

## Epidemiological and mycological profile of superficial mycoses in diabetic patients

Fatiha Bousnina \*, Oumaima Nassiri, Oumaima kharkhach, Asmae Rhoubi, Samira Laghzaoui and Aziza Hami

*Laboratory of parasitology-mycology, Mohammed VI University Hospital of Oujda. Faculty of Medicine and Pharmacy, Mohammed Premier University. Oujda, Morocco.*

World Journal of Biology Pharmacy and Health Sciences, 2024, 17(03), 067–074

Publication history: Received on 25 January 2024; revised on 02 March 2024; accepted on 04 March 2024

Article DOI: <https://doi.org/10.30574/wjbphs.2024.17.3.0075>

### Abstract

**Introduction:** Superficial mycoses, especially those of nails, scalp and skin, are very common in various countries. The presence of specific terrain, as well as other factors, can affect the prevalence of these. In this sense, diabetic patients are more susceptible to infections especially fungal infections because of changes in the immune system and macro and microvascular changes. The objective of our study was to determine the epidemiological and mycological profile of superficial mycoses in diabetic patients at the Mohammed VI Hospital in Oujda (Morocco).

**Material and methods:** This is a retrospective, observational and descriptive study spread over 34 months, from January 2021 to October 2023 at the Parasitology-Mycology laboratory of the Mohammed VI Hospital Center of Oujda. We collected demographic and mycological data for the included patients. In the laboratory, upon reception of the samples, a direct examination (DE) of the sample was obtained after clarification by KOH to better visualize the mycelial filaments or yeasts and culture on both media: Sabouraud with chloramphenicol and Sabouraud with chloramphenicol and cycloheximide is systematically carried out.

**Results:** We collected 136 diabetic patients referred to our laboratory for suspicion of superficial mycosis. The latter was confirmed in 46 of the 136 diabetic patients (33.82%) referred to our laboratory, which corresponds to 50 lesions among the 166 sites taken, a prevalence of 30.12%. At the mycological examination, the DE allowed the confirmation of the diagnosis of mycoses in 64% (n=32) and showed most often mycelial filaments (n=21; 65.62%) especially at the level of the feet and yeasts were observed in particular at the level of the nails of the hands (n=11; 34.37%). While culture was positive in all cases without exception (100%; n=50), dermatophytes were the most frequently isolated fungi (n=30; 60%), followed by yeasts (n=20; 40%). By species, *T. rubrum* (n=30; 60%) was the only isolated dermatophyte species, followed by *Candida albicans* (n=17; 34%).

**Conclusion:** In recent years, we have seen an increase in the prevalence of superficial mycoses in diabetic patients which is why we conducted this study. The results show on the one hand that the most isolated fungi in the superficial mycoses of these patients are dominated by dermatophytes, as *T. rubrum* represents the most frequently encountered species. Furthermore, the high prevalence of the latter demonstrates the significance of implementing important prevention measures.

**Keywords:** Superficial mycoses; Diabetic patients; Fungi, yeasts; Mycological examination.

### 1. Introduction

Superficial mycoses, particularly those of the nails, scalp and skin, are very common in various countries [1]. They are particularly common in tropical and subtropical zones, where the climate is hot and humid. These fungal infections

\* Corresponding author: Fatiha Bousnina

occupy an important place in clinical pathology and are a common reason for consultation. The high prevalence, the similarity of these infections with other conditions, especially dermatological ones, and the atypical clinical picture have underlined the importance of this subject to finding an informative means of diagnosis. For this mycological examination should be necessary. According to the literature, the most common agents involved in these infections are dermatophytes and yeasts, especially of the *Candida* genus.

The prevalence of superficial mycoses varies according to several factors not least the presence of a particular predisposing terrain. In this respect, diabetic patients are more susceptible to infections, particularly fungal ones, due to changes in the immune system and macro- and micro-vascular changes [2]. Diabetes mellitus is a chronic metabolic and degenerative disorder characterized by chronic hyperglycemia. It causes numerous long-term complications and is becoming one of the major emerging threats to public health in the 21st century. In 2017, the global prevalence of adult-onset diabetes was nearly 425 million [2]. It is currently recognized that diabetes mellitus is both a factor in promoting and aggravating mucocutaneous lesions [3]. Although these infections are generally benign, they can have a major impact on the vital and functional prognosis, as well as on the quality of life, of diabetic patients.

Our objectives in this study were to determine the epidemiological and mycological profile of superficial mycoses in diabetic patients and to gain a better understanding of the prevalence, clinical aspects and pathogens responsible, to enable appropriate therapeutic and prophylactic management.

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## 2. Materials and method

This is a retrospective, observational and descriptive study spread over 34 months, from January 2021 to October 2023 at the Parasitology-Mycology laboratory of the Centre Hospitalier Mohammed VI d'Oujda. In our study, we included all diabetic patients who had undergone a metabolic work-up at the central laboratory of the Mohammed VI University Hospital in Oujda, with a glycated hemoglobin level above 6.5% during the study period, and who presented lesions suggestive of mycotic involvement: Onychosis, Epidermophytosis, tinea capitis and any other suspicious clinical aspect. We excluded all patients undergoing antifungal treatment on the day of sampling or who had not respected a therapeutic window: 15 days for local treatment and one month for per os treatment or after application of a film-forming solution, as well as all patients who had not undergone a metabolic work-up in the biochemistry laboratory. All patients underwent a thorough history-taking and clinical examination. In the case of any suspicious lesion, one or more samples were taken for mycological examination, which generally involved 3 stages: direct examination, culture and identification of the incriminating agent. Firstly, a good-quality sample must be taken, respecting asepsis rules and the recommended quantity. Then, a direct examination (DE) of the sample obtained must be carried out after KOH clarification for all samples rich in keratin to better visualize mycelial filaments or yeasts. A culture is systematically performed on both media: Sabouraud with added chloramphenicol and Sabouraud with added chloramphenicol and cycloheximide, incubated in an oven at 27°C and read twice a week for 4 weeks. Where filamentous fungi were isolated, identification was based on macroscopic (color, recto/verso appearance, relief) and microscopic (size, macroconidia/microconidia, presence, or absence of chlamydoconidia/ornaments) cultural criteria. In addition, in the presence of yeasts, identification is based first and foremost on macroscopic (color, size) and microscopic (size and shape of blastospores, presence of pseudofilaments/chlamydoconidia) cultural criteria, so a subculture on chromogenic medium was carried out from the colony isolated in culture to identify the species. A filamentation test is sometimes required to identify *Candida albicans*. Data processing was carried out using Microsoft® Office Excel®.

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## 3. Results

During the study period, 136 diabetic patients were referred to our laboratory for suspected superficial mycosis, to confirm the diagnosis by mycological examination. Our patients were predominantly male (n=70; 51.47%) with an average age of 57. A total of 166 specimens were taken from all these patients an average of 1.2 specimens per patient, reflecting the possible existence of several simultaneous mycotic foci in a single patient. The distribution of these samples was as follows: (n= 144; 86.74%) samples from the hands and feet, (n=19; 11.44%) samples of different kinds (skin samples, ear samples and esophagus biopsy) and only (n=3; 1.80%) scalp samples were taken on suspicion of Tinea capitis.

Superficial mycosis was confirmed in 46 of the 136 diabetic patients (33.82%) referred to our laboratory, corresponding to 50 lesions among the 166 sites sampled, with a prevalence of 30.12%. In this population, a slight female predominance was noted (n=24; 52.17%), with an average age of 55. In terms of diabetes type, type 2 diabetic patients (n=37; 80.43%) were the most affected, with an average duration of diabetes in these patients of 5 years. The majority of these patients were on insulin (n=30; 65.21%), with an average hbA1c level of 9.13%.

Clinically, we found two main groups: dermatophytes in (n=30; 60%) and candida in (n=20; 40%). Dermatophytes are mainly represented by unguinal dermatophytoses (Onychosis) (n=27; 54%), followed by dermatophytes of the feet and hands (intertrigos and plantar keratodermias) (n=3; 6%). Tinea capitis and corporis were absent in our series (n=0; 0%). Candidiasis was distributed as follows: unguinal candidiasis, particularly of fingernails (n=12; 24%), candidiasis of the esophageal mucosa (n=3; 6%), otomycosis (n=3; 6%) and candidiasis of the cutaneous and nasal mucosa in only 2 cases (4%). In terms of lesion location, among confirmed dermatomycoses, mycoses of the feet (onychomycoses, plantar keratoderma and intertrigo-toe) were the most common (n=27; 54%), followed by onychosis of the hands (n=15; 30%). (GRAPH 1)

On mycological examination, DE confirmed the diagnosis of mycosis in 64% (n=32), while culture was positive in all cases without exception (100%; n=50). The DE showed mycelial filaments most frequently (n=21; 65.62%), particularly on the feet, while yeasts were observed particularly on the fingernails (n=11; 34.37%). In terms of culture results, dermatophytes were the most frequently isolated fungi (n=30; 60%), followed by yeasts (n=20; 40%). By species, *T. rubrum* (n=30; 60%) was the only dermatophyte species isolated from toe and fingernail samples. *Candida albicans* (n=17; 34%) was the main agent isolated from fingernails. (GRAPH 2)

Of the 166 samples taken, culture was positive in 50 cases (30.12%), giving us a direct examination sensitivity of 60%, a specificity of 88.46%, a positive predictive value of 71.42% and a negative predictive value of 82.14%. Considering the type of mycosis according to the direct examination result, we found that the sensitivity of this examination was 70% for *T. rubrum* (n = 30), 52.94% for *Candida albicans* (n = 17) and 50% for *Candida glabrata* (n = 2). (TABLE 1)

#### 4. Discussion

Diabetes is currently one of the most widespread chronic non-transmissible diseases in Morocco, due to its increasing prevalence, morbidity and mortality [4]. It also has a major impact on human health, notably through the occurrence of multiple infectious complications, particularly fungal infections. In our study, the prevalence of superficial mycoses confirmed through mycological examination was 33.82%, which is in line with data in the literature where the frequency of superficial mycoses in diabetics varies from 24 to 75% of cases [5-7]. On the other hand, 136 diabetic patients were suspected of having superficial mycosis. This discrepancy between clinical and mycological examination has been found in most studies [3,8], which may be explained by the similarity of the various clinical aspects of dermatological disorders. Of all patients with superficial mycoses, there was a slight predominance of women, which is similar to the data in the literature [8-11]. The dress, aesthetic, and domestic habits of women can explain the latter. [9]. Thus, type 2 diabetes was identified as the most frequent contributing factor, accounting for 80.43% of cases. Our findings are in line with the literature, which reports that dermatological problems occur more frequently in patients with type 2 diabetes [3,9,12]. The role of diabetes mellitus as a factor promoting the occurrence of superficial mycoses can be illustrated by the presence of an inadequate immune response. Indeed, the chemotactic of polynuclear and macrophages is diminished, as are their phagocytic and intracellular bactericidal faculties [2,8]. These phenomena were accentuated by the high glycemic level (10-11 mmol/l) [3,13-15]. The majority of our patients had a mean hbA1c level of 9.13%. In this sense and given that glycemic control has often been judged on fasting plasma glucose levels and not on mean glycated hemoglobin values [16], we cannot conclude that the latter had any impact on our study.

Superficial mycoses are benign infections most often caused by dermatophytes, yeasts and molds (pseudodermatophytes++). In our study, unguinal dermatophytoses were the most prevalent, with a prevalence of 54% (n=27), in line with the literature [3,9,17]. However, in a study by N. El Fékih and al [18], involvement of the inter-toe spaces was the main site of lesions. The main site of superficial mycoses was the foot (n=27; 54%) in all samples taken. This is consistent with data in the literature [3,8-10,18]. This last can be explained by the wearing of often unsuitable footwear and insufficient drying of the feet after the five daily ablutions [3]. Foot damage can also be explained by alteration of the nail plate as a result of structural and biochemical changes caused by diabetes, and in some cases by the presence of arteriopathy and peripheral neuropathy [9].

The symptomatology of these infections is often common to other conditions, and in some cases can be highly atypical. In such cases, clinical examination alone is insufficient to establish the diagnosis. Mycological examinations should be used to establish the involvement of a pathogenic fungal agent in the appearance of these lesions. Classical mycological examination remains the gold standard, as it is more informative, easier to perform and also the only examination capable of isolating and identifying the pathogenic agent, provided it is performed under the right conditions to obtain the best results [16].

The mycological examination involves 4 essential steps: a good quality sample, a meticulous direct examination, culture on appropriate media and, finally, identification of the isolated fungus [16,19]. The quality of a mycological examination

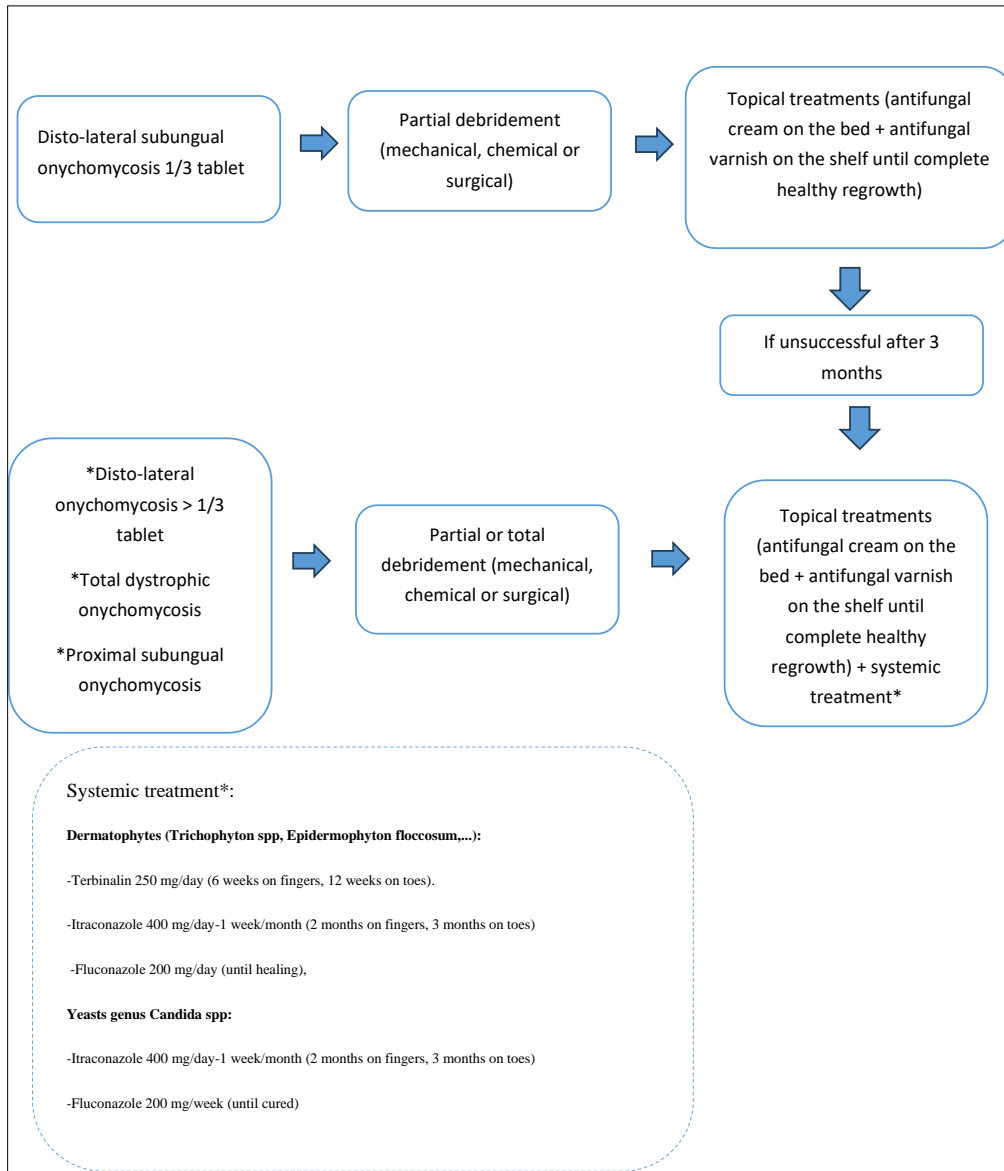
depends first and foremost on the quality of the sample. A good-quality sample must be collected in sufficient quantity in a sterile petri dish, and taken at a distance from any application of antifungal agents: 1- 3 months therapeutic window after prescription of a film-forming solution or varnish, or use of an antifungal agent such as terbinafine per os, for nail examination, and 4 weeks after application of another antifungal agent for skin or scalp lesions [19]. It varies according to the location of clinical involvement. In our study, the foot was the most frequently sampled site (scales/nails), in line with a study carried out in Tunisia [3].

DE is essential to establish and confirm the presence of the fungus in a parasitic state within the lesion [19]. It is performed between slide and coverslip, and read under a light microscope with an objective ( $\times 40$ ), either directly or after diluting the sample in physiological saline. In the case of thick samples (dander), it is performed after the addition of a liquid that dissociates keratinocytes (30-40% potash, chlorazol E black) [19]. It enables the fungus to be visualized: the mycelial filaments and spores of filamentous fungi, or the blastospores and pseudofilaments of yeasts. It can also be used to assess the vitality and abundance of the fungus [16]. In our series, DE most frequently showed mycelial filaments (n=21; 65.62%) and yeasts (n=11 ;34.37%), which is similar to a study by S. Cheikhrouhou and al [9].

Samples are preferably cultured in tubes on Sabouraud agar media. Two tubes are usually inoculated. The first tube is supplemented with antibiotics (Chloramphenicol or Gentamycin) to limit bacterial growth. The second tube is supplemented with antibiotics and actidione (cycloheximide) to limit mold growth [19]. The tubes are placed in an oven at 27 ° C. They are read within two days to four weeks of incubation, depending on the fungus. In our study, the culture was positive in (30.12%) of all samples taken, which is in line with data from a study carried out in Tunisia by S.Cheikhrouhou et al [9], and another by R. Bouguerra [3] on hospitalized diabetic patients. The prevalence found in our study is similar to that found in a study carried out among diabetics aged over 60 living in rural areas in Brazil [20]. Higher figures (73.23%) were noted in a study by J. Issouani and al [6]. Dermatophytes were the most frequently isolated fungi in our study (n=30; 60%), as well as in other studies [3,6,9,10,16,17,20]. By species, *T. rubrum* (n=30; 60%) was the only dermatophyte species isolated from the various samples taken. *Candida albicans* (n=17; 34%) was the main agent isolated, particularly from fingernails (n=12; 24%), in line with the literature [3,6,8,9-11,20-23].

Comparison of direct examination with culture in our series showed a DE sensitivity of 60%, and a specificity of 88.46%. However, the study carried out by R. Bouguerra [3] showed a sensitivity of 85.3% and a specificity of 79.5%. Concerning the sensitivity of DE in species identification, and according to several studies carried out in this field [3,15], *C. glabrata* represents the most misunderstood candidal species of DE in the absence of pseudomycelium formation, which is in line with our result.

Treatment options for superficial mycoses vary according to the clinical picture and the causative agent. Onychosis can be treated locally in cases of minor or moderate involvement, using an antimycotic nail varnish after scraping, grinding or chemical keratolysis of the infected area [24]. Atraumatic nail abrasion with a 40% urea ointment has a beneficial effect on healing. Terbinafine is the most effective systemic treatment for severe dermatophyte onychomycosis, administered orally at a dose of 250 mg per day for 6 weeks for fingernails, and 12 weeks for toenails [25]. Terbinafine or itraconazole is the safest and most effective antimycotic agent for treating onychomycosis, particularly in children (FIGURE 1). Treatment of primary *Candida* spp onychomycosis may be local if the condition is monodactylic or simple onycholysis, or systemic in the event of failure or multi-dactylic disease with paronychia, and is taken for 4 to 6 weeks. The latter should be combined with local treatment and applied 4 to 6 times a day until cured. Effective local antifungal agents include azole derivatives, ciclopiroxolamine, and possibly topical terbinafine. In addition, the most widely used systemic antifungal agents are fluconazole (150-300 mg/d one day a week) and itraconazole (400 mg/d one week a month) [24,26]. Treatment of esophageal candidiasis is immediately systemic. The first-line antifungal agent is fluconazole at doses of 200-400 mg (3-6 mg/kg) administered for 14-21 days. Alternatives to fluconazole, particularly in cases of resistance, are primarily itraconazole and posaconazole [27]. The most commonly used antifungal treatment for *Candida* otomycosis mainly comprises 2 molecules, which can be administered locally for at least 2 weeks (adapted ear drops) or systemically: polyenes (amphotericin B, nystatin) and azoles (ketoconazole, fluconazole and itraconazole) [28,29].



**Figure 1** Treatment of dermatophyte onychomycosis (26)

Superficial mycoses are common fungal infections worldwide. A series of preventive measures must be applied and respected to reduce their prevalence. Prevention starts with good blood sugar control, with regular screening of diabetics, and hygiene measures. These measures are based on controlling the source of contamination by treating infected animals and their entourage, disinfecting premises (e.g. swimming pools) and, in the event of a recurrence of lesions, rapidly resuming treatment. In the case of mycosis of the feet, these measures are based on careful and regular washing of the feet [30], as well as the use of personal sandals and leather soles. And don't forget to avoid walking barefoot and traumatizing your nails by cutting them short [31].

## 5. Conclusion

In recent years, we have seen an increase in the prevalence of superficial mycoses in diabetic patients. According to the literature, the frequency of these mycoses in diabetics varies from 24 to 75% of cases, which is why we carried out this study. The results show that the fungi most frequently isolated from superficial mycoses in these patients are dominated by dermatophytes, with *T. rubrum* being the most frequently encountered species, particularly in foot infections, followed by *Candida albicans*, which was the main agent isolated from fingernails. Moreover, the high prevalence of the latter proves the importance of implementing crucial preventive measures, including blood sugar control and strict adherence to hygiene rules.

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

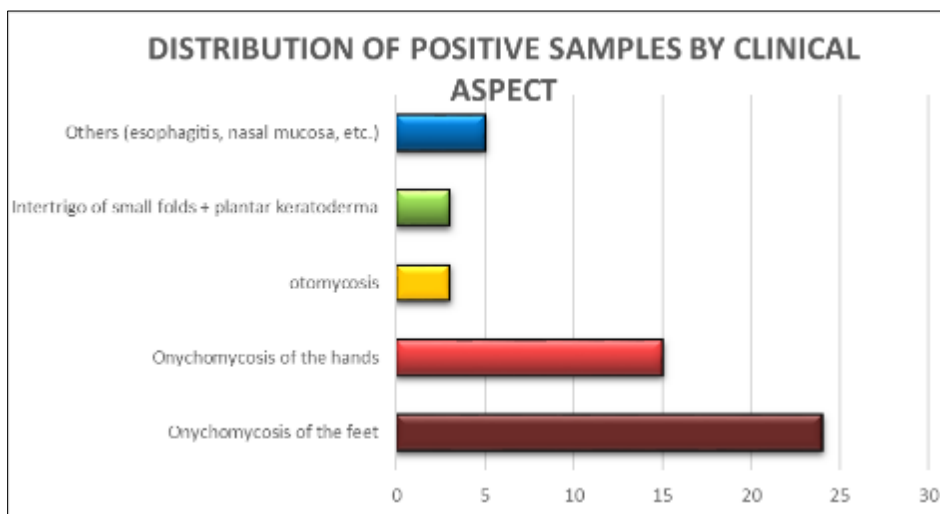
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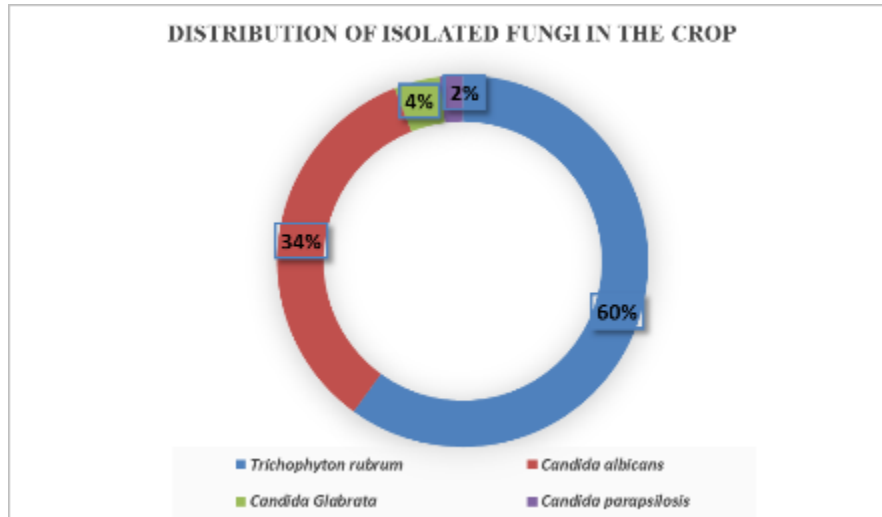
**Annexes**



**Graph 1** Distribution of positive samples according to clinical appearance (n=50).

**Table 1** Correlations between direct examination and culture (n=166)

Direct exam \ Cultural	Positive		Negative	
	n	%	n	%
Positive	30	18,04	20	12,04
Negative	12	7,22	92	55,42
Non conclusive	12	7,22	0	0



**Graph 2** Distribution of fungi isolated at crop level (n=50)