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Biological activities of *prosopis juliflora* and *zilla spinosa* extracts against *spodoptera littoralis* (boisd.)

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The HPLC-fingerprint of aqueous methanol (80%) extracts of *Prosopis julifera* and *Zilla spinosa* were performed. The biological activities of both extracts and their fractions were studied against the cotton leafworm, *Spodoptera littoralis*. Both *P. juliflora* and *Z. spinosa* extracts possess behavioral effects and post-ingestive toxicity on the developmental stages of tested insect. The total extract (Methanol) of *P. juliflora* gave the highest larval mortality (93.8%) followed by Ethyle acetate (75.0%). The highest pupal mortality recorded for the *Z. spinosa* Methanol, Hexane and Ethyle acetate extracts was 15 %, 5 % and 5% respectively. Both plant extracts had latent biological effects on percentage of adult emergency treated previously as larvae it was recorded (5%) in the case of Methanol and ethyl acetate, respectively. While adult survival reached 80 % in control. Data indicated the superior ovicidal activity of *Prosopis* extract fractions on the viability of egg masses of *S. littoralis* aged 24 hrs than *Zilla* extracts. *Z. spinosa* tested extracts stronger deterrent activity than *P. juliflora* extracts against *S. littoralis* larvae. The superior extract as a deterrent was Methylene chloride of Zilla (97.2%). *Prosopis* extracts gave considerable protection from *S. littoralis* attack and can be used in the Integrated Pest Management Program.

Keywords: HPLC, biological activities, ovicidal activity, antifeedant, cotton leafworm

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Biosd.) Lepidoptera: Noctuidae has been known as the major economic pest of many fields, vegetables and ornamental crops in Egypt (Dahi, 2005; Amin, 2007 and Ismail, 2014). These caterpillars are noxious polyphagous, giving rise to important economic losses in both greenhouses and open field (Alford, 2000 and Ghoneim et al., 2015). Abundant problems are correlating with the use of synthetic insecticides, in addition to the disturbance, of ecological balance, insect resistance and increased pollution hazards. Therefore, the use of natural pest control agents, especially those derived from plants, may offer better promise in cotton leaf worm control (Abd El-Aziz and Sharaby, 1997; Abd El-Aziz and Ezz El-Din, 2007; Abd El-Aziz et al., 2007; Vanichpakorn et al., 2010; Abd El-Aziz et al., 2015).

Honey mesquite *P. juliflora*, (Leguminosae: Mimosaceae), secondary metabolites investigated. Tannins, flavonoids, saponins, and alkaloids are isolated (Ibrahim, et al*.,* 2013; Vedak and Raut, 2014). Also, *P. juliflora* methanolic crude extract of green leaves succeeded in inhibiting the growth of all tested bacteria

(Raghavendra et al*.,* 2009). Moreover, *P. juliflora* provides antifungal activity (Deepa et al., 2013), and Anti –Tumor (Sathiya and Muthuchelian, 2011).

Zilla spinosa plant, (Brassicales: Brassicaceae) uses in the folk medicinal as a drink against kidney stone (Heneidy and Bidak, 2001) and also shows a potent insecticidal effect (Malik et al. 1983). Previous photochemical study of *Z. spinosa* led to the isolation of glucosinolates of free sinapine, progotrin, goitrin, flavonoids, triterpenes, carbohydrates and sterols (Karawya et al., 1974; El-Menshawy et al., 1980), some of which have a wide range of biological activities including antioxidant, antifungal, hepatoprotective and antiviral activities (Karawya et al*.,* 1974; El-Menshawy et al., 1980).

The effect of plant extracts on insects can be demonstrated in several manners including toxicity, antifeedant, suppression of reproductive behavior, reduction of fecundity and fertility and growth inhabitation (Rachid et al., 2006; Ladhari et al., 2013). Aqueous extracts of the leaves of *Alternanthera intermedia* and *Alternanthera sessilis* affected the development of *Plutella xylostella* in all stages of the life cycle, causing mortality in the larval or pupal stages. Treatments with *A. intermedia* and *A. sessilis* extracts caused the lowest fecundity and the number of hatched larvae Hikal et al., (2017). Larvicidal activity, antifeedant activity and some biochemical studies of garlic and lemon essential oils on *S. littorals* larvae by leaf dipping method were evaluated by Ali, et al., (2017).

The present work elucidates the High-performance liquid chromatography (HPLC) fingerprint of aqueous methanol (80%) extracts of *P. julifera* and *Z. spinosa*. The latent biological effects, antifeedant and ovicidal effects of both *P. juliflora, Z. spinosa* and their fractions experimented against the cotton leafworm *S. littoralis* to detect new natural products for controlling this economic pest through the Integrated Pest Management Program.

MATERIALS AND METHODS

1. Extraction

The aerial parts of the selected plants (*Prosopis julifera* (1.2 kg) and *Zilla spinosa* (750 gm) were powdered and extracted with aqueous methanol (80%) at room temperature. Combined extracts were evaporated *in vacuo* at 45˚C to yield circa 114.75 and 79.50 g of a dark brown residue of *P. julifera* and *Z. spinosa* respectively. Initial separation was performed by means of successive liquid-liquid extraction of the crude extract with hexane, CH_2Cl_2, EtOAc, and BuOH. The total alcoholic extract of the plants, as well as the fractions, were tested as the latent biological effects.

High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) of aqueous methanol (80%) extract from *P. julifera* and *Z. spinosa* was performed on an Agilent pump equipped with an Agilent 1200 HPLC instrument with a variable wavelength UV detector at 210 nm using analytical column YMC Pack ODS-A (250 9 4.6 mm i.d.) (Fig. 1& 2).

The mobile phase was composed of Methanol (A) and 0.05% phosphoric acid in water (B). The conditions of solvent gradient elution were 1–5% (A) in 0–10 min, 5–10% (A) in 10–15 min, 10–15% (A) in 15–20 min, 15–30% (A) in 20–25 min, 30-35% (A) in 25–30 min, 35-40% (A) in 30–35 min, 40-45% (A) in 35–40 min, 45-50% (A) in 40–50 min, 50-65% (A) in 50–60 min, 65-75% (A) in 60–70 min, 75-85% (A) in 70–80 min, 85-100% (A) in 80–90 min, 100% (A) in 90–120 min, at a flow rate of 0.7 mL/min., (Fig. 1& 2).

Insect maintenance:

A standard laboratory culture of *Spodoptera littoralis* was maintained in the laboratory of Pests and Plant Protection Dept. National Research Centre, Dokki, Giza, Egypt. *S. littoralis* was reared on castor leaves *Ricinus communis* (Malpighiales, Euphorbiaceae) at a temperature of 28 ±2°C and 65 ± 5 % R.H.

4. Latent biological effects:

Twenty-second instar larvae of *S. littoralis* were left for 24 hrs. on leaf discs of castor plant (4 cm diameter) treated with one of the tested extracts *P. juliflora* and *Z. spinosa*. The dipping technique was followed and five replicates were used for each tested extract. After feeding, the larvae were kept in clean glass jars provided with untreated leaves. Survivors were repeatedly transferred to new jars and supplied with fresh leaves every 2 days till pupation.

Daily records were taken for the percentages of larval mortality, pupal mortality, pupal malformation and pupal weight. Emergence and malformation of moths were also recorded. Pairs of moths were kept each in a glass jar (1-liter capacity) with a strip of paper for egg deposition. A piece of cotton wool soaked in a 10 % honey solution was also provided for moth feeding.

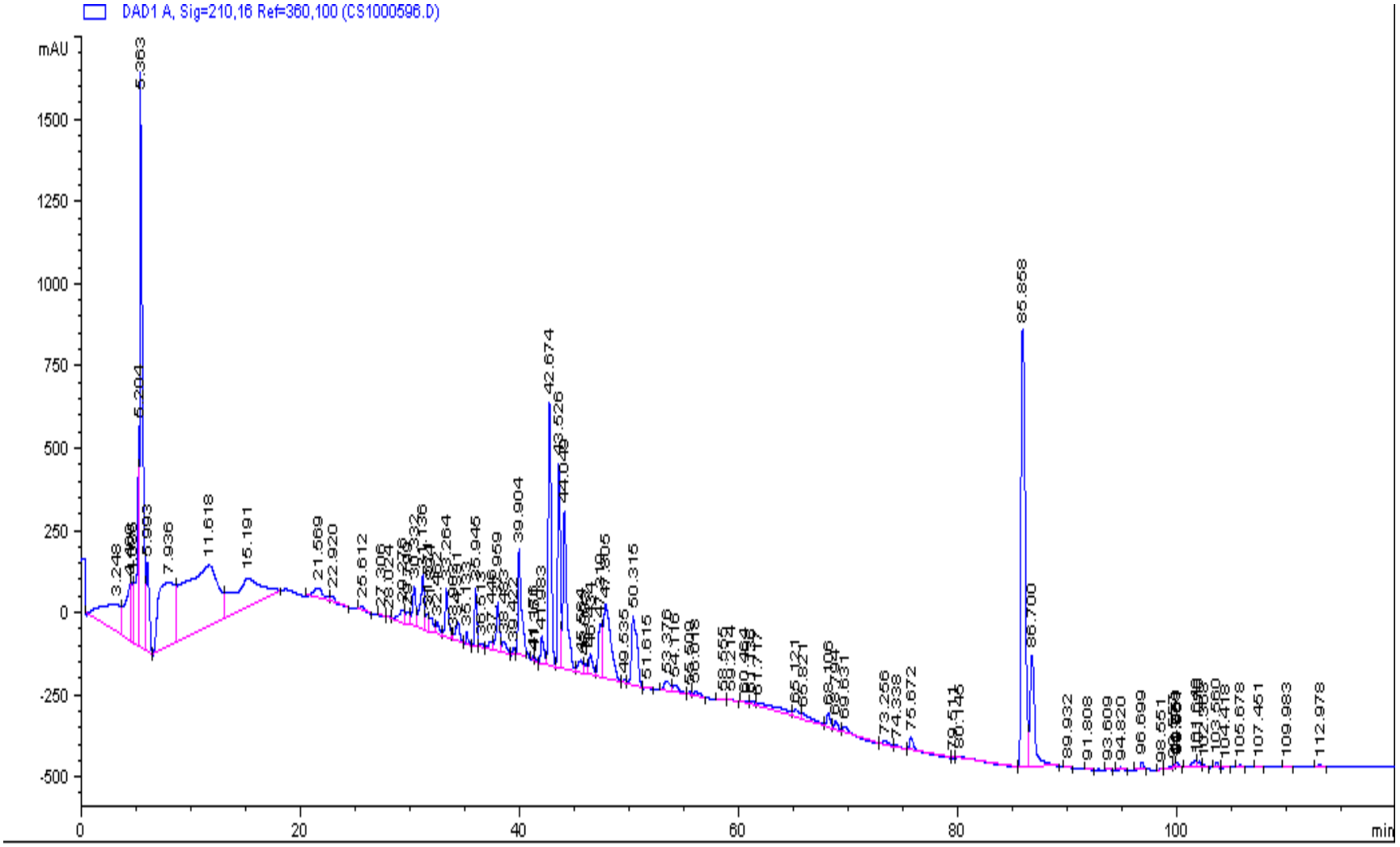

Figure 1. HPLC-fingerprint of aqueous methanol (80%) extract of *Prosopis julifera*

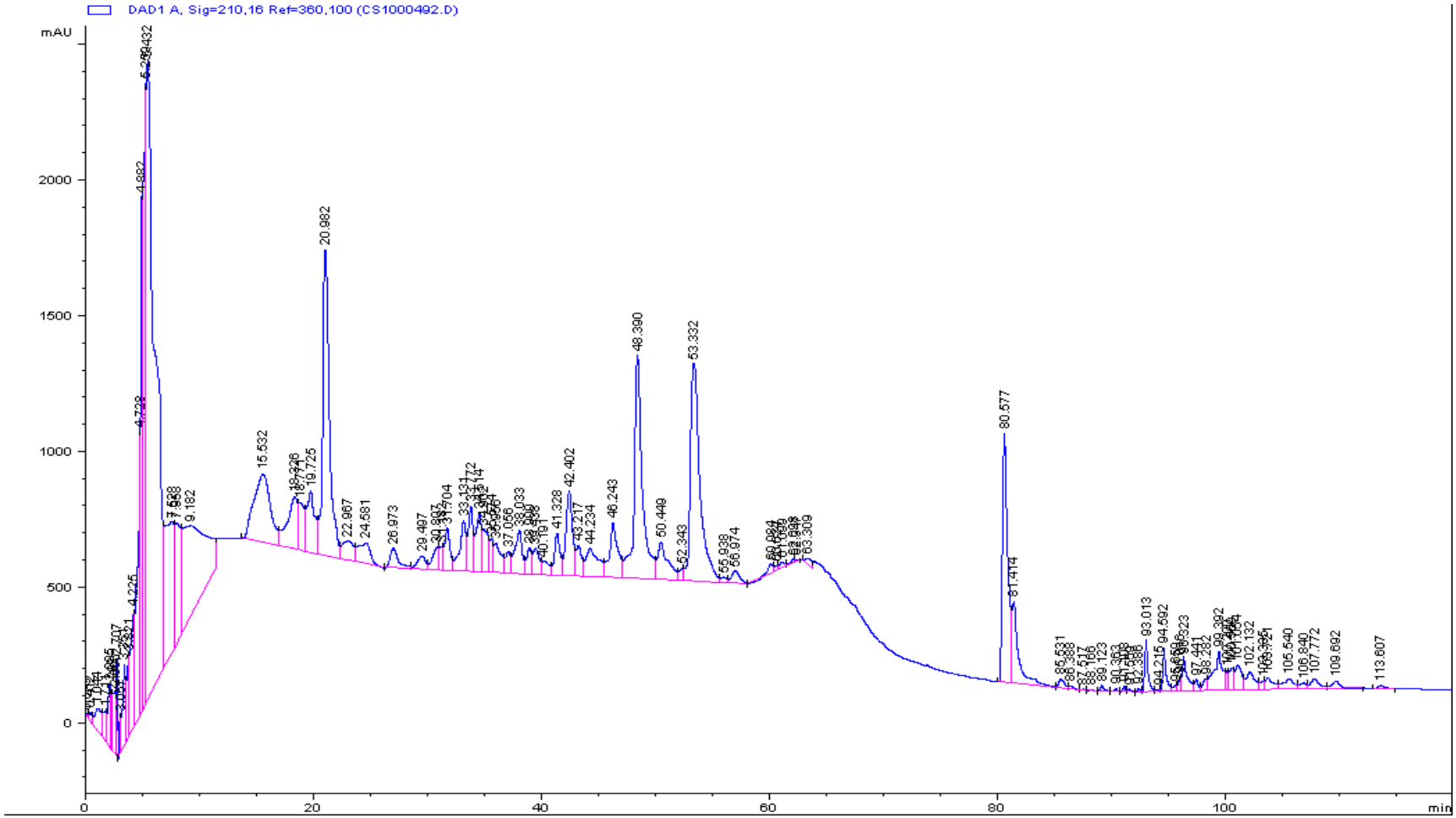

Figure 2. HPLC-fingerprint of aqueous methanol (80%) extract of *Zilla spinosa*

Records on the fecundity of moths were taken. Eggs deposited were incubated at 28 ± 2°C and the percent egg hatchability was recorded.

Ovicidal tests:

The ovicidal effects of *P. juliflora* and *Z. spinosa* plant extracts were tested against *S. littoralis* egg masses, aged 24hrs. The egg masses were dipped in extract emulsions of tested plants. Five egg masses were used and the experiment was repeated two times. Another five groups of eggs of the same ages were dipped in water and emulsifier as a check.

After dryness, the egg masses were transferred to Petri-dishes on moist filter paper to avoid desiccation throughout the experimental period and incubated at 28 ± 2°C until hatching. The incubation period and the percentage of hatchability were recorded.

Antifeedant activity (no choice test):

Third instar larvae of the tested insect were starved for 3 hrs. Each larva was provided with a leaf disc of castor oil plant (5x5 cm diameter) sprayed with one of the tested extract**s** *P. juliflora* and *Z. spinosa*. The dried treated discs were placed in Petri-dishes (15 cm diameter) on moist filter paper to avoid desiccation throughout the experimental period. After 24, 48 hrs. the feeding activity was determined by placing the leaf disc on a graph paper and counting the number of squares through the feeding holes. Twenty replicates were used for testing each extract. Untreated leaf discs (control) were sprayed with water and emulsifier.

Antifeedant activity = (C-T)/C X 100

Where's: T and C represent the mean of leaf area consumed per larva of the treated and control sets, respectively. The percentages of mortality were corrected using the Abbott formula (Abbott, 1925**).** Data were subjected to analysis of variance (ANOVA) and means were conducted using (L.S.D.).

RESULTS

Latent biological effects:

Reduction in the considered biological aspects differed according to plant species and kind of extract used. The total extract (Methanol) of *P. juliflora* gave the highest corrected larval mortality (93.8%) followed by (75.0%) in the case of the fraction (Ethyl acetate) (Table 1). However, the total extract (Z-Methanol) gave the corrected mortality (75.0%) followed by (70.0%) in case of the extract (Z- Methylene chloride). The pupal weight (mg) was ranged from 256.0 mg to 341.3 mg in both Methanol and Ethyle acetate of *Prosopis* extracts, respectively. While, Methylene chloride and Ethyle acetate of *Zilla* were the most effective on reduction of pupal weight (240.3 and 248.3 mg, respectively in comparison with control (319.2 mg). The highest pupal mortality recorded (15 %, 5 %, and 5%) in the case of Methanol, Hexane and Ethyle acetate of *Zilla*, respectively. Both *Prosopis* and *Zilla* extracts (Table 2) had latent biological effects on % of adult emergency of *S. littoralis* treated previously as larvae and recorded (5%) in case of Methanol and ethyl acetate, respectively. While it reached 80 % in control. Also, Methylene chloride of *Prosopis* gave the most effect on % Hatchability (7.1%) of eggs.

Both Methanol and Hexane gave the same effects (10.0 and 10.2 %) of egg hatchability percentage. While Hatchability percentage was 100% in control. In the adult stage the results in a table (2) cleared that Pre-oviposition days was elongated from 2.00 infraction Zilla- Methylene chloride to 5.7 in crude extract Zilla- Methanol. While oviposition days ranged from 1.00 to 3.2 days in total extract Zilla- Methanol and control, respectively.

Ovicidal tests

Data concerning the ovicidal activity of the tested plant extracts clearly indicate the important role of eggs age, nature of tested plant extract and solvent of extraction in determining the ovicidal activity against *S. littoralis*. The efficiency of the fractions of *Prosopis* extracts against *S. littoralis* can be arranged according to egg Hatchability % in a descending order as follows: Butanol > Ethyl acetate > Hexane > Methylene chloride, Table (3). Both Methanol and fraction of Methylene chloride of aerial parts completely suppressed the % of egg hatchability (zero %) (Table 3). While the efficiency of *Zilla* extracts fractions against *S. littoralis* can be arranged according to egg Hatchability % in a descending order as follows: Butanol > Methanol > Methylene chloride > Ethyle acetate> Hexane, (Table 3). Data indicated the superior ovicidal activity of *Prosopis* extract fractions on the viability of egg masses of *S. littoralis* aged 24 hrs than *Zilla* extracts.

Table 1. Effect of *Prosopis juliflora* and *Zilla spinosa* extracts on latent biological aspects of *Spodoptera littoralis.*

Tested Plant	Extract	Corrected larval Mortality (%)	Larval duration(days) ±S.E. (range)	Pupal duration (days) ±S.E. (range)	Pupal weight (mg) ±S.E. (range)	Pupal Mortality (%)
Prosopis juliflora	Methanol	93.8	7.00±1.3 (1-15)	8.00±0.00 (0-8)	256.0±9.1 (220-295)	0.0
	Hexane	62.5	8.00±0.9 (1-5)	7.00±0.40 (6-8)	287.0±11.5 (252-336)	0.0
	Methylene chloride	50.0	12.00±2.0 (1-12)	8.50±0.20 (9-10)	281.6±12.0 (230-315)	1.0
	Ethyl acetate	75.0	10.50±2.2 (1-14)	6.50±0.50 (5-7)	341.3±11.6 (297-395)	0.0
	Butanol	56.3	10.30±1.7 (2-14)	6.50±0.30 (5-7)	298.3±13.3 (245-355)	1.0
Zilla spinosa	Methanol	75.0	6.70± 1.2 (1-13)	8.25±0.53 (6-10)	290.3±19.1 (210-298)	15.0
	Hexane	50.0	8.30±1.5 (1-16)	7.90±0.48 (6-10)	267.6±9.6 (219-307)	5.0
	Methylene chloride	70.0	6.85±1.7 (1-19)	6.00±0.45 (5-7)	240.3±8.3 (218-270)	0.0
	Ethyl acetate	30.0	10.40±1.3 (1-18)	7.80±0.45 (5-10)	248.3±10.7 (171-310)	5.0
	Butanol	35.0	10.30±1.3 (1-17)	7.77±0.23 (6-9)	257.8±9.8 (205-320)	0.0
Control		00.0	8.00±1.1 (3-10)	9.00±0.50 (3-10)	319.2±12.3 (270-365)	0.0
LSD		-	2.7	0.87	24.5	-

Table 2. Effect of *Prosopis juliflora* and *Zilla spinosa* extracts on latent biological aspects of *Spodoptera littoralis.*

Tested plant	Extract	Adult				Egg		
		Adult emergency %	Pre-oviposition ±S.E. (range)	Oviposition Days ±S.E. (range)	Post-oviposition Days ±S.E. (range)	No. egg ±S.E.	No. Hatched ±S.E.	Hatchability %
Prosopis juliflora	Methanol	5	1.0±0.0 (1-1)	0.0±0.0 (0-0)	0.0±0 (0-0)	500±91.3	50±13.5	10.0
	Hexane	20	1.0±0.0 (1-1)	1.0±0.0 (1-1)	1.0±0 (1-1)	245±30.9	25±6.1	10.2
	Methylene chloride	30	2.7±0.3 (2-3)	2.0±0.2 (0-2)	1.0±0.3 (1-2)	425±78.9	30±4.6	7.1
	Ethyl acetate	20	3.3±0.3 (3-4)	1.0±0.0 (1-1)	1.0±0 (1-1)	275±80.9	150±46.9	54.6
	Butanol	45	3.3±0.3 (3-4)	1.0±0.0 (1-1)	1.0±0 (1-1)	900±214.5	700±250.3	77.8
Zilla spinosa	Methanol	20	5.7±0.9 (4-7)	1.0±0.6 (0-2)	2.7±0.5 (0-5)	195±25.9	39±3.3	20.0
	Hexane	30	3.9±0.6 (1-6)	1.7±0.6 (0-3)	3.0±.4 (2-4)	475±70.3	125±5.5	26.3
	Methylene chloride	20	2.0±0.3 (1-3)	2.3±0.3 (2-3)	2.8±0.3 (2-3)	700±77.9	275±7.7	39.3
	Ethyl acetate	5	2.8± 0.5 (1-6)	2.8± 0.4 (1-4)	2.4±0.2 (2-3)	300±33.9	110±1.7	36.7
	Butanol	45	3.0±0.3 (2-4)	1.9±0.3 (1-3)	2.8±0.2 (2-4)	490±43.3	325±8.9	66.3
Control		80	4.2±0.3 (3-7)	3.2±0.2 (2-4)	2.2±0.1 (2-3)	2400±414.3	2400±414.3	100
LSD		-	0.8	1.2	0.9	480.6	17.8	-

Table 3. Effect of *P. juliflora* and *Z. spinosa* extracts on *S. littoralis* egg masses (24hrs)

Tested Plant	Extract	Incubation period ±S.E. (range)	Hatchability %
Prosopis juliflora	Methanol	5.0±0 (5-5)	0.0
	Hexane	5.0 ±0.3 (4-5)	8.0
	Methylene chloride	5.0± 0 (5-5)	0.0
	Ethyl acetate	4.0±0.6 (2-5)	66.7
	Butanol	5.0±0.6 (2-5)	86.7
Zilla spinosa	Methanol	2.0±0.9 (0-3)	30.0
	Hexane	0.0 (0-0)	0.0
	Methylene chloride	2.7±0.8 (0-5)	21.0
	Ethyl acetate	1.0±1.0 (0-3)	6.0
	Butanol	4.0±0.6 (0-5)	38.0
Control		4.0±0.6 (3-5)	85.3
LSD		2.1	-

Table 4. Percent of Antifeedant activity of 3rd larvae of *S. littoralis* after treatments (non choice treatments).

Tested plant	Extracts	Mean area consumption (mm^2) ±S.E.		Antifeedant activity %	
		24hr.	48hr.	24hr.	48hr.
Prosopis juliflora	Methanol	72.0±15	27.0±11	23.4	76.9
	Hexane	24.0±7	19.0±6	74.5	83.8
	Methylene chloride	87.0±9.7	22.0±6	7.5	81.2
	Ethylacetate	63.0±14	37.0±7	32.9	68.4
	Butanol	78.0±16	70.0±10	17.0	40.2
Zilla spinosa	Methanol	38.3±8.6	7.5±3.2	59.3	93.6
	Hexane	11.8±2.01	12.5±4.3	87.4	89.3
	Methylene chloride	17.8±4.5	3.3±2.6	81.1	97.2
	Ethyl acetate	22.5±5.6	7.5±0.9	76.1	93.6
	Butanol	23.0±9.6	11.3±5.5	75.5	90.3
Control		94.0±10	117.0±15	-	-
LSD		2.9	2.3	-	-

Antifeedant activity (no choice test):

The mean area (mm^2) that was consumed by *S. littoralis* differed according to feeding period and tested extracts. The mean area (mm^2) treated with Methanol extract of *P. juliflora* that *S. littoralis* consumed ranged from 72 mm^2 to 27 mm^2 while, it was from 94 to 117 mm^2 when larvae were fed on the control for 24hrs and 48hrs, respectively, (Tabl 4). However, the mean area (mm^2) that *S. littoralis* consumed from Z – Methanol was 38.3 mm^2 and 7.5 mm^2 and it was 94 and 117 mm^2 when larvae were fed on the control for 24hrs.and 48hrs, respectively. *Z. spinosa* tested extracts had strong deterrent activity than *P. juliflora* extracts against *S. littoralis* larvae. The superior extract as a deterrent was Methylene chloride of *Zilla* (97.2%). The lowest antifeedant activity (40.2%) was recorded in case of Butanol of *Prosopis*.

The efficiency of the fractions of *P. juliflora* extracts against *S. littoralis* can be arranged according to % Antifeedant activity in a descending order as follows: Hexane > Methylene chloride > Methanol > Ethyle acetate > Butanol for aerial part (After 48 hrs.). However, the efficiency of the fractions of *Zilla* extracts against *S. littoralis* can be arranged according to Antifeedant activity % in a descending order as follows: After 48 hrs. Zilla- Methylene chloride > Zilla- Methanol =Z-

Ethyle acetate >Z- Butanol.

DISCUSSION

Different plant extracts proved to be antimicrobial, antifungal and antitumor and toxic to insects. Also, it was an insecticidal effect or antifeedant activity (Abd El-Aziz and Sharaby, 1997; Abd El-Aziz and Ezz El-Din, 2007; Sabbour and Abd El-Aziz, 2010; Sharaby et al., 2014; Ismail et al., 2015, 2016). The foregoing results indicated that the tested plant extracts resulted in an obvious retardation in development and reduced the productivity of *S. littoralis,* and this may be correlated to the chemical constituents of these plants. The high content of alkaloids was found in *P. juliflora* plant and had various biological activities including antifungal activity against seed-borne fungi. Alkaloid extract of *P. juliflora* was amended with all the chemical fungicides (Preeti et al., 2015). Moreover, *P. juliflora* leaves include several chemicals including tannins, flavonoids, steroids, hydrocarbons, waxes and alkaloids, Pasiecznik et al., 2001. The juliprosopine (juliflorine), prosoflorine and juliprosine, were showed Gram-positive antibacterial activity against *Micrococcus luteus*. The effect of plant extracts on insects can be demonstrated in several manners including toxicity, antifeedant, suppression of reproductive behavior, reduction of fecundity and fertility and growth inhabitation (Rachid et al., 2006; Ladhari et al., 2013). Ali et al., 2017 was evaluated the larvicidal activity, antifeedant activity and some biochemical studies of essential oils on *Spodoptera littorals* larvae by leaf dipping method.

The foregoing results indicated that the Both Methanol and fraction Methylene chloride of aerial parts of *Prosopis and Zilla* completely suppressed the egg hatchability percentage (zero %) of egg masses (24h). These results in agreement with (Sharaby et al., 1994 b) they founded that newly deposited eggs of *S. littoralis* were more susceptible than the older ones, This may be attributed to the inhibition of embryonic development of eggs. This the favors the necessity of treating newly laid eggs of the insect without time passing which may lead to weakening the effect of these extracts. Saxena and Sharma (1972) showed that the terpenoids, carvacrol, citral, citronellal, eugenol, farnesol, and geraniol inhibited embryonic development and prevented hatching in a high percentage of eggs of *Aedes aegypti*. As the eggs advanced in age their susceptibility to inhibition declined. Hikal et al. (2017) stated that the aqueous extracts were affected by the development of *P. xylostella* in all stages of the life cycle, causing mortality in the larval and pupal stages. Treatments with extracts caused the lowest fecundity and the number of hatched larvae.

The mean area (mm^2) that was consumed by *S. littoralis* differed according to feeding period and tested extracts. These findings are in agreement with many authors who attributed the activity of different naturally occurring compounds to the presence of special chemicals, Abd El-Aziz and Sharaby, 1997; Abd El-Aziz and Ezz El-Din (2007). Abd El-Aziz and Ezz El-Din (2007) studied the biological and phytochemical screening of four Egyptian wild plants/weed against cotton leafworm. The highly significant antifeedant activity was recorded in case of *Maytenus senegalensis* (95.52%) followed by *Cleom amblyocarpa* (45.23%) and *Solenostemma argel* (32.12%) against 3rd larval instar of *S. littoralis.* Many research work has shown that the secondary plant substances considerably reduce feeding, growth reproduction and survival of insect species (Sharaby et al., 1994 a). Plant metabolites make toxic substances if ingested resulting in the rejection of the host plant by insects (Russel and Lane, 1993). Garlic possesses many secondary metabolites such as saponins, tannins, alkaloid steroids and glycosides that may influence the antifeedant activity (Arekemase et al., 2013 and Ali et al., 2017). Also, Chinnamani and Jeyasankar 2018 Evaluated that the antifeedant activity of plant extracts against *Spodoptera litura* and *Helicoverpa armigera* (Lepidoptera: Noctuidae). Three plants showed significant antifeedant activity viz., *Pseudocalymma alliaceum* (81.55 and 79.44 %), *Solanum pseudocapsicum* (76.32 and 74.66%) and *Barleria buxifolia* (73.23 and 70.66%) in ethyl acetate extracts against *Spodoptera litura* and *Helicoverpa armigera*, respectively.

CONCLUSION

Based on these finding, it can be concluded that Methanol extract of *Prosopis* gave the highest corrected larval mortality (93.8%). *Zilla* extracts were the most effective on reduction of pupal weight and the highest pupal mortality. Both *Prosopis* and *Zilla* extracts had latent biological effects on % of adult emergency of *S. littoralis* treated previously as larvae. While, *Prosopis* extracts recorded the highest reduction in % of egg hatchability (7.1, 10.0 and 10.2 %). *Prosopis*

extract fractions had the superior ovicidal activity on the viability of egg masses of *S. littoralis* aged 24 hrs than *Zilla* extracts. *Zilla* tested extracts had strong deterrent activity than *P. juliflora* extracts against *S. littoralis* larvae. *Prosopis* extracts gave considerable protection from *S. littoralis* attack and can be used in the Integrated Pest Management Program.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

Shadia E. Abd El-Aziz, S.S. Ahmed, and R.S., Abdel-Rahman designed and performed the experiments, collection data, wrote the manuscript, and analysis data, A.E.El-Gohary, M.E.F.Hegazy and T.A. Mohamed, collection plant, performed extracts, wrote the manuscript, and analysis data. All authors read and approved the final version.

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