

**EFFECT OF HAPLOPHYLLUM TUBERCULATUM EXTRACTS
ON AN INSECT PEST, SPODOPTERA LITTORALIS AND THE
PREDATOR, COCCINELLA UNDECIMPUNCTATA**

[71]

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ABSTRACT

Petroleum ether and chloroform extracts of *Haplophyllum tuberculatum* at 5% concentration caused 20 and 40% mortality among tested 4th instar larvae of *Spodoptera littoralis*, respectively. Petroleum ether extract also induced 20% malformation among emerged adults. Both extracts significantly reduced larval food consumption and mean weight gain, while chloroform extract revealed significant reduction in ECI ($P > 0.05$) and also imbalance in sex ratio. Both extracts had no obvious effects on the duration of both larval and pupal stages of *C. undecimpunctata*, but the adult longevity was shortened significantly than the check. Significant reduction in predation capacity among 2nd, 4th larval instar as well as adult stage was detected. Generally chloroform extract of *H. tuberculatum* showed more deleterious effects than petroleum ether extract.

Key words: Biological control, Plant extracts, *Haplophyllum tuberculatum*, *Spodoptera littoralis*, *Coccinella undecimpunctata*

INTRODUCTION

Many research programs had emerged to search for plant derived products that produce deleterious effects on insects. These included many plant families and classes (Lane *et al* 1985 and Saxena *et al* 1986). Some plants have been evaluated to contain insect repellents (Jacopson, 1975). Other plants have been found to act as feeding inhibitors for several insect species (Reese and Beck, 1976; Klock and Chan 1982; Mansour *et al* 1990; Salem *et al* 1994; Amr *et al* 1995 and Amr, 2001).

Mohsen *et al* (1989a) mentioned that petroleum ether extract of the aerial parts of the herb, *Haplophyllum tuberculatum* (Forssk.) (Family : Rutaceae) contained mainly alkaloids. This herb has insecticidal and nematocidal activity. Ethanolic extract of this plant was found to have strong insecticidal activity against *Gambusia affinis* and mosquito as well. The same author in (1989b) found that alkaloids, lignans and flavonoids, from this extract had also insecticidal activity against mosquitoes, *Culex quinquefasciatus*. Moreover, the nematocidal effect of alkaloids of *H. tuberculatum* against

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the plant parasitic nematode, *Meloidogyne javanica* was proved by Stephan *et al* (1989). It was found that over the 7 days period, plant extract killed significantly greater number of eggs and larvae.

This study was selected and schemed to test the impact of such extracts on the mortality and important biological activities of *Spodoptera littoralis* and the predator *Coccinella undecimpunctata* as well.

MATERIAL AND METHODS

The Herb, *Haplophyllum tuberculatum* (Forssk.) A. Juss. (Family: Rutaceae), was obtained from Wady Hagol, region near the Suez Governorate, northeast of Cairo, Egypt, and was identified by Dr. M. El-Gebali, Department of Natural Products, National Research Centre, Dokki, Cairo, Egypt.

Preparation of the extracts

Air-dried powder of aerial part of *H. tuberculatum* herb was extracted by petroleum ether followed by chloroform in Soxhlet apparatus. The extracts were evaporated under reduced pressure till complete dryness. One gram of each dried extract (petroleum ether or chloroform) was mixed thoroughly with 0.1 ml of Tween-80 then proper amount of distilled water was added to obtain emulsion containing 5.0% of the botanical extract.

Effects of both extracts on certain biological aspects of the cotton leafworm, *S. littoralis* larvae were accomplished in two sets of experiments.

Insecticidal and biological effects

Fourth instar larvae were selected as test insects. Three groups, each comprised twenty newly moulted larvae from the stock culture, were kept individually in vials. The first group (check) was fed on discs of fresh (untreated) castor leaves, the 2nd group of insects, fed on discs of castor leaves dipped in 5% chloroform of *H. tuberculatum* extract, while the 3rd group of insects fed on discs of castor leaves dipped in 5% petroleum ether of *H. tuberculatum* extracts. The larvae were checked daily till pupation and adult emergence. Larvae fed on treated leaves for 7 days. The larval period, percent mortality of larvae and pupae, percent deformations in adults and sex ratio were calculated in each group.

The whole experiment were conducted under controlled laboratory conditions of 25±5°C and 70±5% R.H.

Effect on metabolic parameters of *Spodoptera littoralis*

Sixty newly moulted fourth instar larvae of the same weight were divided into three equal groups, each larva was kept individually in vials. The first group (check), was fed on untreated castor leaf discs of known weight, the second group fed on castor leaf discs dipped in 5% chloroform of *H. tuberculatum* extract, while the last group fed on castor leaf discs dipped in 5% petroleum ether of *H. tuberculatum* extract.

Both control and treated larvae were examined daily, faeces were carefully separated from uneaten diet, weighed and dried to constant weight. The uneaten parts of discs (residual food) were collected daily and dried to constant weight.

Twenty samples of identical weight of treated and untreated discs were dried to constant weight. Dry weight of exuviae and faeces was estimated. Twenty newly moulted fourth instar larvae were dried to constant weight to be used in calculation of the initial dry weight of larvae. Seven days after the experiment, larvae were weighed and dried to constant weight. The metabolic parameters were calculated according to Waldbauer (1968) and Slansky and Scriber (1982). All calculations were based on the dry weight.

The following equations were applied:

$$AD \% = \frac{\text{Amount of food consumed} - \text{faeces}}{\text{Food consumed}} \times 100$$

$$ECD \% = \frac{\text{Dry weight gain in mg.}}{\text{Food consumed} - \text{faeces}} \times 100$$

$$ECI \% = \frac{\text{Dry weight gain}}{\text{Food consumed}} \times 100$$

Where:

AD = Approximate Digestibility

ECD= Efficiency of conversion of digested food to the body matter

ECI = Efficiency of conversion of ingested food

Coccinella undecimpunctata treatments

It is well known that adult and larvae of the ladybird beetle fed on eggs and the newly hatched larvae of many lepidopterous insects when they were prevailing in the target crops (Ibrahim, 1955 a&b). So, the author wish to fined out the side

effects of such extracts on larvae and/or adults of the ladybird.

Effect of the extracts on the predator

Egg treatment

Three groups (twenty-five newly laid eggs / each) were used, two groups were sprayed with 5% concentration of either extract, and the third one (check) was received water only. After 10 minutes, the excess extract or water was removed using filter paper, then treated eggs were incubated till hatching. Daily observations were carried out. The experiment was replicated three times.

Adult treatment

For each extracts as well as check, three groups of adult ladybird (males & females) each comprised 25 individuals, were used (replicated three times). In treated groups, adults were preyed on the corresponding treated *S. littoralis* eggs for three days, then fed on fresh aphids renewed daily till death. The 3rd group offered only untreated batches of eggs. Daily observations were carried out for adult mortality.

Larval treatment

Three groups (twenty-five each) of newly hatched 1st larvae were used for both extracts and the check as well (replicated three times). The 1st instar larvae in each group offered the corresponding treated cotton leafworm eggs for three days, then fed on fresh aphids renewed daily. Daily observations were continued for the next larval instars, pupation then adults till death.

Data were subjected to analyses of variance applying "One Way Anova" test to evaluate the significance between treatments using "Microstat" Computer program.

RESULTS AND DISCUSSION

Biological criteria

The effect of *Haplophyllum tuberculatum* extracts on the mortality and development of 4th instar *Spodoptera littoralis* larval

Data presented in Table (1) clarified the effect of chloroform and petroleum ether extracts of *H. tuberculatum* on some biological criteria of the cotton leafworm. It is obvious that the two extracts have no effects on larval duration as lasted nearly the same period from 4th instar till the

pupal formation being insignificantly different ($F=3.045$, $P=0.0759$). Chloroform extract showed a pronounced mortality (40%), while petroleum ether extract caused only 20% mortality. These differences are significantly different between each other and the check as well ($F=20.00$, $P=0.0000$). Although larvae treated with petroleum ether extract showed less mortality but they showed high percentage of adult malformation (20%) which indicates latent effects of the petroleum ether, while no malformation was detected in either chloroform or the check.

These results are in agreement with the finding of Mansour *et al* (1990 and 1994) and El-Gengaihi *et al* (1996) who mentioned that some phenolic compounds e.g., resorcinol, catechol and gallic acid and the different extracts of harmful seeds at 5% concentration reduced survival, slowed and elongated the development of *S. littoralis*.

Table 1. Biological criteria of *Spodoptera littoralis* 4th instar larvae treated with *Haplophyllum* extracts

Criteria	Control	Chloroform	Petroleum ether	F-value
Larval duration (days) (from 4 th instar to pupal formation)	12.0±0.58 a	12.2±0.87 a	10.0±0.58 a	3.054 ^{NS}
% Larval mortality	0 c	40.0±1.41 a	20.0±2.0 b	20.00**
% Pupal mortality	0	0	0	
% Malformation	0	0	20	
Sex ratio (female : male)	1 : 2	(male only)	1 : 3	

** : Highly significant

NS : Not significant

Metabolic Parameters

The effect of *H. tuberculatum* extracts on some metabolic parameters *S. littoralis* larvae

Data presented in Table (2) clarified the effect of chloroform and petroleum ether extracts of *H. tuberculatum* on some nutritional parameters of *S. littoralis* larvae.

It is obvious that larvae fed on castor leaves treated with 5% petroleum ether extract consumed less amount of food than those fed on either leaves treated with 5% chloroform extract or untreated leaves (check) (being 38.54, 61.23 and 89.45mg, respectively). Meanwhile, larvae fed leaves treated with chloroform extract consumed food, significantly less than check larvae. This reduction in food

consumed by treated larvae caused a significant reduction in the total body weight gain with regard to check larvae. This, however, might be attributed to the effect of some alkaloids are responsible for that as mentioned by (Amr, 2001), who found similar results when *S. littoralis* larvae fed leaves treated with 5% chloroform extract of *Salvia aegyptiaca*. Salem *et al* (1994) obtained similar effects with phenolic active substances on *Agrotis ipsilon*. On the other hand, when larvae of *S. littoralis* fed on leaves treated with 3% chloroform extract of *Nerium oleander*, they gained more weight than check larvae (Amr *et al* 1995).

It could be concluded that chloroform and petroleum ether extracts of *H. tuberculatum* may have a phago-deterrent activity as they caused a pronounced reduction in food consumption.

Table 2. Effect of *Haplophyllum* extracts on nutritional indices of *Spodoptera littoralis* larvae

Treatment	Food consumed (mg)	Mean weight gain (mg)	AD (%)	ECD (%)	ECI (%)	% of faeces with respect to food consumed
Control	89.45±	6.15±	76.83±	13.69±	9.30±	14.72
	10.53 a	0.37 a	5.11 ab	2.79 a	1.48 a	
Chloroform	61.23±	2.56±	68.12±	7.76±	5.09±	19.80
	6.67 b	0.28 b	4.68 b	1.36 a	0.85 b	
Petroleum ether	38.54±	2.59±	78.38±	10.25±	7.57±	20.13
	2.75 c	0.18 b	2.24 a	1.33 a	0.82 ab	
F-Value	10.35**	48.38**	3.29*	2.28 ^{NS}	3.75*	
LSD at 5%	21.45	0.83	10.02	—	4.35	

** Highly significant

* Significant

NS= Not significant

Means in a column followed with the same letter(s) are not significantly difference at 5% probability.

AD = Approximate digestibility

ECD = Efficiency of conservation of digested food

ECI = Efficiency of conservation of ingested food

With respect to the ability of the larvae to digest food, data showed that chloroform extract had a significant effect to reduce the approximate digestibility (AD) of the larvae compared with those treated with petroleum ether extract. These results were confirmed by measuring the ability of larvae to utilize the digested food to convert it into body tissue (ECD). The ability of larvae when treated with chloroform extract to convert digested food into body tissues was not significantly low but it still less than those fed on untreated leaves or leaves treated with petroleum ether extract.

The ability of larvae to convert ingested food into body tissue was significantly low when larvae fed leaves treated with chloroform extract, this result proved the aforementioned hypothesis that chloroform extract has a deterrent effect towards *S. littoralis* larvae, as it was 5.09% compared to 9.30% in the check. The amount of faeces with respect to the food consumed in both treated

groups was higher than the check group which confirm the ability of treated insects to convert both digested and ingested food was less than that in the check.

The previous results may be attributed to the amount of energy spent by the larvae in order to detoxify the extract (Schoonhoven and Meerman, 1978 and Dow *et al* 1983).

From the data in Tables (1 & 2) it is clear that chloroform extract of *H. tuberculatum* has obvious deterrent effects on the cotton leafworm larvae.

Effect of *H. tuberculatum* extracts on *C. undecimpunctata*

The obtained results indicated that petroleum ether extract of *H. tuberculatum* induced 40% mortality among 2nd instar larvae of *C. undecimpunctata*, while chloroform extract induced 40 and 10% mortality among the 2nd and the 3rd instar larvae, respectively (Table 3).

Table 3. Effect of *Haplophyllum* extracts on the mortality and the duration of the ladybird beetle

Treatment		Larval instar				Pupa	Adult
		1 ^a	2 nd	3 rd	4 ^b		
Chloroform	Avg. Duration (days)	3.8±0.1 (3-4)	3.2±0.3 (2-4)	3.6±0.2 (3-4)	3.4±0.2 (3-4)	3.4±0.2 (3-4)	13.6±1.9 (8-20) b
	Mortality %	--	40	10	--	--	--
Petroleum ether	Avg. Duration (days)	3.6±0.2 (3-4)	3.3±0.2 (3-4)	3.0±0.5 (2-4)	3.3±0.3 (2-4)	3.2±0.2 (3-4)	12.3±2.0 (7-19) b
	Mortality %	--	40	--	--	--	--
Control	Avg. Duration (days)	4.0±0.0 (4-4)	3.1±0.1 (3-4)	3.9±0.1 (3-4)	3.2±0.1 (3-4)	3.6±0.2 (3-4)	20.9±0.8 (18-25) a
	Mortality %	--	--	--	--	--	--
F-value		2.70 ^{NS}	0.386 ^{NS}	3.381 ^{NS}	0.223 ^{NS}	1.440 ^{NS}	12.426 ^{**}
LSD at 5%		--	--	--	--	--	4.147

Numbers between brackets indicated the range

** Highly significant

NS = Not significant

Also, data presented in Table (3) clarify that the duration of larval and pupal stages in the treated groups were insignificantly different from the check group. The adult longevity was sharply shortened by more than one week, being significantly different from the check. The longevity of the adult was nearly the same in both of the two extract groups.

Data in Table (4) explained how far both extracts affected the predation activity. Both 1st and 3rd instar larvae in treated groups were nearly the same as the check group, being insignificantly different. On

the other hand, it was found that the 2nd and 4th instar larvae as well as adults were sharply affected. Statistical analysis showed that the predation activity being significantly less than the check group (control). Regarding the 2nd instar larvae, statistical analysis showed no significant difference between the inhibitory effects of both *H. tuberculatum* extracts in reducing the predation activity. While in case of 4th instar larvae or adult stage there was a significant difference between both extracts and also between both extract and the check too.

Table 4. Effect of *Haplophyllum* extracts on food consumed by the ladybird beetle

Treatment	Mean number of aphid consumed				Adult
	Larval instar				
	1 st	2 nd	3 rd	4 th	
Chloroform	8.75±0.85	16.33±2.03b	21.67±0.88	30.25±2.14 c	7.25±1.11c
Petroleum ether	8.25±1.11	12.67±1.20 b	25.67±3.38	41.25±3.84 b	12.75±0.85b
Control	9.25±1.75	22.67±1.45 a	26.67±1.76	59.80±2.87 a	19.25±1.11a
F-value	0.149 ^{NS}	10.015**	1.370 ^{NS}	25.507**	33.961**
LSD at 5%	—	5.532	—	9.529	3.298

** Highly significant

NS= Not significant

Means in a column followed with the same letter(s) are not significantly difference at 5% probability

From the aforementioned results, it could be concluded that treatment with both extracts did not significantly affected the duration of larval and pupal stages of *C. undecimpunctata*, but latent effects particularly, that of chloroform extract became obvious

on the longevity of emerged adults.

Finally, it could be concluded that, field application of such extracts should be cautiously undertaken when *C. undecimpunctata* are prevailing in the cotton fields.

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تأثير مستخلصات نبات " هابلوفيليم " على يرقات دودة ورق القطن ويرقات المفترس أبى العيد ذو الاحدى عشرة نقطة

[٧١]

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نسبة تشوه فى الحشرات الكاملة (الفراشات) بلغت ٢٠%.

ومن ناحية اخرى لم يكن لتلك المستخلصات آثار جانبية (ضارة) على فترة طول العمر لليرقات والعدارى لحشرة أبى العيد، فى حين تأثرت فترة حياة الحشرات الكاملة بدرجة معنوية. وقد تأثرت الكفاءة الاقتراسية سلبيا بدرجة معنوية فى يرقات العمر الثانى والرابع وأيضا فى أفراد الطور الكامل. وبصفة عامة وجد أن مستخلص الكلوروفورم كان أكثر تأثيرا وفاعلية من مستخلص الاثير البترولوى.

تم اختبار تأثير مستخلص الاثير البترولوى ومستخلص الكلوروفورم لنبات *Haplophyllum tuberculatum* تحت الظروف المعملية على يرقات العمر الرابع لحشرة دودة القطن، وكذلك يرقات حشرة أبى العيد ذو الاحدى عشرة نقطة. دلت النتائج على أن كلا المستخلصين ليس لهما تأثير معنوى على طول فترة العمر اليرقى. كذلك وجد أن مستخلص الاثير البترولوى قد أحدث نسبة وفيات بلغت ٤٠%، فى حين أحدث مستخلص الكلوروفورم نسبة موت بلغت ٢٠% أعقبها

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