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Impact Of Nutritional Factors In *Microsporum canis* Using Response Surface Methodology

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Abstract: The three genera of dermatophytes —*Trichophyton, Microsporum*, and *Epidermatophyton*—are a group of keratinophilic fungi that can penetrate keratinized tissue and result in dermatophytosis. One of the most casual agents of tinea peds, tinea corporis, and tinea capitates is *Microsporum canis*. The present inquiry aimed to isolate and identify *Microsporum canis* at the Dermatology and Venereology Hospital in Mansoura and evaluate the optimal growth medium required for the sporulation and maximum biomass production of the isolated fungi. *Microsporum canis* was identified morphologically by conventional methods and the effects of various nutritional factors on biomass production were investigated. The significance of eleven independent variables given was investigated by PB design with a set of twenty experiments using different combinations of factors, the impact on fungal growth represented by peptone, yeast extract, maltose, and fructose, with P-value 0.00, followed by Response Surface Methodology using CCD. CCD was employed to assess and optimize the ideal conditions of the four independent variables and the interactions among input variables. The findings of the 31 run trials demonstrated that the yield varied significantly per fungus with the four variables. The maximum biomass was accomplished at run 8 with peptone 15 g/l, yeast extracts 15 g/l, maltose 10 g/l, and fructose 10 g/l with a yield of 19.480 g/l.

Keywords: Dermatophytes, *Microsporum canis*, PBD, RSM.

Introduction

The three genera of dermatophytes that cause dermatophytosis a superficial fungal infection are *Trichophyton, Microsporum, and Epidermophyton* [(1)], which belong to the family: Arthrodermataceae[(2)]. Dermatophytes have been divided into three groups: anthropophile, zoophile, and geophile based on ecological distribution[(3)]**.** Dermatophytes are often identified by a combination of clinical symptoms, colony features, and microscopy [(5)]. The physical similarities among species make identifications difficult and time-consuming. [(3)]. Macroconidia are the essential elements needed to identify dermatophytic fungi in standard laboratory settings[(6)]. Numerous vegetative structures, including spiral hyphae, racquet hyphae, nodular organs, and faith and elders, may also be generated. These are useful for identifying the three dermatophyte genera[(7)]. Dermatophyte colonies can be classified into three forms[(8)]**:** A) The membranous form (faviform, glabrous, waxy, and humid) has compact masses of vegetative mycelium, and no aerial mycelium e.g. *T. concentricum, T. violaceum, M. ferrugineum, T. schoenleinii,* and *T. verrucosum.* B) The filamentous form, which can be woolly, velvety, fluffy, hairy, or cottony: The aerial mycelium is dense and relatively high e.g. *M. canis, E. floccosum, M. audouinii, M. nanum, M. distortum,* and *T. rubrum.* C) The granular-powdery form is distinguished by abundant conidia and the absence of aerial filamentous components e.g. *T. mentagrophytes, M. equinum, and T. megnini* [(9)]**.** Dermatophyte infections are confined to a part of the epidermis, although they can be invasive and cause significant

infections in immunocompromised patients [10]. Dermatophytic fungi have been shown to have keratinolytic, proteolytic, and lipolytic activities[11], that capable of hydrolyzing keratin[12], which can used as a carbon source[13]. The development of dermatophytosis symptoms is induced by metabolic changes occurring in host tissues [14]. Most dermatophytes are similar and cause similar symptoms; therefore, dermatophyte infections in humans are often classed based on the portion of the body that they affect[15]. *Microsporum canis* is a fungus that causes tinea capitis, a disorder characterized by hair loss and intense scalp itching, red scaly papules around hair shafts, in both people and animals [16]. *M.canis* is the principal agent for dermatophytosis cases in domestic cats as well as tinea capitis in humans in various parts of Europe [17].

Materials and Methods

Manipulation of fungal isolates

Media Used for Isolation and Identity of Fungi:

Sabourad Dextrose Agar Medium (SDA).

Dermatophyte Test Medium (DTM).

Sabourad Dextrose Broth.

Christensen's urea agar.

Specimens collection

All specimens were acquired from our hospital's Department of Dermatology. This investigation was carried out in April 2023. The samples comprised skin scales, hair, and nails. The lesions were cleansed with 70% alcohol, and samples were collected in a sterile paper fold and labeled with the patient's data. All gathered samples were submitted to direct microscopy and culture.

Fungal culture

Under sterile conditions, the collected specimens from the nail, skin, and hairs were cultured on Sabouraud's dextrose agar, containing chloramphenicol (0.05g/l) and cycloheximide (0.5g/l) (SCC). The plates were incubated in a warm incubator at 28◦C for 14 days. The inoculation plates were tested every three days for the presence of dermatophytes. *M. canis* isolates were recognized by their culture morphology and microscopic characteristics [18].

Direct microscopic examination

The acquired samples were tested for the presence of fungal components using a 20% potassium hydroxide mount before being examined under direct microscopy. A few drops of KOH were placed on a clean, grease-free glass slide. The sample (skin scraping or hair plucking) was immersed in KOH on the slide, cover slip was placed on top and squeezed to prevent air bubbles from forming. Let in KOH and inspect after 5 - 8 minutes. Slides were examined at low(10×), and high (40×) magnifications for the presence of hyphae and/or arthroconidia [19].

Identification of isolated fungi

Dermatophyte identification was made based on the colony characteristics and microscopic features of the fungal isolates according to the methods described by Rippon (1988) and Larone (1995) (20). The macroscopic identification was based on the development rate, texture, topography, and pigmentation of the front and reverse sides of the culture (21).

Statistical optimization of mycelium growth rate

The Plackett-Burman Design (PBD) is a great instrument for investigating the influence of medium composition and is particularly useful for filtering the most essential variables based on their principal effects. PBD results do not describe the interaction among these variables; however, they are utilized to screen and analyze the variables that have a substantial impact on response[22] [23]. The effects of nutritional parameters nitrogen and carbon sources on the mycelium growth of two species of dermatophytes were applied to examine. The different factors were prepared in two levels: -1 for low concentration and +1 for high concentration[24]. The experimental results were examined by the first-order model using the following equation:

$$Y = \beta 0 + \sum \beta i \chi i \qquad (eq1)$$

Where Y is the response, β_0 is the model intercept, βí is the linear coefficient and χí is the level of the independent variable [25].

The effect of each variable was determined by Equation 2

$$Y = \sum(+1)/n\ (+1) - \sum \frac{-1}{n(-1)} \quad (\text{eq2})$$

RSM improved the significant factors reported by PBD utilizing central composite design (CCD) with Minitab 19 statistical software.

Response Surface Methodology(RSM)

(RSM) is a combination of mathematical and statistical approaches that are useful for the modeling and analysis of issues in which a response of interest is influenced by numerous variables and the purpose is to maximize this response[26] [27]. After the identification of variables affecting the mycelium growth, a central composite design CCD was conducted and an asset of 4 variables for *Microsporum canis,* maltose, yeast extract, fructose, and peptone was determined. The experimental design consisted of 30 runs, with the independent variables studied at two levels factorial. All experiments were performed in triplicate, and the average dry mass obtained was used as the dependent variable or response (Y). The following second-order polynomial equation was used to calculate the relationship between different variables and the response.

$$Y = \beta 0 + \sum \beta\ ixi + \sum i\ j\ \beta\ ijxixj + \sum \beta\ iixi$$

(eq3)

Where Y is the predicted response, Xi, Xi 2, and Xj are variables in coded values; β0 is the constant; bi is the linear effect; β ii is the squared effect and β ij is the interaction effect. The analysis of results was performed with statistical and graphical analysis software (Design Expert, Version 8.0.0). Design Expert software was used for regression analysis of the data obtained and to estimate the regression equation coefficient [28].

Determination of mycelial dry weight (biomass)

After incubation, cultures were harvested by filtration (Whatman no.1), and the mycelial mats (in triplicate) were thoroughly washed with sterile distilled water. The biomass was dried in a hot-air oven set at 60°C until it reached a constant weight. The dry weight of the biomass per liter of culture media was used to quantify fungal growth [29].

Data analysis

The dermatophyte data from each trial was analyzed using the statistical program Minitab 19 for Plackett-Burman and experimentation. To determine the model's statistical significance, an analysis of variance (ANOVA) was performed.

3. Results

Diagnosis of skin infections

This study focuses on a total of 36 patients diagnosed with fungal infections of different ages affecting various body regions, including, hair, nails, skin, and feet with a diverse dimorphic group of young (n=9), Male (n=15), and females (n=12). Clinically, the infected areas showed red circular patches on the skin with raised scaly edges and the infection was combined with intense itching, peeling, and dryness of the infected sites, signs of hair loss on the head and chin, a scaly bald patch on the scalp and a black dot was observed. Between the toes, it is characterized by peeling, redness, intense

itching and deep cracks appear obviously.

The highest incidence was found in males aged (31-63) followed by females aged (26-57), and then children aged <20 years. Fungal structures were directly observed in samples by 20 % KOH with methylene blue and examined under low (10×) and high (40×) magnification. Out of all collected specimens, 26 displayed positive

results against the 20% KOH test, while in the remaining isolates, 10 specimens showed negative results. Sabouraud dextrose agar (SDA) with gentamicin (8mg/liter) was used for the initial culturing of the specimens, followed by a dermatophyte test medium (DTM). All cultures were incubated at 25 ^{o}C for 2 weeks.

From The results of microscopic and macroscopic characteristics, it can be seen that a total of 24 isolates from hair or skin scrapings from clinically affected 17 isolates were identified as dermatophytes while 9 samples were identified as non-dermatophytes.

Identification of fungal strains by conventional methods

Morphological characteristic

The colonies' texture has a white flat dense, cottony surface with radial grooves and a tufted center, and have a bright white to yellow color on the reverse side Fig (1). Macroconidia are spindle-shaped, with an asymmetrical apical knob, multicellular, long, rough, and have thick outer cell walls Fig (2), macroconidia are elongated with thick cell walls, and have a terminal knob. The unicellular pyriform microconidia are rare, and septate hyphae were seen. These morphological features suggest that the isolate is closely related to *Microsporum canis.*

Table.1. isolation and distribution of dermatophytes infection among different genera.

Sites of infection	Gender			Microscopy KOH mount	
	Male Age group	Females Age group	young	Positive	Negative
	31-63	25-57	Age= <20		
Hair	5	6	2	9	4
Skin	4	4	2	6	4
Feet	4	1	3	8	0
Hands	0	1	1	0	2
Nails	2	0	1		2
Total	15	12	9	23	12

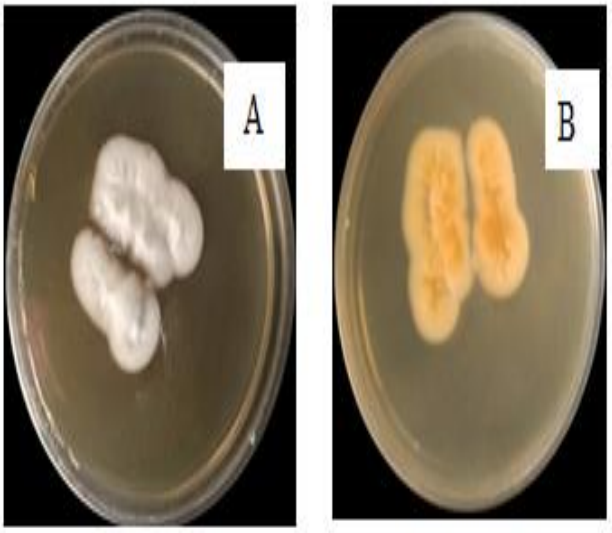


Figure 1. A Macroscopic view of fungus on SDA media shows a white cottony appearance and B shows yellow pigment on the reverse side

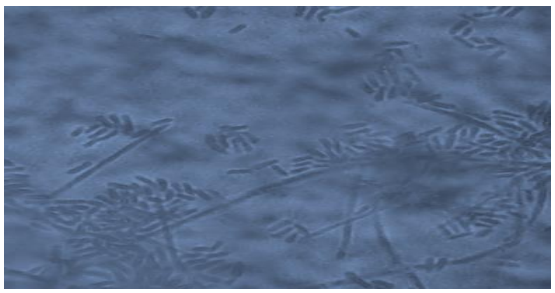


Figure 2. Microscopic view using methylene blue staining under light microscopic at 4X magnification power showing hyphae and abundant microconidia

Effect of different nutrition factors on mycelial growth of dermatophytes

Plackett–Burman Design (PBD)

To find out the effects of various nutritional factors on biomass production of Microsporum canis, the significance of eleven independent variables given in Table (2) was investigated by PB design with a set of twenty experiments using different combinations of factors, PB design examined the significance of eleven independent variables to screen the main media constituents. Each independent variable had two levels: high (+) and low (-). The primary effect was evaluated by subtracting the sum of responses at the high (+1) and low (−1) levels for each component. The following steps were performed to evaluate the data; - Regression analysis and ANOVA of the PBD were conducted in Table (3), and the coefficients t- State, p-value, and confidence level were recorded. The P-values are used as a tool to figure out the significance of each of the coefficients, which, in turn, reflect the statical significance of factors and interaction, helping build a precise model for optimizing the response and analyzing the effect of different variables. The lower the P values, the more significant the related coefficient. Analysis of the measured response variables enabled us to obtain standardization. Normal probability Fig (3) and, Pareto charts Fig (4) chart display the significant effects of each factor that are most important to the process or design optimization study. Factors with bares exceeding the red reference lines were identified as significant, with their effects being either positive or negative. The dummy variables showed low effects, suggesting that no essential variable(s) were omitted from the analysis. From the normal plot and half-

normal plot, the positive coefficients (red bars) suggest an incremental effect on fungal growth while, non-significant variables with negative coefficients blue bars, imply a decremented effect on fungal growth. From the experimental design, the biomass of Microsporum canis ranged from a minimum yield of 0.2g/l at run 20 to the highest of biomass yield 8.1g/l at run 12, with the optimal medium containing glucose, lactose, maltose, mannitol, and fructose, starch, peptone, yeast extract, casein, sodium chloride, and ammonium carbonate. A multiple regression mathematical model was applied to analyze the relation between the independent variables and the fungal biomass production. Based on the findings of the regression model and analysis of the variance, Table (3) illustrates the considerable impact on fungal growth represented by peptone, yeast extract, maltose, and fructose, with a *P*-value of 0.00 (less than 0.05). As for the substances that were shown to be inversely correlated with fungal biomass, glucose, mannitol, casein, lactose, starch, ammonium chloride, and sodium carbonate had P values greater than 0.00 and were deemed not significant. The R^2 and modified R^2 values are 98.67% and 96.40%, respectively, indicating a strong correlation between observed and projected values, with only 1.33% of the total variation remaining unexplained.

Table 3. Statistical Analysis of Varian's (ANOVA) for Plackett–Burman Design.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	12	82.0780	6.8398	43.35	0.000
Linear	12	82.0780	6.8398	43.35	0.000
Glucose	1	0.3765	0.3765	2.39	0.166
Lactose	1	0.3297	0.3297	2.09	0.192
Maltose	1	22.7612	22.7612	144.25	0.000
Mannitol	1	0.0609	0.0609	0.39	0.554
Fructose	1	11.1721	11.1721	70.81	0.000
Starch	1	0.2464	0.2464	1.56	0.252
peptone	1	18.9579	18.9579	120.15	0.000
Yeast extract	1	26.8378	26.8378	170.09	0.000
Casein	1	0.1043	0.1043	0.66	0.443
Ammonium chloride	1	0.4609	0.4609	2.92	0.131
Sodium carbonate	1	0.0871	0.0871	0.55	0.482
Dummy	1	0.6830	0.6830	4.33	0.076
Error	7	1.1045	0.1578		
Total	19	83.1825			

Table 2. Plackett-Burman design of selected carbon and nitrogen sources that affect mycelium dry weight g/l.

Std Order	Run Order	Glucose	Lactose	Maltose	Mannitol	Fructose	Starch	peptone	Yeast extract	Casein	Ammonium chloride	Sodium carbonate	Dummy	Mycelium dry weight (g/L)
1	1	10	2	10	10	1	1	1	2	8	1	5	-1	2.4
2	2	10	10	2	10	10	1	1	2	0.5	5	1	1	2.104
3	3	1	10	10	2	10	10	1	2	0.5	1	5	-1	4
4	4	1	2	10	10	1	10	10	2	0.5	1	1	1	3.99
5	5	10	2	2	10	10	1	10	10	0.5	1	1	-1	6.132
6	6	10	10	2	2	10	10	1	10	8	1	1	-1	4.1
7	7	10	10	10	2	1	10	10	2	8	5	1	-1	4.182
8	8	10	10	10	10	1	1	10	10	0.5	5	5	-1	7.042
9	9	1	10	10	10	10	1	1	10	8	1	5	1	6.162
10	10	10	2	10	10	10	10	1	2	8	5	1	1	2.922
11	11	1	10	2	10	10	10	10	2	0.5	5	5	-1	3.32
12	12	10	2	10	2	10	10	10	10	0.5	1	5	1	8.1
13	13	1	10	2	10	1	10	10	10	8	1	1	1	3.84
14	14	1	2	10	2	10	1	10	10	8	5	1	-1	7.832
15	15	1	2	2	10	1	10	1	10	8	5	5	-1	2.542
16	16	1	2	2	2	10	1	10	2	8	5	5	1	2.704
17	17	10	2	2	2	1	10	1	10	0.5	5	5	1	1.796
18	18	10	10	2	2	1	1	10	2	8	1	5	1	2.496
19	19	1	10	10	2	1	1	1	10	0.5	5	1	1	3.94
20	20	1	2	2	2	1	1	1	2	0.5	1	1	-1	0.2

Figure 3. Normal probability of standardized effects.

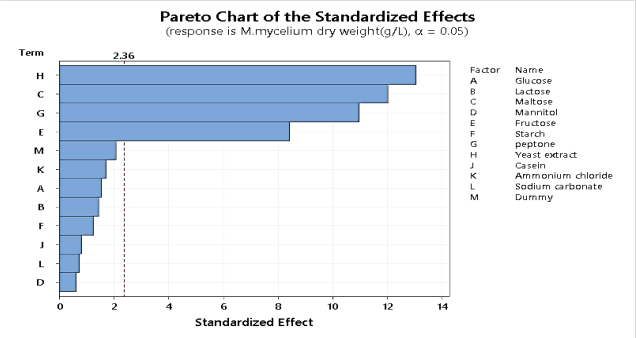
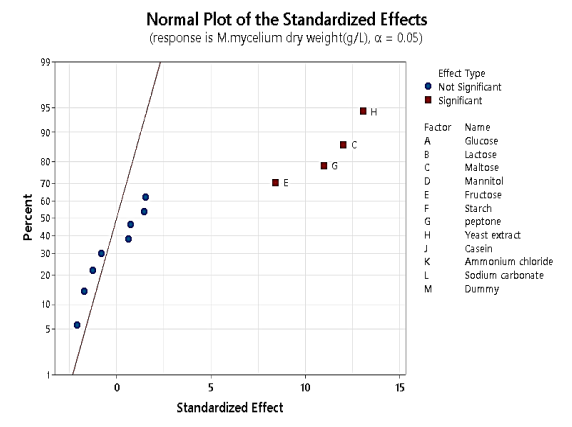


Figure 4. The Pareto chart represents the estimated effect of variables in the Plackett–Burman Design on mycelium dry weight.



Response Surface Methodology

Process optimization for mycelium dry weight by CCD-RSM

After selecting the most significant factors affecting fungal growth by placket Burman design, the Response Surface Methodology using central composite design CCD was employed to assess and optimize the ideal conditions of the four independent variables and the interactions among these input variables. The findings of the 31 run trials demonstrated that the yield varied significantly per fungus with the four variables. The interaction between the four variables is shown in table (4). The maximum of biomass was accomplished at run 8 with peptone 15g/l, yeast extract15 g/l, maltose10g/l, and fructose 10g/l along with a yield of 19.480g/l for *Microsporum canis*, while the minimum biomass production was detected at the run 20. Analysis of variance table (5) (ANOVA) was done by the Fisher's statistical test, the high F-value and very low *P*-value < 0.05 indicate a significant effect of variables on mycelium biomass yield. The adequacy of the polynomial model equation was judged statistically and the fit of the model can be checked by the determination coefficient (R^2) and correlation coefficient (R). The model gave goodness of fit at the coefficient of determination R^2 98.69%, adjusted R^2 97.55%, and predicted R^2 92.80%.

Table. 4. CCD for the significant variables affecting the growth of *M.C.*

Std Order	Run Order	Yeast extract	Peptone	Fructose	Maltose	M.mycelium dry weight(g/L)
1	7	8	8	10	10	7.418
2	22	15	8	10	10	9.1
3	18	8	15	10	10	12.008
4	8	15	15	10	10	14.98
5	13	8	8	25	10	7.8
6	11	15	8	25	10	7.62
7	26	8	15	25	10	5.992
8	12	15	15	25	10	8.002
9	14	8	8	10	25	8.088
10	30	15	8	10	25	8.621
11	31	8	15	10	25	10.19
12	2	15	15	10	25	12.354
13	17	8	8	25	25	11.992
14	19	15	8	25	25	11.836
15	24	8	15	25	25	8.818
16	29	15	15	25	25	9.97
17	23	4.5	11.5	17.5	17.5	10.401
18	10	18.5	11.5	17.5	17.5	11.754
19	20	11.5	4.5	17.5	17.5	7.248
20	5	11.5	18.5	17.5	17.5	7.628
21	27	11.5	11.5	2.5	17.5	12.513
22	25	11.5	11.5	32.5	17.5	10.09
23	4	11.5	11.5	17.5	2.5	7.744
24	28	11.5	11.5	17.5	32.5	9.215
25	21	11.5	11.5	17.5	17.5	13.601
26	1	11.5	11.5	17.5	17.5	13.582
27	3	11.5	11.5	17.5	17.5	13.603
28	15	11.5	11.5	17.5	17.5	13.606
29	9	11.5	11.5	17.5	17.5	13.964
30	16	11.5	11.5	17.5	17.5	13.491
31	6	11.5	11.5	17.5	17.5	13.774

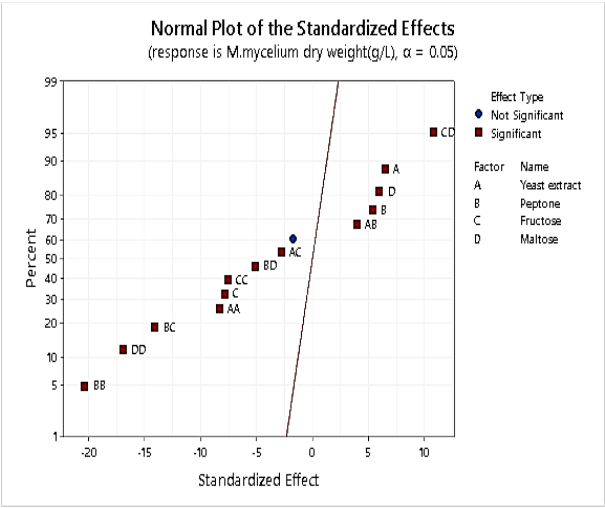


Figure 5. Normal probability of standardized effects

Table5. Analysis of Variance Anova for CCD of carbon and nitrogen levels

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	197.195	14.0854	86.26	0.000
Linear	4	27.595	6.8988	42.25	0.000
Yeast extract	1	6.915	6.9155	42.35	0.000
Peptone	1	4.681	4.6808	28.66	0.000
Fructose	1	10.108	10.1075	61.90	0.000
Maltose	1	5.891	5.8915	36.08	0.000
Square	4	109.556	27.3889	167.73	0.000
Yeast extract*Yeast extract	1	11.312	11.3123	69.28	0.000
Peptone*Peptone	1	67.715	67.7153	414.68	0.000
Fructose*Fructose	1	9.388	9.3876	57.49	0.000
Maltose*Maltose	1	46.739	46.7388	286.22	0.000
2-Way Interaction	6	60.044	10.0074	61.28	0.000
Yeast extract*Peptone	1	2.575	2.5752	15.77	0.001
Yeast extract*Fructose	1	1.280	1.2797	7.84	0.013
Yeast extract*Maltose	1	0.487	0.4869	2.98	0.103
Peptone*Fructose	1	32.407	32.4074	198.46	0.000
Peptone*Maltose	1	4.253	4.2529	26.04	0.000
Fructose*Maltose	1	19.042	19.0423	116.61	0.000
Error	16	2.613	0.1633		
Lack-of-Fit	10	2.463	0.2463	9.87	0.006
Pure Error	6	0.150	0.0250		
Total	30	199.808			

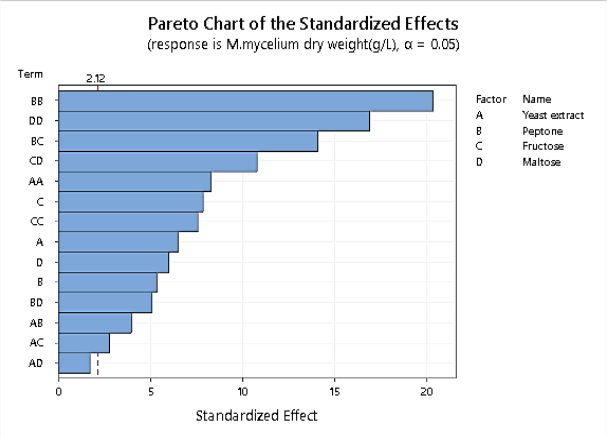


Figure 6. The Pareto chart represents the estimated effect of variables in CCD on mycelium dry weight

The relationships between the variables and the response are depicted by the three-dimensional response surfaces and their corresponding contour plots. The color gradient indicates the dry weight of mycelium g/l, where blue color indicates low growth, green color refers to moderate growth, and dark green reveals high growth Figure (7-a) illustrates how the dry weight of *M*. mycelium increases significantly when peptone and yeast extract levels rise, up until a point where an excess of these substances reduces efficiency because of inhibitory effects, the maximum dry weight (>12) is achieved with peptone at 8-16g/l and yeast extract at 7.5-18g/l while fructose maintained at 17.5g/l and maltose maintained at 17.5g/l.Fig (7-b) shows the combination of fructose with 10-15 g/l and yeast extract with 12.5-15g/l, while peptone and maltose were fixed at 11.5 and 17.5 g/l respectively to achieve fungus yield with (>14 g/l). Fig (7-c) shows a positive increase of the dry weight(>12g/l) to a certain point with increasing of maltose 10-25 g/l and yeast extract 7.5-18g/l while peptone and fructose were fixed at 11.5 and 17.5 respectively. Fig (7-d) shows, that the maximal fungus dry weight of (>12.5) g/l could be observed where the concentration of fructose at 5-25g/l and peptone at 10-18g/m while yeast extract and maltose were maintained at 11.5 and 17.5 respectively. The highest dry weight was observed to be (>12g/l) at 10-25g/l of maltose and 8-15g/l of peptone, while yeast extract and fructose were held at 11.5 and 1.5 respectively as shown in Fig (7-e). At 5-25g/l of maltose and 5-28g/l of fructose, while yeast extract and peptone fixed at 11.5g/l, the obtained dry weight was > 12g/l in Fig (7-f).

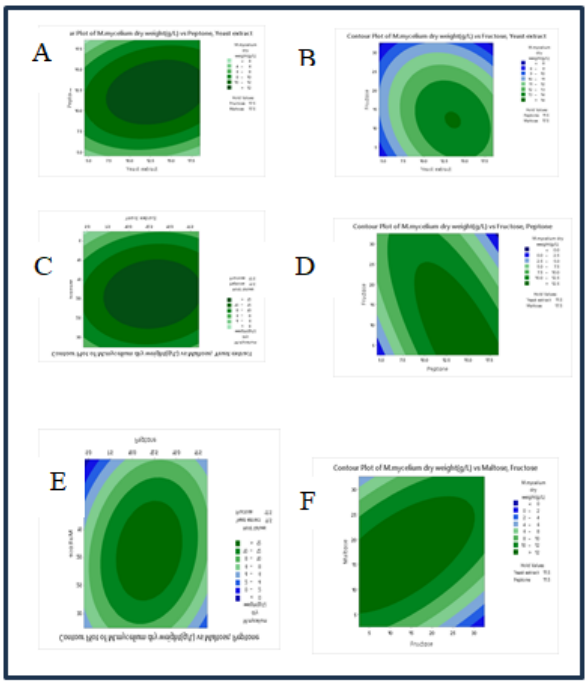


Figure 7. Contour plot described by the model on mycelial dry weight. (A) fructose and peptone (B) Lactose and Peptone. (C) maltose

and peptone. (D) Fructose and Lactose. (E) Maltose and Lactose (F)Maltose and Fructose for optimum growth levels of *Microsporum canis*

4. Discussion

It is estimated that superficial fungal infections affect about 20-25% of the world's population, cutaneous mycoses, which are primarily caused by dermatophyte fungi, are among the most common fungal infections globally, affecting a variety of age groups and negatively impact the quality of life of infected patients [31]. This study focuses on a total of 36 patients diagnosed with fungal infections of different ages affecting various body regions, including, hair, nails, skin, and feet with a diverse dimorphic group of young (n=9), male (n=15), and female (n=12). The highest incidence was found in males aged (31-63) followed by females aged (26-57), and then children aged <20 years. This agrees with (31) whose study revealed that out of 53 KOH-positive cases, the majority were male 33(62.2%) whereas 20 (37.73%) were only female patients. A similar study was done by [32] which showed that the male percentage was slightly higher than the females. The reason for the larger number of males may be due to more outdoor exposure and added physical work, and it may be one of the reasons why the rural population results in increased sweating and less cosmetic consciousness than females, but this disagrees with [33] who showed that The most common types of dermatophytosis were tinea corporis, and onychomycoses followed by tinea capitis, tinea cruris, and tinea pedis. Also, [34] investigated that dermatophytosis is more common in young adults aged 21-30 years in our study the most common clinical forms were tinea pedis followed by tinea capitates and this agreed with [35] who investigated that Tinea pedis was the most common clinical form. Identification of fungal agents and their species causing dermatophytosis is important for epidemiology and mostly for the therapeutic point of view when treatment is advised for a longer time. In our study, traditionally, the identification of fungi relies mainly on colony characteristics, microscopic spores, and mycelium morphology**.** In our study, the colonies' texture has a white flat dense, cottony surface with radial grooves and a tufted center, and have a bright white to yellow color on the reverse side. The macroconidia are elongated with thick cell walls, spindle shape, and have a terminal knob. The unicellular pyriform microconidia are rare, and spectated hyphae were seen, this agrees with [36] and [37] who showed that colonies of *M. canis* are white to cream-colored with reverse pigment ranging from golden-yellow to brownish-yellow. The topography is usually flat and spreading with radial grooves and the texture is cottony to wooly. *M. canis* can produce different types of conidia including spindle-shaped macroconidia and microconidia. Macroconidia have thickened cell walls. Unlike previous research that relied primarily on conventional methodologies, we adopted advanced designs-placket-Burman for factor screening and response surface methodology for optimization of the most influenced factors affecting fungal growth. Our study represents the first application of Placket-Burman and RSM methodologies in the context of *Microsporum canis*. This method minimized experimental runs while maximizing the reliability of results. Our study reveals that specific nutrients such as carbon-source maltose, fructose, and nitrogen source peptone, play a pivotal role in enhancing fungal growth. The placket-Burman Design is effective. Pinpointed these components as critical factors. Furthermore, RSM optimization was demonstrated.

Adjusting these nutrients within specific ranges resulted in significant improvements in fungal biomass. [38], showed that media containing a standard glucose concentration (20 g 1-') and with a reduced glucose content of 0.1-2.0g 1-' did not induce sporulation when provided to the fresh isolates.

Conclusion

This study investigated the impact of carbon and nitrogen factors on the growth of *Microsporum canis* using the Plackett-Burman design to identify significant variables. The results revealed that four of these factors had a substantial effect on fungal growth at specific concentrations, highlighting their importance in fungal development. After identifying the key factors, Response Surface Methodology (RSM) was employed to determine the optimal concentrations, enabling precise optimization of

growth conditions. These findings suggest that manipulating these factors can significantly influence the growth of *Microsporum canis*, which can be useful both for scientific research and for developing therapeutic or preventive strategies against fungal infections. These results represent an important step toward a deeper understanding of the environmental factors that affect fungal growth, which may aid in the development of new tools and techniques for controlling fungal infections in clinical and industrial settings.

5. References

1. Elavarashi E, Kindo AJ, Rangarajan S. (2017) Enzymatic and non-enzymatic virulence activities of dermatophytes on solid media. *Journal of Clinical and Diagnostic Research*.;**11(2)**:DC23–5.
2. Vandewoude S, Moskaluk AE, Vandewoude S. (2022) Current Topics in Dermatophyte Classification and Clinical Diagnosis. Pathogens.;**11(9)**.
3. Cafarchia C, Iatta R, Latrofa MS, Gräser Y, Otranto D. Molecular (2013) epidemiology, phylogeny and evolution of dermatophytes. Infection, Genetics and Evolution;20(September):336–51.
4. Jamin F, Estuningsih S, Pribadi ES, Handharyani E, Medicine V, Syiah U, et al. (2020) Dermatophyte Infection Pathogenesis on New Zealand White Rabbit Skin, Bogor, West Java, Indonesia.;**20(2)**:7657–62.
5. Bouchara JP, Nenoff P, Gupta AK, Chaturvedi V. Dermatophytes and dermatophytoses. Bouchara JP, Nenoff P, Gupta AK, Chaturvedi V, editors. (2021) Dermatophytes and Dermatophytoses. Cham: Springer International Publishing;. 1–519 p.
6. Yüksel T, IlkIt M. (2012) Identification of rare macroconidia-producing dermatophytic fungi by real-time PCR. Med Mycol.;**50(4)**:346–52.
7. Shah N. (2014) Clinico-Mycological Study of Dermatophytoses Diagnosed at Medical College, Mehbubnagar (Andhra Pradesh), India.;(October):464–7.
8. Refai M, Abo El-Yazid H, El-Hariri M. (1994) Monograph On Dermatophytes A guide for isolation and identification of dermatophytes, diseases and treatment.;**30**:1–73.
9. Gautam SS, Navneet, Babu N, Kumar S. (2021) Current Perspective of Dermatophytosis in Animals.;(June):93–104.
10. Sahoo A, Mahajan R. (2016) Management of tinea corporis, tinea cruris, and tinea pedis: A comprehensive review. *Indian Dermatol Online J*.;**7(2)**:77.
11. Kaufman GIL, Horwitz BA, Duek LEA, Ullman Y, Berdicevsky I. (2007) Infection stages of the dermatophyte pathogen Trichophyton: Microscopic characterization and proteolytic enzymes. Med Mycol. Jan;**45(2)**:149–55.
12. Akcaglar S, Ener B, Toker SC, Ediz B, Tunali S, Tore O. (2011) A comparative study of dermatophyte infections in Bursa, Turkey. Med Mycol.;**49(6)**:602–7.
13. Author C, Munir: Al-Bashan M, Al-Bashan MM. (2016) General Assessment of the Occurrence of Dermatophytes and Other Keratinophilic in the Camels (Camelus dromedarius) Rearing of the Taif Governorate Soil Regions, KSA. *World Appl Sci J*.;**34(8)**:1054–9.
14. Gnat S, Nowakiewicz A, Łagowski D, Zięba P. (2019) Host- and pathogen-dependent susceptibility and predisposition to dermatophytosis. *J Med Microbiol*.;**68(6)**:823–36.
15. Thebo NK, Abro H, Soomro AQ, Anwer J, Suhail M (2006). Isolation and identification of dermatophytes from Sindh, Pakistan. Pak *J Bot*.;**38(2)**:493–5.
16. Aljuhani S, Rizwana H, Aloufi AS, Alkahtani S, Albasher G, Almasoud H, et al. (2024) Antifungal activity of Carica papaya fruit extract against Microsporum canis: in vitro and in vivo study. Front Microbiol.;**15**(May):1–12.
17. Moskaluk A, Darlington L, Kuhn S, Behzadi E, Gagne RB, Kozakiewicz CP, et al. (2022) Genetic Characterization of Microsporum canis Clinical Isolates in the United States. *Journal of Fungi*.;**8(7)**:1–17.
18. Khanipour Machiani M, Jamshidi S, Nikaein D, Khosravi A, Balal A. (2024) The inhibitory effects of zinc oxide nanoparticles on clinical isolates of

Microsporum canis in dogs and cats. Vet Med Sci.;**10(1)**:1–9.
19. Namidi MH, Ananthnaraja T, Satyasai B. (2021) Antifungal Susceptibility Testing of Dermatophytes by ABDD and E-Test, a Comparative Study. Open *J Med Microbiol*.;**11(03)**:129–43.
20. Ganguly S, Para PA, Praveen PK. (2017) Investigation of Dermatophytosis from the Skin Scrapings Collected from a Cow: A Case Study. *Int J Curr Microbiol Appl Sci*.;**6(3)**:640–3.
21. Sachin K, Seema B. (2016) Clinico-mycological profiles of dermatophytosis in Jaipur, India. *Afr J Microbiol* Res.;**10(35)**:1477–82.
22. Chen J, Lan X, Jia R, Hu L, Wang Y. (2022) Response Surface Methodology (RSM) Mediated Optimization of Medium Components for Mycelial Growth and Metabolites Production of Streptomyces alfalfae XN-04. Microorganisms.;**10(9)**.
23. Darani KK, Farahani EV, Shojaosadati SA. (2003) Application of the Plackett-Burman Statistical Design to Optimize Poly(β-hydroxybutyrate) Production by Ralstonia eutropha in Batch Culture. *Iran J Biotechnol.*;**1(3)**:155–61.
24. Hegazy T, Ibrahim M, Abdel Hamid H. (2015) Plackett-Burman Design of Environmental and Nutritional Parameters for Petroleum Bioremediation by Penicillium chresogenum. Scientific *Journal for Damietta Faculty of Science*.;**5(2)**:40–4.
25. Abdelwahed NAM, Ahmed EF, El-Gammal EW, Hawas UW. Application of statistical design for the optimization of dextranase production by a novel fungus isolated from Red Sea sponge. 3 Biotech. 2014;4(5):533–44.
26. Kumar J, Kushwaha RKS. (2012) Optimization of media composition for keratinase production on feather by Acremonium strictum RKS1. Advances in Applied Science Research.;**3(5)**:3233–42.
27. Prajapati H V, Minocheherhomji FP. (2021) Optimization of Laccase Enzymes Production Through Response Surface Methodology By Trametes Elegans H6. *J Adv Sci* Res.;**12(2)**:301–8.
28. Othman AM, Elsayed MA, Elshafei AM, Hassan MM. (2017) Application of response surface methodology to optimize the extracellular fungal mediated nanosilver green synthesis. *Journal of Genetic Engineering and Biotechnology*.;**15(2)**:497–504.
29. Ali TH, El-Gamal MS, El-Ghonemy DH, Awad GE, Tantawy AE. (2017) Improvement of lipid production from an oil-producing filamentous fungus, Penicillium brevicompactum NRC 829, through central composite statistical design. Ann Microbiol.;**67(9)**:601–13.
30. Kumawat TK, Sharma A, Bhadauria S. (2016) Effect of culture media and environmental conditions on mycelium growth and sporulation of chrysosporium queenslandicum. *Int J Chemtech* Res.;**9(11)**:271–7.
31. Jha B, Bhattarai S, Sapkota J, Sharma M, Bhatt CP. (2019) Dermatophytes in Skin, Nail and Hair among the Patients Attending Out Patient Department. *J Nepal Health Res Counc*.;**16(41)**:434–7.
32. Farooq U, Altaf A, Singh S, R Sharma S, Sharma V. (2020) Occurrence of dermatophytes and other fungal agents from clinically suspected cases of superficial mycoses. IP *International Journal of Medical Microbiology and Tropical Diseases*.;**6(2)**:92–5.
33. Das S, Goyal R, Bhattacharya SN. (2007) Laboratory-based epidemiological study of superficial fungal infections. *Journal of Dermatology*.;**34(4)**:248–53.
34. Rezusta A, de la Fuente S, Gilaberte Y, Vidal-García M, Alcalá L, López-Calleja A, et al. (2016) Evaluation of incubation time for dermatophyte cultures. Mycoses.;**59(7)**:416–8.
35. Didehdar M, Shokohi T, Khansarinejad B, Ali Asghar Sefidgar S, Abastabar M, Haghani I, et al. (2016) Caractérisation des dermatophytes cliniquement importants dans le nord de l'Iran en utilisant la PCR-RFLP de la région ITS. *J Mycol Med*.;**26(4)**:345–50.
36. Chen L, Shi GY, Wang MM, Zhao LL, Huang YY, Chen XL, et al. (2013) Morphological and molecular identification of two strains of

dermatophytes. *Journal of Huazhong University of Science and Technology - Medical Science.*;**33(6)**:917–22.
37. Nasimuddin S, B A, P S, CR S. (2014) Isolation, Identification and comparative analysis of SDA and DTM for dermatophytes from clinical samples in a tertiary care hospital. IOSR *Journal of Dental and Medical Sciences.*;**13(11)**:68–73.
38. Mavroudeas D, Velegraki A, Leonardopoulos J, Marcelou U. (1996) Effect of glucose and thiamine concentrations on the formation of macroconidia in dermatophytes. Occurrence of dysgonic Microsporum canis strains in Athens, Greece. Mycoses.;**39(1–2)**:61–6.