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Biological activity of extracts of *Tephrosia nubica* (Boiss) Baker against *Spodoptera littoralis* (Boisd.)and *Agrotis ipsilon* (Hufn.)

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Abstract

Keywords: *A. ipsilon*, Biological activity, *S. littoralis*, plant extracts, *T. nubica*.

The biological activity of *Tephrosia nubica* extracts was tested against the cotton leaf-worm, *Spodoptera littoralis* and greasy cutworm, *Agrotis ipsilon*. The methanolic and chloroformic extracts induced serious chronic effects on the larvae, pupae and adults. They reduced percentages of pupation and moths emergence, prolonged larval and pupal periods, caused reductions in egg production and egg hatchability and increased percentage of sterility. Phytochemical, chromatographic and spectral analysts of the methanolic extract, which was the most effective one, revealed the presence of three flavonoidal glycosides (kaemferol 3,7-dirhamnoside, quercetin 3-galactoside 7-rhamnoside and quercetin 3,7-dirhamnoside), rotenones and deguelin. However, four prenylated flavones (semiglabrin, Pseudosemiglabrin, apollinine and lanceolatin A) were isolated from the chloroformic extract.

1 Introduction

Several naturally occurring plant insecticides, such as nicotine, rotenone and pyrethrin, had been investigated as highly toxic for pest control. However, the plant kingdom is a rich source of chemicals that may prove useful in this respect. These chemicals must be active against specific organisms, biodegradable to nontoxic products and suitable for use in integrated pest management. Thus many investigators have made large screening efforts for plants that have physiological effects on pests (McMillan et al. 1969, Jacobson et al. 1978; Rembold et al. 1980 and Foon and Guany, 1984).

The leguminous herb *Tephrosia nubica* is wildly found in Egypt at the border between Egypt and Sudan. It is used as an insecticide, fish poison and in folk medicine (Ammar and Jarvis, 1986). The present work aimed to investigate the biological activity of *T. nubica* extracts against *Spodoptera littoralis* and *Agrotis ipsilon* and to identify the chemical constituents responsible for such activity.

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2 Materials and Methods

2.1 Plant material :

Samples of *Tephrosia nubica* were collected from Gabal Elba and authenticated by Dr. Loutfy Boulos, Professor of Taxonomy at the National Research Centre, Cairo.

2.2 Experimental apparatus :

UV spectra were determined in MeOH solution on a Perkin-Elmer model 552 spectrophotomter. IR spectra were determined in $CHCL_3$ on a Perkin-Elmer model 183 spectrophotometer. ^{1}H-NMR spectra were determined in deuteriochloroform on an IBM-200 MHz spectrometer with tetramethylsilane as an internal standard. Mass spectra were determined on a VG 707E mass spectrometer. Column chromatography was used on silica gel 60 F_{254} 60.25 mm. thickness, E. Merck, Darmstadt, Germany.

2.3 Insect breeding :

Susceptible strains of *S. littoralis* and *A. ipsilon*, which were reared for several years under laboratory conditions away from insecticidal contamination, served as test insects. The larvae were fed on castor leaves, reared and used in assaying the extracts at $27 \pm 2^{\circ}C$.

2.4 Extraction and isolation procedures

150g of the dried powdered material was defatted in a continous extraction apparatus with petroleum ether. The defatted powder was successively extracted with chloroform then 70 % methanol in Soxhlet apparatus. The extracts were concentrated under a low pressure and the resulting residues were subjected to a preparative column chromatography using silica gel as an adsorbent, the column was eluted with chloroform, followed by chloroform containing gradient amounts of methanol. The collected fractions were further purified by a preparative TLC and finally by passing over Sephadex LH-20 in methanol. The isolated compounds were identified by physical and spectral analysis (mpt. TLC, IR, UV, ^{1}H-NMR, 2D-NMR and MS). The obtained data were compared with published data (AMMAR AND JARVIS, 1986).

The glycosides were hydrolysed to their respective aglycones and sugars with 2 NHCl, which were identified by comparison with authentic samples.

2.5 Ovicidal test :

Egg masses (1,2 and 3-days old) were dipped for 10 seconds in 5 and 10 % solvent extract in water mixed with one drop of treton X 100 as an emulsifier. The extract was at first diluted with acetone 1 ml/g extract. Control eggs were treated with water and treton X 100 only. Five hundred eggs were used for each test and percentage of egg unhatchability was recorded l8 h after hatching of untreated eggs.

2.5 *Larvicidal test*

Fresh castor leaves were dipped for 10 seconds in 5 % extract in water mixed with treton x 100 left to dry and offered to fourth instar larvae. for 24 h. Five replicates with 5 larvae each were used for each treatment and mortality was recorded after 48 h Abbot's (1925) formula was used for corrected mortalities.

2.6 *Antifeeding test*

Leaf disks (3.5 cm^2) of castor were dipped for 10 seconds in 5 % extract in water with two drops of treton X 100. Water with treton X 100 was used as a control. The disks were left until the solvent had evaporated. Each disk was offered to one fourth instar larva in a petri-dish (7.5 cm^2). After 24 h, the disk was removed and weighed to determine the percentage consumed by each larva. Ten larvae were used for each test. The antifeeding activity of the tested extracts was calculated by using the formula of Saleh et al. (1986) as follows :

$$\text{Antifeeding activity} = 1 - \frac{\text{\% of treated disk eaten}}{\text{\% of untreated disk eaten}} \times 100$$

2.7 *Biotic potential test*

Second instar larvae were left to feed for 24 h on castor leaves treated with 5 % tested extracts by dipping for 10 seconds. Then the survivors were allowed to complete their life cycle in glass jars containing untreated castor leaves. In case of *A. ipsilon* third instar larvae were separated individually to avoid cannibalistic habits. Results obtained in all treatments were compared with those of the control. Percentage of pupation, pupal weight and percentage of moths emergence were recorded. Larval and pupal periods, percentage of egg hatchability, fecundity and sterility of females were also estimated. Percentage of sterility was calculated by using the formula modified by Toppozada et al. (1966) as follows :

$$\text{\% Sterility} = 100 - \frac{a \times b}{A \times B} \times 100$$

Where: a = Number of eggs laid/female in treatment; b = Percentage of egg hatchability in treatment; A = Number of eggs laid/female in control and B = Percentage of egg hatchability in control.

3 Results and Discussion

3.1 *Ovicidal action*

Data in Table 1 shows clearly that the methanolic extract was the most effective one against eggs of *S. littorlais*, followed by the chloroformic and lastly the petroleum ether extract. There was a proportional correlation between the percentage of egg unhatchability and the concentration used. Three-days old eggs were the most affected, while

1-day old ones were the least affected. This finding may be due to the inhibitory effect of methanol extract on emberyonic development.

Table 1: Effect of extract of *T. Nubica* on egg unhatchability of *S. Littoralis*.

Solvent extract	Age of eggs (days)					
	Concentration (%)					
	1		2		3	
	% Egg unhatchability					
	5	10	5	10	5	10
Pet. Ether	80	100	35	45	30	60
Chloroform	10	20	10	40	20	60
Methanol	10	20	30	50	30	70
Control	0	0	0	0	0	0

The same previous trend could be applied to the eggs of *A.ipsilon* (Table 2). However, the chloroformic extract was the most effective one, followed by the methanolic then the petroleum ether extract.

Table 2: Effect of extract of *T. Nubica* on egg unhatchability of *A. ipsilon*.

Solvent extract	Age of eggs (days)					
	Concentration (%)					
	1		2		3	
	% Egg unhatchability					
	5	10	5	10	5	10
Pet. Ether	30	80	40	65	5	20
Chloroform	0	10	10	20	30	50
Methanol	0	2	5	10	10	30
Control	0	0	0	0	0	0

The methanolic and chloroformic extracts were more effective on eggs of *S. littoralis* than on *A. ipsilon*. The percentages of unhatched 3-days old eggs of *S. littoralis* were 60 and 70 % at 10 % chloroform and methanol, respectively, while the corresponding values were *50* and 30 % for those of *A. Ipsilon*. El-Sayed (1982-1983) found that the viability of *S. littoralis* eggs was reduced as a result of dipping them in aqueous suspension of the neem seeds *Azadirachta indica* A. Juss. About a rate of 90 % unhatched eggs was obtained when eggs were treated with 2 % of suspension. There were no differences between 0-24 h and 24-48 h old eggs in their response to neem treatment.

3.2 Larvicidal. action :

Table 3 shows that the chloroform or methanol extract caused only 30 % larval mortality for *S. littoralis*, while they induced little toxic effects on *A. ipsilon*.

Saxena et al. (1974) found that the minimum dosage of a petroleum ether extract of *Tephrosia purpurca* Def. seeds required for 100 % mortality was 5 X 10^4 g/cm^2 for *Musca domestica* (L) and *Sitophilus oryzae* (L.) 7.8 x 10^2 g/cm^2 for *Aedes aegyptii* (Linn.) and 7.1775 g/cm^2 for *Tribolium castaneum* (Herbst.).

Table 3: Mortality percentage of fourth instar larvae of *S. Littoralis* and *A. Ipsilon* fed for 24 h on castor leaves treated with extracts of *T. nubica*

Solvent extract	% Mortality	
	S. littoralis	*A. ipsilon*
Pet. Ether	5	0
Chloroform	30	10
Methanol	30	5
Control	0	0

3.3 Antifeeding action

All extracts caused antifeeding activity against the two insect species (Table 4). Extracts of petroleum ether, chloroform and methanol caused 35, 60 and 64 % antifeeding for *S. littoralis*, respectively. The corresponding percentages for *A. ipsilon* were 60, 80 and 80 %.

Table 4: Antifeeding activity of extracts of *T. Nubica* against *S. Littoralis* and *A. Ipsilon*

Solvent extract	% Antifeeding activity	
	S. littoralis	*A. ipsilon*
Pet. Ether	35	60
Chloroform	60	80
Methanol	64	80
Control	0	0

Bentley et al. (1987) isolated two antifeedants, tephrosin and isopongaflone, from *Tephrosia elata* Deffers. Tephrosin displayed high activity against the African crop pest, *Spodoptera exempta,* whereas isopongaflone was very active against *Maruca testulalis* and *Ebdana saccharina*. Rotenone was also an active antifeedant against these insects.

Lwande et al. (1985) isolated hildecarpin from the healthy roots of *Tephrosia hildebrandtii*, (Vatke) . This compound is petrocarpan phytoalexin formed as a result of

microbial infection of the cowpea and may constitute a basis for induced resistance in the plant against *Maruca testulalis*. Hildecarpin proved to be an antifeedant against this insect.

3.4 Biotic potential action

Table 5 shows that larval treatment with 5 % petroleum ether, chloroform or methanol extract of *T. nubica* induced serious chronic effects on the larvae, pupae and adults of *S. littoralis* and *A. ipsilon*. The most effective extract was the methanolic, followed by the chloroformic then the petroleum ether extract. All extracts reduced significantly the percentage of pupation and moth emergence, prolonged larval and pupal periods caused significant reductions in egg production and egg hatch-ability and increased percentage of sterility.

Table 5: Effect of extract of *T. Nubica* on the biotic potential of *S. Littoralis* (*S.i*) and *A. Ipsilon* (*A.i*)

Biological criterion	Solvent extracts						Control	
	Pet. ether		Chloroform		Methanol			
	S.i	*A.i*	*S.i*	*A.i*	*S.i*	*A.i*	*S.i*	*A.i*
	Means ± S.E.							
% Pupation	70	60	50	50	60	60	85	90
Pupal weight (mg)	215 ± 0.27_B	220 ± 1.44_b	155 ± 1.15_C	110 ± 0.14_c	110 ± 0.22_C	150 ± 0.37_c	345 ± 2.15_A	350 ± 1.14_a
% moths emergence	50	60	30	50	30	20	95	80
Laval duration (days)	19 ± 0.15_B	15 ± 0.22_b	25 ± 0.22_C	20 ± 0.21_c	29 ± 0.12_D	25 ± 0.28_d	10 ± 0.37_A	11 ± 0.55_a
Pupal duration (days)	7 ± 0.21_A	8 ± 0.12_a	8 ± 0.17_B	8 ± 0.22_a	8 ± 0.22_B	6 ± 0.12_A	6 ± 0.12_A	8 ± 0.22_a
No. of eggs laid ♀	340 ± 1.12_B	115 ± 1.35_b	0	62 ± 1.01_c	0	30 ± 0.02_c	1125± 2.18_A	220 ± 1.13_a
% egg hatchability	60	40	0	20	0	50	95	80
% moth sterility	81	96	0	99	0	99	0	0

Means followed by the same letter in horizontal rows are insignificantly different (P>0.05) Duncan's multiple range test.
A, B, C, D: letters for *S. Littoralius*; a, b, c, d: letters for *A. ipsilon*

Bahai El-Din (1987) showed that larval treatment with ethyl acetate extract of *Saliconia fruticosa* (L.) and *Tamarix tetragyna* (Ehrenb) induced significant reduction in fecundity and a high degree of sterility of the resulting female moths of *S. Littoralis*, *S. fruticosa* and *T. tetragyna* with a LD_{50} induced 34.4 and 43 % reduction in egg output, and 72 and 74.1 % sterility respectively.

The phytochemical, chromatographic and spectral analysis of *T. nubica* revealed the presence of flavones and isoflavones. Four prenylated flavones, semiglabrin, pseudosemiglabrin apollinine and lanceolatin-A were detected in the chloroformic extract. However three favonoidal glycosides (Kaemferol 3, 7-dirhamnoside, quercetin 3,7-dirhamnoside and quercetin 3-galactosdie 7-rhamnoside), rotenone and deguelin were isolated from the methanolic extract.

Previous studies on *T. nubica* revealed the presence of prenylated flavones (AMMAR AND JARVIS, 1986) and glycosides of kaemferol (AMMAR et al., 1992).

Kiuchi et al. (1989) identified lanceoiatin-B, Toxicarol, deguelin, tephrosin, 0-methyl obovatin and dehydrodeguelin from the aerial part of *Tephrosia purpurea*. However, they isolated pongamol, rotenone, rotenolone, methyl pongamol and flavanones from the root.

Ehriich and Raven (1964) concluded that flavonoids and other secondary substances play the leading role in determining the plants, upon which the larvae feed. Therefore, co-evolutionar patterns have developed making a given flavonoid attractive to on insect, repugnant to another, toxic to a third and completely uninteresting to the rest.

Flavones and isoflavones are considered to be toxic to insects (ANJANEXULU AND RAMACHANDRAROW, 1964). It was postulated that a pyrone ring containing the structure Co-C=C-0 is a toxophore (WILLAMAN, 1955). The toxicity of rotenoids, rotenone and deguelin on insects has long been known (HARBORNE AND MABRY, 1982). Rotenoids were widely employed at one time as useful insecticides because of their low toxicity to man (HARBORNE 1973). Toxic activity is almost due to the resemblance in structure between rotenoids and steroidal saponins. The isoflavonoid molecule shows some correspondence in molecular shape with the steroid nucleus and therefore, certain isoflavonoids are physiologically active. (EHRLICH AND RAVEN, 1964).

Rotenone and deguelin are principal insecticidal constituents, which are toxic to many insects. They are used as potent insecticides grubicides have been used for domestic manage and rotenone may be considered as a precursor in the synthesis of insecticides of other prenylated flavones (HARBORNE, 1982).

The rotenoids are also extremely potent inhibitors of mitochondrial oxidation in insects (FUKAMI AND NAKAJIMA, 1971). Rotenone degradation in house flies proceeds, at least in part, through the same pathway in vitro. It may be concluded that *T. nubica* proved to possess a potent insecticidal activity against *S. littoralis* and *A. ipsilon* due to presence of flavones and isoflavones.

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