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## ANTIFUNGAL EFFECTS OF TANNIN-RICH PLANT (*ACACIA NILOTICA*) IN-VITRO AND IN-VIVO STUDIES

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### ABSTRACT :

This study was undertaken in a trial to evaluate the antifungal effect of tannins and tannin-rich plants. The research was conducted in two parts, the first one was *in-vivo* study where 25 male baladi goats were randomly classified into five groups (5 per each). The first group was left as a control while the second, third, fourth and fifth were received 5, 10, 15 and 20% *Acacia nilotica* leaves, respectively. Over one month, two faecal and ruminal samples were collected weekly to study the pH and total fungal count. The obtained results revealed that there was no effect of *Acacia nilotica* on pH of the ruminal or faecal samples. On the other hand, the total fungal count was drastically reduced only in the fourth and fifth groups that received 15 and 20% *A. nilotica*, respectively. However, the inhibition percentages of the ruminal fungal count were 12.7 and 43.3% in the fourth and fifth groups, respectively while they were 46.5 and 62.9% in the faecal matter in the fourth and fifth groups, respectively. The second part was the *in-vitro* study where three concentrations of *Acacia nilotica* leaves viz. , 0.25, 0.50 and 0.75% were added to fungal suspension and the count was determined over two hours at 30 minutes intervals. Four fungal species including *Aspergillus fumigatus*, *A. flavus*, *A. niger* and *Fusarium solani* were tested. The results showed that there was no effect on all fungal species at 0.25%, while the inhibition was directly increased by the increase in concentration of *A. nilotica*. The results illustrated that inhibition was also directly proportional to the time. It was revealed that *A. flavus* was highly sensitive to *A. nilotica* where its inhibition percentages were up to 77.5 and 89.1% at 0.50 and 0.75%, respectively, followed by *Fusarium solani* and *A. fumigatus*. On the other hand, *A. niger* was the most resistant fungal species to the tannin-rich plant where it was only inhibited by 24.0 and 68.4% at 0.50 and 0.75%, respectively. From the obtained results one can safely conclude that the tannin and tannin-rich plants have strong antifungal properties which are directly proportional with the concentration of tannins and their time of contact with the fungus. In this respect tannins could be used as a tool to reduce the fungal count and protect the animal from their direct harmful effect on its health.

## INTRODUCTION :

Colonization of microfungi in the rumen have an important implication in growth and health status of ruminants. Extremely slow growth rate of sheep in the summer and autumn was attributed [1], to the presence of pathogenic fungi among the ruminal flora of these animals.

Apart from the indirect effect of fungi by their mycotoxins on animal's health, they also have direct harmful effects on animal's tissues. Since fungi have the ability to grow on the feeding substrate, mouldy hay, straw and cereals are considered the main sources of ruminal and air fungi [2]. Since many fungal species can grow under the ruminal conditions, so, their presence in animal's rumen must be expected [3].

Plants produce a great variety of defensive chemicals which may give resistance to the fungal diseases [4]. There are many specific and non-specific biologically active substances that have the potential to inhibit fungal growth. These substances occur commonly as constituents of the plant tissues. Polyphenolic compounds particularly tannins constitute the major class of the secondary plant metabolites which have the antifungal characters. Protection of plant tissues against fungal attack was attributed to the presence of tannins. Presence of polyphenols, phenolic acids, aldehydes, quinones and other flavonoids gives the antifungal properties to the plant tissues [4]. Due to the heterogeneous distribution of polyphenolic compounds between different plants and within the different tissues of the same plant, they are thought to have profound effects on the distribution of fungal

species and structure of communities [5]. In 1947 Gilliver [6] tested water extracts of 1915 plant species and the results showed that about 23% contained antifungal substances. These antifungal compounds are particularly widespread in the woody plant species[7].

Natural plant tannins are amorphous, water soluble polyhydroxy phenolic compounds of molecular weight (500-3000 daltons). They contain sufficient number of hydroxyl groups (1-2/100 daltons) enabling the molecule to form an effective cross-linking with proteins and other macro-molecules under the appropriate conditions [8,9]. Tannins were classified into two main groups, hydrolysable and condensed forms [10,11].

The antimicrobial effects of tannins are attributed to their affinity to react with proteins and other macromolecules, as well as , membrane structure, microorganisms [8,12-14]. This occurs mainly , through hydrogen bonding between hydroxyl groups of tannins and free amino groups of the proteins [8-10,15]. Moreover, hydrogen bonds may be formed between hydroxyl groups of tannins with carboxyl groups of other polymers[16-19]. The antibacterial effects of tannins have been demonstrated [20,21,23]. Great efforts have been devoted to study the effects of chemically defined polyphenols of low and high molecular weight on the phyto-pathogenic fungal and disease development.

As early as 1911, Cook & Taubenhous[24], showed that 18 species of saprophytic and pathogenic fungal species were inhibited when crude extract of tannins were incorporated into the culture media. Subsequent studies

were undertaken to demonstrate the antifungal effect of tannins [4,25-32].

Mode of antifungal properties of tannins and other polyphenolic compounds can be attributed to their ability to inactivate respiratory enzymes and some extracellular enzymes of fungi [33-35]. Inhibition of extracellular cellulase, xylanases and pectinase by phenolics seems to be associated with the presence of enzyme -SH (sulfhydryl) groups. Normally (-SH) groups function to activate the enzyme but they tend also to react with oxidised phenols and so become inactivated [29]. Moreover, Goldstein & Swain [36], stated that the inhibitory actions of tannins are probably due to the readiness with which they tan or form complexes with extracellular enzymes. Since there is no available literature about the *in-vivo* effects of plant tannins on fungi of ruminal juice and faecal matter, this work was performed. Moreover, the work includes also an *in-vitro* effect of raw leaves of *Acacia nilotica* (tannin-rich plant) at different concentrations on most dominant fungal species demonstrated during the *in-vivo* studies.

## EXPERIMENTAL :

### Materials & Methods :

#### 1)- In-vivo studies :

##### A) Animals:

This work was conducted at the clinic of Veterinary Medicine, Assiut University where five groups (5 per each) of male balady goats (3-5 years of age with an average of 19-21 kg

body weight) were used. The animals were clinically examined to ensure their soundness before starting the experiment.

##### B) The plant :

The leaves of *Acacia nilotica* (as a tannin-rich plant) were freshly plucked, air dried and finally grinded.

##### C) Experimental procedures :

In order to determine the antifungal effect of the plant material, various amounts were added to the normal ration of the animals where the first group was left as a control throughout the experiment. The second, third, fourth and fifth groups received their rations with 5, 10, 15 and 20% of the prepared plant material, respectively.

##### D) Sampling schedules :

Two samples of the faecal and ruminal juice were collected weekly during the preliminary period as well as the experimental period. The samples were collected under complete aseptic conditions. About 90 g of faecal matter were collected in sterile plastic bags hanged to the hind quarters of each animal by clips. On the other hand, 50-100 ml of ruminal juice were collected in sterile flask by a sterile stomach tube. After calming down the animal, a sterile stomach tube was introduced through the animal's mouth to the rumen. However, the stomach tube was moved to and fro to obtain a representative sample from different strata of the rumen. The collected ruminal juice samples were immediately kept in the refrigerator for their mycological examination.

#### E) Mycological examination:

Basic dilutions (1:10) of faecal matters were prepared by addition of 10 grams of faecal matter to 90 ml sterile saline solution. The faecal matter was thoroughly mixed in the saline by using a sterile blender and then sieved through a sterile gauze. Moreover, 1:10 basic dilution of the ruminal juice samples was also prepared where 1 ml of the filtered juice was mixed with 9 ml sterile saline solution. Ten fold serial dilutions were prepared from both faecal and ruminal samples.

#### F) Fungal counting and identification :

For enumeration and identification of fungi, replicate plates of Sabroud dextrose agar medium (SDAM) were inoculated and were incubated at 25°C for 7-10 days. The growing colonies were identified and were counted according to their macro- and microscopic characters [2,37-42].

#### G) Determination of pH :

pH values were determined directly in the ruminal juice samples by using the pH meter (Orion pH meter model 250 A). On the other hand, pH values of 5% suspension of both faecal matter and ruminal juice samples (in distilled water) were also determined.

### 2)- In- vitro studies:

#### A) Fungal suspension:

The tested fungi (*A. niger*, *A. fumigatus*, *A. flavus* and *Fusarium solani*) were first inoculated onto SDAM (Difco) and incubated

at 25°C for two weeks, then the fungal growth was harvested by scraping the plate with sterilized spatula to minimize the amount of the agar carryover. The cells were immediately suspended in 20 ml sterile saline solution (0.85 NaCl, W/V). Sterile glass beads were added and the fungus was uniformly distributed on a rotatory platform shaker at 120 rpm for 15 minutes [30].

#### B) Antifungal effect of the raw plant material :

Three concentrations (0.25, 0.50 and 0.75%) were prepared from the *Acacia nilotica* leaves. The concentrations were prepared by addition of 50, 100 and 150 mg of the prepared plant materials to 19 ml of sterile saline solution and 1 ml of the fungal suspension. On the other hand, control test was prepared by addition of 1 ml of the fungal suspension to 19 ml of the physiological saline solution without plant material.

At time interval, fungal count was conducted by pour plate technique [43]. The inoculated plates were incubated at 25 °C for two weeks. Growing colonies were counted and the total fungal count was calculated as colony forming units (CFU)/ml (ruminal juice) and CFU/g (faecal matter). The inhibition percentages were calculated by the equation according to Klindworth et al.[44] :

$$\text{Inhibition \%} = (A-B)100/A$$

where :

A = Control plate count .

B = Test plate count .

## RESULTS AND DISCUSSION :

Data presented in table (1) revealed that, under conditions of the experiment the mean pH values of ruminal samples ranged from  $6.44 \pm 0.05$  to  $6.61 \pm 0.07$  (in the raw samples) while ranged from  $6.41 \pm 0.04$  to  $6.54 \pm 0.13$  (in 5% ruminal suspension). On the other hand, the pH values of the control samples were  $7.18 \pm 0.04$  (raw ruminal samples), and  $7.11 \pm 0.03$  (in 5% ruminal suspension), respectively. The obtained results showed that pH values of the ruminal liquor was within the normal range. These results more or less are in agreement with the results recorded [45-48], where the pH values of ruminal samples ranged from 5.5-7.9 under different rations. The slight fluctuation of pH values in our study may be attributed to some factors as stirring the samples, method of collection and type of the rations whether rich in carbohydrates or in nitrogenous feed [45, 49,50]. However, the mean pH values of 5% faecal samples were between  $8.19 \pm 0.07$  and  $8.24 \pm 0.08$ , while it was  $7.9 \pm 0.05$  in the control samples. The results revealed that there is no significant effect of the plant materials on pH values of the faecal or ruminal juice.

Aerobic fungi might be expected in the rumen because of their ubiquitous presence in animal's feed stuffs and the ability of some of them to grow anaerobically under the ruminal conditions [3]. Table (2) and Fig. (1) revealed that, the total fungal count in the ruminal

juice was reduced from  $1.2 \times 10^5$  in the control group to  $6.8 \times 10^4$ /ml in the fifth group. The count was higher than that recorded by Abdel-Salam [48], who found that the total fungal count in the ruminal juice was ranged from  $1.6 \times 10^4$  to  $3.9 \times 10^4$  according to the feed stuffs used. However, the total count was drastically reduced only in the fourth and fifth groups where the fungal inhibition was increased from 12.7% in the fourth group to 43.3% in the fifth group, while the count in the second and third groups was fluctuated. Moreover, the same results were recorded in the faecal matter where the total count was reduced from  $1.7 \times 10^5$  (control group) to  $6.3 \times 10^4$  in the fifth group with 62.9 inhibition percentage. A significant reduction of the fungal count was only showed in the fourth and fifth groups. The percentage of inhibition was increased from 46.5% (in the fourth group) to 62.9% (in the fifth group). This could be attributed to the tannins content incorporated in the rations performed to these animal's groups (table 2). This confirm the theory that antimicrobial effect of phenolic compounds are directly proportional to their concentration [30,51-53]. Fluctuation of the fungal count both in ruminal juice and faecal matter in the second and third groups could be attributed to the lower content of tannins. Since the source of fungi in lower intestine is from ruminal content, so reduction of their total count in the rumen by tannins will in turn reduce their count in the faecal matter.

Table (1): Mean pH values in both ruminal and faecal samples.

Animal groups	Examined Samples		
	Ruminal		Faecal
	Raw	5% Suspension	5% Suspension
1 <sup>st</sup> group (control)	7.18± 0.04	7.11±0.03	7.9±0.05
2 <sup>nd</sup> (5% <i>Acacia nilotica</i> )	6.59± 0.07	6.53±0.03	8.19±0.09
3 <sup>rd</sup> (10% <i>A. nilotica</i> )	6.61± 0.07	6.54±0.13	8.24±0.08
4 <sup>th</sup> (15% <i>A. nilotica</i> )	6.49± 0.04	6.46±0.06	8.22±0.08
5 <sup>th</sup> (20% <i>A. nilotica</i> )	6.44± 0.05	6.41±0.04	8.19±0.07

Table (2): Mean total fungal count in both ruminal and faecal samples.

Animal groups	Fungal count			
	Ruminal samples		Faecal samples	
	Count	Inhibition %	Count	Inhibition %
1 <sup>st</sup> group (control)	1.2x10 <sup>5</sup>	-	1.7x10 <sup>5</sup>	-
2 <sup>nd</sup> group	9.9x10 <sup>4</sup>	17.5	3.5x10 <sup>5</sup>	*
3 <sup>rd</sup> group	2.3x10 <sup>5</sup>	*	2.5x10 <sup>5</sup>	*
4 <sup>th</sup> group	9.4x10 <sup>4</sup>	12.7	9.3x10 <sup>4</sup>	45.3
5 <sup>th</sup> group	6.8 x10 <sup>4</sup>	43.3	6.3x10 <sup>4</sup>	62.9

\* There is no inhibition as the count was higher than that in the control group.

Concerning differential fungal count, tables (3&4) illustrated that Aspergilli represent the most frequent species where ten species of the genus were identified in the ruminal liquor while eight species were in the faecal matter. The Aspergilli represent more or less fifth fungal counts in the ruminal juice (17.7%) while it represented 83.3% of the total count in the faecal matter. These results disagree with that recorded [48], who revealed that 72.2% of the total fungal count in the ruminal juice was Aspergilli. However, *Aspergillus niger* and *A. fumigatus* were the most dominant species of Aspergilli both in ruminal and faecal samples. The high incidence of Aspergilli in the faecal matter may be attributed to their resistance to

the unfavourable conditions in the gastrointestinal tract. The results in table (3&4) showed that the number of many fungal species decreased by the effect of tannin-containing plant while other species were completely disappeared during the subsequent investigation. This disappearance may be due to their sensitivity to the phenolic compounds as *Aspergillus terreus*, *A. candidum*, *A. ustus*, *Fusarium oxysporum*, *F. solani*, *Penicillium species*, *Scopulariopsis brevicaulis*, *Cladosporium*, *Curvularia species*, *Trichoderma species*, *Paecilomyces species* and some yeast species. The majority of yeast cells were obviously destroyed during their passage through the alimentary tract, whereas large quantities of

fungi could be excreted in a viable state[54]. Some fungal species were recorded again after their disappearance as *A. fumigatus*, *A. glaucus*, *A. clavatus* which may be attributed to their introduction again with the mouldy feeding stuffs [2,3].

It was revealed that the percentages of the total Aspergilli in the ruminal juice were 17.7, 30.0, 11.3, 97.8 and 93.1% in the first, second, third, fourth and fifth groups, respectively (table 3). Moreover, the percentage of Aspergilli in the faecal matter was 83.3% (in the control group), while they were 91.4, 80.0, 98.9, and 98.4% in the second, third, fourth and fifth groups, respectively (table 4). These results indicated that the percentages of Aspergilli were increased in the ruminal juice and faecal matter by increasing concentration of tannins incorporated. This could be attributed to the relative resistance of Aspergilli to the unfavourable conditions in the gastro intestinal tract which in turn increase their percentages of occurrence[54]. Moreover, many of Aspergilli species are relatively resistant to the effect of tannins in relation to the other fungal species. This in turn increases the percentages of Aspergilli by increasing the tannins in the animal's feed. From hygienic point of view, many of the isolated fungi are pathogenic and have the ability to induce direct and indirect harmful effects on the animal's tissue as aspergillosis, aspergilo-toxicosis, hepatic degeneration, liver cancer, internal haemorrhage, intestinal phycomycosis, hyperkeratosis as well as mycotic abortion [2,55-58].

Concerning the *in-vitro* effect of *Acacia nilotica* on some fungal species, table (5) and figure (2 & 3), showed that the percentages of inhibition of the fungal species are directly proportional to the concentration of the tannins content and to the time of contact. In this respect it was revealed that a concentration of 0.25% had no effect on any fungal species under test and their count was fluctuated within the normal range as that in the control count. On the other hand, the percentage of inhibition was increased by the time both at 0.50% (100 mg/20 ml) and 0.75% (150 mg/20 ml). Table 5, illustrated that the antifungal effect of tannins varied according to the fungal species under test. However, *A. flavus* was highly inhibited by addition of *A. nilotica* than other species where 77.5% and 89.1% inhibition was noted by addition of 0.50% and 0.75%, respectively. Moreover, *Fusarium solani* was inhibited by 30.8% and 82.5% at 0.50% and 0.75% of the plant material, while 25.0% and 79.6% inhibition was noted in the *A. fumigatus* when *A. nilotica* was added by 0.50% and 0.75%, respectively. On the other hand, *A. niger* was the most resistant fungal species to the phenolic compounds where it was only inhibited by 24.0% and 68.4% at 0.50% and 0.75%, respectively. More or less similar results were recorded [14,26,30,32,52,59], where an antifungal effect was showed by using tannins from variable sources. No antifungal effect was reported [60], when they used tannin fragments as some flavonoid materials.

Table (3): Differential fungal count in the ruminal samples.

Fungal isolates	Animal groups				
	1 <sup>st</sup> group	2 <sup>nd</sup> group	3 <sup>rd</sup> group	4 <sup>th</sup> group	5 <sup>th</sup> group
<i>Aspergillus niger</i>	2.8x10 <sup>4</sup> ±2.4x10 <sup>2</sup>	9.4x10 <sup>3</sup> ±8.9x10 <sup>3</sup>	1.9x10 <sup>4</sup> ±4.2x10 <sup>3</sup>	4.2x10 <sup>4</sup> ±2.4x10 <sup>4</sup>	2.6x10 <sup>3</sup> ±4.0x10 <sup>2</sup>
<i>A. flavus</i>	1.8x10 <sup>3</sup> ±6.0x10 <sup>2</sup>	2.5x10 <sup>4</sup> ±2.4x10 <sup>3</sup>	1.4x10 <sup>4</sup> ±5.8x10 <sup>3</sup>	4.5x10 <sup>4</sup> ±1.2x10 <sup>4</sup>	1.8x10 <sup>2</sup> ±4.7x10
<i>A. fumigatus</i>	3.8x10 <sup>3</sup> ±2.4x10 <sup>2</sup>	0	0	0	2.4x10 <sup>4</sup> ±1.2x10 <sup>3</sup>
<i>A. glaucus</i>	3.5x10 <sup>3</sup> ±2.2x10 <sup>3</sup>	0	1.3x10 <sup>4</sup> ±2.4x10 <sup>3</sup>	1.8x10 <sup>3</sup> ±5.9x10 <sup>2</sup>	1.9x10±0.7x10
<i>A. clavatus</i>	1.4x10 <sup>2</sup> ±2.4x10	0	0	2.1x10 <sup>2</sup> ±1.6x10 <sup>2</sup>	1.3x10±0.05x10
<i>A. sydowi</i>	2.7x10 <sup>2</sup> ±1.3x10 <sup>2</sup>	2.4x10 <sup>2</sup> ±3.2x10	1.6x10 <sup>2</sup> ±2.7x10	2.3x10±0.5x10	1.1x10±0.6x10
<i>A. versicolor</i>	1.3x10 <sup>3</sup> ±2.1x10 <sup>2</sup>	4.2x10 <sup>3</sup> ±1.8x10 <sup>2</sup>	3.4x10 <sup>2</sup> ±2.2x10	1.7x10 <sup>2</sup> ±1.8x10	1.0x10 <sup>2</sup> ±1.2x10
<i>A. terreus</i>	1.0x10 <sup>2</sup> ±0.8x10	1.7x10±0.8x10	0	0	0
<i>A. candidum</i>	6.4x10±1.2x10	2.3x10±1.4x10	0	0	0
<i>A. ustus</i>	3.4x10±1.1x10	0	0	0	0
Total <i>Aspergilli</i> count	3.9x10 <sup>4</sup> (17.7%)	3.9x10 <sup>4</sup> (30 %)	4.7x10 <sup>4</sup> (11.3%)	8.9x10 <sup>4</sup> (97.8 %)	2.7x10 <sup>4</sup> (93.1 %)
<i>Geotrichum candidum</i>	1.8x10 <sup>5</sup> ±2.1x10 <sup>3</sup>	2.8x10 <sup>4</sup> ±1.1x10 <sup>3</sup>	1.4x10 <sup>5</sup> ±2.3x10 <sup>3</sup>	3.4x10 <sup>2</sup> ±1.7x10 <sup>2</sup>	3.4x10 <sup>2</sup> ±1.7x10 <sup>2</sup>
<i>Fusarium oxysporum</i>	6.8x10 <sup>2</sup> ±4.2x10 <sup>2</sup>	4.3x10 <sup>2</sup> ±7.1x10	0	0	0
<i>F. solani</i>	5.2x10±0.7x10	2.1x10±0.05x10	0	0	0
<i>Mucor spp.</i>	7.3x10 <sup>2</sup> ±5.1x10 <sup>2</sup>	6.2x10 <sup>4</sup> ±8.2x10 <sup>3</sup>	4.3x10 <sup>4</sup> ±1.3x10 <sup>4</sup>	1.5x10 <sup>3</sup> ±2.4x10 <sup>2</sup>	1.5x10 <sup>3</sup> ±2.4x10 <sup>2</sup>
<i>Penicillium spp.</i>	3.1x10 <sup>2</sup> ±1.7x10 <sup>2</sup>	1.2x10 <sup>2</sup> ±4.1x10	0	0	0
<i>Scopulariopsis brevicaulis</i>	4.2x10 <sup>2</sup> ±1.1x10 <sup>2</sup>	3.7x10 <sup>2</sup> ±4.7x10	5.2x10±0.7x10	0	0
<i>Cladosporium spp.</i>	4.9x10±0.3x10	0	0	0	0
<i>Curvularia spp.</i>	6.2x10±0.5x10	0	0	0	0
<i>Trichoderma spp.</i>	4.3x10 <sup>2</sup> ±1.8x10 <sup>2</sup>	2.3x10±0.7x10	0	0	0
<i>Pacelomyces spp.</i>	2.7x10±0.08x10	1.1x10+±0.02x10	0	0	0
Yeast spp.	2.1x10 <sup>2</sup> ±3.4x10	0	0	0	0
Sterile mycelium	3.6x10±1.2x10	0	1.8x10±0.5x10	0	0
Total	2.2x10 <sup>5</sup>	1.3x10 <sup>5</sup>	2.1x10 <sup>5</sup>	9.1x10 <sup>4</sup>	2.9x10 <sup>4</sup>

Table (4): Mean fungal count in the faecal samples.

Fungal isolates	Faecal samples				
	1 <sup>st</sup> group (Control)	2 <sup>nd</sup> group	3 <sup>rd</sup> group	4 <sup>th</sup> group	5 <sup>th</sup> group
<i>Aspergillus niger</i>	3.0x10 <sup>4</sup> ±1.2x10 <sup>2</sup>	7.0x10 <sup>4</sup> ±9.8x10 <sup>3</sup>	6.2x10 <sup>4</sup> ±2.3x10 <sup>4</sup>	5.4x10 <sup>3</sup> ±4.2x10 <sup>3</sup>	3.8x10 <sup>3</sup> ±1.7x10 <sup>3</sup>
<i>A. flavus</i>	5.7x10 <sup>2</sup> ±6.2x10	1.9x10 <sup>5</sup> ±7.1x10 <sup>4</sup>	6.7x10 <sup>4</sup> ±1.3x10 <sup>4</sup>	2.3x10 <sup>4</sup> ±1.5x10 <sup>3</sup>	4.6x10 <sup>3</sup> ±2.1x10 <sup>2</sup>
<i>A. fumigatus</i>	1.0x10 <sup>5</sup> ±2.3x10 <sup>2</sup>	2.1x10 <sup>4</sup> ±9.3x10 <sup>3</sup>	6.3x10 <sup>4</sup> ±1.9x10 <sup>4</sup>	5.7x10 <sup>4</sup> ±2.7x10 <sup>4</sup>	5.1x10 <sup>4</sup> ±4.1x10 <sup>2</sup>
<i>A. sydowi</i>	4.3x10 <sup>2</sup> ±1.2x10	2.7x10 <sup>3</sup> ±8.1x10 <sup>2</sup>	1.7x10 <sup>2</sup> ±3.2x10	2.3x10 <sup>3</sup> ±1.1x10 <sup>2</sup>	1.9x10 <sup>2</sup> ±3.2x10
<i>A. versicolor</i>	1.8x10 <sup>2</sup> ±3.1x10	3.4x10 <sup>4</sup> ±2.1x10 <sup>2</sup>	5.2x10 <sup>3</sup> ±6.1x10	4.3x10 <sup>3</sup> ±2.1x10 <sup>2</sup>	2.8x10 <sup>3</sup> ±7.2x10
<i>A. clavatus</i>	7.0x10 <sup>3</sup> ±4.1x10 <sup>2</sup>	0	0	0	0
<i>A. ochraceus</i>	1.2x10 <sup>4</sup> ±1.3x10 <sup>3</sup>	0	0	0	0
<i>A. candidum</i>	1.8x10 <sup>2</sup> ±3.7x10	0	0	0	0
Total <i>Aspergilli</i> count	1.5x10 <sup>5</sup> (83.3%)	3.2x10 <sup>5</sup> (91.4%)	2.0x10 <sup>5</sup> (80%)	9.2x10 <sup>4</sup> (98.9%)	6.2x10 <sup>4</sup> (98.4%)
<i>Fusarium solani</i>	4.7x10±0.7x10	0	0	0	0
<i>F. oxysporum</i>	2.1x10±0.3x10	0	0	0	0
<i>Mucor spp.</i>	1.3x10 <sup>3</sup> ±5.1x10 <sup>2</sup>	3.1x10 <sup>4</sup> ±3.7x10 <sup>3</sup>	5.5x10 <sup>4</sup> ±6.4x10 <sup>3</sup>	3.1x10 <sup>2</sup> ±4.2x10	3.6x10 <sup>2</sup> ±2.1x10 <sup>2</sup>
<i>Curvularia spp.</i>	1.2x10±0.5x10	0	0	0	0
<i>Penicillium spp.</i>	1.0x10 <sup>2</sup> ±5.2x10	0	0	0	0
<i>Trichoderma spp.</i>	2.8x10 <sup>2</sup> ±1.5x10	0	0	0	0
<i>Geotrichum candidum</i>	2.3x10 <sup>4</sup> ±7.2x10 <sup>2</sup>	1.3x10 <sup>2</sup> ±4.1x10	0	0	0
Yeast spp.	1.2x10 <sup>3</sup> ±3.2x10 <sup>2</sup>	0	0	0	0
Sterile mycelium	7.3x10 <sup>2</sup> ±0.6x10 <sup>2</sup>	4.1x10 <sup>2</sup> ±1.0x10	7.3x10 <sup>2</sup> ±2.0x10	4.6x10 <sup>2</sup> ±7.0x10	5.2x10 <sup>2</sup> ±2.0x10
Total	1.8x10 <sup>5</sup>	3.5x10 <sup>5</sup>	2.5x10 <sup>5</sup>	9.3x10 <sup>4</sup>	6.3x10 <sup>4</sup>

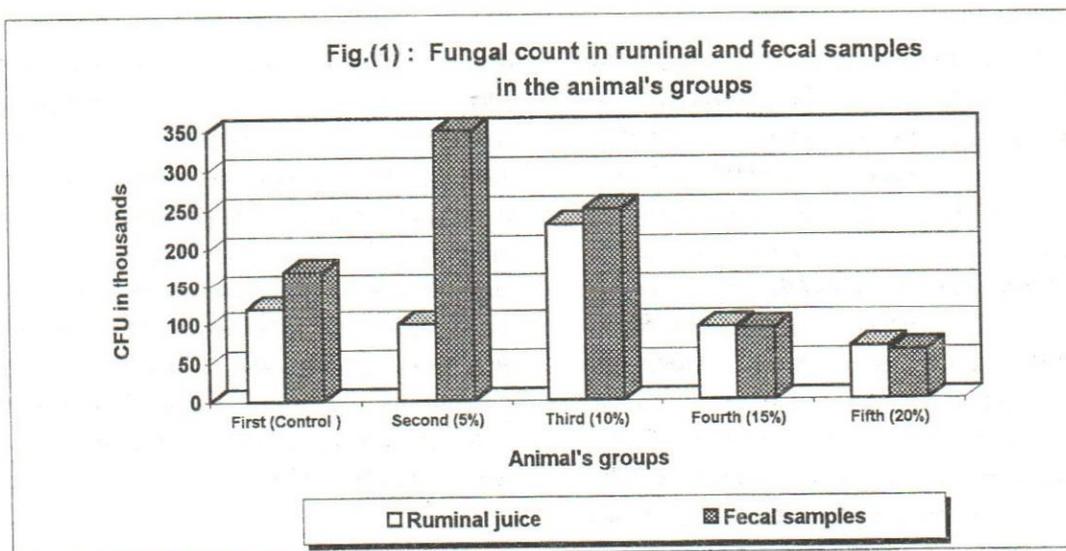
Table (5) and figures (2&3) revealed also that, the inhibition of fungal species was directly proportional to the time of contact between fungal species and tannins. It was found that the inhibition percentages of *A. flavus* were increased at 0.50% *A. nilotica* from 3.4% after addition of the plant material to 10.3, 32.2, 50.0 and 77.5% after 30, 60, 90 and 120 minutes, respectively. Moreover, similar results were recorded on all fungal species under test both at 0.50% and 0.75% concentration of the plant material. This confirms the theory that the antimicrobial

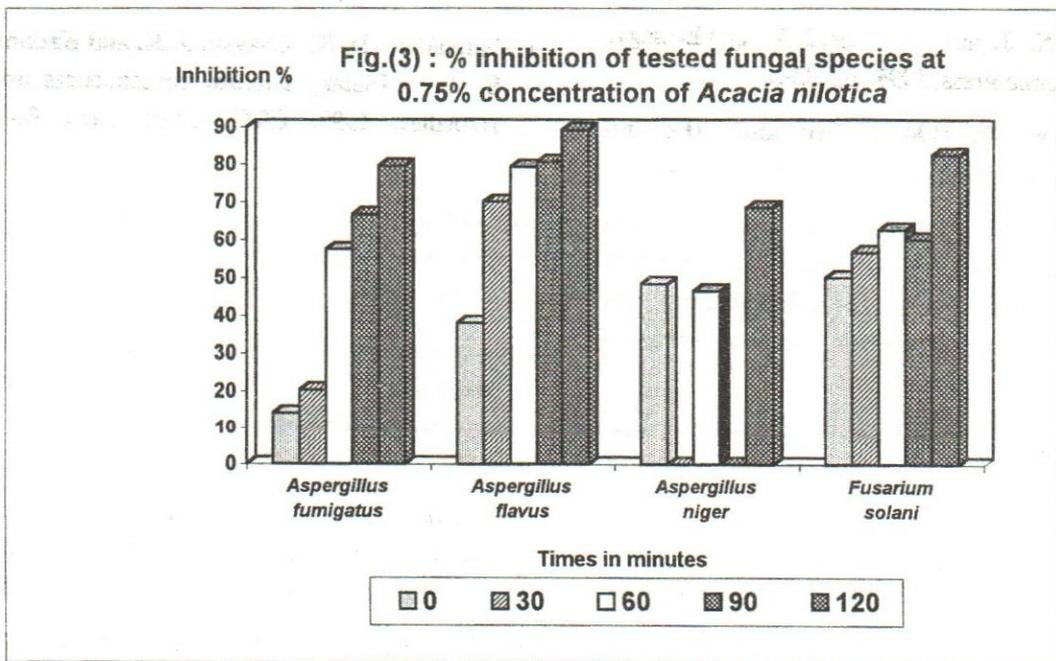
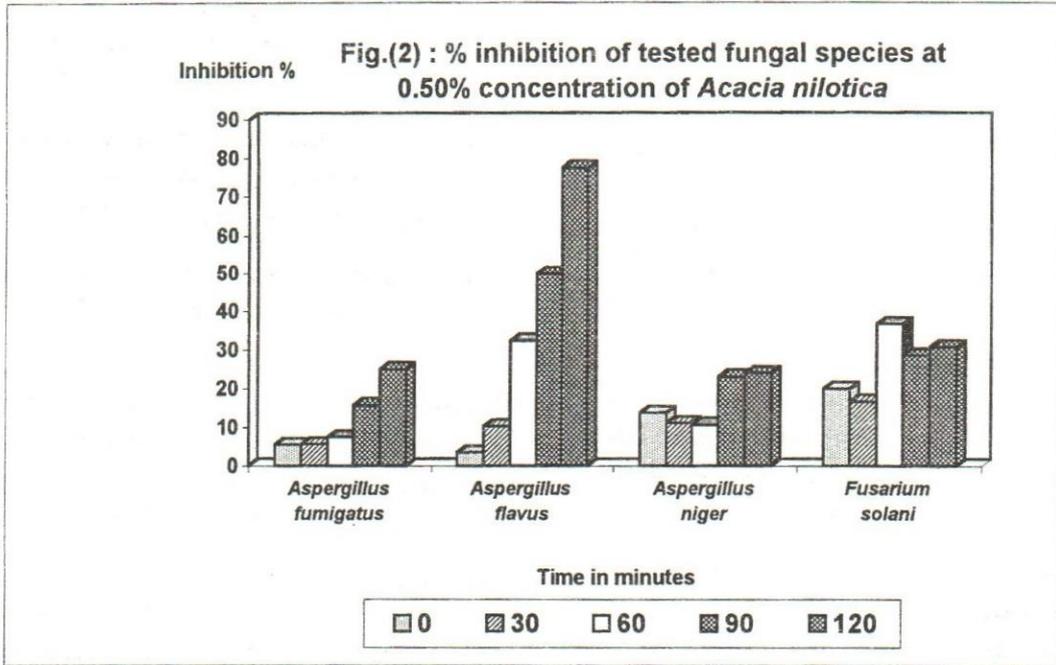
effect of tannins is directly proportional to the time of contact with the organism[23,61]. From the obtained results one can safely conclude that tannins and tannin-containing plants are strong antifungal material and such plant materials could be used to reduce the fungal count in the animal's gut. However, since *Acacia nilotica* is widely distributed throughout tropical and subtropical countries, it could be performed to animals as a feeding material and at the same time it protects it against fungal species.

Table (5): in-vitro effect of raw plant materials on some selected fungi.

Fungal spp.	Exam. time	Control	Count and % of inhibition in relation Conc. of plant material		
			0.25%	0.50%	0.75%
<i>Aspergillus fumigatus</i>	T <sub>0</sub>	5.6x10 <sup>7</sup>	5.4x10 <sup>7</sup> (3.6%)	5.3x10 <sup>7</sup> (5.4%)	4.8x10 <sup>7</sup> (14.3%)
	T <sub>30</sub>	5.4x10 <sup>7</sup>	6.2x10 <sup>7</sup> (**)	5.1x10 <sup>7</sup> (5.6%)	4.3x10 <sup>7</sup> (20.4%)
	T <sub>60</sub>	5.4x10 <sup>7</sup>	5.1x10 <sup>7</sup> (5.6%)	5.0x10 <sup>7</sup> (7.4%)	2.3x10 <sup>7</sup> (57.4%)
	T <sub>90</sub>	5.1x10 <sup>7</sup>	5.7x10 <sup>7</sup> (**)	4.3x10 <sup>7</sup> (15.7%)	1.7x10 <sup>7</sup> (66.7%)
	T <sub>120</sub>	4.8x10 <sup>7</sup>	5.1x10 <sup>7</sup> (**)	3.6x10 <sup>7</sup> (25%)	9.8x10 <sup>6</sup> (79.6%)
<i>A. flavus</i>	T <sub>0</sub>	2.9x10 <sup>7</sup>	3.1x10 <sup>7</sup> (**)	2.8x10 <sup>7</sup> (3.4%)	1.8x10 <sup>7</sup> (37.9%)
	T <sub>30</sub>	2.9x10 <sup>7</sup>	3.2x10 <sup>7</sup> (**)	2.6x10 <sup>7</sup> (10.3%)	8.7x10 <sup>6</sup> (70.0%)
	T <sub>60</sub>	3.1x10 <sup>7</sup>	3.0x10 <sup>7</sup> (3.2%)	2.1x10 <sup>7</sup> (32.3%)	6.4x10 <sup>6</sup> (79.3%)
	T <sub>90</sub>	3.2x10 <sup>7</sup>	3.0x10 <sup>7</sup> (6.3%)	1.6x10 <sup>7</sup> (50%)	6.2x10 <sup>6</sup> (80.6%)
	T <sub>120</sub>	3.2x10 <sup>7</sup>	2.8x10 <sup>7</sup> (12.5%)	7.2x10 <sup>6</sup> (77.5%)	3.5x10 <sup>6</sup> (89.1%)
<i>A. niger</i>	T <sub>0</sub>	2.9x10 <sup>5</sup>	3.1x10 <sup>5</sup> (**)	2.5x10 <sup>5</sup> (13.8%)	1.5x10 <sup>5</sup> (48.3%)
	T <sub>30</sub>	2.7x10 <sup>5</sup>	3.0x10 <sup>5</sup> (**)	2.4x10 <sup>5</sup> (11.1%)	3.8x10 <sup>5</sup> (**)
	T <sub>60</sub>	2.8x10 <sup>5</sup>	2.7x10 <sup>5</sup> (3.6%)	2.5x10 <sup>5</sup> (10.7%)	1.5x10 <sup>5</sup> (46.4%)
	T <sub>90</sub>	2.6x10 <sup>5</sup>	2.7x10 <sup>5</sup> (**)	2.0x10 <sup>5</sup> (23.1%)	3.1x10 <sup>5</sup> (**)
	T <sub>120</sub>	2.5x10 <sup>5</sup>	2.5x10 <sup>5</sup> (0%)	1.9x10 <sup>5</sup> (24%)	7.9x10 <sup>4</sup> (68.4%)
<i>Fusarium solani</i>	T <sub>0</sub>	2.0x10 <sup>6</sup>	1.9x10 <sup>6</sup> (5.0%)	1.6x10 <sup>6</sup> (20%)	1.0x10 <sup>6</sup> (50.0%)
	T <sub>30</sub>	1.8x10 <sup>6</sup>	1.8x10 <sup>6</sup> (0%)	1.5x10 <sup>6</sup> (16.7%)	7.8x10 <sup>5</sup> (56.7%)
	T <sub>60</sub>	1.9x10 <sup>6</sup>	2.0x10 <sup>6</sup> (**)	1.2x10 <sup>6</sup> (36.8%)	7.1x10 <sup>5</sup> (62.6%)
	T <sub>90</sub>	1.4x10 <sup>6</sup>	2.1x10 <sup>6</sup> (**)	1.0x10 <sup>6</sup> (28.6%)	5.6x10 <sup>5</sup> (60.0%)
	T <sub>120</sub>	1.2x10 <sup>6</sup>	1.3x10 <sup>6</sup> (**)	8.3x10 <sup>5</sup> (30.8%)	2.1x10 <sup>5</sup> (82.5%)

(\*\*) There was no effect on the colony count





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## التأثير المثبط للنباتات الغنية بالتينينات (السنط) على نمو الفطريات.

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قسم صحة الحيوان - كلية الطب البيطرى - جامعة أسيوط - ج ٥٠م ٥

تم إجراء هذا البحث بهدف تقييم تأثير المركبات الفينولية [التينينات] على الفطريات وفى هذا الإطار تم تقسيم البحث إلى جزئين الأول منهما تم إجراؤه تطبيقياً على عدد من ذكور الماعز البلدى حيث تم تقسيم الحيوانات إلى خمسة مجموعات [خمس فى كل مجموعة] تركت المجموعة الأولى بدون معاملة (ضابطة) والمجموعات الثانية والثالثة والرابعة والخامسة أضيف إلى علائقها العادية ورق نبات السنط بنسب ٥ ، ١٠ ، ١٥ ، ٢٠٪ على التوالى . ثم أخذت عينتان أسبوعياً من عصير الكرش والبراز لمدة شهر من كل المجموعات بهدف دراسة العد الطبقي القياسى للفطريات وكذلك الأس الهيدروجينى تحت ظروف التجربة. أوضحت النتائج أن إضافة النبات بأى من النسب السابقة لم يكن له تأثير على الرقم الهيدروجينى فى كلا من عصير الكرش والبراز فى حين كان له تأثير مثبت قوى على الفطريات عند ١٥ ، ٢٠٪ ولكنه لم يكن له ثمة تأثير عند تركيز ٥ ، ١٠٪ . من خلال هذه الدراسة أتضح أن مجموعة الأسبرجلس كانت هى الأكثر تكراراً فى عينات الكرش والبراز .

الجزء الثانى تم إجراؤه معملياً وفيه تم دراسة تأثير إضافة نبات السنط على بعض العترات الفطرية مثل: *Aspergillus fumigatus*, *A. flavus*, *A. niger* and *Fusarium solani*. عند ثلاثة تركيزات مختلفة وهى ٢٥٪ ، ٥٠٪ ، ٧٥٪. ثم حساب العد الطبقي القياسى للفطريات كل نصف ساعة على مدى ساعتان . أوضحت النتائج أن إضافة النبات عند تركيز ٢٥٪ لم يكن له أى تأثير على حيوية الفطريات فى حين وجد أن إضافته بنسب ٥٠٪ ، ٧٥٪ أدى إلى قتل الفطريات بنسبة كبيرة . كما وجد كذلك أن نسبة قتل الفطريات تتناسب طردياً مع وقت الفحص . فى هذا الإطار وجد أن فطر الأسبرجلس فلافس كان أعلى حساسية للنبات وقد بلغت نسبة قتله إلى ٧٧,٥٪ ، ٨٩,١٪ بعد ساعتين عند تركيز ٥٠٪ ، ٧٥٪ على التوالى وجاء فطر الفيوزاريوم سولانى فى المرتبة الثانية ثم تلاه فطر الأسبرجلس فيوميغاتس فى المرتبة الثالثة . كما أظهرت النتائج أن فطر الأسبرجلس نيجر كان أقل الفطريات تأثراً بإضافة النبات وبلغت نسبة قتله إلى ٠,٢٤٪ ، ٦٨,٤٪ بعد ساعتين عند تركيز ٥٠٪ ، ٧٥٪ على التوالى . من هذه النتائج يمكن أن نخلص إلى أن التينينات لها تأثير مثبت قوى على الفطريات ويمكن استخدامها فى هذا الغرض لتجنب آثار الفطريات الضارة على صحة الحيوان .



## ANTITERMITE PRINCIPLES ISOLATED FROM THE WILD HERB, *PSORALEA PLICATA* Del.

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### ABSTRACT :

The total extract and seven compounds isolated from the indigenous herb *Psoralea plicata* ,Leguminosae ,were evaluated against *Amitermes desertorum* which causes wide range of damage for trees and woody household furniture in upper Egypt. The study revealed the promising activity of plicatin-B as termiticidal within one week. On the other hand, plicatin-B was proved to be potent antibacterial against the bacteria isolated from the gut of the termite , where the insect is dependable on digestion of its food.

### INTRODUCTION :

The wild herb *Psoralea plicata* Del. which is known in Arabic as مرميد "Marmeed" or "Mermeed", is commonly grown in Allaqi area south east of Aswan . The plant is widely eaten by grazing animals in the area for its palatable green leaves [1].

It has been used in folk medicine as skin photosensitizer, anthelmintic, antipyretic, analgesic, anti-inflammatory, diuretic, diaphoretic and it is also useful in bilious infections, in liprosy and in menstrual disorders [2,3] .

Phytochemical studies on the plant revealed the presence of interesting compounds including furocoumarins, chromenes ,isoflavonoids, phenolic cinnamates, cinnamate dimers, flavonoid glycosides, monoterpenoids and triterpenoids in addition to tocopherol [4-10].

The subterranean termite *A. desertorum* (Desneux), builds its nests underground or in tree hearts ,and attacks dead wood and live trees [11]. It has been reported that *A. desertorum* is a very adaptable species and has a wide distribution in Egypt, especially in arid

and semi-arid localities [12-14]. The same species has been recorded to infest living trees and shrubs in Aswan governorate [15].

From the current literature, plant extractives, have been bioassayed for one or more antitermitic effects including toxicity to the insect and/or gut symbionts, feeding deterrence, non-preference or repellency against one or more termite species [16-18]. The above mentioned facts arouse our interest to study the effect of *Psoralea plicata* extractives and isolated components against *A. desertorum*.

## EXPERIMENTAL :

### Materials and Methods :

#### Termites :

A colony of the termites was collected from infected *Ficus* tree at Kima area in Aswan city. Termite workers (externally undifferentiated insects beyond the 3rd. stage) from that colony of subterranean termite *Amitermes desertorum* (desneux) was used as test insect.

#### Extraction and isolation of plant constituents :

Air-dried aerial parts of *Psoralea plicata* Del. herb (2 kg), collected from Wadi Allaqi (Aswan, Egypt), were powdered and exhaustively extracted with 75% MeOH by maceration. The alcohol extract was concentrated under reduced pressure to a syrupy consistency (179 g). The solvent free residue (50 g) was mixed with 200 ml water and 100 ml MeOH, transferred to a separatory funnel and partitioned between hexane, ethyl-acetate and

n-butanol respectively. Each fraction was dried over anhydrous sodium sulphate and condensed to syrupy residue (10 g hexane residue; 3.6 g ethylacetate residue and 5 g n-butanol residue).

Each fraction was subjected to flash silica gel column chromatography, with hexane-ethylacetate gradient. Plicatin-B, angelicin, psoralen and chromene compounds were isolated from the hexane soluble fraction. Plicatin-A, p-dimethylcoumaric acid and  $\alpha$ -duplicatin-B were isolated from the ethyl-acetate soluble fraction and roseoside, daidzin (isoflavonoid), psoralic acid and isovitexin (flavonoid) were isolated from the n-butanol soluble fraction.

#### Isolation of the bacteria from the termite gut :

A culture medium was prepared by dissolving 31g of nutrient agar powder in 1 L. distilled water, autoclaved at 120°C for 15 min. Slands were prepared from this medium and inoculated by the organisms isolated from the termite gut by homogenization in sterilized distilled water. The slands were incubated at 30°C for 24 hrs and were investigated [19].

#### Antimicrobial effect of the isolated compounds :

Small discs of filter paper were immersed in different concentrations of the selected compounds, then placed in petri dish containing nutrient agar medium and inoculated with the isolated organisms and incubated at 30°C for 24 hrs, then the clearing zones were inspected.

#### Force-feeding test :

A round plastic container of 5 cm in diameter and 3.5 cm in height was packed with 50 g of sterile sand moistened with 7 ml of distilled water to keep relative humidity near saturation. Five levels of seed extracts were tested (15, 30, 75, 150 and 300 ppm). Three replicates of small pieces of filter paper (3 cm in diameter) were dipped in the methanolic extracts for each level for a period of 10 sec., and dried at room temperature for evaporation of the solvent. The treated papers were set up on sand surface and 100 worker termites were added to each container. Untreated filter papers were fed to termites as a control. The containers were incubated at 25°C for 5 weeks and the number of surviving termites was recorded in each replicate.

Filter papers treated with two concentration levels of the isolated compounds of *Psoralea plicata* (75 and 175 ppm), were evaluated against termites and the number of surviving termites was taken at 2, 6, 10, 14 and 18 days respectively.

The results were statistically analysed and the means were compared according to both the t-test and Duncan's multiple range test [20].

#### RESULTS AND DISCUSSION :

The first part of the present work shows the bioassay experiments of crude extract from *Psoralea plicata*, on feeding and survival of *A. desertorum*, which depends in its digestion on

some symbiotic organisms (either bacteria or protozoa) living in their gut.

The mean survival of *A. desertorum* feeding on filter papers treated with different levels (ppm) of crude extract of *Psoralea plicata* during 5 weeks is given in Table (1). It was observed that the mean survival was level and exposure period dependent. The termite lived approximately for two weeks only after feeding on two levels (75 and 150 ppm), and the remaining insects died before the end of the third week; but other levels (15 and 30 ppm), showed weak anti-termite effect and the mean survival was not significantly different during the same period.

Increasing the concentration of the plant extract to 300 ppm, does not significantly increase the mean of mortality relative to other concentrations (75 or 150 ppm) till the end of the second week, but during the third and fourth week, the mean survival values was greatly decreased and 100% mortality was observed by the end of the fourth week. On the other hand, it was observed that the termites avoid eating the papers with concentration level of 300 ppm, and hide under the sand.

Some of the isolated compounds from *Psoralea plicata* viz., plicatin B, flavonoids, psoralen, angelicin, isovitexin, chromene E,  $\alpha$ -duplicatin B and total extract were tested for anti-termite effect.

Data presented in table (2) showed the mean survival percentages of *A. desertorum*

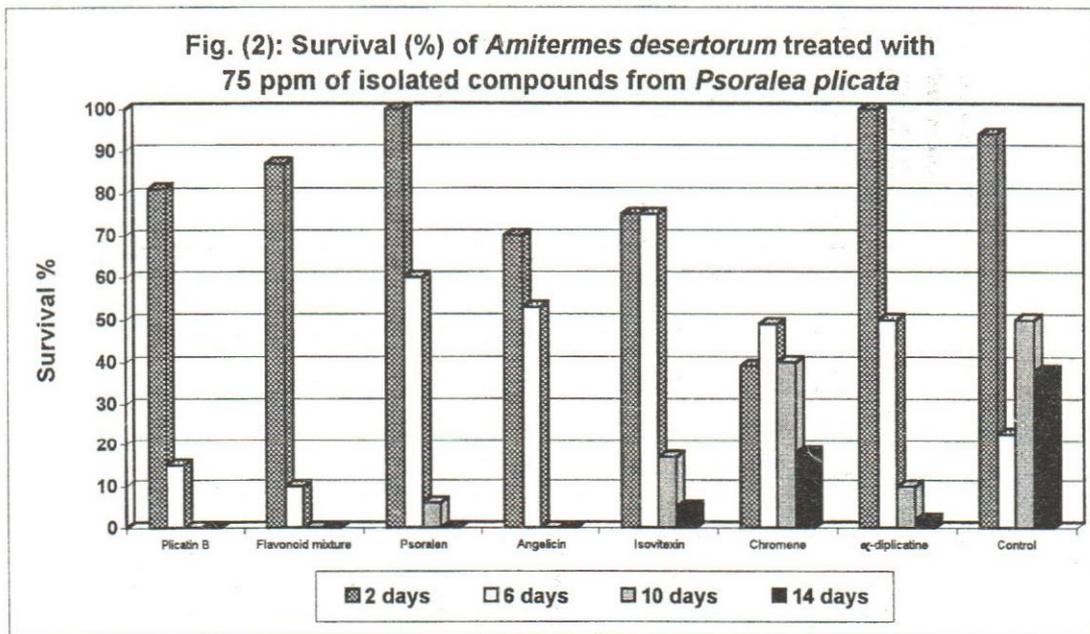
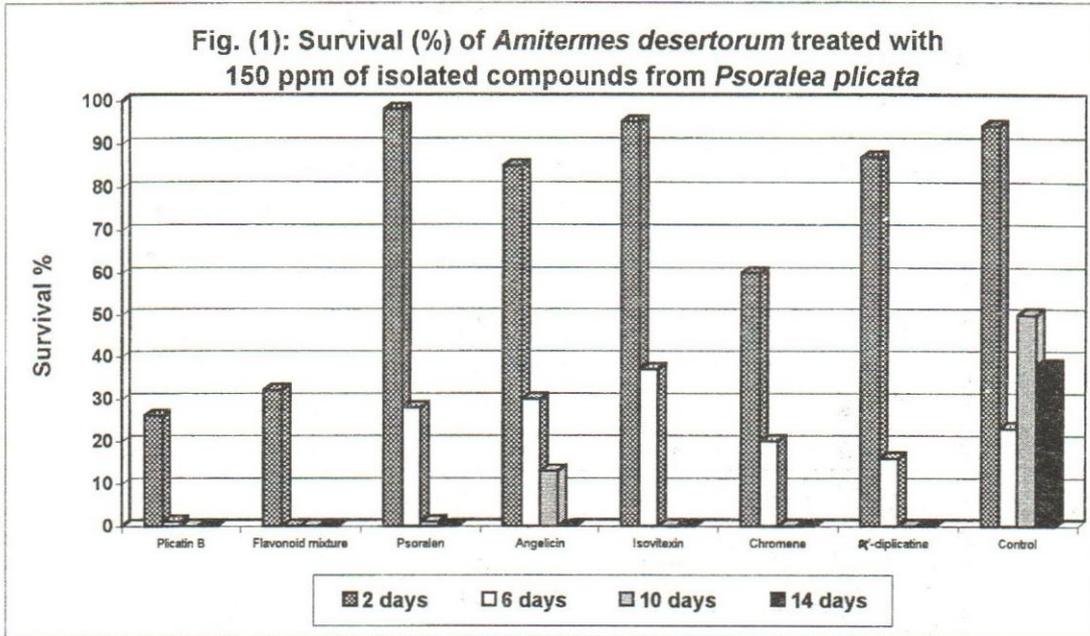
feeding on filter papers treated with two concentration levels (75 and 150 ppm) of the isolated compounds during 18 days. Figures 1&2 showed that, plicatin B, was the most effective compound since it showed significant decrease in the survival of the termites after two days at the two applied levels. After six days of treatment, most of the termites are

died at the level of 150 ppm, then no survival was recorded after 7 days at the same concentration. A clear reduction was noticed in the individuals size, probably due to the dehydration effect of the applied compound after two days of feeding ; at the same time the activity of the termites had greatly reduced at the same period.

Table (1) : Survival % of workers of *Amitermes desertorum* after exposure to *Psoralea plicata* extract for 5 weeks at five ppm conc. levels.

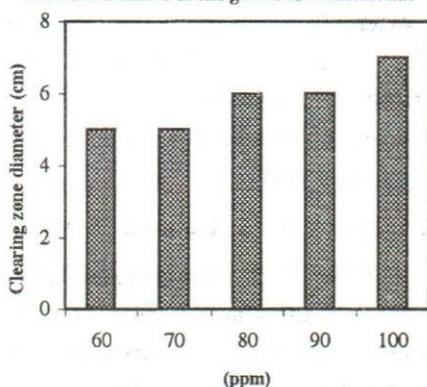
Level ppm	Exposure Week	Survival %					
		I	II	III	Total	Mean	±SD
0	1	100	100	100	300	100	0
	2	91	92	93	276	92	0.82
	3	68	87	81	236	78.7	7.93
	4	25	73	11	109	36.3	26.5
	5	6	7	1	14	4.7	0.66
15	1	98	91	86	275	91.7	4.92
	2	98	88	76	262	87.3	8.99
	3	75	71	62	208	69.3	5.44
	4	43	15	6	64	21.3	15.76
	5	1	2	1	4	1.3	0.47
30	1	98	81	97	276	92	7.79
	2	78	67	88	233	77.7	8.58
	3	12	63	65	140	46.7	24.53
	4	11	1	22	34	11.3	8.85
	5	5	0	7	12	4	-
75	1	12	11	13	36	12	0.82
	2	5	1	1	7	2.3	1.90
	3	0	0	0	0	0	-
	4	0	0	0	0	0	-
	5	0	0	0	0	0	-
150	1	35	18	17	70	23.3	8.26
	2	22	15	12	49	16.3	4.19
	3	0	0	0	0	0	-
	4	0	0	0	0	0	-
	5	0	0	0	0	0	-
300	1	100	88	87	275	91.7	5.91
	2	85	81	77	243	81	3.27
	3	6	23	11	40	13.3	7.13
	4	3	13	5	21	7	4.32
	5	0	0	0	0	0	-





In case of flavonoid mixture, the mean survival of the termites was greatly decreased after two days of feeding at the two concentration levels 75 and 150 ppm, but the survival percent was much decreased at level 150 ppm comparatively to level 75 ppm. After nine days, all the individuals were dead at the two levels. It was observed that most of the termites started to avoid feeding on the filter papers and hide under damp sand after two days of exposure to the treated papers.

Fig. (3): Effect of Plicatin B in-vitro on the bacteria isolated from gut of *A. desertorum*



In case of chromene compound, the concentration level of 150 ppm, was more effective than the 75 ppm level, since all the termites were killed after nine days of exposure. The same effect was noticed with concentration levels 75 and 150 ppm in the first two days, but differ after six days.

Psoralen and angelicin, were similar in their effects on termites survival during feeding at levels 75 and 150 ppm.

Among the tested compounds of *Psoralea plicata*, only plicatin B, flavonoids and chromenes respectively showed the strongest termiticidal activity at concentration level of 150 ppm. Unlike these three *Psoralea* compounds, it was found that the other compounds are more or less similar in action on termites with apparent weak antitermite efficiency.

The statistical analysis of the obtained data indicated that the survival of termites is significantly different according to the concentration of the compound applied (in case of plicatin B and flavonoids), and the survival difference was clear with other compounds (psoralen, angelicin, isovitexin and  $\alpha$ -diplicatin B) especially at level 150 ppm after two days of feeding.

The action of plicatin B on the anaerobic bacteria isolated from the gut of *A. desertorum* was demonstrated by clear zone, when it is applied to nutrient agar medium inoculated with the respective bacteria after 24 hrs.

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## المواد المقاومة للنمل الأبيض والمفصولة من نبات " البسوراليا بليكاتا "

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تم في هذه الدراسة إجراء التجارب الحيوية على مقاومة خلاصة النبات وسبعة من المركبات المفصولة من نبات " البسوراليا بليكاتا " المعروف باسم المرميد على النمل الأبيض ، الذي يسبب تدميراً شديداً للأشجار والأثاث المنزلى فى منطقة مصر العليا.

وقد أثبتت التجارب فاعلية مركبات البليكاتين - ب وكذلك المواد الفلافونية على نمو النمل الأبيض ، حيث قضت تماما على الحشرات فى فترة لا تتجاوز أسبوع واحد .

كما تأكد بالتجارب فاعلية مادة البليكاتين ب فى القضاء على البكتيريا المفصولة من أمعاء الحشرة خلال أربعة وعشرون ساعة ، والتي تعتمد عليها الحشرة فى هضم الغذاء .

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**THE SURVEY OF ORDER ISOPTERA III : ON ASWAN BOTANICAL ISLAND AND CONTROL OF *AMITERMES DESERTORUM* BY CRUDE EXTRACT AND SOME PURE COMPOUNDS ISOLATED FROM *ARTEMISIA ARGENTEA* L'HER.**

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**ABSTRACT :**

A study of the trees on Botanical Island at Aswan recorded seven species belonging to four families which were infested by the subterranean termite *Amitermes desertorum* (Desneux). This termite was recorded as the only species in this island representing the order Isoptera.

Many structurally different compounds from the root bark of *Artemisia argentea* L' Her (*Asteraceae*), including sesquiterpene lactones, arborescin and argentiolid  $\beta$  [1,2], a group of lignan compounds especially sesamine and yangambin and the crude extract were tested against the workers of the subterranean termite, *A. desertorum* (Desneux). The crude extract showed significant insecticidal activity against either feeding or survival after one week at all tested levels, and the compounds argentiolid  $\beta$  and sesamine gave a high effect at concentration level 25% after two days. The crude extract was applied on two species of trees on this island.

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## INTRODUCTION :

In connection with our interest in biologically active natural products [3], we investigated the chemical constituents of the root bark of *Artemisia argentea* L' Her . A large number of sesquiterpene lactones and tetrahydrofurofuran lignans were previously reported from over hundred species of *Artemisia* [1,2,4-9]. Argentiolid  $\beta$ , sesamine, yangambin and arborescin were evaluated against *Amitermes desertorum* which damage trees and woods in Upper Egypt .

The subterranean termite species are increasing in its importance in temperate regions [10,11], especially those belonging to family Termitidae [12]. Some researchers [13,14] have observed that the most destructive termites belong to the genera *Amitermes* and the frequently most recorded termite in Aswan Governorate is *A. desertorum* (Desneux) which builds its nests underground or in the heart of trees and attacks both dead wood and live trees. In 1982, Ali et al, [15] stated that this species is very adaptable and has a wide distribution on Egyptian timber. Plant extractives have been bioassayed for one or more antitermitic effects, including toxicity to the insects and/or gut symbionts, feeding deterrence, non-preference or repellency, against one or more termite species .

Aswan Botanical Island is situated in the middle of the river Nile in Aswan, Egypt, and covers an area of 17 feddans. In this garden there are many different species of tropical and subtropical plants. The present study indicated that *A. desertorum* infested many

trees species specially *Eugenia jambolana*, Family *Myrtaceae* (mambosia) and *Roystonea regia*, Family *Palmaceae* (Royal Palm).

The aim of this work is to determine the biological value of the root bark extract of *A. argentea* and some pure compounds against the subterranean termite *A. desertorum* in the laboratory and field.

## EXPERIMENTAL :

### Materials and Methods :

#### Plant material :

The root bark of *Artemisia argentea* plants cultivated in the experimental station of medicinal plants, Faculty of Pharmacy, Assiut University was collected during April and May 1996.

#### Extraction and isolation :

See reference [9].

#### Field visits :

Twenty-four field visits were carried out during one year from April 1997- April 1998. The number and species of infested trees were recorded. Superficial examination of the trees was made in order to discover any symptoms due to termites infestation.

#### Laboratory investigation:

Wood samples of termite-infested trees were collected from the close vicinity of roots and transferred to the laboratory. The

termites species were identified by the Plant Protection Department, Faculty of Agriculture, Assiut University.

#### Termites :

A colony of the *A. desertorum* was collected from infested mambosia trees.

#### Force-feeding test :

A round plastic container of five cm in diameter and 3.5 cm in height was packed with 50 gm. sterile sand moistened with 7 ml. distilled water to keep the relative humidity near saturation. Five levels of crude extracts were tested (5,10,25,50 and 100 conc. %). Filter papers (1 cm in diameter) were dipped in the methanolic extracts for each level for 10 seconds, and dried at room temperature for evaporation of the solvent. The treated papers were placed on the sand and 100 worker termites were added to each container. Untreated filter papers were supplied to termites as a control. The containers were incubated at  $25\pm 1^{\circ}\text{C}$  for five weeks and the number of surviving termites were recorded in each replicate. Filter papers treated with five concentration levels of the isolated compounds of *Artemisia argentea* (10, 15, 20, 25 and 30 concentration %), were evaluated against termites and the number of surviving termites were taken at 2, 6, 10 and 14 days, respectively.

#### Field application of *Artemisia* extract:

An observation of the trees revealed the existence of tunnels on their surface. These tunnels were removed and cleaned, then the trees were painted with the extract solution (extract soluble in water). The quantity of crude extract which was brushed on each tunnels (7 m. length) equals 260 mg. and observed weekly. After two months the experiment was repeated. To spread the crude extract solution through the colony or the nest of the termites a frame of wood (20×20 cm) full of blocks (1×1×2 cm) of the same treated trees was burried beneath the tree near its root after dipping in the plant extract solution for ten seconds. The frame was also observed after two months.

#### Statistical analysis:

Data were statistically analyzed using F-test.

#### RESULTS AND DISCUSSION :

This paper is the third of a series on the insect distribution of *Isoptera* species in Aswan Botanical Island [16,17]. The pest infested 7 tree species with subterranean termite *A. desertorum*. This higher termite (Termitidae) was the only species that represents order *Isoptera* (Table 1). The first part of this work shows the bioassay of crude extract with four pure compounds from *Artemisia argentea* L' Her on feeding and survival of *Amitermes desertorum* which depend in their digestion on some symbiotic organisms (bacteria), living in their gut.

Table 1: A list of wooden trees in the Botanical Island, Aswan and the number of infested trees by *Amitermes desertorum*

Family	Species name	Common name	Total number of trees	Number of infested trees	Infested trees %
Palmaceae	<i>Borassus flabelliformis</i>	Deiolib palm	6	1	16.7
Palmaceae	<i>Phoenix dactylifera</i>	Date palm	48	2	4.2
Palmaceae	<i>Roystonea regia</i>	Royal palm	143	2	1.4
Myrtaceae	<i>Eucalyptus rostrata</i>	Kafour	3	1	33.3
Myrtaceae	<i>Eugenia janbolana</i>	Mambosia	34	15	44.1
Anacardiaceae	<i>Mangifera indica</i>	Mangoes	14	2	14.3
Moraceae	<i>Pleiogynium solandri</i>	Gambosia	2	1	50

Tables 2 and 3 show the mean survival of *A. desertorum* feeding on filter papers treated with different levels (%) of crude extract during five weeks and the pure compounds during two weeks. It was observed that the percentage of survival was level and exposure dependent. The results represented in table 2 guide to the range of compounds concentration, these ranged between 10-30%. Table 3 indicated that the termites lived approximately two weeks only after feeding on arborescin and yangambin at two levels (10 and 15%) and

the remaining were dead within 10 days for both argentiolid  $\beta$  and sesamine. The activity of sesamine and argentiolid  $\beta$  started from the concentrations 20-30% after two days and increased gradually until becoming maximum at 25% after six days. But all levels (10-20%) showed a weak anti-termitic effect similar to untreated samples and the survival was not significantly different after two days except for compounds argentiolid  $\beta$  and sesamine which gave significant effect.

Table 2 : Mean survival of workers of *Amitermes desertorum* after exposure to *Artemisia argentea* as a crude root extract for five weeks at five concentrations

% Conc. (ppm)	1 week	2 weeks	3 weeks	4 weeks	5 weeks
5 (15)	73	42	13	2	1
10 (30)	14	3	0.0	0.0	0.0
25 (75)	17	0.3	0.0	0.0	0.0
50 (150)	6	0.0	0.0	0.0	0.0
100 (300)	0.0	0.0	0.0	0.0	0.0
Control	100	96	81	69	61

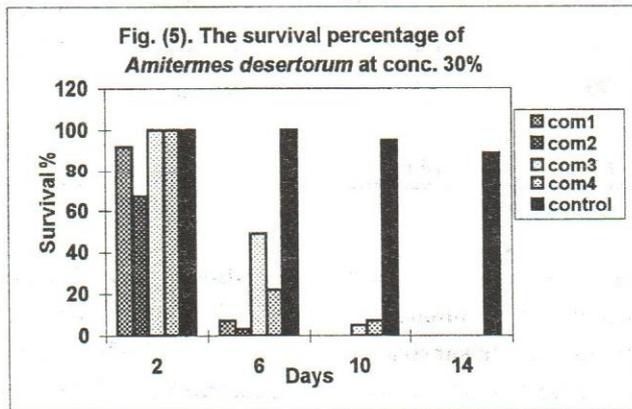
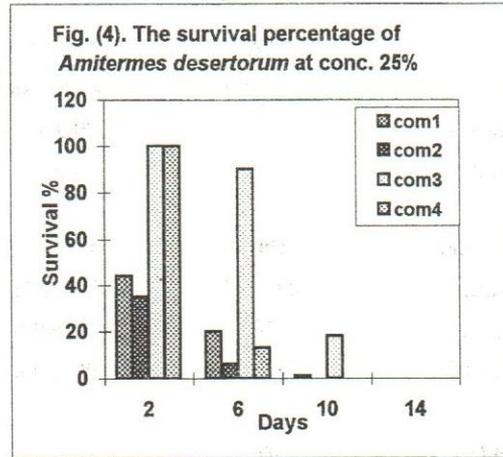
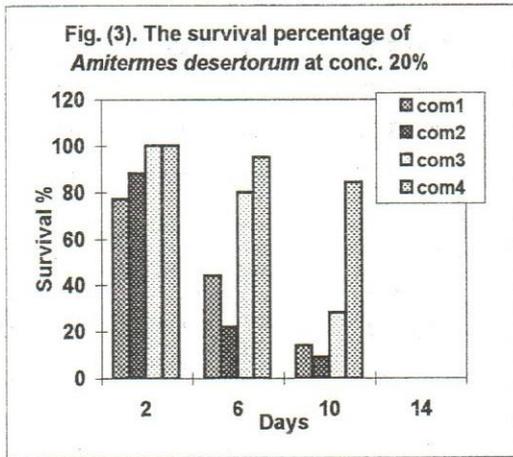
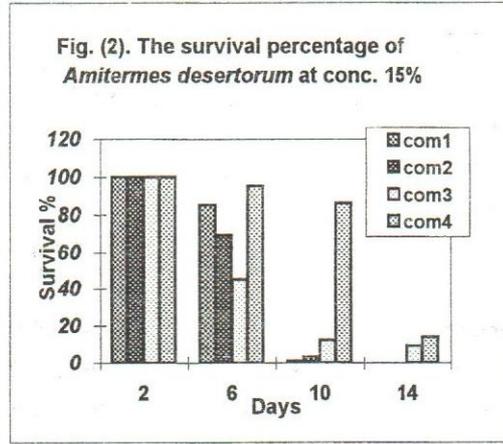
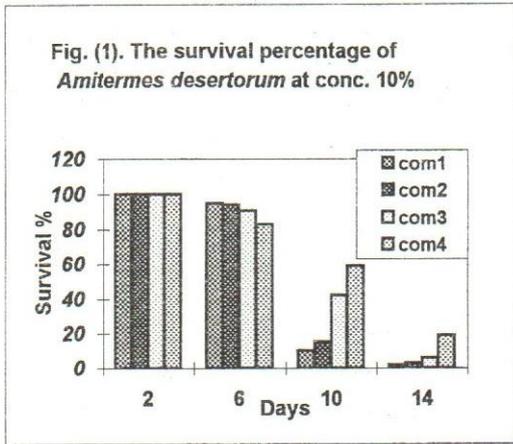
**Table 3 : The survival of *Amitermes desertorum* workers fed on filter papers with five concentrations of four compounds isolated from *Artemisia argentea* root bark .**

Compounds	ppm (conc. %)	Mean survival $\pm$ SD			
		after 2 days	after 6 days	after 10 days	after 14 days
Argentiolid $\beta$	762	100 $\pm$ 0.0	95 $\pm$ 1.26	10 $\pm$ 0.5	2.0 $\pm$ 0.96
Sesamine	433 (10)	100 $\pm$ 0.0	94 $\pm$ 2.94	15 $\pm$ 3.40	3.0 $\pm$ 1.71
Yangambin	714	100 $\pm$ 0.0	91 $\pm$ 3.32	42 $\pm$ 17.17	6.0 $\pm$ 2.63
Arborescin	1300	100 $\pm$ 0.0	87 $\pm$ 7.44	59 $\pm$ 2.63	19 $\pm$ 4.32
Argentiolid $\beta$	1144	100 $\pm$ 0.0	85 $\pm$ 5.44	1.0 $\pm$ 0.5	0.0 $\pm$ 0.00
Sesamine	650 (15)	100 $\pm$ 0.0	69 $\pm$ 5.44	3.0 $\pm$ 1.26	0.0 $\pm$ 0.00
Yangambin	1071	100 $\pm$ 0.0	45 $\pm$ 2.94	12 $\pm$ 1.26	9.0 $\pm$ 1.63
Arborescin	1950	100 $\pm$ 0.0	95 $\pm$ 2.87	86 $\pm$ 4.08	14 $\pm$ 3.56
Argentiolid $\beta$	1526	77 $\pm$ 3.74	44 $\pm$ 5.35	14 $\pm$ 3.30	0.0 $\pm$ 0.00
Sesamine	867 (20)	78 $\pm$ 5.80	22 $\pm$ 3.27	9.0 $\pm$ 0.82	0.0 $\pm$ 0.00
Yangambin	1428	100 $\pm$ 0.0	80 $\pm$ 5.91	28 $\pm$ 2.45	0.0 $\pm$ 0.00
Arborescin	2600	100 $\pm$ 0.0	95 $\pm$ 2.50	84 $\pm$ 3.77	0.0 $\pm$ 0.00
Argentiolid $\beta$	1906	44 $\pm$ 4.65	20 $\pm$ 5.44	1.0 $\pm$ 0.50	0.0 $\pm$ 0.00
Sesamine	1083 (25)	35 $\pm$ 4.50	6 $\pm$ 2.50	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00
Yangambin	1786	100 $\pm$ 0.0	90 $\pm$ 2.94	18 $\pm$ 5.25	0.0 $\pm$ 0.00
Arborescin	3250	100 $\pm$ 0.0	13 $\pm$ 1.89	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00
Argentiolid $\beta$	2287	92 $\pm$ 4.19	7.0 $\pm$ 4.55	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00
Sesamine	1300 (30)	67 $\pm$ 2.83	3.0 $\pm$ 2.06	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00
Yangambin	2143	100 $\pm$ 0.0	49 $\pm$ 8.34	5.0 $\pm$ 3.20	0.0 $\pm$ 0.00
Arborescin	3900	100 $\pm$ 0.0	22 $\pm$ 3.30	7.0 $\pm$ 1.71	0.0 $\pm$ 0.00
Control	0.00	100 $\pm$ 0.0	100 $\pm$ 0.0	95 $\pm$ 3.10	89 $\pm$ 3.30

F=358.08, P=0.000 (ANOVA)

Figures 1-5 show that argentiolid  $\beta$  and sesamine were the most effective compounds, since a significant decrease in the survival of the termites was shown after two days at two applied levels (20-25%). After six days of

treatment most of the termites died at the level of 25 - 30%. No survival was recorded after 10 days at 15-30 %. It was observed that at concentration 30% termites avoid to eat the treated papers and hide beneath sand surface.



There are three possible causes of death:

- 1- The effect of the crude extract and the pure compounds on the derm of the termite;
- 2- Death of bacteria which live in the gut of the termite;
- 3- A combination of the effect on the derm and on the gut bacteria.

This requires further specialized study in order to determine the exact cause. Many investigators world wide have made large screening efforts for plants possessing physiological effects on pests [3,18-22]. The large scale application of common insecticides has a great threat to the environment. The application of the plant extracts to the control of insect pests can be a possible alternative[23].

The second part of our research deals with the application of plant extracts to control of termites on *Eugenia jambolana* (mambosia) and *Roystonea regia* (Royal Palm) trees on Aswan Botanical Island. The data in Table 1 shows about 50% of mambosia trees were infested by termites and we selected also the royal palm tree because it is a rare tropical trees.

In the case of *E. jambolana*, after painting the infested trees with *Artemisia argentea* crude extract, it was observed that the crude extract protected the treated trees from termite attack for two months and prevented the formation of tunnels by termites on the surface of the trees. After two months, the tunnels appeared again, so the experiment was repeated again and the same results were obtained. That, the infestation occurred again

may be due to the infestation of other trees or the crowdy nest by increasing individuals of termite [24]. But in the case of *R. regia*, the crude extract successfully prevented the return of tunnel by termites from April 1997 to April 1998.

Generally, the application of crude extracts or pure compounds derived from the root bark of *Artemisia argentea* offers a desirable alternative to chemical insecticides for the control of the subterranean termite *A. desertorum*, which infests wooden trees in Aswan city. Also of special merit is the testing of other plant extracts against insect and pests, including the termites and the microorganisms inhabiting their guts.

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## حصص حشرات رتبة متساوية الأجنحة . ٣. فى جزيرة النباتات بأسوان مع استخدام المستخلص الخام وبعض المركبات النقية المفصولة من نبات أرتيميزيا أرجنتيا فى مقاومة النمل الأبيض أميترمس ديزرتورم

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سجلت الدراسة والفحص للأشجار فى جزيرة النباتات بأسوان سبعة أنواع تنتمى لثلاثة عائلات كانت مصابة بالنمل التحت أرضى أميترمس ديزرتورم ( ديزنو) . تم تسجيل هذا النوع من النمل الراقى كنوع وحيد ممثل للرتبة متساوية الأجنحة فى هذه الجزيرة .

تم فصل العديد من المركبات المختلفة من قشر جذور نبات أرتيميزيا أرجنتيا - عائلة أستريسي، مشتملة على كل من السيستيترين لكتونات ( أرجنتيوليد ب و أربوريسين ) ومجموعة من مركبات الليجانز خاصة ( سيسامين و يانجامين ) حيث تم تحديد تركيبهم الكيمايى عن طريق مقارنتها بعينات سبق فصلها من أوراق نفس النبات .

تم اختبار الخصائص الإبادية للمستخلص الخام للنبات وأربعة من المركبات النقية المفصولة على شغالات النمل التحت أرضى أميترمس ديزرتورم .

أوضحت النتائج أن للمستخلص الخام نشاط واضح كمبيد لحشرة النمل الأبيض فى كل من اختبارى التغذية والبقاء وذلك بعد أسبوع واحد من التجربة عند كل مستويات الاختبارات ، كما أعطى مركبى الأرجنتيوليد والسيسامين تأثيراً مرتفعاً عند مستوى تركيز ٢٥٪ وذلك بعد يومين .

كما تم التطبيق الحقلى لمستخلص نبات أرتيميزيا أرجنتيا الخام على نوعين من الأشجار فى جزيرة النبات .



## FATTY ACIDS COMPOSITION, NEUTRAL LIPIDS AND PHOSPHOLIPIDS FRACTIONATION IN EXTRACTED LIPIDS OF FOUR PLANT SEED VARIETIES

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### ABSTRACT :

The study was carried out on the extracted lipids of four different varieties of plant seeds viz., *Sehouwia purpurea*; *Cassia senna*; *Cassia italica* and *Cassia holosericea* . The chemical characteristics and lipid classes composition of these lipids were studied.

The total lipids content of plant seeds ranged from 6.7 % to 29.87% , depending on the variety. The total lipids extracts were fractionated into neutral lipids, phospholipids and glycolipids by column chromatography. The neutral lipids varied from 95.62% to 96.28%, phospholipids from 2.04% to 2.78% and glycolipids from 1.06% to 1.6% . Thin layer chromatography ( TLC ) revealed that triglycerides constituted the major fraction of the neutral lipids , for all studied varieties and accounted from 90.78% to 92.70% . TLC showed the presence of seven fractions of phospholipids. The main component of phospholipids was phosphatidyl choline accounted from 36.72 % to 40.62 % of the total phospholipids .

The fatty acid composition of total lipids was carried out by gas liquid chromatography (GLC). The plant seed lipids contained high percentage of unsaturated fatty acids consisted mainly of oleic and linoleic acids. Therefore, the studied plant seeds could be used successfully as a source of unsaturated fatty acids, and offer a promising source of oil for nutritional purposes .

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## INTRODUCTION :

The shortage of oils and fats in several parts of the world has become more acute with the rapid increasing of population. Possible practical procedures to overcome this problem are the cultivation of new crops and/or plant breeding to increase the oil content of traditional oil crops .

The chemical composition of different seeds was affected by the variety of the seed and the environmental conditions of planting [1] .

Oil quality depends solely upon the fatty acid composition of the oil. The previous studies revealed the presence of palmitic, stearic, arachidic and behenic acid among the saturated fatty acids, and palmitoleic, hexadecadienoic, oleic, linoleic, linolenic, and erucic acid from the unsaturated fatty acids in the oil of gobhi sarson (*Brassica napus*)[2] .

Correlation studies revealed that erucic acid was negatively associated with oleic, linoleic and linolenic acids. It is suggested that the variation for fatty acids in gobhi sarson and nature of association amongst fatty acids can be exploited to develop genotypes with better oil quality [2].

Comparative physical and chemical characteristics of chinese sarson (*Brassica chinensis*) seeds oil and other mustard varieties indicated that the chinese sarson seeds are low in oil but high in protein content. Besides, the specific gravity and saponification values of

Chinese sarson oil are slightly higher compared to other Brassicae seed oils . The oil contained more erucic acid and less linolenic acid than other varieties [3].

Lajolo et al.,[6] reported that the chemical composition, oil characteristics, and glucosinolate, sinapine, and phytic acid contents were studied in low glucosinolate *Brassica napus* varieties. Lipids (43- 45%) with an erucic acid content lower than 1% and proteins (18-20%) were the main components. The rape seed varieties introduced in Brazil showed adequate agronomic and chemical characteristics and still offer a promising source of oil and protein for nutritional purposes .

Therefore, the potential for utilizing some plant seeds for oils production appears to be favorable. This study was designed to shed light upon the lipids and phospholipids fractionation in the oils of four Saudian plant seeds. The fatty acid composition of the extracted lipids were studied as well .

## EXPERIMENTAL :

### Materials and Methods :

#### Materials :

Representative samples of four varieties of Saudian plant seeds namely: *Sehouwia purpurea*; *Cassia senna*; *Cassia italica* and *Cassia holosericea* were collected from Taif area, kingdom of Saudi Arabia. The seeds were crushed and dried in an oven at 40°C.

### Analytical methods :

The crushed seeds (2g) were extracted with petroleum ether in a soxhlet apparatus at 40-60°C for 16 hrs. For quantitative determination of crude oil, according to AOAC method [5].

Total lipids of the ground seeds were extracted using a solvent mixture of chloroform methanol (2:1 v/v) according to the method described by Folch et al. [6].

Acidity, iodine, saponification, peroxide values and unsaponifiable matters were determined according to AOAC methods [5].

### Fractionation of lipid classes:

The extracted lipids were fractionated by silicic acid column chromatography, using sequential elution with chloroform to obtain the neutral lipid. The glycolipids and phospholipids were eluted with acetone and methanol, respectively [7,8].

The eluted fractions were collected in preweighed flasks, solvents were removed using a rotary evaporator at 40°C. Each eluted fraction was determined gravimetrically as a weight percentage of the total lipids .

### Fractionation of neutral lipids and phospholipids :

This was carried out on a thin layer of silica gel G plate using hexane : diethyl ether : acetic acid (80: 20:1 v/v) and chloroform:

methanol: water (85: 10 : 5 v/v), respectively, according to the methods of Malins and Mangold and of Radwan [9,10] .

For quantitative analysis of neutral lipids and phospholipids, the chromatograms were scanned using Shimadzu TLC scanner (C-S-910). The area under each peak was measured by the triangulation method [11] . The percentage of each fraction was calculated with regard to the total area .

### Estimation of fatty acids :

The methyl esters of fatty acid composition of total lipids of each variety were prepared as described by Rossell et al. [12] using 3% H<sub>2</sub>SO<sub>4</sub> in absolute methyl alcohol .

A Perkin Elmer gas chromatograph (GC-4CM-Shimadzu), with a flame ionization detector was used in the presence of nitrogen as a carrier gas. The separation was carried out at 150-240°C (temperature rate 5°C min on a (3 M × 0.3 mm) glass column packed with silar 5 cp on chromo (80-100) mesh. Both the injector and detector temperatures were 270°C. The nitrogen, hydrogen and air flow rates were 20, 1 and 0.5 ml min, respectively. The chart speed was 5 mm min .

Peak identifications were established by comparing the retention times obtained with standard methyl esters. Quantitative results were obtained with the aid of an HP computing integrator .

**RESULTS AND DISCUSSION :**

Table(1) illustrates the chemical characteristics of plant seed lipids. The data

showed that the chemical characteristics of the lipids varied slightly according to the variety of the seeds .

**Table (1) : Chemical characteristics of plant seed lipids .**

Characteristics	Seed varieties			
	<i>Schouwia purpurea</i>	<i>Cassia senna</i>	<i>Cassia italica</i>	<i>Cassia holosericea</i>
Iodine value	109	101	118	124
Acid number	0.9	0.4	0.6	0.4
Peroxide value	0.1	0.1	0.2	0.3
Saponification number	185	192	190	188
Unsaponifiable matter %	1.0	0.9	1.2	1.1

The iodine value of plant seed lipids were 109, 101, 118 and 124 in *Schouwia purpurea*; *Cassia senna*; *Cassia italica* and *Cassia holosericea* varieties, respectively. The extracted lipids showed relatively low acid numbers and peroxide values, indicating their high stability against deterioration. This clearly indicated that the studied plant seed lipids might have low levels of oxidative and lypolytic activities or could have high contents of natural

antioxidants. As shown in the tabulated data, the saponification number and the unsaponifiable matter for all studied samples differed slightly .

**Fractionation of Lipid Classes :**

The relative percentages of the major lipid classes are presented in table (2).

**Table (2): Total lipids and their classes from different varieties of plant seeds \***

Constitatuents	<i>Schouwia purpurea</i>	<i>Cassia senna</i>	<i>Cassia italica</i>	<i>Cassia holosericea</i>
A- Total lipids content %	29.87	9.05	11.65	6.70
B- Lipid classes as % of total lipid :				
1- Neutral lipids	95.62	96.04	96.18	96.28
2- Phospholipids	2.78	2.66	2.04	2.62
3- Glycolipids	1.46	1.20	1.60	1.06
Column recovery	99.86	99.90	99.82	99.96

\* On dry weight basis .

The neutral lipids represented 95.62%, 96.04 %, 96.18% and 96.28% of the total lipids of *Schouwia purpurea* ; *Cassia senna* ; *Cassia italica* and *Cassia holosericea* seed lipids, respectively.

However, phospholipids and glycolipids recorded(2.78% and 1.46%), (2.66% and 1.20%), (2.04% and 1.60%) and (2.62 %, and 1.06%) of the total lipids of the same studied varieties, respectively .

The qualitative and quantitative data of the individual lipid classes of the studied plant

seed lipids are shown in table (3) and Figs (1 and 2) .

The tabulated data showed that the neutral lipids revealed the presence of seven fractions (Fig. 1).

Triglycerides constituted the major percentage of the neutral lipids, and accounted for 91.82% , 90.78% , 92.70% and 91.0% of *Schouwia purpurea*, *Cassia senna*, *Cassia italica* and *Cassia holosericea* respectively.

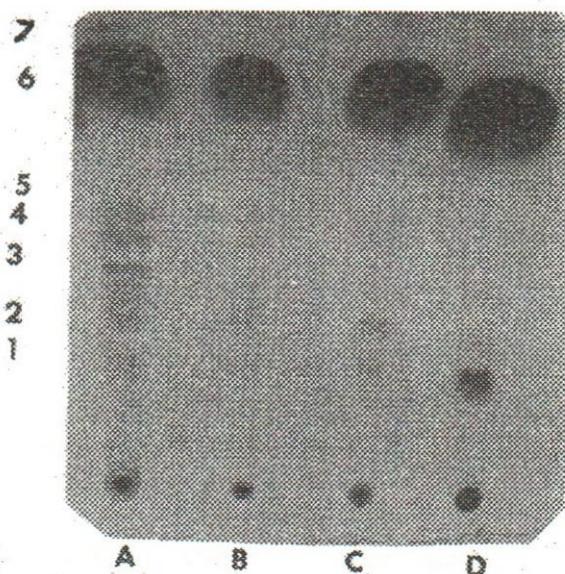


Fig. (1) : Thin layer chromatogram of neutral lipid fractions of plant seeds.

A= *Schouwia purpurea*  
C= *Cassia italica*

B= *Cassia senna*  
D= *Cassia holosericea*

1- Monoglycerides  
3- sterols  
5- Free fatty acids  
7- Sterol esters + Hydrocarbons .

2- 1,2 & 2, 3 - diglycerides  
4- 1,3 diglycerides  
6- Triglycerides

The data showed that the lowest percentage of triglycerides was found in *Cassia senna* lipids (90.78%), indicating that the lipid might have been hydrolyzed, which resulted in higher proportions of free fatty acids (3.62%).

Similar trend of results that reported in the literature concerning *Brassica juncea* lipids [13].

Table (3): The densitometric analysis of the major lipid fractions of plant seed lipids .

Lipid	<i>Schouwia purourea</i>	<i>Cassia senna</i>	<i>Cassia italica</i>	<i>Cassia holosericea</i>
<b>Neutral Lipids :</b>				
Monoglycerides	0.62	0.78	0.50	0.44
1,2 & 2,3-diglycerides	1.24	1.02	1.68	1.82
Sterols	1.02	1.26	0.82	0.96
1,3 diglycerides	1.34	1.42	1.06	1.06
Free fatty acids	2.48	3.62	2.04	2.92
Triglycerides	91.82	90.78	92.70	91.00
Sterol esters+ Hydrocarbons	1.48	1.12	1.20	1.80
<b>Phospholipids :</b>				
Phosphatidyl serine	5.62	6.45	5.44	5.82
<b>Lyso-phosphatidyl choline</b>				
Lyso-phosphatidyl choline	3.45	2.98	3.06	3.41
Phosphatidyl inositol	9.82	8.82	7.43	9.22
Phosphatidyl choline	36.72	38.04	40.62	37.33
Phosphatidyl ethanolamine	29.41	27.66	28.50	29.62
Unidentified	11.26	12.63	12.14	11.97
Phosphatidic acid	3.72	3.42	2.81	2.63

On the other hand, variations were detected in the minor lipid fractions among the different varieties of the studied plant seed lipids .

The phospholipid class showed seven fractions (Fig. 2). Six of these fractions were identified. The data indicated that the main component of phospholipids was phosphatidyl choline, amounting to 36.72%, 38.04%,

40.62% and 37.33% of the total phospholipids in the above studied samples, respectively. It was followed by phosphatidyl ethanol amine, unidentified, phosphatidyl inositol, phosphatidyl serine and Lysophosphatidyl choline, respectively .

However, rather slight variations in phospholipid components were observed among all the studied varieties .

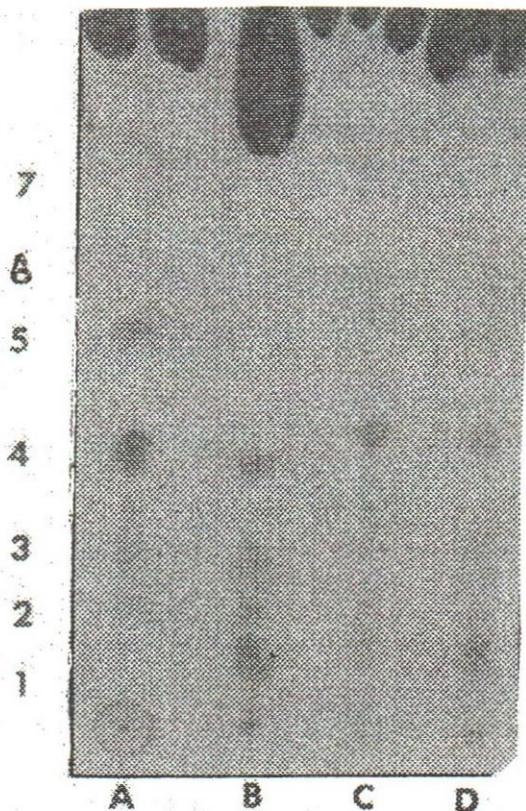


Fig (2) : Thin layer chromatogram of phospholipid fractions of plant seeds.

A= *Schouwia purpurea*  
C= *Cassia italica*

B= *Cassia Senna*  
D= *Cassia holosericea*

1- phosphatidyl serine  
3- phosphatidyl inositol  
5- phosphatidyl ethanolamine  
7- phosphatidic acid

2-lyso-phosphatidyl choline  
4- phosphatidyl choline  
6- unidentified

### Fatty acids composition :

The fatty acids composition of the total lipids are presented in Table (4).

The tabulated data showed that *schouwia purpurea* seed lipids contained high percentage

of unsaturated fatty acids (53.417%) of the total fatty acids , consisted mainly of oleic acid (25.705%) followed by linoleic acid (15.474%) and linolenic acid (12.238%). The main saturated fatty acid was arachidic acid (18.059%).

Table (4) : Fatty acids composition of total lipids in some plant seeds (% of the total) .

Fatty acids %	Carbon	<i>Schouwia purpurea</i>	<i>Cassia senna</i>	<i>Cassia italica</i>	<i>Cassia holosericea</i>
	Chain Length				
Hendeconic	C <sub>11</sub> : 0	0.332	0.489	N.D.*	N.D.*
Lauric	C <sub>12</sub> : 0	13.004	4.014	1.156	3.968
Trideconic	C <sub>13</sub> : 0	0.784	N.D.*	0.129	N.D.*
Myristic	C <sub>14</sub> : 0	1.988	1.264	0.491	0.663
Pentodeconic	C <sub>15</sub> : 0	4.591	8.043	N.D.*	4.886
Palmitic	C <sub>16</sub> : 0	7.829	40.424	29.812	18.856
Oleic	C <sub>18</sub> : 0	25.705	41.773	29.323	20.011
Linoleic	C <sub>18</sub> : 0	15.474	1.452	39.089	51.616
Linolenic	C <sub>18</sub> : 0	12.238	N.D.*	N.D.*	N.D.*
Arachidic	C <sub>20</sub> : 0	18.059	2.541	N.D.*	N.D.*
Total saturated fatty acids		46.583	56.775	31.588	28.373
Total unsaturated fatty acids		53.417	43.225	68.412	71.627

\*N.D. = Not detected

In addition, the data showed that total lipids of *Cassia holosericea* seemed to contain high levels of total unsaturated fatty acids (71.627%) followed by *Cassia italica* variety (68.412%), consisted mainly of linoleic acid (51.616% and 39.089%) followed by oleic acid (20.011% and 29.323%) respectively. The variation in unsaturated fatty acids in all studied samples may account for the differences in the iodine value among the varieties. The main saturated fatty acid was palmitic as its percent was 40.424%, 29.812% and 18.856% in *Cassia senna*, *Cassia italica* and *Cassia holosericea*, respectively. These trends of results are in agreement with those previously reported by Singh et al. [11].

From this study it could be noticed that the four varieties of Saudian plant seeds had a

high unsaturated fatty acids content and they can be used successfully, as a source of these fatty acids, and offer a promising source of oil for nutritional purposes .

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## دراسة تركيب الأحماض الدهنية ، تفريد مكونات الليبيدات المتعادلة والفوسفوليبيدات في المستخلص الليبيدي لأربعة أنواع من البذور النباتية

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أجريت الدراسة على الليبيدات المستخلصة من أربعة أنواع من البذور النباتية . تم دراسة الخصائص الكيميائية لليبيدات الكلية المستخلصة ، كذلك قسمت هذه الليبيدات إلى ثلاث أجزاء هي :

الليبيدات المتعادلة - الفوسفوليبيدات - الجليكوليبيدات ، وذلك باستخدام التحليل الكروماتوجرافي بالأعمدة . وإستخدمت كذلك طريقة التحليل الكروماتوجرافي بالطبقة الرقيقة (TLC) لتفريد مكونات الليبيدات المتعادلة والفوسفوليبيدات .

تم كذلك دراسة تركيب الأحماض الدهنية في الليبيدات الكلية باستخدام التحليل الكروماتوجرافي الغازي (GLC) .

وقد أظهرت نتائج الدراسة ما يلي :

- 1 - تراوحت نسبة المحتوى الليبيدي في البذور المختلفة من ٦,٧٪ - ٢٩,٨٧٪ .
- 2 - تراوحت نسبة الليبيدات المتعادلة من ٩٥,٦٢٪ - ٩٦,٢٨٪ بالنسبة لليبيدات الكلية ، بينما كانت نسبة الفوسفوليبيدات من ٢,٠٤٪ - ٢,٧٨٪ ، والجليكوليبيدات من ١,٠٦٪ - ١,٦٠٪ .
- 3 - أظهرت نتائج التحليل الكروماتوجرافي بالطبقة الرقيقة وجود ٧ مكونات لليبيدات المتعادلة ، أكثرها الجلسريدات الثلاثية حيث تراوحت نسبتها من ٩٠,٧٨٪ - ٩٢,٧٠٪ بينما تم تفريد الفوسفوليبيدات إلى ٧ مكونات أيضاً ، وكان أكثرها تركيزاً مركب الفوسفاتيديل كولين حيث كانت نسبته من ٣٦,٧٢٪ - ٤٠,٦٢٪ من التركيز الكلي للفوسفوليبيدات .
- 4 - أوضحت نتائج التحليل الكروماتوجرافي الغازي أن الليبيدات المستخلصة من البذور موضع الدراسة تحتوي على نسبة مرتفعة من الأحماض الدهنية غير المشبعة وبصفة أساسية أحماض الأوليك واللينولينيك .

على ضوء هذه الدراسة يمكن التوصية بأنه يمكن استخدام البذور النباتية موضع الدراسة الحالية كمصدر للزيوت النباتية غير المشبعة حيث أن نسبة الزيوت بها مرتفعة وكذلك لإرتفاع محتوى هذه الزيوت من الأحماض الدهنية غير المشبعة ذات الأهمية من الناحية الغذائية .