



Prevalence of Yeast Infections in Small in Ruminants with Particular References to their Treatment by some Natural Herbal Extracts

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ABSTRACT

A total of 400 samples were collected from different farms including diseased and apparently healthy cases of sheep and goats (200 samples of each species) as well as 50 samples of each of soil and feed from pastures and animal houses. The samples included vaginal swabs, faeces, nasal swabs and milk samples (25 of each) for each species of animals of the diseased and apparently healthy. All samples were subjected to examination for yeast species. The results revealed that the vaginal swabs that collected from cases of the reproductive disorders of sheep and goat were the major sources of yeasts where, *C. albicans* was recovered at percentages of 40.0% and 20%, respectively) but not isolated from apparently healthy sheep, while it was recovered from 8% of apparently healthy goats. Whereas, the *C. albicans* was recovered from 20% of cases of diarrhea (fecal samples) in sheep and goat, while it was only recovered from 4% of cases of both apparently healthy sheep and goat. On the other hand, the mastitic milk yielded nearly similar intensive infection with *C. albicans*, where it was isolated from 32% and 24% of mastitis cases of sheep and goat, respectively. Whereas, it was recovered from nasal swabs of sheep and goat suffered from respiratory disorders (20% and 16%), respectively. The lower infection with *C. albicans* were reported in nasal swabs of apparently healthy cases (12% of sheep and 4% of goat). It was interesting to report that *Cr. neoformans* was detected in 4% of milk of mastitic sheep and 4% of nasal discharge of sheep suffered from respiratory disorders while, *Cr. neoformans* was not recovered from samples of apparently healthy sheep. Also, it was observed that, the total *Candida* species recovered from the soil collected from goat houses was in percentages of (66%) which is higher than that in goat pastures (10.0%). On the other hand, *Candida* species were isolated only from the soil collected from sheep houses (6%) but isolated from 41% of diseased goat houses. The utilized feeds by such animals yielded different types of yeasts particularly *C. albicans* and *Cr. neoformans* which recovered from (30% and 6%) of feeds used by diseased goat but only they were isolated from 2% of feeds of apparently healthy goat. While, *C. albicans* present in 50% of feed samples used by diseased sheep, the *Cr. neoformans* not detected in feeds. Other yeast spp. was also isolated in a lower frequency. Several natural herbal oils and extracts were evaluated as antifungal agents for controlling these yeast infections including Clove, Garlic and Onion oils, in addition to *L. termis* extract. These herbal oils and extracts were tested using disc diffusion technique. All tested oils were effective in inhibiting the growth of yeast, for instance the zone of inhibition of Clove oil on *C. albicans* and *C. krusei* were (15±0.51 mm and 13±0.12 mm at concentration of 3%) which was more pronounced than that observed in *Cr. neoformans* and *R. rubra* (6.0±0.2 mm and 6.3±0.02 mm at conc. 3%). Whereas, stronger effect was obtained by oil of onion at MIC of 1% that ranged from 5.0±0.03 mm for *R. rubra* to 6±0.2 mm for *C. albicans*. Although, the lack of an effective fungicidal regimen as well as the development of antifungal resistant strains suggest that continued investigation is necessary to devise immunotherapeutic strategies and / or drug targets to combat fungal infection for future use in animal topically, locally, prophylactically or as treatments systematically with taking on consideration toxicity of clove oil.

INTRODUCTION

The fungal infections particularly yeasts infections represent the widest spread and prevalent mycotic diseases of man and animals [1]. The incidence and prevalence of serious mycoses continue to be a public health problem. Despite aggressive treatment with new or more established licensed antifungal agents, these infections are important causes of morbidity and mortality, especially in immunocompromised patients [2]. Transmission of mycotic infection from man to man or animal to man has not been documented. Occasionally, there has been a known or presumed exposure to weathered contaminated environmental sources which often contains the yeast including soil, air, feeds likely to be contaminated with yeast very infrequently [3]. Generally, the normal adult human or animal has a strong innate immunity to infection by *C. albicans* which lives as a commensal yeast on the body surface and mucous membranes. However, it is considered as opportunistic yeast, as well as an emerging pathogen especially in immunocompromised hosts [4].

Actually, *C. albicans* has been isolated from different disease conditions in various animal species which indicates the deleterious effect of candidosis on the economical impact of animal production [5]. Moreover, there is an emergence of *C. albicans* strains resistant to antifungal agents so as the rapid and

reliable identification of this species is important in the clinical microbiology laboratories as the management of candidemia and disseminated candidosis depends on an unambiguous identification [6]. On the other hand, *Cryptococcus neoformans*, one species of yeasts which particularly associated with immunosuppressed states most notably during human immunodeficiency virus (HIV) infection, and has been isolated from clinical and environmental sources throughout the world. While, *Cr. neoformans* var. *gattii* is virtually restricted to tropical and subtropical regions [7] and infection occurs almost exclusively in immunocompetent patients. The *Cr. neoformans* var. *neoformans* has been isolated from many sources including soil and pigeon faeces. Septicaemia caused by *Rhodotorula* has been reported in immunocompromised patients, where the yeast multiplied rapidly in various organs. On the other hand, species of genus *Trichosporon* are either pathogenic (white piedra and nail infection) or opportunistic producing septicaemia in compromised patients [4].

The study of a disease in a population requires an understanding of the relationships between organisms; hosts and their environment. Moreover, the study of a disease's ecology is frequently a part of epidemiological investigations and this has two objectives, an increase in the understanding of the pathogenesis, maintenance and for infectious agents transmission of disease also when and where a disease may occur to enable the development of suitable control techniques where, the delay in epidemiological interference discovery is probably due to the lack of long-term surveys on the incidence of infections as reported [8]. Therefore, antimicrobial agents are needed to prevent and overcome severe fungal infections. Based on their previous experiences and that of others, Preuss *et al.* [9] postulated that herbal essential oils, such as those of origanum, and monolaurin offer such possibilities.

Hence, our trials may offer an idea or draw the attention to the possibilities of using the natural antifungal preparations in several pharmaceutical forms for treatment where they are used either alone as reported by Khaldun [10], Prashar *et al.* [11], Thamburan *et al.* [12] and Braga *et al.* [13], or together with natural antifungal preparations as a combined treatment for achieving the synergistic action as reported by Postoienco *et al.* [14], Al-waili [15], Braga *et al.* [13] and Fu *et al.* [16] or together with the commercial synthetic conventional ones for achieving the synergistic action as reported in several recent researches by Maruyama *et al.* [17] and Guo *et al.* [18]. They may be even used as a new alternative treatment in the near future if they are shown to have more potent antifungal activity than synthetic conventional ones as observed by Chaieb *et al.* [19] and Tedeschi *et al.* [20]. The present work aimed to offer as far as possible for a comprehensive understanding about the ecology and epidemiology of the isolated yeasts from various apparently healthy and pathological cases of animals as well as from the surrounding environment. Also, evaluation of herb oils and extracts in order to follow the ideal and correct hygienic measures for the protection from different diseases transmitted to man, animals and birds.

MATERIAL AND METHODS

Material:-

Samples from diseased and apparently healthy animals

A total of 400 samples were collected from different farms including diseased and apparently healthy cases of sheep and goats (200 samples of each species of animal). The samples which collected from animals included vaginal swabs, faeces, nasal swabs and milk samples (25 of each) of diseased and apparently healthy animals for each species of animals. The vaginal swabs were taken from cases with endometritis and other reproductive disorders. The faecal samples were collected from cases suffered mainly from diarrhea. Whereas, the nasal swabs taken from animals with respiratory disorders including mainly coughing and nasal discharges. On the other hand, milk samples were collected from mastitic or apparently healthy animals that may harbor sub clinical mastitis. All samples were collected in tubes containing SD. Broth and transferred to laboratory as rapid as possible where they incubated at 37°C.

Soil and feed Samples: One hundreds of feed and soil samples (50 of each) were collected from pastures and houses of sheep and goats. All samples were collected and brought to the laboratory under complete sterile conditions.

Herbal oils and extracts:-

1. Herbal oils: The following oils were purchased from markets of herbs in crude forms ready for use as Clove, Garlic and Onion oils.

2. Extracts: as the extracts of lupinus termis seeds obtained by the use of ethyl or chloroform in their extraction of seeds.

3. Control: A known antifungal was used as **Itraconazol** which purchased from Al Gomhouria pharmaceutical comp.

Isolation and identification of Yeasts:

After incubation of broth tubes of different samples, loopfuls were streaked onto plates of Sabouraud's dextrose agar with chloramphenicol, incubated at 37°C and examined after 48 hours for the presence of yeast like colonies. The isolated yeast colonies were identified as recommended by [21,22].

Effect of some herbal oils and extracts on the growth of isolated yeasts:-

Preparation of herbal oils and extracts:-

A. Herbal oil as Clove, Garlic and Onion oils were used in crude or diluted forms or diluted before testing against yeast.

B. Extracts of Lupinus termis [23]: as the extracts of Lupinus termis seeds obtained by the use of ethyl in their extraction of seeds. For 25 grams of finely ground seeds, 50 ml ethyl alcohol were added and vigorously mixed. The extracts containing the active principle was evaporated in water bath till dryness and the residues were refrigerated till used. All the tested herbal oils or extracts were mixed with the Sabouraud's dextrose agar medium in concentrations of 1%, 2% and 3%.

Evaluation of the effect of herbal oils or extracts on the growth of *Candida albicans*, *Rhodotorula* and *Cryptococcus neoformans* [24]

The spore suspension of the yeast was incorporated in the medium of Sabouraud's dextrose agar (SDA medium). The oil or extract was absorbed by filter paper disc as described by the following techniques:-

Disc diffusion technique [24]: Filter paper discs of 5 mm Φ soaked with the oils or extracts and dried, were placed on the surface of the SDA plates, incorporated with 10^5 spore of yeasts. The plates then were incubated at 35 °C for 24 hrs and the plates were tested for inhibitory zones around the discs.

Statistical Analysis

The results (Mean \pm S.E) were statistically analyzed by one-way classification throughout analysis of variance (ANOVA) as explained by Petrie and Watson [25]. Significant differences between means at ($p \leq 0.05$) using *SPSS 14* [26].

RESULTS AND DISCUSSION

The yeast infections were reported as potential pathogens and caused different disease conditions in human and animals, particularly after prolonged antibiotic therapy, [4]. The epidemiological studies of the clinical and the environmental isolates are important to determine their origin and ways of transmission [27].

The current results in Table (1) and Fig. (1) revealed that the different types of yeasts were recovered from clinical samples that collected from diseased cases of sheep and goats, in this respect the vaginal swabs that collected from cases suffered from abortion were the major sources of yeasts as reported by Chengappa et al. [28]. One good example for that is *C.albicans*, where it was recovered from the vaginal swabs of the diseased sheep and goats in percentages of (40.0% and 20%), respectively (Table 1 & Fig. 1), and not recovered from apparently healthy sheep while it was recovered from 8% of apparently healthy goats (Table 2), respectively. Whereas, the *C. albicans* was recovered from 20% of cases of diarrhea (fecal samples) in sheep and goats (Table 1 & Fig. 1), while, it was only recovered from 4% of cases of both apparently healthy sheep and goat (Table 2 & Fig. 2). On the other hand, the mastitic milk yielded nearly similar intensive infection with *C. albicans*, where it was isolated from 32% and 24% of mastitis cases of sheep and goats, respectively. However, it was recovered from nasal swabs of cases of sheep and goats suffered from respiratory disorders (20% and 16%), respectively. Whereas, the lower infection with *C.albicans* were reported in nasal swabs of apparently healthy cases (12% of sheep and 4% of goat).

Table (1): Prevalence of yeasts species in clinically diseased Sheep and Goats

Types of yeasts	Sheep								Goats							
	Vag. Swab		faeces		Nasal swab		Milk		Vag. swab		faeces		Nasal swab		Milk	
	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%
<i>C.albicans</i>	10	40	5	20	5	20	8	32	5	20	5	20	4	16	6	24

<i>C. krusie</i>	6	24	3	12	1	4	3	12	3	12	5	20	2	8	2	8
<i>C.parapsilosis</i>	8	32	2	8	2	8	2	8	2	8	1	4	0	0	1	4
<i>Cr.neoformans</i>	0	0	0	0	0	0	1	4	1	4	0	0	1	4	1	4
<i>G.candidum</i>	3	12	0	0	0	0	0	0	0	0	0	0	1	4	0	0
<i>R. rubra</i>	2	8	0	0	4	16	3	12	0	0	2	8	2	8	3	12
<i>T.cutanium</i>	1	4	0	0	0	0	1	4	0	0	1	4	0	0	0	0
<i>M.gyposum</i>	1	4	1	4	0	0	0	0	0	0	1	4	0	0	0	0

N.B.: 100 samples of each species of animal included vaginal swabs, faeces, nasal swabs and milk samples (25 of each)

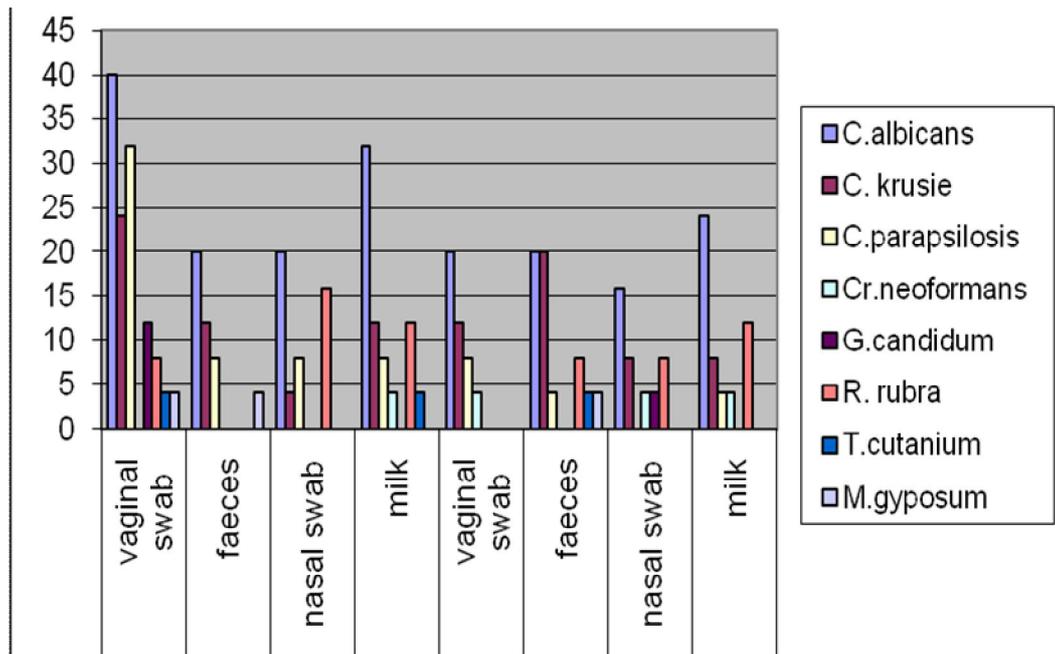


Fig. (1): Prevalence of yeasts species in clinically diseased cases of Sheep and Goats.

Nearly similar results were obtained by [29], who isolated *C. albicans* from some cases of diseased and apparently healthy cases of sheep and goat. It was noticed that, the yeasts were isolated from the diseased cases especially from the diarrheic cases at higher rates than that from cases with other digestive troubles especially *Candida* species, Such results go parallel with the work of Donskey [30] and Hassan et al. [29], who reported that *C. albicans* was the aetiological agent of candidosis in two calves.

Table (2): Prevalence of yeasts species in apparently healthy cases of Sheep and Goats.

Types of yeasts	Sheep								Goat							
	Vag. Swab		feces		Nasal swab		Milk		Vag. swab		feces		Nasal swab		Milk	
	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%
<i>C.albicans</i>	0	0	1	4	3	12	2	8	2	8	1	4	2	4	2	8
<i>C. krusie</i>	2	8	1	4	1	4	1	4	1	4	5	20	3	12	0	0
<i>C.parapsilosis</i>	1	4	1	4	1	4	0	0	1	4	1	4	2	8	0	0
<i>Cr.neoformans</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>G.candidum</i>	0	0	1	4	0	0	0	0	0	0	1	4	1	4	1	4
<i>R. rubra</i>	0	0	2	8	2	8	1	4	1	4	0	0	1	4	1	4
<i>T.cutanium</i>	0	0	1	4	0	0	1	4	0	0	0	0	0	0	1	4
<i>M.gyposum</i>	0	0	0	0	0	0	0	0	0	0	1	4	0	0	1	4

N.B.: 100 samples of each species of animal included vaginal swabs, faeces, nasal swabs and milk samples (25 of each)

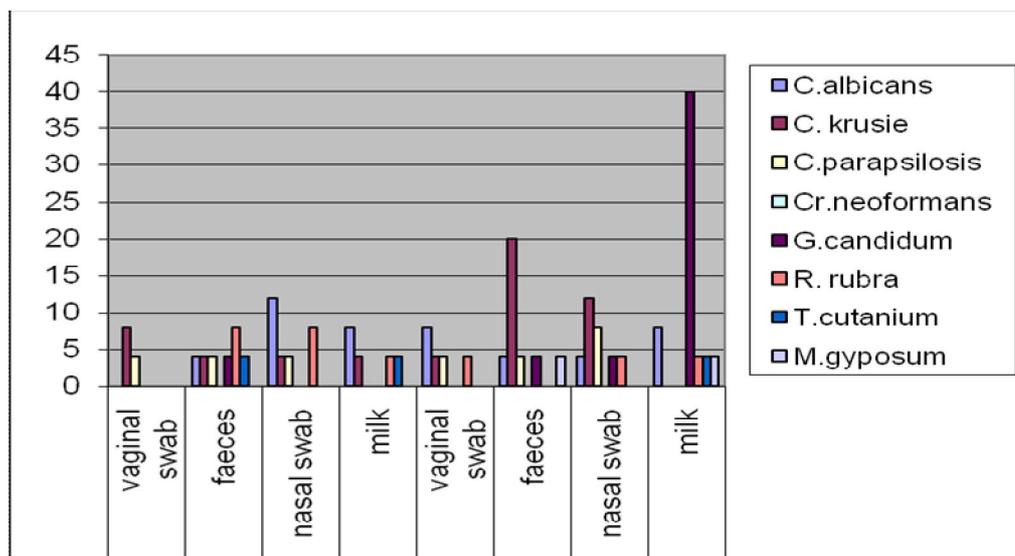


Fig. (2): Prevalence of yeasts species in apparently healthy cases of Sheep and Goats.

Also, Sebryakov et al. [31], described visceral mycoses that occurred alone or with diarrhoea in calves reported to be infected with *C. tropicalis*. Elad et al. [32], who reported that, *C. glabrata* may be involved in enteric pathogenic process, where *C. glabrata* shedding seemed to be associated with diarrhoea and Donskey [30], who published that, the intestinal tract provides an important reservoir for many pathogens, including *Candida* species and some bacteria species. The disruption of normal barriers such as gastric acidity and the indigenous microflora of the colon, facilitate overgrowth of pathogens. Factors such as faecal incontinence and diarrhoea contribute to the subsequent dissemination of pathogens into the health care environment.

It was interesting to report here that *Cr. neoformans* was detected in 4% of milk of mastitis sheep and 4% of nasal discharge of sheep suffered from respiratory disorders. Whereas, *Cr. neoformans* was not recovered from apparently healthy sheep. [29] were not recovered this yeast from their samples collected from diseased and apparently healthy cases of sheep and goats.

Yeasts reach the respiratory tracts of different animal species mainly through inhalation of the contaminated dusts, decaying organic substances or desiccated bird droppings from the soil contaminated with the yeasts obtained from animal secretions and excretions as a result of bad hygienic measures in the animal's environment. Also the environmental events such as construction or renovation act as being specific risk factors for invasive fungal diseases as a result of bad ventilation as mentioned by [33]. In case of fungal pneumonias which are most frequently caused by *Cr. neoformans* especially in an enlarging immunocompromised population while, both *Cr. neoformans* and *Cryptococcus bacillisporus* often causing pulmonary mass lesions as reported by Jenney et al. [34]. On the other hand, several authors recovered *Cr. neoformans* from goats suffering from predominantly severe pulmonary disease [35-36]. Moreover, *Cr. neoformans* was recovered from goats with chronic pneumonia as reported by Baro et al. [37] and Gutierrez and Garcia Marin (38). On the other hand, (39) had been recorded in sheep and goats, septicemia form with pulmonary involvement and encephalomeningitis. It is interesting to note that, *C. albicans* is highly pathogenic to the lactating goat mammary gland even without immunosuppression or antibiotic treatment, resulting in severe irreversible tissue damage and nearly complete agalactia as reported by Singh et al. [40].

It is noticed that, *Cr. neoformans* was isolated from the mastitis goats milk as also reported by Abdellah [41] and Pal and Randhawa [42], who isolated *Cr. neoformans* from caprine mastitis while, Singh et al. [40] isolated the organism after experimental infection of goats with *Cr. neoformans*. The yeast of *R. rubra* is considered the most second common yeast isolated frequently from milk samples of all the studied animal species after *Candida* species. On the other hand, each of *Rhodoturula*, *Geotrichum* and *Trichosporon* were isolated by Costa et al. [43] from milk samples of normal, clinical and subclinical mastitic quarters from dairy herds.

From the current results shown in (Table 3 and Figs3) it was observed that, the total *Candida* species were recovered from the soil collected from goat houses in a percentages of (66%) which is higher than that isolated from goat pastures (10.0%). On the other hand, they were isolated only from the soil

collected from sheep houses (6%) but it was isolated from 41% of diseased goat houses. We might find an explanation from the fact that animal houses are subjected to various contaminated animal secretions and excretions (in a heavy breeding density) more than that in the animal farms or pastures (as the animal houses enable the animals to be restricted in their movements in confined places) that such houses being continuously receive secretions of the animals such secretions are rich in yeasts (especially those coming from the diseased cases as vaginal and nasal discharges, milk or excretion of animals as the faecal matters as well as the decaying organic matters in the soils). All these polluting factors in addition to the bad hygienic measures may offer a good source for environmental contamination by *Candida* species, *Cr. neoformans*, *G. candidum* and other yeasts and bacteria which may be pathogenic or non pathogenic in addition to the contaminated silage used in many farms which may be subjected to spoilage by the action of various yeasts, fungi and bacteria as a result of bad storage and contaminated dirty places causing digestive infections.

These findings of low contamination of soil with *Cr. neoformans*, may provide an explanation for this low incidence that due to the direct germicidal action of sunshine on *Cr. neoformans* and also of the rare occurrence of this fungus in pigeon droppings in unsheltered sites in natural habitats as reported by Hubalek and Prikazsky [44]. However, in sheltered sites that are not in contact with the soil, the organism if found in large numbers, its survival is enhanced by increased relative humidity, while direct exposure to sunlight especially in summer months, soon sterilizes the habitats of yeast, however, it is maintained in similar locations during winter as reported by Rippon [45]; Baro *et al.* [37] and Horta *et al.* (46).

Table 3: Prevalence of yeasts species in soil and feeds of diseased and apparently healthy sheep

Types of yeasts	Source of soils								Feeds			
	Pastures				Animal houses							
	Diseased animal		Apparently healthy		Diseased animal		Apparently healthy		Apparently healthy		Diseased animal	
	No. +ve	%	No. +ve	%	No. ve	%	No. +ve	%	NO. ve	%	NO. ve	%
<i>C.albicans</i>	34	66	7	14	3	6	2	4	10	20	25	50
<i>C. krusie</i>	3	6	2	4	10	20	3	6	2	4	5	10
<i>C.parapsilosis</i>	0	0	8	16	7	14	6	12	0	0	2	4
<i>Cr.neoformans</i>	4	8	0	0	5	10	0	0	0	0	0	0
<i>G.candidum</i>	5	10	0	0	2	4	0	0	0	0	1	2
<i>R. rubra</i>	0	0	1	2	3	6	1	2	1	2	10	20
<i>T.cutanium</i>	0	0	0	0	3	6	0	0	0	0	2	4
<i>M.gyposum</i>	0	0	0	0	3	6	0	0	0	0	1	2

One hundred of soil samples (50 of each of pastures and animal houses) and fifty samples of feeds were examined

The utilized feeds by such animals yielded different types of yeasts, particularly *C. albicans* and *Cr. neoformans* which recovered from (30% and 6%) of feeds used by diseased animals but only isolated from 2% of feeds of apparently healthy goat. While, *C. albicans* present in 50% of feed samples used by diseased sheep. Whereas, *Cr. neoformans* not detected in feeds of diseased and apparently healthy cases. Other yeast spp. were also isolated in lower frequency (Table 4 and Figs 4). Nearly similar results were obtained by [47,48] who recovered different spp. of yeasts from feeds in association to animal diseases in sheep, goats and cattle.

It is noteworthy to mention that this infection may be explained as it could be resulted from polluted water, food, manure and silage, so gain access to the animals and birds during eating, drinking, breathing in a such mixed form. The mixed yeast infection was reported by Slavikova and Vadkertiova [49], who isolated 181 yeast strains out of 180 soil samples. The isolated yeasts were *Cr. laurentii*, *R. aurantiaca* and *T.cutaneum*. Moreover, the mixed yeast infection was obtained by Slavikova and Vadkertiova [50], who isolated 111 yeast strains out of 60 agricultural soil. The isolated yeasts were *Cr. Laurentii*, *C. maltosa* and other yeast species.

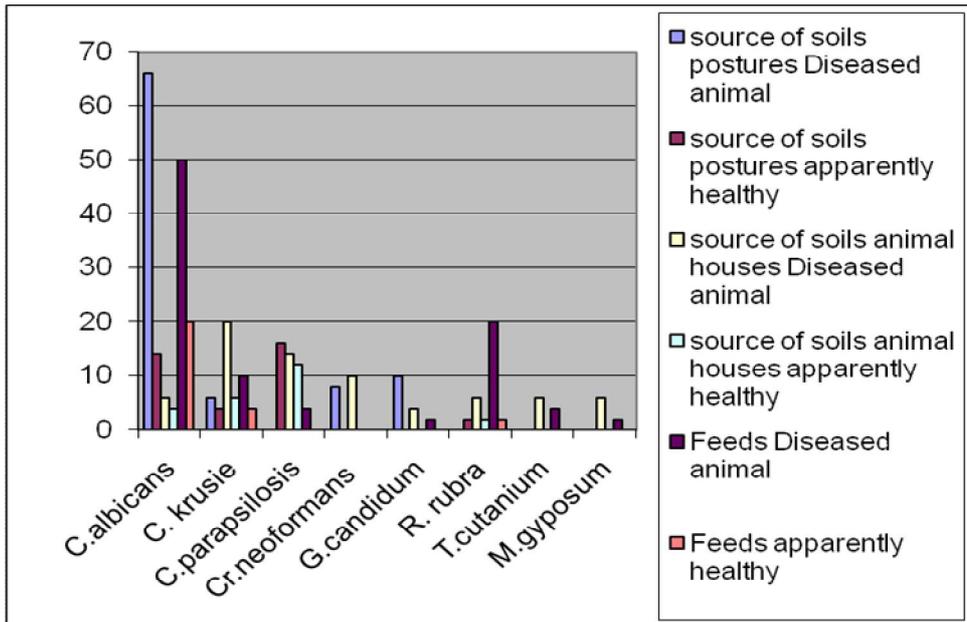


Fig. (3): Prevalence of yeasts species in soil and feeds of diseased and apparently healthy sheep.

On the other hand, medication with antibiotics does not eliminate the yeast infections but cause the flourishing of yeasts, also steroid treatment, cytotoxic drugs and treatment with surgical operations as well as mal nutrition , pregnancy, endemic diseases, adverse environmental conditions as bad hygienic measures and over crowding in both animal and bird farms may predispose to infection [51]. All these factors seriously suppress the animal immune response and favor the development and further dissemination of the yeast infection and development of a diseased condition.

The spread of multidrug resistant strains of fungus and the reduced number of drugs available makes it necessary to discourses new closes of antifungal and compounds that inhibit these resistant mechanisms. This has led to a search for therapeutic alternatives, particularly among medicinal plants and compounds isolated from them used for their empirically antifungal properties. In these national sources, a series of molecules with antifungal activity against different strains of fungus have been found, which are of greet importance to animals, humans and plants so we discuss the significance of using such national sources as proposed antimycotics.

Table (4): Prevalence of yeasts species in soil and feeds of diseased and apparently healthy goats.

Types of yeasts	Source of soils								Feeds			
	Postures				Animal houses				Apparently healthy		Diseased animal	
	Diseased animal		Apparently healthy		Diseased animal		Apparently healthy		Apparently healthy	Diseased animal	Apparently healthy	Diseased animal
	No. +ve	%	No. +ve	%	No. +ve	%	No. +ve	%	NO. +ve	%	No +ve	%
C.albicanse	5	10	2	4	82	41	42	82	1	2	15	30
C. krusie	0	0	1	2	4	8	12	24	3	6	2	4
C.parapsilosis	0	0	3	6	41	41	82	00	0	0	1	2
Cr.neoformans	0	0	0	0	0	0	0	0	1	2	3	6
G.candidum	5	10	0	0	0	0	2	4	2	4	1	2
R. rubra	5	10	0	0	0	0	1	2	3	6	7	14
T.cutanium	0	0	0	0	0	0	0	0	0	0	2	4
M.gyposum	0	0	0	0	1	2	0	0	0	0	1	2

One hundred of soil samples (50 of each of pastures and animal houses) and fifty samples of feeds were examined

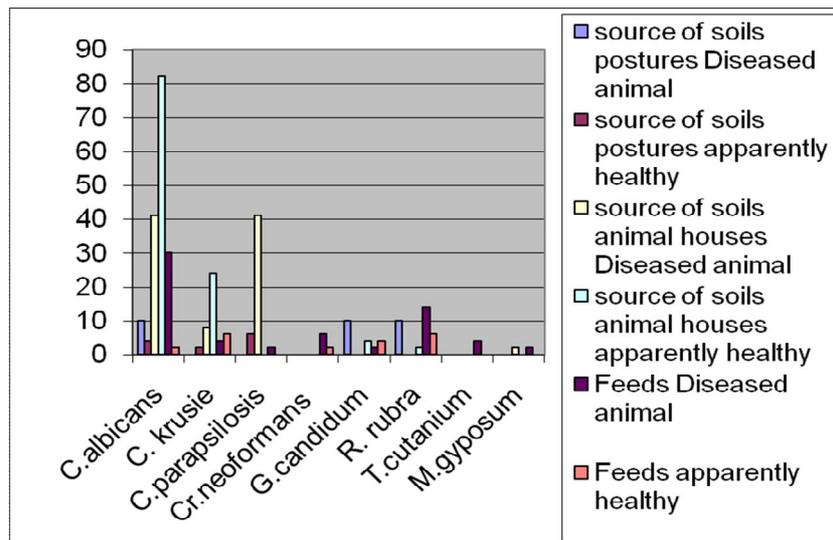


Fig. (4): Prevalence of yeasts species in soil and feeds of diseased and apparently healthy goats.

Moreover, the changes in treatment strategies and the increased use of antifungal prophylaxis [52] and the lack of an effective fungicidal regimen as well as the development of antifungal resistant strains suggest that continued investigation is necessary to devise immunotherapeutic strategies and / or drug targets to combat fungal infection [53].

In the present study, several natural herbal oils and extracts were evaluated as antifungal agents for controlling fungal infections including Clove, Garlic and Onion oils, in addition to *L. termis* extract. These herbal oils and extracts were tested on several *C. albicans*, *C. krusie* and *Cr. neoformans*, *R. rubra* strains isolated from the present samples of this study. Hamal and Koukalova [54], reported significant differences in susceptibility of yeasts to antifungal and distribution their between groups of yeasts isolated from dogs, cows and pigs. They attributed these differences of yeast susceptibilities to antifungal agents perhaps due to different environmental conditions and the endogenous origin of the yeast isolates. Similar results also were obtained by [55, 56, 57], who found that, eighteen among thirty plant oils tested showed anti-Candida activity, where the substantial antifungal activity shown by these plant oils suggested their potential against infections caused by *C. albicans*. The studies reported by various authors on clove oil revealed interesting findings. Briozzo *et al.* [58] reported that, essential oil of Clove dispersed (0.4% v/v) in a concentrated sugar solution had a marked germicidal effect against various bacteria and *C. albicans*, Khaldun [11], detected the inhibitory effect of Clove oil on *C. albicans*. Braga *et al.* [14] and He *et al.* [59] tested the anticandidal activity of Eugenol, main phenolic component of Clove oil on *C. albicans*. The molecules of oil altered the morphogenesis of the envelope of yeast, where, they affected major virulence factors such as adhesiveness and the morphological transition of *C. albicans* to hyphal form and it displayed potent activity against *C. albicans* biofilms in vitro with low cytotoxicity and therefore has potential therapeutic implication for biofilm-associated candidal infections.

Similar findings were obtained in our study, where the inhibitory effect of Clove oil on *C. albicans* and *C. krusie* (zone of inhibition were 15 ± 0.51 mm and 13 ± 0.12 mm at concentration of 3%) which was more pronounced than that observed in *Cr. neoformans* and *R. rubra* (6.0 ± 0.2 mm and 6.3 ± 0.02 mm at conc. 3%) (Table,5). Whereas, stronger effect was obtained by oil of onion at MIC of 1% that ranged from 5.0 ± 0.03 for *R. rubra* to 6 ± 0.2 for *C. albicans*.

However, the Allicin, one of the active principles of freshly crushed garlic homogenates, had a variety of antimicrobial activities. Allicin in its pure form was found to exhibit antifungal activity, particularly against *C. albicans* [55, 60, 12]. Similar findings were obtained in our study as the inhibitory effect of Garlic oil on all yeast were nearly equal in zone of inhibition at concentration of 2%.

The antifungal activity of "Allium" vegetable was investigated by [61], they reported that garlic (*Allium Sativum* L.), Onion (*Allium Cepa* L.) and Leek (*Allium Potum* L.) were investigated against *Aspergillus niger*. Minimal inhibitory concentration (MIC) and minimal fungicides concatenations (MFC) of aqueous ethyl alcohol and acetone extracts were determined by disc diffusion and broth dilution methods in the test tubes. Onion extract with ethyl alcohol (275 mg/ ml, MFC) and aqueous Leek-extract (900 mg / ml MFC) found to be the most inhabitory against *A. niger*.

Whereas, the an aqueous formulation of lupinus terrmis seed extract was tested against Pythium aphanidermatum and Rhizoctonia Soloni both in vitro and in vivo (in a food poison test), the highest concentration of seed extract (9 mg/ml) resulted in a complete inhibition of P.phanidermatum myelial growth and a 77% suspersion in the linear growth of R.soloni [62]. The action of lupinus terrmis seed extract as antimicrobial and as antifungal due to some types of proteins which showed such effect [63]. Also, alkaloid Profiles of two lupinus species growing naturally in Egypt were studied which indicated the presence of quinolizidine, bipiperidyl and proto-indole alkaloid in various plant organs, demonstrating for antimicrobial activities of these plant extracts showing substantial activity against Candida albicans, Aspergillus flavus and Bacillus subtilis [64].

Table5: Inhibitory zone of MIC of herbal oil against isolated yeast species using disc diffusion technique.

Yeast species	Herbal oils or extracts														
	Clove oil			onion			Garlic			L. terrmis			Itraconazol (control)		
	1%	2%	3%	1%	2%	3%	1%	2%	3%	1%	2%	3%	1%	2%	3%
C. albicans	R	R	15±0.51**	6±0.2*	8±0.4*	8.5±0.1	R	5±0.2	5.7±0.3	R	7.1±0.1***	9±0.1	11.5±0.5	17±1.0	17.5±2.0
C.krusie	R	R	13±0.12*	R	10±0.5*	11.2±0.2**	R	6.1±	6.5±0.3**	R	8±0.15**	R	R	15±0.8	16±0.2
C.neofomas	R	R	6±0.2*	R	9±0.1	11±0.5	R	5.8±0.1	6.5±0.3**	R	9.1±0.2*	10±0.5	R	12±0.5	13±0.1
R.rubra	R	R	6.3±0.02**	5.0±0.03*	7±0.2	8.5±0.1	R	2.5±0.1	4±0.2	R	5.0±0.3	6±0.5	R	13.5±0.1	14±0.2

All figures expressed as the area of the zone of fungal inhibition (mm Ø)

Significance at * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ (ANOVA), R = resistant

Therefore, these trials may offer an idea or draw the attention to the possibilities of using these natural antifungal preparations in several pharmaceutical forms for treatment where they are used either alone as reported by [10, 11,12] and Braga *et al.* [13], or combined with natural antifungal preparations (garlic and onion oils) as a combined treatment for achieving the synergistic action as reported by Postoienco *et al.* (14 , Al-waili [15] , Braga *et al.* [13] or co administered with the commercial synthetic conventional ones for achieving the synergistic action as reported in several recent researches [17,16] . The current antifungal oil and extract may be even used as a new alternative treatment in the near future if they are shown to have more potent antifungal activity than synthetic conventional ones throughout future studies or use it alone as prophylactic or local or topical applications combination (synthetic and natural)

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