; Mordue, 2004; Nassar, 2000; Negahban & Moharramipour, 2007; Weaver & Subramanyam, 2000).

The plant *Ipomoea carnea* belongs to family Convolvulaceae, it was used in ancient system of medicine in many countries but not to great extent. The fact is that the plant had immense potential as an anti-inflammatory activity, antioxidant activity, antidiabetic activity, antimicrobial activity, wound healing activity, Immunomodulatory activity, cardiovascular activity, embryo toxic effect, antifungal activity, hepatoprotective activity, inhibition activity and anxiolytic properties (Sharma & Bachheti, 2013).

The most important of these constituents are alkaloids, terpenoids, steroids, phenols, saponins and tannins chemicals and there may be an alternative source of insect control agents (Wink, 1993). Pest control strategies, especially those that are effective, cheap and environmentally non-hazardous are needed. Hence, crude plant extracts have played an important role in this aspect (Mahadevan, 1982).

Thus, the present study was planned to experimentally assess the effect of *I. carnea* extracts and chlorpyrifos insecticides against fourth instar larvae of *S. littoralis*. Also, it was undertaken to know the chemical constituents of the acetone extract of *I. carnea* using GC-mass and its effect on deformation abnormalities of different stages of *S. littoralis*.

2. Materials and methods

2.1. Plant extracts of Ipomoea carnea

Plant has been defined according to the Research Department and Classification of Plant Flora, Institute of Horticultural Research Center, Cairo, Egypt as *Ipomoea carnea* (Jacq).subsp. Fistulosa from the Convolvulaceae family.

Fresh aerial part of *I. carnea* was collected from local gardens of Abu Hammad sharkia, Egypt during December 2012.The freshly collected aerial part was spread to dry at normal room temperature (28°C ± 2°C) in the shade. Upon drying, the aerial part was pounded using mortar and pestle into smaller particles and the powder was blended and then subjected to successive extraction using hexane, acetone and ethanol organic solvents according to the method of Nassar (2000). Extracts were filtrated and then evaporated to dryness using rotary evaporator (Model 349/2, Corning Limited) maintained at 40°C and the dried substance was stored in refrigerator until further use.

2.2. Insecticide

Chlorpyrifos (48% EC) was obtained from Plant Protection Research Institute, Dokki, Giza.

2.3. Rearing technique of Spodoptera littoralis

Spodoptera littoralis strain used in this study is a laboratory susceptible strain reared in the Plant Protection Research Institute, Zagazig, Sharkia Governorate. The culture was maintained under

optimum condition (27°C ± 2°C and 70% ± 5% RH) and reared on fresh castor bean leaves until the fourth larval instar which was used in this study according to El-Defrawi, Tappozada, Mansour & Zaid (1964) with some modifications.

2.4. Bioassay

Chlorpyrifos insecticide and plant extracts of *Ipomoea carnea* were assessed against fourth instar larvae. Serial successive concentrations of each extract were used 3%, 5% and 8% (w/v) and chlorpyrifos concentration was prepared using distilled water. Disks (9 cm diameter) of caster bean leaves were dipped in the tested concentration for 10 seconds, left to dry and offered to larvae, which starved for 4–6 hours before treatment (Merdan, 1968). Larvae were placed into glass jars and each treatment was replicated five times (10 larvae per each). Control disks were dipped in distilled water only. The larvae were allowed to feed on treated disk for 48 hours. Mortality percentages recorded after 24 hours for Chlorpyrifos and after 72 hours for all tested plant extracts according to (Anonymous, 2013). Mortality was corrected according to Abbott's (1925) formula. The dosages mortality regression lines were statically analysed by probit analysis (Finney, 1971). Toxicity index was calculated according to Sun (1950) equations:

$$\text{Toxicity index} = \frac{LC_{50} \text{ or } LC_{90} \text{ of the efficient compound}}{LC_{50} \text{ or } LC_{90} \text{ of the other compound}} \times 100$$

2.5. GC–MS analysis

Depending on LC_{50} values, the acetone extract of the plant was used for GC–MS analysis depending on LC_{50} values in the Regional Center for Mycology and Biotechnology, Al-Azhar University.

2.6. Statistical analysis

The significance of the main effects was determined by analysis of variance. The significance of various treatments was evaluated by Duncan's multiple range test ($p < 0.05$) (Snedecor & Cochran, 1980). Data were subjected to statistical analyses using a software package CoStat® Statistical Software (2005), a product of Cohort Software, Monterey, California.

2.7. Morphogenic abnormalities

Due to the effect of *Ipomoea carnea,* some deformation was obtained which was counted and photographed.

3. Results

3.1. Effects of *I. carnea extracts on fourth larval instar of Spodoptera littoralis*

Different extracts of *I. carnea* were assessed against fourth instar larvae of *S. littoralis* after 72 hours post treatment. The results were compared to the obtained for recommended organophosphorus (Chlorpyrifos) as reference standard after 24 hours as shown in Table 1.

Table 1. Susceptibility of the 4th instar larvae of *S. littoralis* to *Ipomoea carnea* extracts and chlorpyrifos

Compounds	LC_{50} ppm; Fiducial limits (Lower–Upper)	LC_{90} ppm; Fiducial limits (Lower–Upper)	Slop	Toxicity index
Chlorpyrifos	9.497 ppm (7.806–11.391)	91.126 ppm (69.41–137.493)	1.305	100
Acetone extract of *I. carnea*	24.622 ppm (13.659–37.158)	164.947 ppm (121.52–205.76)	1.552	38.571
Hexane extract of *I. carnea*	232.677 ppm (154.125–582.430)	15,377.590 ppm (12,487.08–20,419.20)	0.454	4.082

According to LC_{50} and LC_{90} values, Chlorpyrifos was the most effective that recorded 9.497 and 91.126 ppm, respectively, followed by acetone extract (24.622 and 164.947 ppm) and finally, hexane extract (232.677 and 15,377.590 ppm), respectively. Meanwhile, the ethyl alcohol extract of *I. carnea* which doesn't reveal any toxic effect until 5 days post treatment.

3.2. Chemical constituents of the acetonic extract of I. carnea

Data in Table 2 and Figure 1 showed the chemical composition of the acetone extract of the aerial parts of *I. carnea* as analysed using GC–Ms. The major constitution of *I. carnea* was isopropyl hexadecanoate (Palmatic) (44.025%) at retention time 30.967 minutes, followed descendingly by (Silan) [[(3 alpha.,5. alpha.,20R)-pregnane-3,20-diyl]bis(oxy)] [trimethyl] (11.455%) at 33.001 minutes, pederone (9.325%) at 26.438 minutes, 2-benzyl-imidazoline (Tolazoline) (8.375%) at 20.777 minutes, Iso-octyl phthalate (7.725%) at 39.257 minutes, (3 beta) lanostane-7,11-dione, 3-(acetyloxy)—cyclic 7-(1,2-ethanediyl mercaptole) (5.025%) at 41.184 minutes, Bistrimethylsilyl 3 beta., 11. Dihydroxy-androst-5 ene-17-one methoxime (4.815%) at 38,117 minutes, Cyclododecane (3.795) at 27.439 minutes, Octadecane,2,6,10,14-tetramethyl-(CAS)2,6,10,14-tetramethylloctadecane at (3.445%) at 25.280 minutes and finally, 3B, 7a, 12 B trihydroxy-5B chlolan-24-oic acid methyl ester (2.015) at 34.814 minutes.

Table 2. GC–MS data of the acetonic extract *Ipomoea carnea* compounds

Peak no.	Name of compound	Retention time (Minute)	Area (%)
1	2-benzyl-imidazoline (Tolazoline)	20.777	8.375
2	Octadecane,2,6,10,14-tetramethyl-(CAS) 2,6,10,14-tetramethylloctadecane	25.280	3.445
3	Pederone	26.438	9.325
4	Cyclododecanone	27.439	3.795
5	Palmatic (iso propyl—hexadecanoate)	30.967	44.025
6	(Silan) [[(3 alpha., 5. alpha., 20 R) pregnane-3, 20-diyl]bis(oxy)] [trimethyl]	33.001	11.455
7	3B,7α,12B trihydroxy-5B cholom-24-oic acid methyl ester	34.814	2.015
8	Bistrimethylsilyl 3.beta.,11.Dihydroxy-androst-5-ene-17-one methoxime	38.117	4.815
9	Iso—octyl phthalate	39.257	7.725
10	Lanostane-7,11-dione,3-(acetyloxy)-,cyclic7-(1,2-ethanediyl mercaptole), (3.beta.)	41.184	5.025

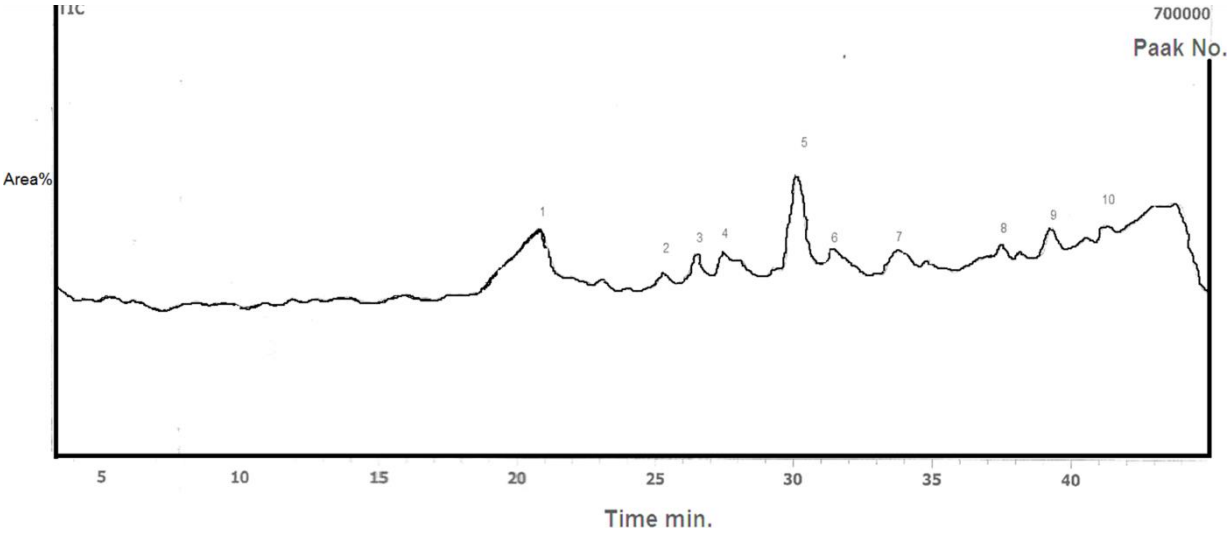

Figure 1. GC–MS for acetonic extract of *Ipomea carnea*

3.3. Deformations of larvae, pupae and adult malformations

Deformations of larvae, pupae and malformations of adult resulted from fourth instar larvae of *S. littoralis* fed on caster bean oil leaves treated with acetone extract of *I. carnea* at two tested concentrations LC_{25} and LC_{50} and chlorpyrifos at LC_{50}. Deformations and malformations were recorded based on the external morphological characters and illustrated in Figures 2–6.

The larval deformations represented in Table 3 were recorded 10% and 16% for acetone extract of *I. carnea* at LC_{25} and LC_{50}, respectively, while chlorpyrifos and controls larvae did not show any larval deformations. The pupal deformation was manifested (4, 6 and 0) for *I. carnea* at LC_{25}, LC_{50} and LC_{50} of chlorpyrifos, respectively. While adult malformations gave (12.00%, 10% and 0%) for *I. carnea* at LC_{25} and LC_{50} and chlorpyrifos (LC_{25}), respectively. Controls did not cause any morphogenic effects (Table 3).

Table 3. Deformation and malformation percentages of *S. littoralis* after treating fourth instar treated with the tested treatments

Treatment	Larval deformation (%)	Pupal deformation (%)	Adult malformation (%)
LC_{25} of *I. carnea*	10.00	4.00	12.00
LC_{50} of *I. carnea*	16.00	6.00	10.00
$LC_{50}$of chlorpyrifos	0.00	0.00	0.00
(-ve) control	0.00	0.00	0.00
(+ve) control	0.00	0.00	0.00

The different types of deformations include:

1. Larvae is being shrinked (dwarf) (Figure 2)
2. Prepupae could not be able to discard the old cuticle (Figure 2).
3. Larval-pupal intermediates (that closes in shape to larva features than pupa) and contains:
   - Larval with small part of pupal cuticle (Figure 3A).
   - Larvae with pupal head (Figure 3B).
   - Larvae mouth parts attached to pupal cuticle (Figure 3C).

4. Pupal-larval intermediates: which have the external characters of pupa than larva that include:
   - Pupae with larval head and thoracic legs (Figure 4A).
   - Pupae with larval head (Figure 4B).
5. Partial emergence of adults (adults adhered to the pupal exuvium) (Figure 5A–C).

*Finally, adults with malformed wings ranged from slight, moderate and severe as shown in Figure 6A–C, respectively.

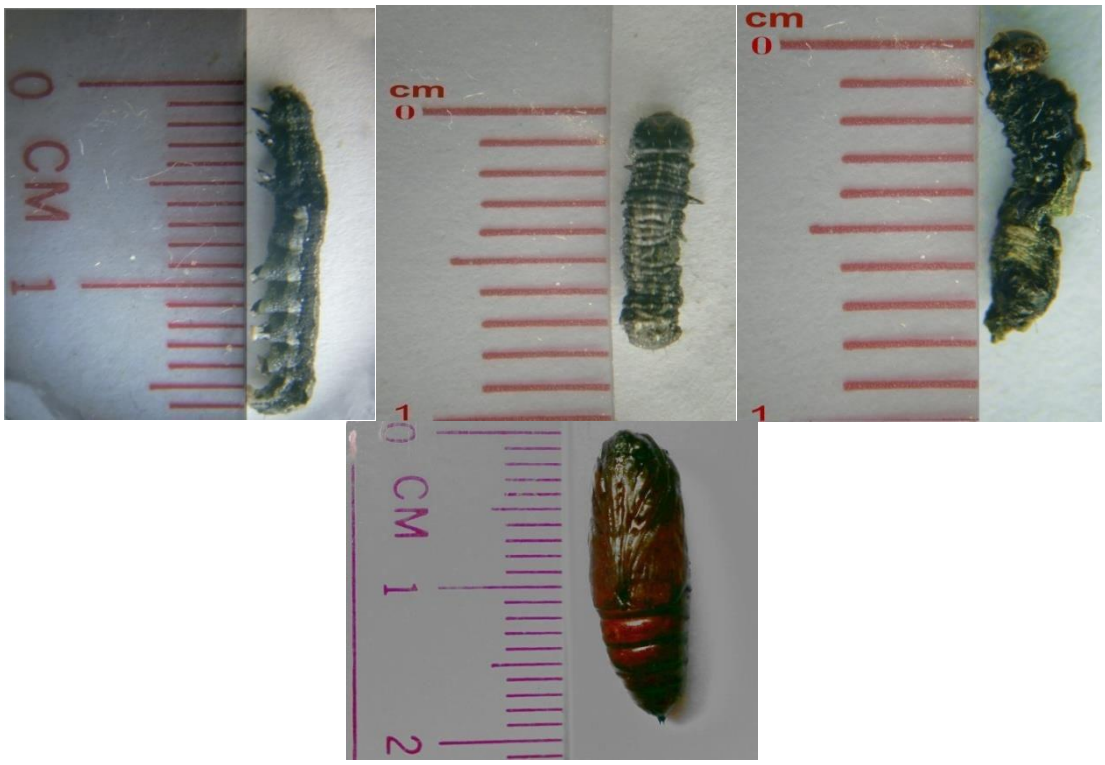

Figure 2.

Figure 3. Larval-pupal intermediates

Figure 4. Pupal-larval intermediates

Figure 5. Adults adhering to pupal exuvium

Figure 6. Adult malformations

4. Discussion

4.1. Susceptibility activities of Ipomoea carnea *extracts and chlorpyrifos insecticide against* Spodoptera littoralis

A wide variety of plant extracts were investigated against *Spodoptera littoralis* (Guriguis, Gouhar, Watson & Salama, 1989; Hafez, 2001; Nugroho et al., 1997). Most reports advised the use of plant extracts as a way of controlling *S. littoralis.* Additionally, the tested plant *I. carnea* had antioxidant, antibacterial and antifungal activities (Kumar et al., 2012). Therefore, dual benefit of using *I. carnea* was achieved as they can be used as *S. littoralis* agents as well as against other organisms causing plant diseases.

Based on LC_{50} values, the type of solvent used for extraction seemed to be considerably the effect of *I. carnea* and the same finding was obtained by Zidan, Gomaa, Afifi, Fam and Ahmed (1993). The acetone extract was more effective than hexane and ethyl alcoholic extracts. This result is in agreement with that of Hafez (2001) when testing the acetone and petroleum ether extracts of *Iberis amara* seeds and *Antholyza aehtiopica* leaves against first and fourth instar larvae of *S. littoralis.* Also, Gaur, Kori, Tyagi, Sharma and Tripathi (2009) and Khatiwora, Adsula, Torane, Deshpande and Ashalkar

(2011) found that acetone extract of *I. Carnea* showed higher antioxidant activity than ethyl acetate and methanol extract.

In the present investigation, chlorpyrifos was tested against fourth instar larvae of *S. littoralis* as a reference standard for the activity of plant extracts. The highly toxic effect of chlorpyrifos suggests that the insecticide is effective similarly recorded by Gaaboub, Halawa and Rabiha (2012) and Osman, Fetoh and Mohammad (2012) where they found that chlorpyrifos proved as the most effective insecticide against fourth instar larvae of *S. littoralis* comparing to other insecticides and plant extracts.

4.2. Chemical constituents of the acetonic extract of I. carnea

Several authors demonstrated that, the plant *I. carnea* had immense potential as an antioxidant, anti-inflammatory, anti-microbial, antifungal and inhibition activities (Khatiwora, Adsul, Kulkarni, Deshpande & Ashalker, 2013; Kumar et al., 2012; Sharma & Bachheti, 2013). The primary chemicals identified from acetone extract of the aerial part of *I. carnea* were the major compound isopropyl hexadecanonate (Palmatic acid) (44.025%) and the steroid compound (Silane) [[(3 alpha., 5. alpha., 20 R) pregnane-3,20-diyl]bis(oxy)] [trimethyl] (11.455%). The result of our analysis are in agreement with the literature of Sharma and Bachheti (2013) that reported palmatic acid is a major chemical constituent in *I. carnea* and may be responsible for various pharmacological medicinal properties. Also, the steroidal compound cholestan-3-one identified from both benzene and chloroform extracts of *I. carnea* and it has a high insecticidal property (Sahayaraj & Ravi, 2008). Furthermore, iso-octyl phthalate (7.725%) a characteristic compound in the acetone extract of *I. carnea* was found to possess antimicrobial as well as potent larvicidal activity, so its derivative might be bioactive (Khatiwora et al., 2013; Sharma & Bachheti, 2013).

In our result, the preliminary phytochemical analysis of *I. carnea* showed the presence of steroids and ketones in acetonic extract while Smita and patil (2014) found that preliminary phytochemical screening revealed the presence of tannins, coumarins, alkaloids and glycosides in the ethanolic extract of *I. carnea*, while the chloroform fraction revealed the presence of sterols and flavonoids. The variations of chemical composition of *I. carnea* extracts may be attributed mainly to the plant part, polarity of solvent and method of analysis (DeBalogh et al., 1990; Krishnamoorthy, Nattuthurai & Dhaslima Nasreen, 2014; Smita and Patil, 2014).

4.3. Deformations of larvae, pupae and adult malformations

Forty-eight hours of feeding larvae of *Spodoptera littoralis* on castor bean oil leaves treated with two different concentrations of *I. carnea* (LC_{25} and LC_{50}) resulted larvae that did not able to complete the molting process and subsequently died. Eid et al. (1992) reported that the injection of *S. littoralis* larvae in laboratory with sub-lethal doses of *lamina minor* extract caused malformation in subsequent life stages. Additionally, Antonious and Rizk (1994) found that feeding inhibition in the growth and development of fifth instar of *S. littoralis* using neem seeds oil as measured by a clear reduction in larval gain weight and accordingly leading to a very poor relatively growth rate (dwarf larvae). Some larvae were highly impaired at ecdysis as exhibited deformations in formed pupae. Also, Marei et al. (2009) found obvious physiological changes on estimation of metabolic parameters, where there was a significant reduction in the efficiency of larvae to convert digested food into body tissues; hence, a reduction in total body weight gain of treated individuals (dwarf larvae). Deformations and malformations take place in the different stages of *S. littoralis* exposed during larval stage to the different plant extracts were also reported by some authors. Dimetry et al. (1998) and Abd El-Aziz and Ezz El-Din (1999) observed malformations of the resulting pupae as affected by petroleum ether of neem fruits *C. propheta* extract. Also, Khedr (2002) showed malformations of resulting larvae, pupae and adults after treating fourth instar larvae of *S. littoralis* with three plant extracts Neem Azal, soybean and Biorepel (garlic extract).

The reason of deformations and malformations may be a result of reduction in protein, transaminase enzymes, carbohydrate hydrolysing enzymes and lipid. The amino transferases, especially GPT is one of the components of oxidative metabolism of protein which in certain insect is utilised during the initial periods of flights (Bursell, 1963). In addition, trehalase has the important function for liberating glucose for chitin build (Ishaaya & Ascher, 1971; Meisner et al., 1978). Moreover,

Downer (1978) reported that the sufficient lipids is present in the thoracic musculature energy for extended period of flight and include important hormones and pheromones. Smagghe and Degheele (1992) suggested that the lack of haemolymph proteins as a cause of unsuccessful pupation.

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