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Full Length Research Paper

The inhibitory effect of camel's urine on mycotoxins and fungal growth

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The effect of urine and camel milk in the inhibition of biological effects of mycotoxins produced by nine isolates of *Aspergillus flavus* and one isolate of *Aspergillus niger* isolated from pulse seeds was studied. Where these toxins lost their ability to inhibit *Bacillus subtilus* growth, milk could not. Also, our study records the effect of camel urine on mycelial growth of some roots rot fungi isolated from seeds of pulses like *Rhizoctonia solani, Fusarium moliniform, Aschocayta* sp., *Pythium aphanidermatum, Sclerotinia sclerotiorum* studies, also included are some storage fungi (*Aspergillus* sp) isolated from coffee beans. Results proved that camel urine at low concentrations has no significant inhibitory effect on fungal growth, while inhibition can be obviously recorded after using high concentrations.

Key words: Camel urine, mycotoxins, mycelial growth, inhibitory effect on fungal growth.

INTRODUCTION

It is mentioned in Islam online that camel's milk and urine have medical effects, so Islam encourages and permits the drinking of camel milk, and camel urine is permitted in case of necessary medical treatment (Al-Bukhhari). The Saheeh Hadeeth says that some people came to Madeenah and fell sick. The Prophet (peace and blessings of Allaah be upon him) told them to drink the milk and urine of camels, and when they drank it they recovered and grew fat. This was narrated by Al-Bukhaari. There are many well known health benefits, with regard to drinking the milk and urine of camels, to the earlier generations of medical science and they have been proven by modern scientific researches. For example swollen abdomen, which may indicate oedema and liver disease (jaundice), or cancer, and thin bodies which indicate extreme weakness, and which often accompanies hepatitis or cancer. This may be due to the effectiveness of camel's urine, as against all other cattle to the active substances contained in desert plants which benefited more of them; this was summed up by the Prophet (peace be upon him). Many researches have been conducted on a variety of desert plants and a strong effect against bacteria, yeast and fungi has been found. Kaul et al. (1976) and Zaki et al. (1984) have conducted researches on the wormwood plant, and results have shown strong effectiveness against bacteria, yeast and fungi.

The chemical composition and nutritional quality of camel milk was studied. Results showed 11.7% total solids, 3.0% protein, 3.6% fat, 0.8% ash, 4.4% lactose, 0.13% acidity and a pH of 6.5. It contains low level of cholesterol and sugar and is rich in the levels of Na, K, Zn, Fe, Cu, Mn, niacin and vitamin C (Knoess, 1979). Besides, camel milk contains low level of protein and high concentration of insulin, and could be safely taken by people who have high sensitivity to lactose and have immune deficiency (Gast, 1969). Camel milk is pure white and sugary. Camels who feed on certain diets may produce salty milk when feed on desert weeds. There are physiological and genetical factors affecting milk production. Percentage of water in camel milk varies according to the doses of water which camel drinks; it may reach 89% in the milk if camels drink water every day, or 91% if camels drink one hour weekly. It seems

that camel loose more water during its deficiency in nature for the benefits of babies or human beings in generally (Farah, 1993; Abu-Lehia, 1989). Camel milk is also used in Kazakhstan as an adjunct to chemotherapy for some cancer treatments, especially those of the digestive tract. Good results were reported in autoimmune diseases such as Crohn's disease and multiple sclerosis (Yagil and van Creveld, 2000). The positive effect of camel milk on diabetic patients has been studied in India (Agrawal et al., 2003). With the consumption of 0.5 l of camel milk per day, the insulin demand decreased in diabetic patients and glycaemia was better balanced. Consumers appreciate camel milk for its medicinal properties: it is reputed to be anti-infectious, anti-cancerous and antidiabetic. More generally, it is regarded as an energy-giving product for convalescents. Camel milk is commonly used to help treat infectious diseases such as tuberculosis in humans. *Shubat* is commonly used as a cure in sanatoriums (Urazakov and Bainazarov, 1974). The camel milk works as a laxative on people unaccustomed to drinking this milk (Rao, 1970). A series of metabolic and autoimmune diseases are successfully being treated with camel milk. In India, camel milk is used therapeutically against dropsy, jaundice, problems of the spleen, tuberculosis, asthma, anemia, piles and diabetes (Rao, 1970).

Urine, although a waste product of the body, nonetheless has many medical practitioners, and as such is used both internally and externally as medicine. The urines of animals such as goat, sheep, buffalo, elephant, horse, camel, donkey etc. were also very much in use as remedies for the treatment of worms, dropsy, abdominal enlargements, flatulence, colic, anaemia, abdominal tumor, loss of appetite, tuberculosis, poison, haemorrhoids, amenorrhea, leucoderma, leprosy, aggravation of kapha and vat and in several other mental diseases (Thakur, 2004). When Amer and Al-hendi (1996) analyzed urine of mature camels of between 5 -10 years old, they found that its relative density ranged from 1.022 to 1.07, while pH values varied to be either acidic or alkaline. Urea level ranged from 18-36 gm/dL. Keratin recorded 0.2 - 0.5 gm/L. Microscopical analysis proved the presence of phosphorus and calcium oxalate and ammonium urate; some epithelial and granular cells appeared. Al-Attas (2008), using neutron activation analysis, estimated some essential elements within milk and urine of camels, and discovered that it contains large amount of Na and K substituting the loss of such elements in the case of diarrhea. Also it contains large amount of Zn which assists in the cure of the infection due to diarrhea. Mycotoxins are diverse range of molecules that are harmful to animals and humans. They are secondary metabolites secreted by moulds, mostly *Penicillium* and *Fusarium*. They are produced in cereal grains as well as forages before, during and after harvest in various environmental conditions. Due to the diversity of their toxic effects and their synergetic properties, mycotoxins are considered risky to the consumers of contaminated foods and feeds (Yiannikouris and Jonany, 2002). Mycotoxins are metabolized in the liver and the kidneys and also by microorganisms in the digestive tract. Therefore, the chemical structure and associated toxicity of mycotoxin residues often excreted by animals or found in their tissues are different from the parent molecule (Ratcliff, 2002). Storage fungi belonging to genus *Penicillium* and *Aspergillus* play an important role in spoiling stored seeds with increasing humidity. Such fungi produce toxins that lead to human liver disfunction, cancer and undesirable mutations (Pereyra et al., 2008). From these fungi, we mentioned the genera, *Colletotrichum, Sclerotinia, Alternaria, Fusarium, Rhzoctonia, Pythium, Ascochyta and Botrytis* (Sweetingham, 1989; Mackie et al., 1999; Zhang and Yang, 2000; Elmer et al., 2001; Wen et al., 2005). AL-awadi and AL-Jedabi (2000) proved an inhibitory and antibiotic activity of camel urine against the growth of *Candida albicans* (yeast), *Aspergillus niger, Fusarium oxysporum* even after it's boiling to 100°C. Our search was aimed at tracing the effect of camel urine on the growth properties of such fungi. The effect of such products (urine and milk) on efficiency of aflatoxins as inhibitors to *Bacillus subtilus* growth, is seen as a primary step fined away to get rid of fungal toxins.

MATERIAL AND METHODS

Samples of *Camelus dromedaries* urine and milk were collected from females feed on wild weeds at the west of Dammam. Samples were collected in sterile bottles and kept at 4°C for not more than 2 weeks for urine.

Fungal isolates

Used fungi isolates (*Rhizoctonia solani, Fusarium moliniform*, *Pythium aphanidermatum, Aschocayta* sp., *Sclerotinia sclerotiorum*, *Aspergillus flavus* and *A. niger*) were extracted from pulse seeds as lupine (*Lupinus albus* L.), cow pea and mung bean (*Vigna radiata* L.), faba bean and field bean (*Vicia faba* L.) and lentil (*Lens culinaris*), chickpea (*Cicer judaicum*), kidney beans (*Phaseolus vulgaris*) (Al-Abdalall, 2008), and five isolates of *A. niger* from coffee beans (*Coffia arabica*). .

Effect of milk and urine on aflatoxins

Filtrates of nine fungal isolates belonging to *A. flavus* and one belonging to *A. niger* were obtained from seed of pulses (mung bean, faba bean, field bean, lupine, and lentil) (Al-Abdalall, 2009). All isolates were individually grown on SMKY liquid medium described by Diener and Davis (1966) containing:200 gm sucrose; 0.5 gm magnesium sulphate; 3 gm potassium nitrate; and 7 gm yeast extract for 10 days at 25°C to obtain the culture filtrate. Then, the filtrate of each isolate was extracted three times with equal volumes of ethyl acetate. The ethyl acetate was removed by evaporation and the residue was brought up in sterilized distilled water. The method described by Lenz et al. (1986) was used as follows: A species of specific bacteria, that is, *B. subtilis,* was

Table 1. Effect of camel urine and milk on aflatoxins of tested fungi on growth of *Bacillus subtilis.*

Isolates of fungi	Cultivar from which, each fungus was isolated	Aflatoxins conc. p.p.m. (Al-Abdalall, 2009)				Inhibition zone (cm^2)			
		B1	B2	G1	G2	control	50%urine + 50% filtrate	25%urine + 25% milk + 50% filtrate	50% milk +50% filtrate
*A. flavus*1	Mung bean 1	251	71.5	5	0	3.71	0	2.83	3.14
A. flavus2	Mung bean 2	109	60	8	0	4.34	0.75	2.99	3.79
A. flavus 3	Field bean 1	238.5	156	0	5.5	2.75	0	1.65	1.9
A. flavus4	Field bean 2	316	71	83	0	3.8	0.5	2.62	3.7
A. flavus5	Faba bean 1	225	37	0	0	3.8	0	0	3.79
A. flavus6	Faba bean 2	496	0	0	0	9.36	3.14	5.96	5.11
A. flavus7	Faba bean 3	337.5	20.5	0	0	10.68	0	7.07	3.85
A. flavus8	Lupine	259.5	56	21	0	3.69	0.71	0.86	3.69
A. flavus9	Lentil	146.5	26	0	0	1.52	0.94	1.17	1.52
A. niger	Field bean	109.5	21	0	0	5.91	0	1.45	2.85
L.S.D. at 0.05%						1.321	0.791	1.631	1.093

obtained from Bacterial Disease Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. Equal disks (cm) of the tested bacterium were prepared from 10-days old bacterial cultures grown on TYG solid medium (Scott and Kennedy, 1975) which consists of 50 g tryptone, 2.5 g yeast extract, one g glucose and 20 g agar dissolved in one liter of distilled water. A liquid medium of TYG was prepared, distributed into 500 ml Erlenmeyer flasks (200 ml/each) and autoclaved. After cooling, one disk (0.5 cm) of bacterial culture was added to each flask. All the flasks were incubated for 48 h at 30°C. Other flasks (250 ml) containing TYG solid medium (100 ml/each) were prepared and autoclaved. After cooling and before solidification, one ml of the previous bacterial suspension was added to each flask and shaken well. The inoculated medium was distributed into Petri dishes (20 cm) at the rate of 10 ml medium/plate. Diffusion through agar pore technique (Rojas et al., 2003) was used to study the effect of camel urine against the efficiency of aflatoxins by using a cork borer (0.3 cm in diameter). A pore was made in the middle of each plate. One ml of the aforementioned filtrate of each tested fungal isolate was added to the pore. The same steps were repeated but the fungal filtrate was mixed with urine, milk, urine and milk together, and all the dishes were incubated for 48 h at 30°C. The diameters of the inhibition zones were measured (in cm^2) as an indicator for aflatoxin production.

Effect of camel urine on fungal mycelium growth

Effect on the fungal mycelium dry weight

Each tested fungus was grown in Erlenmeyer flasks (100ml) containing 50ml of glucose yeast extract broth medium. The medium was autoclaved at 120°C for 20 min. After cooling, flasks were inoculated with 1cm disc of each tested fungus, taken from 7 days old cultures grown on PDA medium. Different concentrations of urine (0.5, 1, 2, 3%) were applied to the fungal growth. Control flasks did not receive urine. All flasks were incubated at 25±2°C for ten days. After incubation, filtration allows isolation of the fungal growth, followed by drying in oven at 75 - 80°C for one day (AL-awadi and AL-Jedabi, 2000).

Effect of urine on the linear growth of fungal mycelia

The PDA medium was prepared as usual and autoclaved. After cooling, camel urine was added to each flask at concentrations 3, 5, 7, 10% respectively. Control flasks did not receive urine. Then the medium was poured into sterilized Petri dishes. The plates were inoculated with equal discs (one cm in diameter) of each tested fungus, taken from fungal cultures (5 days old) that were grown on potato-dextrose agar (PDA) media using a sterilized cork borer. All dishes were incubated at 25+2°C for 10 days. Mycelium growth area was calculated in each dish (Al-Zahrani, 2002). The same experiments were repeated using high concentrations of urine (25, 50%). Results are tabulated in Table 3.

Statistical analyses

Data obtained were statistically analyzed using SPSS Version 6. Treatment averages were compared at the 0.05 level of probability using LSD (Norusis, 1999).

RESULTS

Effect of camel urine and milk on aflatoxins

Results in Table 1 and Figure1 (1A -10D) were successful in inhibition of aflatoxins. Inhibition is measured by the inhibition of *B. subtilis* growth, where camel's urine was found to suspended inhibitory effect of toxins in most samples and weakness in the others compared to several attempts did not succeed in influencing the effectiveness of these toxins. Also Al-Abdalall (2009) did not succeed in inhibiting these toxins by using freezing and sterilizing in autoclave or using microwave rays as treatments for these toxins. Camel milk does not show any inhibitory properties.

Effect of camel urine on mycelium growth

Data obtained are presented in Table 2. The effect of low concentrations of camel urine (0.5, 1, 2, 3%) on mycelial growth of *A. niger*, *A. flavus, R. solani, Fusarium* sp.,

Table 2. Effect of low concentrations of camel urine on dry weight of fungal mycelium.

Tested fungi	Sources of isolates	concentrations of camel urine %				
		0	0.25	0.50	1	3
A. niger1	Coffee beans	0.46	0.38	0.34	0.31	0.28
A. niger2	Coffee beans	0.37	0.26	0.25	0.25	0.21
A. niger3	Coffee beans	0.40	0.30	0.24	0.23	0.17
A. niger4	Coffee beans	0.30	0.25	0.25	0.21	0.17
A. niger5	Coffee beans	0.40	0.28	0.27	0.26	0.22
R. solani	Faba beans	0.07	0.07	0.07	0.07	0.085
F. moliniform	Kidney beans	0.07	0.07	0.05	0.075	0.10
Aschocayta sp.	Chickpea	0.07	0.11	0.09	0.09	0.09
S. sclerotiorum	Mung beans	0.08	0.085	0.09	0.09	0.095
P. aphanidermatum	Mung beans	0.08	0.07	0.07	0.06	0.09
L.S.D		1.05	0.87	0.83	0.798	0.69

Table 3. Effect of low concentrations of camel urine on the mycelial growth of test fungi.

Tested fungi	Sources of isolates	concentrations of camel urine%				
		0	3	5	7	10
A. niger1	Coffee beans	44.36	41.32	41.32	15.49	7.9
A. niger2	Coffee beans	47.6	31.9	16.1	12.56	12.76
A. niger3	Coffee beans	53.48	33.37	27.19	18.44	2.46
A. niger4	Coffee beans	38.67	21.69	16.1	14.23	14.52
A. niger5	Coffee beans	53.48	23.95	15.9	18.09	7.81
A. niger6	Field beans	53.48	28.46	26.01	16.62	4.91
A. flavus	Cowpea	42.49	14.23	10.56	9.09	8.35
R. solani	Faba beans	56.72	56.72	56.72	51.03	25.96
F. moliniform	Kidney beans	56.72	56.72	56.72	56.72	16.62
Aschocayta sp.	Chickpea	56.72	56.72	53.48	45.35	43
S. sclerotiorum	Mung beans	56.72	56.06	54.08	42.995	41.83
P. aphanidermatum	Mung beans	38.67	36.34	35.82	26.88	18.49
L.S.D		2.35	3.1	3.27	3.18	2.81

Pythium aphanidermatum, Aschocayta sp., and *Sclerotinia sclerotiorum* in liquid medium, as well as concentrations (3, 5, 7, 10%) on solid medium (Table 3 and Figure 1 (1-12)) was noticed. There is no significant differences in dry weight in treated or control flasks, although there is a decrease in the dry weight of the mycelial growth with increasing urine concentrations. After using concentrations of urine (25, 50%) a significant decrease in fungal growth (on Petri dishes) was recorded (Table 4 and Figure 1 (1-12). Some treatments showed complete inhibition of the fungal growth, *Aschocayta* sp. is totally inhibited to grow at 25% concentration of camel urine. While complete inhibition of *Aschocayta* sp., *Rhizoctonia solani, Pythium aphanidermatum* is recorded after application of urine at 50%.

DISCUSSION

Abdel Magjeed (2005) mentioned that camel milk can improve some biological aspects after toxication withaflatoxins, including improvement in the level of Keratin, Haemoglobin, triglycrides and blood properties of toxicated rates. Results of the therapeutic groups were compared with other groups treated with either the anticarcinogenic drug or treated with milk camel mixed with small amounts of urine. EL-Elyani and Khalifa (2006) noted that no histopathological changes could be recorded when studying the effect of camel milk or urine on stomach rate, while AL-Kabarity et al. (1988) mentioned that camel urine is used commonly, in alternative medicine, against cancer and respiratory tractinfections. Khalifa, (1999) EL-Elyani (1999) and Khalifa et al. (2005) noted that no pathological signs appeared on liver or kidney tissues after using camel milk or urine. The high salt concentration of the urine causes plasmosis and analysis of mycelium hyphae; this agrees with AL-awadi and AL-Jedabi (2000). Similar results were obtained by Al-Zahrany (2002) who recorded growth

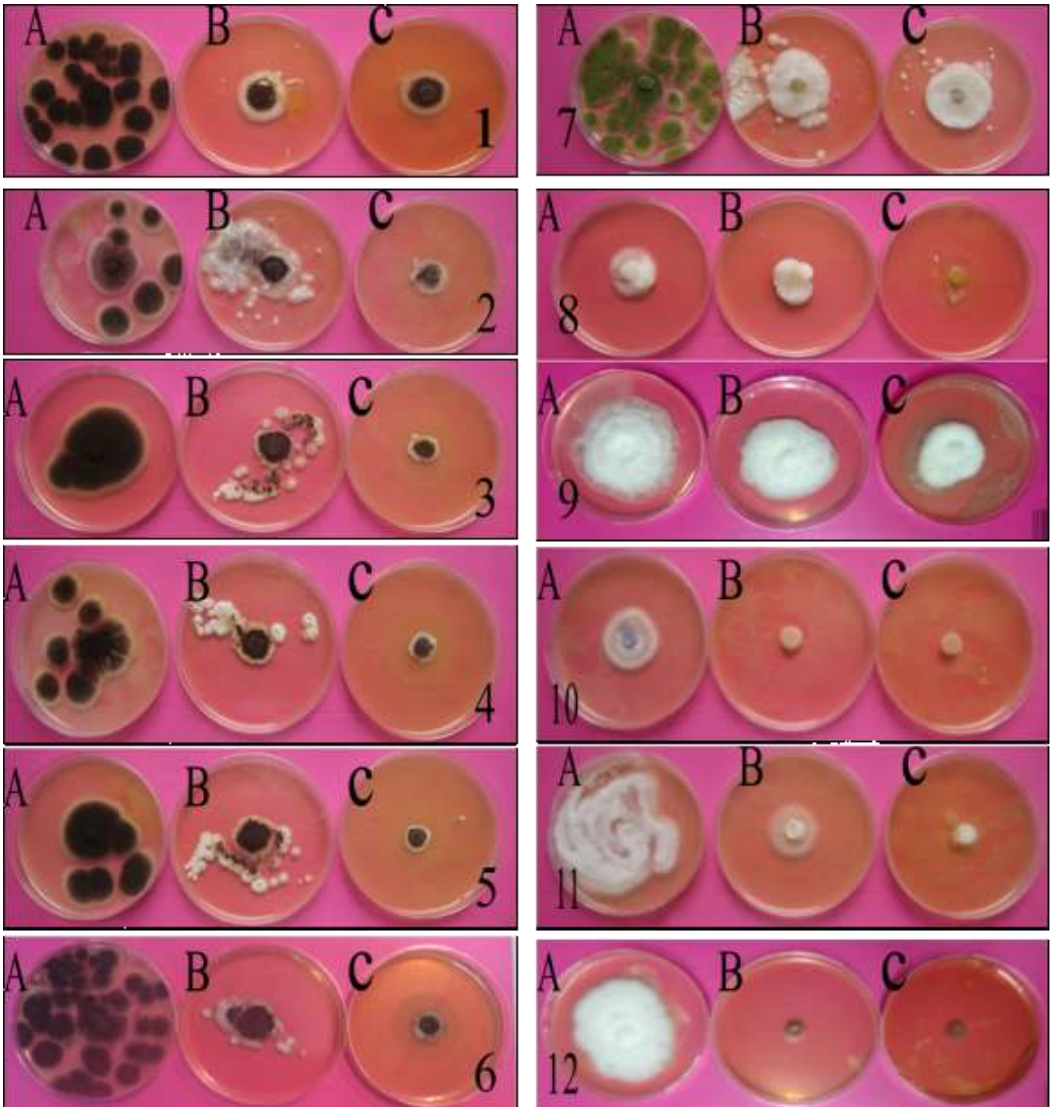

Figure 1. (1-12): Effect of high concentrations of camel urine on the mycelial growth of test fungi. A = control, b = 25% camel's urine, c = 50% camel's urine.

Table 4. Effect of high concentrations of camel urine on the mycelial growth of test fungi.

Tested fungi	Sources of isolates	0	25	50
A. niger1	Coffee beans	56.72	6.61	5.31
A. niger2	Coffee beans	56.72	7.89	2.82
A. niger3	Coffee beans	28.03	8.18	1.34
A. niger4	Coffee beans	27.34	8.36	1.61
A. niger5	Coffee beans	31.61	8.36	1.66
A. niger6	Field beans	19.22	13.35	2.74
A. flavus	Cowpea	56.72	11.95	9.82
R. solani	Faba beans	10.18	4.36	0
F. moliniform	Kidney beans	30.18	21.69	11.09
Aschocayta sp.	Chickpea	12.25	0	0
S. sclerotiorum	Mung beans	53.48	9.35	2.5
P. aphanidermatum	Mung beans	29.79	1.77	0
L.S.D		3.21	1.94	1.5

inhibition of *A. niger* after its treatment with camel urine for 14 - 18 months.

AL-awadi and AL-Jedabi (2000) recorded inhibitory effect on the dry weight of the yeast and fungi. Shoeib and Ba-hatheq (2008) proved through electro microscopic studies, the effect of urine on the morphological properties of some human pathogenic bacteria. The chemical and organic constituents of urine proved to have inhibitory properties against fungal and bacterial growth (Ghosal et al., 1974; Varley et al., 1980; Mura et al., 1987; Amer and Hendi, 1996). Shoeib and Ba-hatheq (2007) mentioned that there is no effect on the deadly bacterial cells for each of the *E.coli* and *P. aeruginosa* when treated with fresh urine effect was two fold, firstly, to stop the proliferation of bacterial cells, plasmids, leading to the production of cells of any kind of cured cells in order to be free of plasmids. This agrees with Rose and Barron (1983), and the continued exposure of cells camel's urine, which led to a second effect: the impact of killer cells. This also resulted in non-disintegration of bacterial cells, bacteriolysis after death and this agrees with Höltje (1998), with regards to the appearance of bacterial chromosome without plasmids.

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