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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 anf 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-virto* 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 pictinase 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 plateform 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±0.05 to 6.61±0.07 (in the raw samples) while ranged from 6.41±0.04 to 6.54±0.13 (in 5% ruminal suspension). On the other hand, the pH values of the control samples were 7.18±0.04 (raw ruminal samples), and 7.11±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 carbo-hydrates or in nitrogenous feed [45, 49,50]. However, the mean pH values of 5% faecal samples were between 8.19±0.07 and 8.24±0.08, while it was 7.9±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 $1.2x10^5$ in the control group to $6.8x10^4$/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 $1.6x10^4$ to $3.9x10^4$ 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 $1.7x10^5$ (control group) to $6.3x10^4$ 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
1st group (control)	7.18± 0.04	7.11±0.03	7.9±0.05
2nd (5% *Acacia nilotica*)	6.59± 0.07	6.53±0.03	8.19±0.09
3rd (10% *A. nilotica*)	6.61± 0.07	6.54±0.13	8.24±0.08
4th (15% *A. nilotica*)	6.49± 0.04	6.46±0.06	8.22±0.08
5th (20% *A. nilotica*)	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 %
1st group (control)	$1.2x10^5$	-	$1.7x10^5$	-
2nd group	$9.9x10^4$	17.5	$3.5x10^5$	*
3rd group	$2.3x10^5$	*	$2.5x10^5$	*
4th group	$9.4x10^4$	12.7	$9.3x10^4$	45.3
5th group	$6.8 x10^4$	43.3	$6.3x10^4$	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 durig the subsequent investigation. This disappearence 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 disappearence 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 proportial 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 repoted [60], when they used tannin fragments as some flavonoid materials.

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

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

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

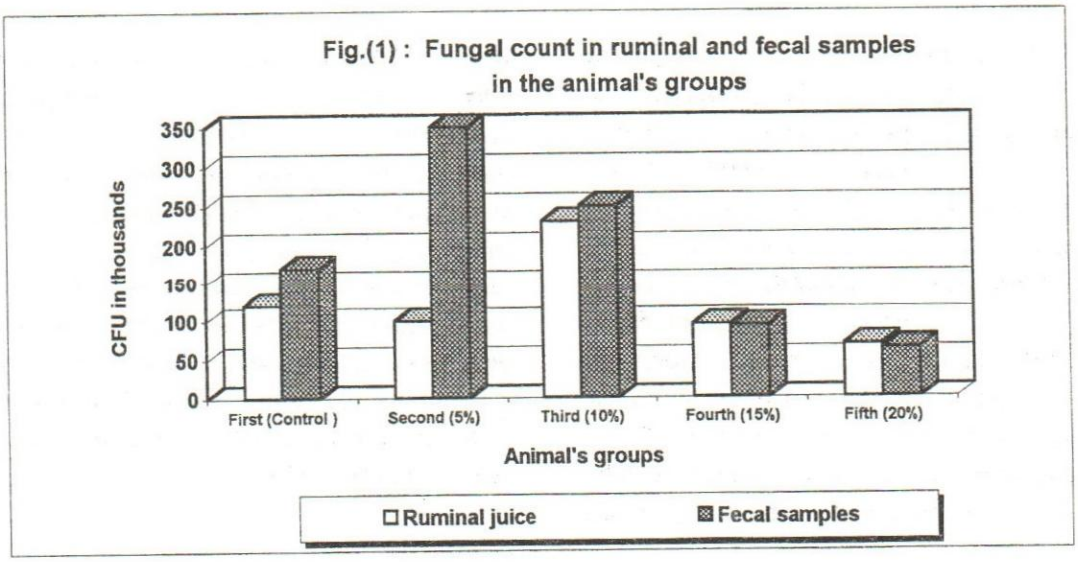
Fungal isolates	Faecal samples				
	1st group (Control)	2nd group	3rd group	4th group	5th group
Aspergillus niger	$3.0x10^4±1.2x10^2$	$7.0x10^4±9.8x10^3$	$6.2x10^4±2.3x10^4$	$5.4x10^3±4.2x10^3$	$3.8x10^3±1.7x10^3$
A. flavus	$5.7x10^2±6.2x10$	$1.9x10^5±7.1x10^4$	$6.7x10^4±1.3x10^4$	$2.3x10^4±1.5x10^3$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

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 imhibition in relation Conc. of plant material		
			0.25%	0.50%	0.75%
Aspergillus fumigatus	T_0	$5.6x10^7$	$5.4x10^7$ (3.6%)	$5.3x10^7$ (5.4%)	$4.8x10^7$ (14.3%)
	T_{30}	$5.4x10^7$	$6.2x10^7$ (**)	$5.1x10^7$ (5.6%)	$4.3x10^7$ (20.4%)
	T_{60}	$5.4x10^7$	$5.1x10^7$ (5.6%)	$5.0x10^7$ (7.4%)	$2.3x10^7$ (57.4%)
	T_{90}	$5.1x10^7$	$5.7x10^7$ (**)	$4.3x10^7$ (15.7%)	$1.7x10^7$ (66.7%)
	T_{120}	$4.8x10^7$	$5.1x10^7$ (**)	$3.6x10^7$ (25%)	$9.8x10^6$ (79.6%)
A.flavus	T_0	$2.9x10^7$	$3.1x10^7$ (**)	$2.8x10^7$ (3.4%)	$1.8x10^7$ (37.9%)
	T_{30}	$2.9x10^7$	$3.2x10^7$ (**)	$2.6x10^7$ (10.3%)	$8.7x10^6$ (70.0%)
	T_{60}	$3.1x10^7$	$3.0x10^7$ (3.2%)	$2.1x10^7$ (32.3%)	$6.4x10^6$ (79.3%)
	T_{90}	$3.2x10^7$	$3.0x10^7$ (6.3%)	$1.6x10^7$ (50%)	$6.2x10^6$ (80.6%)
	T_{120}	$3.2x10^7$	$2.8x10^7$ (12.5%)	$7.2x10^6$ (77.5%)	$3.5x10^6$ (89.1%)
A. niger	T_0	$2.9x10^5$	$3.1x10^5$ (**)	$2.5x10^5$ (13.8%)	$1.5x10^5$ (48.3%)
	T_{30}	$2.7x10^5$	$3.0x10^5$ (**)	$2.4x10^5$ (11.1%)	$3.8x10^5$ (**)
	T_{60}	$2.8x10^5$	$2.7x10^5$ (3.6%)	$2.5x10^5$ (10.7%)	$1.5x10^5$ (46.4%)
	T_{90}	$2.6x10^5$	$2.7x10^5$ (**)	$2.0x10^5$ (23.1%)	$3.1x10^5$ (**)
	T_{120}	$2.5x10^5$	$2.5x10^5$ (0%)	$1.9x10^5$ (24%)	$7.9x10^4$ (68.4%)
Fusarium solani	T_0	$2.0x10^6$	$1.9x10^6$ (5.0%)	$1.6x10^6$ (20%)	$1.0x10^6$ (50.0%)
	T_{30}	$1.8x10^6$	$1.8x10^6$ (0%)	$1.5x10^6$ (16.7%)	$7.8x10^5$ (56.7%)
	T_{60}	$1.9x10^6$	$2.0x10^6$ (**)	$1.2x10^6$ (36.8%)	$7.1x10^5$ (62.6%)
	T_{90}	$1.4x10^6$	$2.1x10^6$ (**)	$1.0x10^6$ (28.6%)	$5.6x10^5$ (60.0%)
	T_{120}	$1.2x10^6$	$1.3x10^6$ (**)	$8.3x10^5$ (30.8%)	$2.1x10^5$ (82.5%)

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



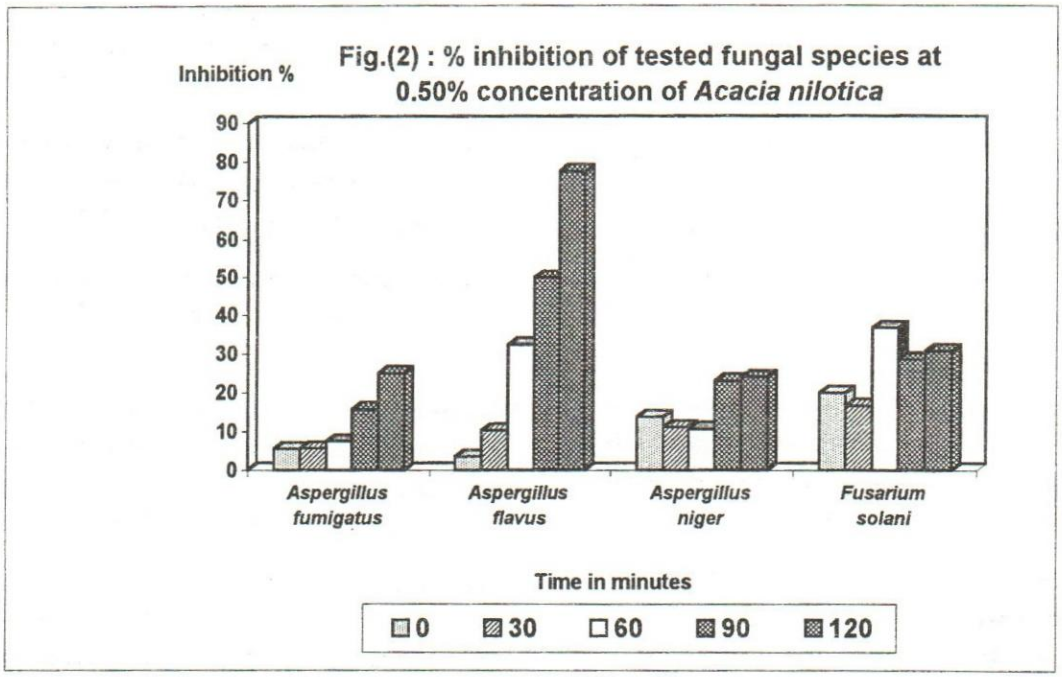


Fig.(2) : % inhibition of tested fungal species at 0.50% concentration of *Acacia nilotica*

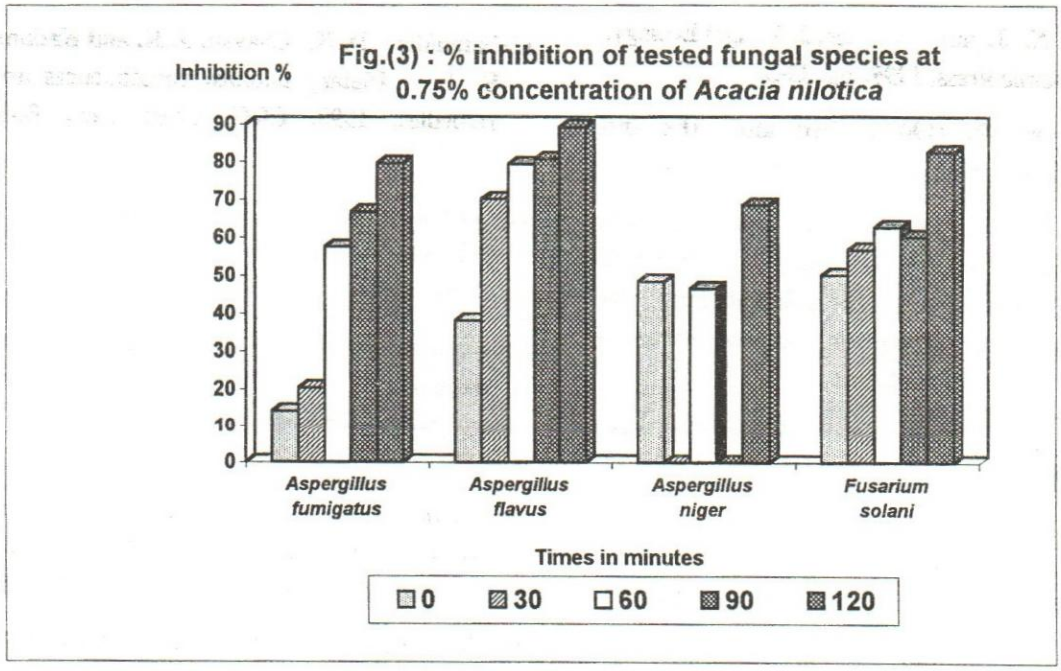


Fig.(3) : % inhibition of tested fungal species at 0.75% concentration of *Acacia nilotica*

REFERRENCES :

1- Biali, V.J. and Pidoplicho, N.M.: On microflora of the rumen of cows suffering from chronic haematurea. Microbiol. Zh., 1962, 24: 3-8.

2- Raper, X.B. and Fennel, D.I.: A manual of the Pencillia. Williams & Wilkins, 1965, Baltimore, U.S.A.

3- Brewer, D. and Taylor, A., : *Aspergillus fumigatus* and *Sporomia minima* isolated from the rumen of sheep., 1969, National Research council of Canada, Atlantic Regional Laboratory, Halifax, Nova Scotia, Canada.

4- Manfield, J. W.: Antimicrobial Compounds. In: Biochemical Plant Pathology (ed., J.A. Callow), J. Wiley & Sons), New York, 1983, pp. 237-265.

5- Dix, N. J. and Webster, J. Fungal Ecology. Academic Press, 1995, pp. 33-55.

6- Gilliver, R. (1947): cited after Dix and Webster, "Fungal Ecology", 1995, pp. 49.

7- Melin, E.: Der Einfluss von waldstreu-extrakten auf das wachstum von Bodenpliz mit besonderer Berucksichtigung der Wurzelpliz von Blumen. symbolae Botanicae Upsalienes, 1946, 8: 1-16.

8- White, T. : "Tannins" their occurrence and significance. J. Sci. food Agric., 8: 372-385 (1957).

9- Swain, T: Secondary compounds as protective agents. Ann. Rev. plant Physiol., 28: 479-501(1977).

10- Bate-Smith, E.C. and Swain, T. : Flavonoid compounds In: Comparative biochemistry, Vol. III (Eds, Florkin, M and Mason, H.S.) Academic Pess, New York, 1962, 755-809.

11- Haslam,E. : Vegetable tannins In: Conn,E.E. (ed.), The biochemistry of plant, Vol., 7, New York, Academic Press, 1981, pp. 527-544.

12- McLeod, M.N. : " Plant tannins " their role in forage quality. Nutr. abstr., 44: 803-815 (1974).

13- Tackechi, M., Tanaka, Y., Takehara, M., Nonaka, G., and Nishioka, I. : Structure and antiseptic activity among the tannins. Phytochemistry, 24: 2245-2250 (1985).

14- Scholz, E., Heinrich, M. and Hunkler, D. : New caffeic acid esters from *Plucha symphytifolia* and their antimicrobial activity. Planta medica, 56: 648-651 (1990).

15- Salunkhe, D. K., Chavan, J. K. and Kadam, S. S. : Dietary tannins: consequences and remedies, 1990, CRC, Press, Inc., Boca Raton, Fla.

16- Gustavon, K. H. : The chemistry of tannin processes, 1954, New York, USA, Academic Press.

17- Cannon, C. B. : The interactions and structure of the COWH- groups in amides and polyamides. Microchem. Acta., 1955, pp. 555.

18- Loomis, W. D. and Battaile, J. : Plant phenolic compounds and the isolation of plant enzymes. Phytochemistry, 5: 423-438 (1966).

19- Van Sumere,C.F., Albercht, J, Dedonder, A., Depooter, H. and Pe, I.: The chemistry and

biochemistry of plant proteins (eds.) Harbone, J. B. and Van Sumere, C.F., Academic Press, New York, 1975, pp. 212,.

20-Booth, G.H.: A study of the effect of tannins on the growth of sulfate-reducing bacteria. J. Appl. Bact., 23: 125-129 (1960).

21-Henis, Y. Tagari,H. and Volcani,R.: Effect of water extracts of carob pods, tannic acid and their derivatives on the morphology and growth of micro-organisms. Appl. Microbiol., 12(3): 201-209 (1964).

22-Grant, W.D. and McMurtry, C. M. : Effect of condensed tannins on growth of micro-organisms. In: Microbial Ecology, Loutit, M.W. and Miles, M. (Eds.), 1978, 427-430, Springer Verlag.

23-Sotohy, A. S., Müller,W. and Ismail, A. A.: In-vitro effect of Egyptian tannin-containing plants and their extracts on the survival of pathogenic bacteria. Dtsch. Tierkrztl. Wschr., 102: 334-348 (1995).

24-Cook, M. T. and Taubenhaus, J. J. : The relations of parasitic fungi to the contents of the cells of host plants. 1: the toxicity of tannins. Bulletin Delaware College Agricul. Exper. Stat., 91, 1-77 (1911).

25-Kundson, L.. Tannic acid fermenta-tion. J.of Biol. Chemistry,14: 159-184 (1913).

26-Lindeberg, J. : Influence of enzymatically oxidised gallic acid on the growth of some hymenomycetes. Svensk Botanisk Tidskrift, 43: 438-447 (1949).

27-Cowley, G.T. and Whittingham, W.F. : The effect of tannins on the growth of selected soil microfungi in culture. Mycologia, 53: 539-542 (1961).

28-Brauch, J.N., Rao, P.R., Janardhanan, K.K. and Ganguly, D. : Fungitoxicity of different polyphenolic fractions of Acacia arabica bark. J.proc. Inst. Chem., 35: 303-306 (1963).

29-Lyr, H. : On the toxicity of oxidized polyphenols. Phytopathologische Zeitschrift, 52: 227-240 (1965).

30-Lewis, J. A. and Papavizas, G.C. Effects of tannins on spore germination and growth of *Fusarium solani f phaseoli* and *Verticillium Albo-Atrum*. Canad. J. Micro. , 13: 1655-1661 (1967).

31-Dix, N. J. : Identification of a water-soluble fungal inhibitor in the leaves of *Acer plantanoides* . Annales of Botany, 38: 505-514 (1974).

32-Megalla, S.E, El-keltawi, N.E.M., and Ross, S. A. : A study of antimicrobial action of some essential oil constituents. Herba polonica, 26 (3): 181-186 (1980).

33-Lyr, H. : Hemmungsanalytische Unter suchungen an einigen Ektoenzymen holzzer Strender pilz. Enzymologia, 23: 231-248 (1961).

34-Basaraba, J.: Effect of vegetable tannins on glucose oxidation by various microorganisms. Can. J. microbiol., 12: 787-794 (1966).

35-Mahadevan, A. and Muthukumar, G.: Aquatic microbiology with reference to tannin degradation. Hydrobiologia, 72: 73-79 (1980).

36-Goldstein, J. L. and Swain, T.: The inhibition of enzymes by tannins. Phytochemistry, 4: 185-192 (1965).

37-De-Vries, G.A.: Contribution to the knowledge of the genus Cladosporium, 1952, Limited Ex. Fr. Viggererij and Drukkerij, Hollanclia Press, Baarn.

38-Gilman. I. C. : A manual of soil fungi, 1957, Iowa state University Press. Ames. Iowa, USA.

39-Zycha, H., Siepmann, R. and Linneman,G.: Mucorales eine Beschreibung aller gattungen und Arten dieser, Pilzgruppe J. Cramer Verlag, pp. 3301 (1969).

40-Booth, C. : The genus fusarium. Common wealth Mcological Institute, Kew, Surrey, England, 1971.

41-Ellis, M. B. : Dematiaceous hypho-mycetes. common Wealth, 1971, Mycological Institut, Kew, Surrey, England .

42-Kezalliewiez, Z. : Aspergillus species on stored product, C. A. B. International Mycological Institute. Mycological papers, No. 116, pp. 188 (1989).

43-Cruickshank, R., Dugid, J. P., Mormison, B. P. and Swain, R. H. : Medical Microbiology, 1981, 12th Ed., Vol. II, reprinted churchill, Livingstone and Robert Stevenson, Edinbergh, EH I, 3AE.

44-Klindworth, K.J., Davidson, P.M., Brekke, C. J. and Branen, A. L.: Inhibition of Cl. Perfringens by butylated hydroxyanisole. J. food Sci., 44 (2): 564-567 (1979).

45-Knoth, M. : Nue Versuche zur zuchtung der in pasen Von Wiederkzuren lebenden Ophryoscoleiden. Z. parasitentunde, 1: 262-270 (1928).

46-Ferber, K.E.: Derzahl und masse der in passen. USW.Z. für Tierzuchtung, 12: 31-38 (1928).

47-Myburgh, S.J. and Quin, J. I. : Studies of the alimintary tract of Merino sheep in south Africa. IX. The H-ion concentration in the forestomach in a fistulated sheep under different experimental conditions. Can. J. Vet. Sci. And Anim. Ind., 18: 119-130 (1944).

48-Abd El-Salam, M. N. : Ruminal fluid picture in sheep feed on field. M.V.Sc., thesis, Assiut University, Assiut, Egypt, 1980.

49-Blood and Henderson : Veterinary medicine, 3rd Ed., Baillier, Tendall & Gassel, London, 1968.

50-Olsen, T.M. : The pH values of the ingesta of the rumen of slaughtered animals. J. dairy Sci., 24: 413-416 (1941).

51-Jurd, L., Stevens, K. L., Ring, A.D. and Michara, K. : Antimicrobial properties of natural phenols and related compounds. II. Cinnamylated phenols and their hydro-genation products. J. pharm. Sci., 60: 1753-1755 (1971).

52-Chang, N.C. and Branen, A.L.:Antimicrobial effects of butylated hydro-xyanisol (BHA). J. food Sci., 40: 349-351 (1975).

53-Zemeck, J., Kosikova, B., Augustin, J. and Joniak, D.: Antibiotic properties of lignin components. Folia microbiology, 24: 483-486 (1979).

54-Lund,A.: Yeast and Moulds in bovine rumen. J. Gen. Microbiol., 81 (2): 353-462 (1974).

55-Wogan, G. N. : Mycotoxins in food stuffs. Cambridge, 1966, Mass. Inst. Technol. Press.

56-Clrak, B. M. : Epidemiology of phycomycosis in Wolstenholme, G. E. W. and Porter, R. (eds) : Systemic Mycosis., 1968, London, Church hill, uk .

57-Grove, M. D., Yates, S. G., Talleny, W.H., Ellis, J.I., Walf, I.A., Kousuri, N.R. and Nicholes,R.E.: Mycotoxin production by Fusarium tricinctum as possible causes of cattle diseases. J. Agr. Food Chem., 18: 734-736 (1970).

58-Emmons, C. W., Binford, C. H., Vtz, J. M. and Chung, K. J. : " Medical Mycology ", Henry Kimpton, 1977, London.

59-Ivancheva, S., Manolova, N., Serkedjieva, J. Dimov, V. and Ivanooska, N. : Polyphenols from Bulgarian medicinal plants with anti-infectious activity. In: Plant polyphenols (ed. Hemingway, R.W. and Laks, P.E., Plenum Press, New York, 1992, pp. 717.

60-Sannmartin, E. I., Barberan, F. A. M., Guirado, A. and Lovente, F.T.: Antibacterial flavonoides from Helichrysum picardii and Helichrysum italicum. Planta medica, 56: 648-649 (1990).

61-Schraegle, R. and Mueller, W.: The influence of selected tannin-containing plant species on the tenacity of pathogenic bacteria in an in-vitro rumen system. J. Vet. Med., 37: 181-186 (1990).

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التأثير المثبط للنباتات الغنية بالتنينات (السنط) على نمو الفطريات.

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تم إجراء هذا البحث بهدف تقييم تأثير المركبات الفينولية [التنينات] 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 [خمسة 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 أسبوعيا من عصير الكرش والبراز لمدة شهر من كل المجموعات بهدف دراسة العد الطبقى 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 مثبط 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 تكرارا فى عينات الكرش والبراز .

الجزء الثانى تم إجراؤه معمليا 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 ٠,٢٥٪ ، ٠,٥٠٪ ، ٠,٧٥٪ ثم حساب العد الطبقى القياسى للفطريات كل نصف ساعة على مدى ساعتان . أوضحت النتائج أن إضافة النبات عند تركيز ٠,٢٥٪ لم يكن له أى تأثير على حيوية الفطريات فى حين وجد أن إضافته بنسب ٠,٥٠٪ ، ٠,٧٥٪ أدى إلى قتل الفطريات بنسبة كبيرة . كما وجد كذلك أن نسبة قتل الفطريات تناسبت طرديا 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 ٠,٥٠٪ ، ٠,٧٥٪ على التوالى وجاء فطر الفيوزاريوم سولانى فى المرتبة الثانية ثم تلاه فطر الأسبرجلس فيوميجاتس فى المرتبة الثالثة . كما أظهرت النتائج أن فطر الأسبرجلس نيجر كان أقل الفطريات تأثرا بإضافة النبات وبلغت نسبة قتله إلى ٠,٢٤٪ ، ٦٨,٤٪ بعد ساعتين عند تركيز ٠,٥٠٪ ، ٠,٧٥٪ على التوالى . من هذه النتائج يمكن أن نخلص إلى أن التنينات لها تأثير مثبط قوى على الفطريات ويمكن استخدامها فى هذا الغرض لتجنب آثار الفطريات الضارة على صحة الحيوان .