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Fenugreek Seeds Extracts as Growth Regulatory and Feeding Inhibitor for the African Melon Lady Bird Beetle *Henosepilachna elaterii* (Rossi)

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Abstract: This study aimed at investigating the effect of the extracts of fenugreek seeds on feeding and development of the African melon ladybird beetle, *Henosepilachna elaterii*. (Rossi). Seeds of fenugreek (*Trigonella foenum-graceum*) were extracted by distilled water and organic solvents (chloroform, acetone and ethanol), and the extracts were tested against the African melon ladybird beetle under laboratory conditions. Both aqueous and organic extracts showed significant growth regulatory activities, mainly prolonging the larval duration. The effect was clear in late instars. The organic extracts showed strong feeding inhibitory activity against the fourth larval instar, where the amounts of food consumed inversely related to dose. The effects induced by acetone and ethanol extracts were greater than chloroform extract. Phytochemical groups present in various fractions were identified. Various aspects of efficacy against the test insect are discussed.

Key words: Fenugreek; *Henosepilachna elaterii*; growth regulatory; feeding inhibitor

INTRODUTION

The increased concern about negative environmental effects caused by large-scale use of broad-spectrum synthetic pesticides, such as emergence of resistant strains, destruction of beneficial insects and toxicity to human and non-target organisms, have dictated the need for effective, bio-degradable and safe alternatives with greater selectivity. This has created world-wide interest in re-evaluation of traditional botanical pest control agents (Heyde *et al*. 1984). Higher plants produce many secondary

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compounds, which possess strong efficacy on other living organisms and could constitute environmentally safe drugs and pesticides (Phillipson 1989). Fenugreek is one of the promising plants which received increased attention during the last decade as a potential source of natural pesticides (El-Mubark 1998; Manzoul 1999; Mahmoud and Abdelbagi 2003). This study was carried out to evaluate the growth regulatory and feeding inhibitory effects of fenugreek seed extracts against the African melon lady bird beetle (*H. elaterii*). The different chemical groups found in various fractions of the extracts were identified to explain possible correlations with biological activity.

MATERIALS AND METHODS

Preparation of extracts

(i) Aqueous extract: Five grammes of fenugreek seed powder were mixed with 100 ml distilled water in a conical flask. The content was stirred for 24 hrs with a magnetic stirrer, and the mixture was filtered through muslin cloth. Three concentrations (v/v) (5%, 2.5% and 1%) were prepared by serial dilution with distilled water.

(ii) Organic extracts: Three hundred grammes of fenugreek seeds powder were consecutively extracted with chloroform, acetone and ethanol in a soxhlet apparatus for eight hours. The solvents used were Analar HPLC grade (purity 99% minimum). Defatted powder was thoroughly dried before extraction with next solvent. The solvents were removed by a rotary evaporator. Four concentrations (w/v) (30%, 20%, 10% and 5%) were prepared. The extracts were diluted with distilled water (95%) and solvent of each extract (5%). Soap (1%) was added to chloroform extract, just before bioassay, as an emulsifier.

Effect of extracts of fenugreek seed on development of *H. elaterii* larvae

Ten (starved) African melon ladybird larvae from each instar (first, second, third and fourth) were placed in Petri-dishes and tested separately with each organic extract. The separate testing of each instar was done because of the high pre-moulting mortalities, noticed among treated larvae. However, for aqueous extracts, the experiment started with the first instar and observation continued until adult emergence. The Petri-

dishes were lined with moist filter paper containing either treated and/or untreated pumpkin leaf discs (42.99 cm^2).

In each set of treatments, leaf discs were dipped in chosen concentrations and left to dry at room temperature. Additional set of leaf discs were treated with the corresponding solvent of each extract (distilled water + solvent 5% for organic extract and distilled water only for aqueous extract) and left to dry in a similar manner as the control checks. Each treatment was replicated three times, and the experimental units were arranged in a completely randomized design. The leaf discs were replaced daily by freshly treated ones.

Effect of organic extracts of fenugreek seeds on larval feeding

Pumpkin leaf discs (42.99 cm) were treated by dipping in prepared extracts and left to dry at room temperature, as previously mentioned. Ten (starved) fourth larval instars were introduced in each Petri-dish. Each treatment was replicated three times, and experimental units were arranged in a completely randomized design. The consumed leaf area was measured daily by graph paper and recorded. The feeding rate was calculated, according to the formula described by Reed *et al*. (1982)

$$\text{Feeding activity} = \frac{\text{Percentage consumed (treated)} \times 100}{\text{Percentage consumed (treated+ untreated)}}$$

Phytochemical analysis of extracts

The previously prepared extracts were subjected to phytochemical analysis to identity the different chemical groups present in each fraction, following the method described by Harborne (1982) and Wanger *et al*. (1984).

(i) Preparation of reagents: Ferric chloride test reagent, potassium hydroxide solution and hydrochloric acid (2N) were prepared according to the method described by Harborne (1982), while ninhydrin was prepared according to the method described by Wanger *et al.* (1984).

(ii) Procedures: Test for the presence of unsaturated sterols and triterpens, flavonoids, saponins, tannins, anthraquinone glycosides, alkaloids and physostigmine (alkaloid) were conducted according to Harborne (1982) methods, and trigonelline were tested by thin layer chromatography (TLC) according to Wanger *et al*. (1984).

RESULTS

The aqueous extract of fenugreek seeds prolonged the larval duration in all concentrations tested (Table 1). The percentage increase in larval duration was 5 and 59 at the concentrations of 1% and 5%, respectively. The effect of various types of organic extracts on the developmental period is summarized in Table 2. Most treatments prolonged the larval duration. Chloroform extract induced a prolongation of larval duration, ranging from 30% to 81%, 31% to 75%, 7% to 134% and 34% to 37% of the first, second, third and fourth larval instars, respectively. However, for acetone and ethanol extracts, clear and consistent prolongation of larval duration was associated with the lower concentrations tested (10% and 5%). Their respective percentage increase in larval period ranged from 24 to 76 for acetone extract and 10 to 85 for ethanol extract. Data in Table 2 generally show that the 4^{th} larval instar appears least affected by all extracts compared with the first three.

The results of the feeding activity of larvae treated with the three extracts indicated that acetone and ethanol extracts had superior activity than chloroform extract (Table 3). All treatments were significantly different from the control, except chloroform extract especially at lower concentrations. At three days post treatments, the feeding rates ranged from 2.11% to 49.86%, 0.33% to 2.62% and 0.007% to 0.38 for chloroform, acetone and ethanol extracts, respectively. The feeding inhibition was positively related to dose.

The results of phytochemical analysis (Table 4) indicate the presence of alkaloids, physostigmine and flavenoids in all extracts although their levels varied, with ethanol extract showing higher contents of alkaloids. All extracts appeared to have no sterols, while saponins and tannins were

detected in acetone and ethanol extracts. Triterpins were detected in the ethanol extract only. Thin layer chromotography (TLC) indicated the presence of trigonelline in acetone and ethanol extracts, while chloroform extract was devoid of it.

DISCUSSION

Lower concentrations of organic extracts of fenugreek seeds prolonged the larval period of all instars. The prolongation was mostly clear in the late instars than in the early ones. This could be due to the reduced tolerance of early instars resulting in their death rather than any growth regulatory effect. The high sensitivity of early instars may also be explained by their thinner cuticle compared with late instars and/or their increased total body surface area which allow more uptake. The effect of fenugreek seed powder and/or extracts on growth of insects was also reported by Barakat *et al.* (1984) and Pemonge *et al. (*1997) who mentioned that fenugreek seed powder and/or extract reduced the longevity of *Tetranychus unticae* Koh and *Acanthoscelides obtectus*. This apparently disagrees with the current findings; the discrepancy from their findings may be explained by the differences in the insect studied and/or method of extraction and bioassay.

Organic extracts of fenugreek seeds greatly reduced the feeding rates of the test insect, with acetone and ethanol extracts being superior to chloroform extract. The order of efficacy can be explained by the high content of tannins and alkaloids in these extracts. Further, these findings indicate that the lethal effect of ethanol and acetone extracts on *H. elaterii* may occur as a result of starvation, beside the direct toxic effects. The lethal effects associated with these fractions may be explained by their higher content of physostigmine, a well known carbamate natural ester with insecticidal activity (Matsumura 1985). Intercropping of fenugreek with musk melon reduces the pest population on the latter as previously reported (Mahmoud and Abdelbagi 2003).

The results indicated that chloroform extract contained alkaloids, flavonoids and trace of physostigmine, while acetone extract contained alkaloids, physostigmine, saponins, tannins, anthraquinone glycosides and

flavonoids. On the other hand, ethanol extract contained alkaloids, physostigmine, saponins, tannins, anthraqoinone glycosides and triterpens. These findings generally agree with those of Afifi *et al*. (1988). The slight discrepancies from their result may be attributed to differences in the analytical procedure and /or fenugreek variety tested.

The current results indicate the promising potential of fenugreek as pest control agent. Its low mammalian toxicity, being a table human food (FAO 1988), supports this argument. Future lines of research should include detailed chemical analysis to identify the active compounds in the extracts and evaluation of the purified extracts against other insect pests in laboratory and field trials.

Table 1. Larval duration (days) of the African melon lady bird beetle (*H. elaterii*) continuously fed on pumpkin leaves treated with aqueous extract of fenugreek seeds

Concentration (%)	Larval duration (days)	Percentage increase in larval duration
5.0	23.88	59.20
2.5	17.88	19.20
1.0	15.67	04.47
0.0	15.00	-
SE±	0.60	-
LSD (0.05)	1.86	-

Table 2. Larval duration (days) of the African melon lady bird beetle *(H. elaterii*) fed on pumpkin leaves treated with organic extracts of fenugreek seeds

Conc. (%)	Chloroform				Acetone				Ethanol			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
30	3.25	4.25	3.50	2.75	1.00	1.50	2.00	4.25	2.00	3.00	3.00	3.25
	(30)	(32)	(8)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
20	3.75	5.00	7.25	3.50	1.00	1.50	3.00	3.25	2.75	2.75	3.50	3.75
	(50)	(55.)	(123)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(4)	(-)
10	5.25	5.75	8.75	7.25	4.77	2.50	5.50	6.25	4.25	3.50	5.00	6.50
	(110)	(78)	(169)	(35)	(36)	(-)	(76)	(30)	(36)	(4)	(48)	(24)
5	4.54	5.67	7.63	7.38	4.33	3.00	5.00	6.50	5.00	6.25	5.00	5.75
	(82)	(76)	(135)	(37)	(24)	(-)	(60)	(22)	(60)	(85)	(50)	(10)
0.00	2.50	3.23	3.25	5.38	3.50	3.50	3.13	5.35	3.13	3.38	3.38	5.25
SE±	0.44	0.35	0.54	0.31	0.31	0.46	0.23	0.36	0.44	0.34	0.23	0.32
LSD	1.34	1.05	1.62	0.94	0.66	1.39	0.69	1.09	1.33	1.01	0.69	0.95

Number between parentheses is the percentage increase in duration

Table 3. Daily feeding rate (%) of African melon lady bird beetle *(H. elaterii*) fed on pumpkin leaf disc treated with organic extracts of fenugreek seeds

Conc. (%)	Chloroform			Acetone			Ethanol		
	1	2	3	1	2	3	1	2	3
30.0	1.58	2.03	2.11	0.55	0.33	0.11	0.60	0.03	0.007
20.0	20.90	21.94	23.67	0.63	0.28	0.17	0.61	0.02	0.04
10.0	30.27	49.14	46.45	1.56	1.30	0.38	1.09	0.21	0.14
05.0	38.93	49.93	49.86	2.56	2.62	0.90	1.18	0.25	0.38
0.0	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
SE±	02.27	03.62	05.34	00.42	00.40	00.16	00.16	00.12	00.04
LSD (0.05)	07.16	11.39	16.84	01.30	01.27	00.52	00.47	00.36	00.12

Table 4. Phytochemical analysis of organic extracts of fenugreek seeds

Extract	Chemical groups								
	Alkaloids	Physostigmine	Saponins	Tannins	Anthraquinone glycoside	Sterol	Triterpenes	Flavonoids	Flavone and flavonon
Chloroform	+	+	_	_	_	_	_	+++	_
Acetone	++	+	+	+	+	_	_	++	+++
Ethanol	+++	++	+++	+++	++	_	+	+	+
Aqueous	++	+	++	++	_	_	_	+	_

_ Negative
\+ Slightly positive
++ Moderately positive
+++ Strongly positive

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مستخلصات بذور الحلبة كمنظم للنمو ومانع لتغذية خنفساء القرعيات الافريقية *Henosepilachna elaterii* (Rossi)

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المستخلص : هدفت هذه الدراسة الي بحث تاثير مستخلص بذور الحلبة على نمو و تغذية خنفساء القرعيات الافريقية *Henosepilachna elateri* (Rossi).
تم استخلاص بّذور الحلبة (*Trigonella feounum-graceum*) بالماء المقطر والمذيبات العضوية (الكلوروفورم والاسيتون و الايثانول)، وجربت المستخلصات علي خنفساء القرعيات الافريقية تحت ظروف المعمل. اظهرت النتائج أن المستخلصات المائية والعضوية لبذور الحلبة لها تاثير معنوي كمنظم لنمو الطور اليرقي، حيث ادت لزيادة معنوية في طول عمر اليرقات. وكان تاثير المستخلصات كبير في الاطوار اليرقية المتقدمة عمريا عن الاطوار اليرقية الصغيرة. اظهرت المستخلصات العضوية تاثيرا قويا كموانع للتغذية على الطور اليرقى الرابع حيث تناسبت كمية الغذاء المستهلك عكسيا مع الجرعه. وقد كان تاثير مستخلصات الاسيتون والايثانول اكثر من مستخلص الكلوروفورم. كذلك شملت الدراسة التعرف علي المجموعات الكيميائية في المستخلصات المختلفة. نوقشت العديد من الجوانب المتعلقة بالفعالية على هذه الحشره.

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