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EFFECTS OF *RICINUS COMMUNIS*, *BRASSICA NIGRA* AND MINERAL OIL KEMESOL ON SOME BIOCHEMICAL ASPECTS OF LARVAE STAGE OF *SPODOPTERA LITTORALIS* (BOISD) (LEPIDOPTERA: NOCTUIDAE)

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Abstract

The third instars larvae of *Spodotera littoralis* were topically treated with two plant oils, *Ricinus communis* and *Brassica nigra* and one mineral oil, Kemesol 95% dissolved in petroleum ether and acetone at concentrations of 0.8, 1.6, 2.0, 3.0 & 4 %. The results revealed that the mean values of the total haemolymph and fat body protein was reduced in larvae treated with *B. nigra* and Kemesol 95 %. A significant decrease was observed in haemolymph and fat body protein contents in larvae treated with all tested compound, the remarked decrease was noticed at the highest dose (4%) in both two solvents.

Key words: *Spodotera littoralis*, *Ricinus communis*, *Brassica nigra*, Kemesol.

Introduction

No doubt, insects of medical, veterinary or agricultural important play destructive role on human welfare (Morsy *et al*., 2001). The development of modern ecological friend compounds received much attention (Al-Mathal and Fouad, 2006). Botanical molluscicides (Shoukry, 2006; Massoud *et al.*, 2007) or insecticide (Abdel Halim and Morsy, 2005) and mineral oils are one of the effective agents for insect control (Reddy et *al.*, 2002). They affect the synthesis of protein, lipids and carbohydrates; consequently any balance of these agents induces confusion in the sequence of metamorphosis and metabolism (Morsy *et al*., 2000; Scott *et al*., 2003, Seth *et al*., 2004; Cespedes *et al*., 2005; Pavela, 2005).

This study aimed at investigation of biochemical effects of two plant oils; *Ricinus communis* and *Brassica nigra* and one mineral oil; Kemesol 95% on larvae of the cotton leaf worm, *Spodotera littoralis* (Boisd). Also, the biochemical changes on haemolymph, fat body, protein, carbohydrate and lipid content were measured as a marker for insecticidal activity.

Materials and Methods

S. littoralis pupae were obtained from a laboratory culture, maintained

in the biology Department, Faculty of Science for Girls, Jeddah.

The plant oils used were: *Ricinus communis* and *Brassica nigra*. The mineral oil was Kemesol 95%. All tested compounds were dissolved in petroleum ether and acetone at concentrations of 0.8, 1.6, 2, 3, & 4%. The different concentrations of each compound were applied topically using automatic pipette.

Two groups of the third larval instars of *S. littoralis* were collected from the stock culture; the first group was topically treated with different doses of the tested compounds dissolved in petroleum ether and the second one treated with the same doses of the three tested compounds dissolved in acetone.

Treated and untreated larvae were incubated at 27±2ºC & 70±2% R.H. Control group was maintained and treated only with the solvent used. Samples of haemolymph and fat bodies were collected from the treated and check groups and values of haemolymph and fat body content of protein, carbo-hydrates and lipids were estimated within 48 hrs after treatments (Singh and Bhathal, 1992).

Statistical analysis: All data were corrected according to Abbott's Formula (1925), and expressed as mean ± standard deviation. Significant differences between individual means were determined by student "t " test for paired observations. Level of significance of each experiment was stated to be non significant ($p>0.05$), significant ($p<0.05$) and highly significant ($p<0.05$).

Results and Discussion

The main values of the haemolymph and fat body, protein content during the 3rd instar of larval stage *S. littoralis* are presented in table 1 and 2. Statistical analysis of results indicated that the haemolymph and fat body protein contents of 3rd larval instars treated with *B. nigra* and Kemesol 95% were significantly decreased as compared with the check group ($P< 0.05$). The effect was dose dependant, i.e. as a dose increased the protein content decreased. On the other hand, *R. communis* treatments caused a significantly higher total protein than that of untreated control group ($P<0.05$), haemolymph, and fat body protein content in all treated and untreated group tended to decrease as a result of extraction of botanical oils by solvents and dissolving the mineral oil.

The carbohydrate content:

Statistical analysis of data in tables 3 and 4 revealed the following significant increase in the haemolymph carbohydrate content which was observed at dose of 0.8%, it reached 49.1 & 48.6 mg/ml. Haem. for *B. nigra* and Kemesol 95% as compared with 23.2 mg/ml Haem. In the control group the dose of 4% of *R. communis* increased the level of haemolymph carbohydrate content as compared with dose obtained by other doses when it increased from 23.4 to 41.5 mg/ml Haem. The dose of 0.8% of *B. nigra* and *R. communis* increased the level of carbohydrate content in fat body as compared with

other doses, where reached 24.5 and 68.5 mg/g fat body.

The lipid content:

Statistical analysis of data in tables (5 & 6) revealed that the following treatments with all tested compounds significantly increased the haemolymph lipid content. Treatments with Kemesol 95% with dosage of 0.8% induced pronounced increase in haemolymph lipid content; it was 26.4, while it was 30.5 mg/ml Haem. when 3rd larval instars treated with Kemesol 95% at dosage of 4%. The highest lipid content for *R. communis* treatments was 25.3 mg/ml Haem. at 4%.

In the present study an increase in haemolymph and fat body lipid contents of treated larval stage of *S. littoralis* was observed. These observations may be explained that botanicals and mineral oil increased the conversion rate of carbohydrate to lipid leading to the haemolymph and fat body of the treated larvae. The tested compounds affected mainly the fat body and this also led to a strong accumulation of carbohydrates in tissues.

The achieved results were in agreement with Mesbah *et al.* (2007) who found that plant flavonoids had been shown by many investigators to have an effect on insect behaviour, growth, and development. Quercetin is one of many bioflavonoids that exist in several fruits and vegetables.

Results indicated that the botanicals and mineral oil inhibited the anabolism of the treated insects. The metabolic activity is mostly of catabolic pattern. Results indicated that petroleum ether was more effective than acetone when used as solvent for both plant oils and mineral oil.

The present data agreed with that of Hegazy *et al.* (1992), Hashem (1994), Shonouda *et al.* (2000), Reddy *et al*. (2002), Scott *et al*. (2003), Pineda *et al*. (2004), Seth *et al*. (2004), Nathan *et al*. (2005). Also, the data agreed with that of Pavela (2005) who tested thirty-four essential oils against larvae of *S. littoralis*, found that these oils were highly toxic of the 3rd larval instars of *S. littoralis* after topical application. The high degree of biodegradation exhibited by most phytocemicals is what makes them ecofriendly and attractive as replacements of synthetic chemicals in the first place. Although the evaluation of phytochemicals is yet in its infancy and much research aims to further characterize promising agents and discover new agents in insect control programs. The present work showed a strong efficiency of the botanical extracts which could be used alone or in combination with sub-lethal doses of certain insecticides to control the cotton leaf worm. Stringer *et al.* (2008) found that successful trapping of female *Thysanoplusia orichalcea* (F.) in either a lure-and-kill or mass trapping system may offer an effective way to manage its population size and Kostic *et al.* (2008) were tested the toxicity and anti-feedant activity of *Osmium basilicum* against second instars gypsy-moth larvae in the laboratory bioassay, they found that all tested solutions showed low to moderate larvicidal effect in both residual toxicity

test and in chronic larval mortality bioassay.

Conclusion

No doubt, the larval stage of the cotton leaf worm, *Spodotera littoralis* (Boisd) hardly affected the human welfare. The outcome results proved that the botanicals extraction (*Ricinus communis* and *Brassica nigra*) and the mineral oil (Kemesol 95%) inhibited the anabolism of the treated insects.

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Table 1: Haemolymph protein content of 3rd instar larvae of *Spodoptera littoralis* (Boisd) treated with different doses of *Brassica nigra*, *Ricinus communis* and Kemesol 95% dissolved in petroleum ether and acetone.

Dose	Solvent used	Haemolymph protein content (mg / ml Haem.) ± S.D		
Conc %		*Brassica nigra*	*Ricinus communis*	Kemesol 95%
4. 0	Pet.ether	15.2 ± 0.73**	71.5 ± 0.36**	21.5 ± 0.24**
	Acetone	14.2 ± 0.86**	68.4 ± 0.45**	19.3 ± 0.63**
3. 0	Pet.ether	16.1 ± 1.01**	63.9 ± 0.63**	23.7 ± 0.45**
	Acetone	15.2 ± 1.71**	61.5 ± 1.82**	21.5 ± 0.60**
2. 0	Pet.ether	26.3 ± 1.01**	55.3 ± 1.68**	28.1 ± 0.67**
	Acetone	22.1 ± 0.41**	51.2 ± 0.46*	24.3 ± 0.86**
1. 6	Pet.ether	29.5 ± 0.68*	48.7 ± 0.86*	31.5 ± 0.16*
	Acetone	27.1 ± 0.60*	44.3 ± 0.46***	26.2 ± 0.46*
0. 8	Pet.ether	32.4 ± 0.99*	38.4 ± 0.92***	33.4 ± 0.56*
	Acetone	30.1 ± 0.86*	36.9 ± 0.91***	31.6 ± 0.34*
control	Pet.ether	40.1 ± 0.90	42.1 ± 0.36	38.4 ± 0.32
	Acetone	39.03 ± 0.86	40.2 ± 0.84	36.4 ± 0.16

Table 2: Fat body protein content of 3rd instar larvae of *S. littoralis* treated with different doses of *B. nigra*, *R. communis* and Kemesol 95% dissolved in petroleum ether and acetone.

Dose	Solvent used	Fat body protein content (mg / g Fat body) ± S.D		
Conc %		*Brassica nigra*	*Ricinus communis*	Kemesol 95%
4. 0	Pet.ether	5.9 ± 0.96**	48.1 ± 0.18**	10.7 ± 1.53**
	Acetone	4.7 ± 0.18**	32.5 ± 0.72**	8.2 ± 0.71**
3. 0	Pet.ether	6.8 ± 0.96**	28.9 ± 0.02**	11.7 ± 0.84**
	Acetone	5.4 ± 0.45**	22.1 ± 0.35**	9.2 ± 0.72**
2. 0	Pet.ether	9.01 ± 1.36*	19.5 ±.0.45*	12.0 ± 1.39*
	Aceton	7.9 ± 0.52**	16.1 ± 0.40*	10.8 ± 0.45*
1. 6	Pet.ether	10.3 ± 0.72*	19.3 ± 0.17*	12.4 ± 0.63*
	Aceton	9.1 ± 1.82*	16.5± 0.89*	11.0 ± 0.46***
0. 8	Pet.ether	12.1 ± 0.53*	14.1 ± 0.86***	13.1 ± 0.47***
	Aceton	9.3 ± 072*	11.5 ± 0.54***	12.1 ± 0.69***
control	Pet.ether	13.2 ± 0.86	14.1 ± 1.72	14.2 ± 1.35
	Aceton	11.2 ± 0.16	11.5 ± 0.96	11.9 ± 0.41

Table 3: Haemolymph-carbohydrate content of 3rd instar larvae of *S. littoralis* treated with different doses of *B. nigra*, *R.communis* and Kemesol 95% dissolved in petroleum ether and acetone.

Dose	Solvent used	Haemolymph-carbohydrate content (mg / ml Haem.) ± S.D		
Conc %		*Brassica nigra*	*Ricinus communis*	Kemesol 95%
4. 0	Pet.ether	34.2 ± 1.23*	41.51 ± 0.13**	29.4 ± 0.53*
	Acetone	30.1 ± 0.15*	36.1 ± 0.19*	28.4 ± 0.89*
3. 0	Pet.ether	37.5 ± 0.16*	31.9 ± 0.54**	32.9 ± 1.32**
	Acetone	36.1 ± 1.32*	28.5 ± 0.31**	30.7 ± 1.52***
2. 0	Pet.ether	45.4 ± 0.56**	32.9 ± 0.98**	38.5 ± 0.71**
	Acetone	41.3 ± 0.72**	31.1 ± 1.32*	36.1 ± 0.81*
1. 6	Pet.ether	47.2 ± 0.15**	36.5 ± 0.45*	44.5 ± 1.21**
	Acetone	42.3 ± 1.38**	34.2 ± 0.69**	41.7 ± 1.65**
0. 8	Pet.ether	49.1 ± 0.16**	35.7 ± 1.62*	48.6 ± 0.72**
	Acetone	45.4 ± 1.23**	32.3 ± 0.91*	43.2 ± 1.36**
control	Pet.ether	25.1 ± 0.96	25.1 ± 1.32	25.6 ± 0.72
	Acetone	23.2 ± 0.72	23.4 ± 1.86	23.7 ± 1.36

Table 4: Fat body carbohydrate content of 3rd instar larvae of *S. littoralis* treated with different doses of *B. nigra*, *R. communis* and Kemesol 95% dissolved in petroleum ether and acetone

Dose	Solvent used	Fat body carbohydrate content (mg/g Fat body) ± S.D		
Conc %		*Brassica nigra*	*Ricinus communis*	Kemesol 95%
4. 0	Pet.ether	25.1 ± 0.12**	22.1 ± 0.72**	15.4 ± 1.5**
	Acetone	23.2 ± 0.12**	18.1 ± 1.32*	13.2 ± 0.45**
3. 0	Pet.ether	42.5 ± 1.32**	52.1 ± 0.71**	8.1 ± 0.2*
	Acetone	37.7 ± 0.69**	49.9 ± 1.36**	7.9 ± 0.5*
2. 0	Pet.ether	38.18 ± 1.32**	82.3 ± 0.96**	15.0 ± 1.36**
	Acetone	36.12 ± 0.92**	79.5 ± 1.32**	13.2 ± 1.5**
1. 6	Pet.ether	36.1 ± 1.52**	78.5 ± 0.92**	9.1 ± 0.878*
	Acetone	34.4 ± 0.91**	71.7 ± 0.83**	9.1 ± 0.86*
0. 8	Pet.ether	24.5 ± 0.13**	68.5 ± 0.32**	7.5 ± 0.53*
	Acetone	14.2 ± 0.86**	61.7 ± 0.82**	5.3 ± 1.4*
control	Pet.ether	4.5 ± 1.56	4.4 ± 1.32	4.6 ± 0.89
	Acetone	4.2 ± 0.31	4.3 ± 0.94	4.1 ± 1.5

Table 5: Haemolymph Lipid content of 3rd instar larvae of *S. littoralis* treated with different doses of *B. nigra, R. communis* and Kemesol 95% dissolved in petroleum ether and acetone.

Dose	Solvent used	Haemolymph Lipid content (mg / ml Haem.) ± S.D		
Conc %		*Brassica nigra*	*Ricinus communis*	Kemesol 95%
4. 0	Pet.ether	15.2 ± 1.23*	25.3 ± 0.75**	30.5 ± 1.23**
	Acetone	13.1 ± 0.13*	24.1 ± 0.31*	29.1 ± 0.15**
3. 0	Pet.ether	18.7 ± 0.16*	20.5 ± 0.64***	22.13 ± 0.53***
	Acetone	15.2 ± 1.32*	19.3 ± 0.86***	20.9 ± 0.89***
2. 0	Pet.ether	20.9 ± 0.56***	22.9 ± 1.32*	24.1 ± 1.51*
	Acetone	18.7 ± 0.72*	21.0 ± 0.96***	22.9 ± 0.81*
1. 6	Pet.ether	28.5 ± 0.15*	20.2 ± 1.63***	26.5 ± 0.96*
	Acetone	26.1 ± 0.14**	17.5 ± 0.91*	17.5 ± 0.72*
0. 8	Pet.ether	26.4 ± 0.96*	20.2 ± 1.63***	28.3 ± 0.71**
	Acetone	22.3 ± 0.72*	19.1 ± 1.86***	22.1 ± 1.32*
control	Pet.ether	20.1 ± 0.71	20.2 ± 1.32	20.15 ± 0.71
	Acetone	18.7 ± 1.36	19.1 ± 1.86	19.2 ± 0.72

Table 6: Fat body Lipid content of 3rd instar larvae of *S. littoralis* treated with different doses of *B. nigra, R. communis* and Kemesol 95% dissolved in petroleum ether and acetone.

Dose	Solvent used	Fat body Lipid content (mg / g Fat body) ± S.D)		
Conc %		*Brassica nigra*	*Ricinus communis*	Kemesol 95%
4. 0	Pet.ether	150.2 ± 0.12**	160.5 ± 0.72**	136.5 ± 1.5**
	Acetone	146.7 ± 0.12**	154.3 ± 1.32**	122.3 ± 0.45**
3. 0	Pet.ether	142.0 ± 1.32**	131.5 ± 0.71*	111.6 ± 0.2**
	Acetone	138.5 ± 0.69**	116.7 ± 1.36*	106.5 ± 0.5**
2. 0	Pet.ether	77.2 ± 1.32***	301.0 ± 0.96**	78.5 ± 1.36*
	Acetone	68.5 ± 0.92***	277.5 ± 1.32**	72.4 ± 1.5**
1. 6	Pet.ether	106.4 ± 1.52**	365.3 ± 0.92**	101.7 ± 0.87*
	Acetone	101.2 ± 0.91**	311.4 ± 0.83**	99.2 ± 0.86**
0. 8	Pet.ether	82.2 ± 0.13*	163.4 ± 1.32**	92.7 ± 0.53*
	Acetone	76.3 ± 1.02*	116.5 ± 0.92*	86.1 ± 1.4**
control	Pet.ether	62.1 ± 0.13	63.4 ± 1.11	62.7 ± 0.53
	Acetone	57.8 ± 1.32	61.2 ± 0.92	36.1 ± 1.4