قسمه: العقاقمير - كلية الصيدلة - جامعة أسيوط. رئيس القسم: أ.د/ أحمد عبد الرحمن علمي.

د راسة التركيب الكيميائي لزيت بذرة نبات بود رة العفريت (ستركيوليا د ايفرسيفوليا د ون ) وتأثيره على انتاج ونوعية البيض في الد جاج )

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تمت دراسة زيت البذرة انبات بودرة العفريت وتبين أنه يحوى أحماض دهنية من نوع السيكلوبروبين (أحماض المالفاليك) وقد تم تعيين نسبتهما في الزيت بطريقتين لونيه ووجد أنها في حدود ٢٠٩٢ الي ٢١٠١٢ بالسيون.

وقد تبين من دراسة البيغى الموضوع من الدجاج بعد تغذيتها بعليقة تحوى نسب مختلفة ومحددة من الزيت أو المح تحول الى اللون الزيتونى وظهر لون محمر في الزلال وكذلك تغييرات في الاس الهيد روجيني ونقص واضح في كمية البيض بالاضافة الى ارتفاع نسبة موت الأجنة ، أما في الديروك فأن معدل الخصوبة قد تناقص.

وتعـــزى كل هذه التغييرات الى وجود أحماض د هتية من نوع السيكلوبروبين في الزيت ولهذا ينصح الباحثان بعدم تواجد مزارع د واجن في أماكن تنموبها أشجار هذا النبات.

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# THE COMPOSITION OF <u>STERCULIA</u> <u>DIVERSIFOLIA</u> DONN SEED OIL AND ITS EFFECT ON EGG PRODUCTION OF HENS

(With 3 Tables)

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#### SUMMARY

Sterculia diversifolia Donn seed oil was prepared and subjected to chemical and biological studies. The chemical study showed the presence of cyclopropene fatty acids in addition to other unsaturated ones. Malvalic acid, the cyclopropenoid fatty acid, was prepared in a pure form. Quantitative determination by colouremetrie and titremetric methods showed that the cyclopropene acids are present in a concentration ranging from 9.979 - 10.12% in the seed oil.

The biological study was achieved on Fayoumi hens and cocks which recieved rations containing 3%, 1.5% 0.5% and 0.0% of the seed oil. A significant and parallel decrease in the egg production was noticed. The laid eggs showed pink whites, olive-green yolks and changes in consistency and pH. The embryonic mortality rate was observed where it ranged between 97 to 100% after 14 or 21 days of incubation of the eggs.

The fertility rate of the male birds remained within normal limits.

#### INTRODUCTION

Sterculta diversifolia Donn (Brachychiton populneum R. Br., Kurajong, bottle tree) is a tree belonging to the Family Sterculiaceae, Order Malvales (BENSON 1970). It was recorded that ingestion of plants of this order to hens resulted in disorders in the laid eggs. These are manifested by the bronze of olive-green colours in the yolks and pink or red colours in the whites (SHERWOOD 1928, LORENZ et al., 1933 & SHENSTONE et al., 1965). RASCOP et al., (1966) noticed that a follicular development, expansion of the sinuosides, fatty infiltration of the corpora lutea and a decrease of the uterus size were produced. They added that reproduction was inhibited completely. LORENZ (1939) FAURE (1956), MACFARLANE et al., (1957) cited that the oils of these plants gave positive Halphen colour test indicating the presence of cyclopropene fatty acids as components of their structures. Sterculic and Malvalic acids are the only fatty acids known to contain a cyclopropene ring in their structures (FAURE 1956, SMITH and Bû LOCK 1965, NUNN 1952, FAWCETT and SMITH 1960). These acids have the following formula:

$$CH_3$$
 -  $(CH_2)_7$  -  $C$   $CH_2$   $C$  -  $(CH_2)_7$  -  $COOH$  Sterculic acid  $CH_3$  -  $(CH_2)_7$  -  $C$   $CH_2$   $C$  -  $(CH_2)_6$  -  $COOH$  malvalic acid

These cyclopropene fatty acids constitute about 50-70% of the seed oils of <u>S. Foetida</u> L. which is a tropical tree and some other species of the same genus and order (NUNN 1952, FAWCETT and SMITH 1960, SHENSTONE <u>et al</u>, 1965, SMITH and Bû LOCK 1965, SHENSTONE and VICKERY 1961, BADAMI <u>et al</u>, 1981).

In the present investigation the seed oil of <u>Sterculia diversifolia</u> Donn cultivated in Egypt (Assiut) was studied by both chemical and biological methods to get an idea about its composition, both quantitative and qualitative effect on eggs laid by hens fed this oil, and possible lethal effect on embryos of incubated eggs.

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# MATERIAL and METHODS

#### I- Preparation of the oil:

The mature seeds of S. diversifolia were collected from trees cultivated in public gardens at Assiut and identified (The late Prof. Dr. F.Y. Amin, Professor of Floriculture and Horticulture, Fac. of Agriculture, Assiut Univ.). The seeds were air - dried and powdered to No. 40 powder. Two kg. powder were exhaustively extracted in a continuous extraction apparatus with petroleum - ether (60-80°C). The obtained extract was concentrated under reduced pressure at 40°C, where an orangeyellow clear oil was obtained (400 g.).

## 2- Chemical study of the oil concerning the cyclopropene fatty acids:

100 g. of the prepared oil were saponified for 2 hours with N potassium hydroxide at 40°C. The free fatty acids were extracted with ether, after meutralisation, and dryress over anhydrous Sodium sulphate in a dessicator. The obtained residue (6.5 g.) was dissolved in the least possible amount of petroleum-ether (40-60°C) and kept at - 20°C for 10 hours, where a precipitate was obtained. Filtration was done at - 20°C in a sentred glass funnel and the filtrate obtained was found to contain the cyclopropene fatty acids (identified by Halphen test.), together with some linoleic and linolenic acids (paper chromatography, What man no.1 impregnated with paraffin, solvent system: 90% glacial acetic acid; the spots of fatty acids were located as reddish - brown spots on a rose baxkground by immersion of the dry chromatogram in 1% aqueous copper acetate solution for 20 minutes, washing and again immersion in 5% aqueous potassium ferrocyanide; R values: oleic 0.64, linoleic 0.78; and a tailed very faint spot of a mixture of components (SMITH 1960, LEDERER 1959). Further cooling and maintainance of the filtrate at lower temperature gave a small residue. The process of cooling and filtration was repeated several times until an amorphous yellowish-white substance was obtained melting at 10 to 10.5°C and gave positive Halphen test and corresponded to malvalic acid (MACFARLANE et al, 1957).

# 3- Estimation of cyclopropene fatty acids in s. diversifolia seed oil:

# a) Purification of the oil:

20 g. of the oil were dissolved in an equal volume of petroleum-ether and passed through a column 50 g. alumina. 100 ml. of the solvent were used for washing the column and lastly it was removed by distillation under reduced pressure.

## b) Colourimetric method for estimation of the cyclopropena acids:

The method described by SHENSTONE and VICKERY (1961), depending on the Halphen colour reaction, was applied with some modifications. A crimson-red colour having a maximum absorption at 515 mm was given by three different samples of the oil and malvalic acid. The developed colour was measured at this wavelength and compared with those of a standard calibration curve obtained from malvalic acid under the same conditions.

#### c) Titremetric method:

The method of SMITH et al, (1960) was adopted. The solution of the purified oil in benzene (1.5 g. in 10 ml.) was titrated directly at 55°C against standard solution of hydrogen bromide in glacial acetic acid. The cyclopropene acids were calculated as malvalic acid.

# 4- Feeding course:

A total of 80-seven months old Fayoumi hens and 12 cooks were supplied by the Faculty of Agriculture, Assiut Univ. The birds were divided into four equal groups: A,B,C and D. The first three groups were fed balanced rations containing 3, 1.5 and 0.5% prepared <u>S. diversifolia</u> seed oil respectively. Group D was considered as control and recieved the ration only. The feeding course was continued for 45 successive days (August to mid September).

Egg production was recorded during the course and for successive 31/2 months (table 1). The quality and PH of both egg yolk and white were observed (table 2), beside their ratio in relation to each other in half the amount of the laid eggs.

The other half was collected directly and incubated. The embryonic mortality was recorded at 7th, 14th and 21 days of incubation.

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The fertilization rate of the males was observed.

#### RESULTS

It is indicated by the chemical study that <u>S. diversifolia</u> seed oil contains the cyclopropene fatty acids, malvalic and sterculic, which were isolated as a mixture under low temperature. From this mixture, malvalic acid could be obtained in a little amount, as a pure substance. Estimation of the cyclopropene acids was accomplished by both colouremetric and titremetric methods. The reusults obtained from the colouremetric method ranged between 10.1 to 10.12% W/W and that of the titremetric method from 9.972 to 9.979% W/W.

A significant and parallel decrease in egg production was noticed during and after the feeding course (table 1). Moreover, changes in the qualities of both egg whites and yolks took place. Table 2 shows that "pink white" eggs were produced by the groups recieving 3% and 1.5% <u>S. diversifolia</u> seed oil in their ration. Meanwhile, these two groups of hens laid eggs with olive-green, pasty or semisolid yolks. Egg size, weight and ratio of yolk to white were not affected by feeding of <u>S. diversifolia</u> seed oil in different concentrations.

The PH values converged rather rapidly, from 6.2 to 8.7 in yolk and from 8.6 to 7.9 in white (table 2).

A complete mortality (100%, table 3) of the embryos was noticed after 14 days of incubation of the eggs.

The fertelization rate of males was not affected by the presence of <u>S. diversifolia</u> seed oil in the ration. The rate ranged from 80-85% in all the groups which is within the normal.

### DISCUSSION

It has been recorded that ingestion of plants of the order Malvales cause disorders in eggs laid by hens as well as physiological disturbances (LORENZ et al, 1933, SHERWOOD 1928, RASCOP et al, 1966). These disorders and disturbances were attributed to the presence of cyclopropenoid fatty acids in the dietary lipids (LORENZ 1939, RASCOP et al, 1966).

The present study deals with <u>Sterculia diversifolia</u> Donn seed oil which belongs to the order Malvales. The presence of cyclopropene acids, malvalic and sterculic, was indicated by the positive Halphen test and confirmed by the isolation of one of them. A sample of malvalic acid was difficultly obtained in a pure form under the available laboratory conditions. Quantitative estimation of these acids in the oil by colouremetric method (SHENSTONE and VICKERY 1961) and titremetric method (SMITH et al., 1960) showed that they are present in the range of 0.972 10.12% W/W in the oil. ALI et al., (1982) studied the seed oil of <u>S. diversifolia</u> Donn and gave no record about the presence of the cyclopropenes. The conditions under which the oil was studied by them was drastic for these labile acids. Moreover, the high proportion folinoleic acid (80%) impaired their isolation and identification by the normal ways.

The production of "pink white" eggs, the olive-green colouration of the yolks, the changes in PH of the eggs beside the significant decrease in their production and the high embryonic mortality rate are all attributed to the biologically-active cycloproene fatty acids in the seed oil (LORENZ et al., 1939, RASCOP et al., 1966).

SHAIBLE and BANDEMER (1946) suggested that the active principles causing the pink white disorders in eggs had a specific effect in increasing the permeability of the vitteline membrane which could account for the uptake of water by the yolk and the changes of the pH values of both yolk, and white. They added that the pink whites are caused by iron difusing from the yolk and chelating with conalbumin in the white is caused by the cyclopropenoids. However, KIRCHER (1964) suggested that the cyclopropenes react with sulfhydryl groups in the physiologically active proteins causing these diverse biological effects.

We recommend here, as a result of this investigation, that the cultivated <u>S. diversifolia</u> Donn could be considered as one of the plants that their oil contains the cyclopropene compounds. It must not be cultivated at any place or farm where poultry breeding is concerned with, to avoid the forementioned complications.

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Table (1)
Egg Production Per 20 hens per month\*

Group Month	A	В	С	D
August	180	213	233	240
September	35	125	196	233
October	6	67	172	225
November	7	40	216	245
December	5	85	225	235

<sup>\*</sup> Egg production was observed also, during the next 31/2 months after the end of feeding course.

# STERCULIA DIVERSIFOLIA DONN PRODUCTION OF HENS

Table (2)
Percentage of embryonic mortality during incubation

Group of hens	Dose of oil (ml%)	Days of incubation				
		7	14	21	total	
A	3	88	12	-	100	
В	1.5	50	35	12	97	
C	0.5	30	70	-	100	
D	-	12	10	7	29	

Table (3)
Qualitative changes in Eggs

Group of hens	egg yolk			egg white		
	pН	colour	consistency	рН	colour	consistency
A	7.7 - 7.8	olive	Semisolid	7.9 - 8.2	pink	viscous
В	7.6 - 8.7	olive	pasty	7.9 - 8.3	pale pink	11
C	6.4 - 7.5	dark orange	viscous	8.2 - 8.8	colourless	и
D	6.2 - 7.2	yellow-orang	e "	8.6 - 9	"	11

