

The difference of fungal growth *Rhizopus* sp. in SDA and PDA Media from toe nail samples of construction workers

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Abstract

Onychomycosis is a fungal infection caused by dermatophyte and non-dermatophyte groups which infect the toenails or fingernails. Onychomycosis infection can lead to the damage of the nails which causes the nail thickness, brittle, and easily destroyed. *Rhizopus* sp. is considered to be one of the fungal species in the non-dermatophyte fungal infection group in the disease Onychomycosis. This study aims to determine the differences in the growth of non-dermatophyte fungi that cause onychomycosis infections on SDA and PDA media in samples of toenails from construction workers in Lamongan Regency. The research method used is descriptive, and the sampling technique used is random sampling. The sample size examined was 15 construction workers in duplicate, 15 planted in PDA media and 15 planted in SDA media. The material for this examination is scrapings from the toenails of construction workers which are planted directly on SDA and PDA media, then followed by macroscopic and microscopic examination. Based on macroscopic examination, it was found that the colonies were white to grayish white, had a cotton-like surface and were spreading. Based on microscopic examination using the addition of 1 drop of LCB dye reagent, fungal elements were obtained which indicated the non-dermatophyte fungus *Rhizopus* sp. namely there are stolon, rhizoid, sporangiospore, sporangium and apophysis. From the results of this research it can be concluded that there is growth of the fungus *Rhizopus* sp. non-dermatophyte group on PDA media and SDA media, there was no difference in the growth of non-dermatophyte fungi that cause onychomycosis in the toenails of construction workers in Lamongan Regency on PDA media and SDA media and there was a difference in the growth of the fungus *Rhizopus* sp. Non-dermatophyte groups causing onychomycosis based on age.

Keywords: Onychomycosis; Dermatophyte; Fungal; *Rhizopus*; Skin disease

1. Introduction

Onychomycosis, a fungal nail infection typically caused by a dermatophyte, is a non life-threatening, but due to its high prevalence (10% of the US population) and associated morbidity, it represents a significant public health concern. Patients may experience discomfort from the illness, which may also negatively impact their ability to work and maintain social relationships. Onychomycosis is classified into four types according to the location and pattern of fungal invasion. The most common pathogens are dermatophyte fungi, although nondermatophyte molds and yeasts, particularly *Candida albicans*, may also be involved (Halvae et al., 2021). Other species that were also found as dermatophyte pathogen was *M. canis*, which causes dermatophytosis in cats and humans, is a dermatophyte that is commonly seen in clinical settings (Moskaluk et al., 2022). In another study, onychomycosis prevalence was high. Molds that were not dermatophytes had an isolation rate that was similar to that of dermatophytes. It is crucial to conduct

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more research on the incidence of onychomycosis, fungi that cause nail infections, and shifts in the species distribution of these agents in Ethiopia (Jaishi et al., 2022).

Onychomycosis can be caused by dermatophytes (*Tinea unguium*), non-dermatophyte molds, and also yeasts (Gupta et al., 2017; Angelo et al., 2017). Almost 75% of fingernail and 90% of toenail onychomycosis are caused by two species of dermatophytes, namely *Trichophyton mentagrophytes* and *Trichophyton rubrum* (Joyce et al., 2019; Youssef et al., 2018). Other cases are caused by *Trichophyton verrucosum*, *Trichophyton violaceum*, *Trichophyton soudanense*, *Trichophyton tonsurans*, *Trichophyton krajdenei*, and *Epidermophyton floccosum* (Falotico et al., 2022; Suzuki et al., 2021). *Trichophyton rubrum* has been identified in multiple reports as the primary dermatophyte species, followed by *Trichophyton mentagrophytes* (Tartor et al., 2019). A study by Jaishi was reported that five cases (4.3%) had dermatophytes isolated from onychomycosis patients. Of them, four cases consisting of *Trichophyton rubrum*, and one case consisting of *Trichophyton mentagrophytes*. Out of the 27 total fungal isolates, 6 cases were consisting of *Cladosporium* species (25%) as the most isolated non-dermatophyte mold that met the criteria as a pathogen. Sweating and poor hygiene were found to be statistically significant in fungal cases that were identified by both culture and KOH. It was becoming clear that both dermatophytes and non-dermatophytes fungi could cause fungal infections. For the diagnosis of fungal infections, direct microscopy and culture followed by LPCB were essential methods (Jaishi et al., 2022).

Most nail infections caused by fungi are not harmful. On the other hand, some people might feel pain or find their nail appearance change. Nail fungus infections can result in thick, fragile, cracked, or discolored nails. It's also possible for the nail to come away from the nail bed. A fungal skin infection on the foot, particularly in the space between the toes, is frequently present in individuals with fungal toenail infections (also known as athlete's foot, ringworm on the foot, or tinea pedis) (Centers for Disease Control and Prevention, 2023). Studies showed that while fingernail type *Candida* is more common in females, toenail involvement is the more common clinical form of onychomycosis in males (Fatahinia et al., 2017). Women had a higher frequency of nail onychomycosis (39.74%) than did men (18.51%). On the other hand, males (81.49%) had a higher frequency of toenail onychomycosis than females (60.26%) (Gregoriou et al., 2020).

The other risk factors include fungal infections elsewhere on the body (particularly tinea pedis), chronic paronychia, prior onychomycosis, wearing occlusive and tight shoes, hyperhidrosis, involvement in physical activities or sports, nail trauma, improper nail care, using public swimming pools, communal bathing, living with family members who have fungal infections, poor health, genetic factors, immunodeficiency (particularly acquired immune deficiency syndrome and transplant patients), diabetes mellitus, obesity, Down syndrome, psoriasis, smoking, peripheral vascular disease, venous insufficiency, hallux valgus, and asymmetric gait nail unit syndrome (Albucker et al., 2023).

Despite being a benign and treatable nail condition, onychomycosis can significantly affect a patient's day-to-day quality of life. Onychomycosis caused physical impairment, decreased functionality, pain or discomfort, and social embarrassment, with psychological and psychosocial effects reaching up to 92% in afflicted patients, according to a systematic review examining the disease's effects on quality of life (QoL) (Stewart et al., 2021). This study aims to elaborate the difference of fungal growth in PDA and SDA media which was isolated from the toe nail of construction workers.

2. Material and Methods

2.1. Study Design and Study Site

The study design used was a descriptive method using fungal culture on SDA and PDA media. As well as using direct microscopy with 20% KOH which aims to determine the presence of fungus on the toenails of construction workers. The population is the toenail scrapings of construction workers in Lamongan Regency. The samples used for the research were 28 samples, taken from scrapings from 10 toenails of construction workers in Lamongan Regency. The research was conducted from May to June 2023 at the Microbiology Laboratory of the Delima Husada Gresik Health Analyst Academy, on Jl. Arif Rahman Hakim No. 2B Gresik, East Java.

2.2. Inclusion and Exclusion Criteria

Sample was taken using primary data taken from construction workers in Lamongan Regency, the sample taken was 15 respondents from the adult and elderly age groups. The sample criteria are divided into 2, namely inclusion and exclusion criteria. Inclusion criteria consisting of: i) samples were taken from adult patients (26-45 years) and elderly patients (46-65 years) in Lamongan Regency; ii) patient willingness to join as participant; iii) the patient that were recruited had experience as a construction worker who had worked for more than 1-2 years. Exclusion criteria

consisting of: i) patients who have injuries in their toes; ii) patients who experience swelling in their toes; iii) patients who have a history of terry nail disease (the appearance of white nails with a narrow pink band at the tip).

2.3. Materials and Equipment

The materials used in this research were construction workers' toenails, SDA media, PDA media, 20% KOH solution, LCB solution, 70% alcohol, and Aquadest. Equipment that were used in this research were plastic bag clips, tweezers, microscope, glass object, deck glass, petridish, round tube, Bunsen flame, Erlenmeyer, stirrer rod, measuring cup, watch glass, pH paper, tissue, gloves, nail clippers. and autoclave.

2.4. Research Procedure

Materials and equipment were sterilized. Then each tool was wrapped in aluminum foil, then put into an autoclave to be sterilized at 121°C for 15 minutes. Research equipment that have been sterilized are left until the hot temperature on the tool drops and the tool is ready to be used. As many as 39 g of powdered PDA with 1000 mL distilled water and 65 g of powdered SDA were dissolved with 1000 mL distilled water using a hot plate. The pH was measured at 5.6 ± 2 . The PDA media was sterilized using an autoclave for 15 minutes at 121°C. After the sterilization process is complete, the SDA media and PDA media are allowed to reach a temperature of 45-50°C then poured into 10 mL sterile petri dishes and allowed to solidify. Before carrying out the examination, the SDA and PDA media that have been made were prepared. Then the sample is taken from the plastic bag clip with tweezers. After that, the samples were planted on SDA and PDA media. Then incubated at 25-30°C for 1 week. After incubation, the results were observed to identify the growth of fungi on SDA and PDA media.

Fungal that had been grown on SDA and PDA media were then sampled. Then the fungal sample is placed on a preparation that has been cleaned with an alcohol swab, after that the sample that has been placed on the preparation is dripped with 1 drop of LCB solution. Then the sample was covered with a cover glass and observed under a microscope with 10-40x magnification.

2.5. Data Analysis

In this study the results obtained were analyzed descriptively and the types of dermatophyte fungi that grew were seen. The data was analyzed using a computer with the Microsoft Excel program and the data was presented in table form.

3. Results

A total of 15 subject were interviewed by questionnaire, which consisting of practical and hygiene question, as shown below

Table 1 The detail of subject response

No.	Sample	Hygiene Practice									
		Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1.	S1	N	Y	N	Y	Y	Y	N	Y	N	N
2.	S2	Y	Y	Y	N	Y	Y	N	Y	Y	N
3.	S3	N	Y	Y	N	Y	Y	N	Y	Y	N
4.	S4	N	N	Y	Y	Y	Y	N	Y	N	N
5.	S5	Y	N	Y	N	Y	Y	N	Y	N	N
6.	S6	Y	N	N	Y	Y	Y	N	Y	N	N
7.	S7	N	Y	N	N	Y	Y	N	Y	N	N
8.	S8	N	N	Y	Y	Y	Y	N	Y	Y	N
9.	S9	Y	N	N	N	Y	Y	N	Y	N	N
10.	S10	Y	N	N	N	Y	Y	N	Y	N	N
11.	S11	N	Y	Y	Y	Y	Y	N	Y	Y	N

12.	S12	N	Y	N	Y	Y	Y	N	Y	N	N
13.	S13	Y	N	N	Y	Y	Y	N	Y	N	N
14.	S14	Y	N	Y	N	Y	Y	N	Y	N	N
15.	S15	N	N	Y	Y	Y	Y	N	Y	Y	N

Table 2 Percentage Distribution of Respondents Based on Hygienic Aspects

No.	Hygiene Practice	Subject Responses			
		Yes		No	
		N	%	N	%
1.	Question 1: Cut nails once a week	7	45%	8	55%
2.	Question 2: Wash your hands frequently with soap	6	30%	9	70%
3.	Question 3: Wash your feet often after work	8	55%	7	45%
4.	Question 4: Wear footwear when working	8	55%	7	45%
5.	Question 5: Use gloves when working	15	100%	0	0%
6.	Question 6: Work all day long	15	100%	0	0%
7.	Question 7: Worked a full month	0	0%	15	100%
8.	Question 8: Shower after work	15	100%	0	0%
9.	Question 9: Use a towel after washing your hands and feet	5	15%	10	85%
10.	Question 10: Using antiseptic after work	0	0%	15	100%

Table 3 Dermatophyte identified in construction workers from Lamongan

No.	Sample	Age	Age Group	Results
1.	S1	59	Elderly	+
2.	S2	48	Elderly	+
3.	S3	58	Elderly	-
4.	S4	28	Adult	+
5.	S5	53	Elderly	+
6.	S6	52	Elderly	+
7.	S7	42	Adult	-
8.	S8	32	Adult	+
9.	S9	43	Adult	-
10.	S10	50	Elderly	+
11.	S11	60	Elderly	+
12.	S12	39	Adult	+
13.	S13	40	Adult	+
14.	S14	54	Elderly	+
15.	S15	60	Elderly	-

Based on Table 3, it shows that respondents who were positively infected with the non-dermatophyte fungus *Rhizopus* sp. from the adult age group (26-45 years old) as many as 4 respondents and elderly age (46-65 years old) as many as 7 respondents. Based on the results of the research carried out, data was obtained that illustrates the presence of nail fungus in the toenails of construction workers in Lamongan Regency in 2023.

Based on Table 3, the results of the identified fungi that cause non-dermatophyte onychomycosis in the toenails of construction workers in Lamongan Regency showed that the fungus *Rhizopus* sp. in adult age (26-45 years) there were 4 respondents and in the elderly (46-65 years) there were 7 respondents. Based on the research that has been carried out, the results of macroscopic and microscopic observations are divided into 2 media, namely SDA media and PDA media. This can be seen based on Table 4 below.

Table 4 Macroscopic and Microscopic Examination of Fungal *Rhizopus* sp.

No.	Sample	Results	
		PDA	SDA
1.	S1	<i>Rhizopus</i> sp.	-
2.	S2	<i>Rhizopus</i> sp	<i>Rhizopus</i> sp
3.	S3	-	-
4.	S4	-	<i>Rhizopus</i> sp
5.	S5	-	<i>Rhizopus</i> sp
6.	S6	-	<i>Rhizopus</i> sp
7.	S7	-	-
8.	S8	-	<i>Rhizopus</i> sp
9.	S9	-	-
10.	S10	<i>Rhizopus</i> sp	-
11.	S11	<i>Rhizopus</i> sp	<i>Rhizopus</i> sp
12.	S12	<i>Rhizopus</i> sp	-
13.	S13	<i>Rhizopus</i> sp	<i>Rhizopus</i> sp
14.	S14	<i>Rhizopus</i> sp	-
15.	S15	-	-

Based on the research results, it shows that the morphology of *Rhizopus* sp. Macroscopically, SDA media and PDA media have a cotton-like texture, spread evenly, and are white to grayish white in color. This can be seen based on figure 1 and figure 2 below:



Figure 1 Macroscopic view of the non-dermatophyte fungus *Rhizopus* sp. on SDA media



Figure 2 Macroscopic view of the non-dermatophyte fungus *Rhizopus* sp. on PDA media

The morphology of *Rhizopus* sp. microscopically: has a round columella, has a collarette on the side of the columella, the sporangium is round and in a whole cluster lining the top of the columella, has an apophysis, the sporangiophore is not insulated and long, at the bottom there are stolons that branch like roots called rhizoids. This can be seen based on figure 3 and figure 4 below:



Figure 3 Microscopic view of the non-dermatophyte fungus *Rhizopus* sp. on SDA media

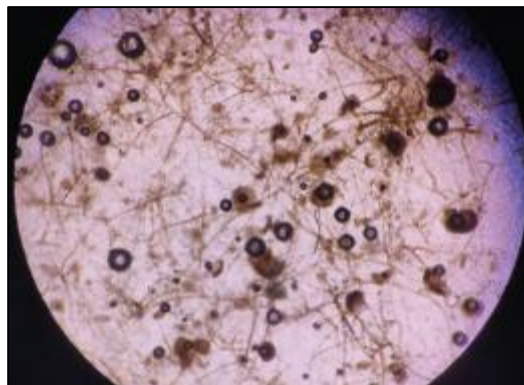


Figure 4 Microscopic view of the non-dermatophyte fungus *Rhizopus* sp. on PDA media

4. Discussion

Based on the research results, it shows that several respondents were positively infected with the non-dermatophyte fungus *Rhizopus* sp. Nail infections or onychomycosis are not only caused by non-dermatophyte fungi such as *Rhizopus* sp., but there are several other fungi that can infect dermatophyte nails such as *Tricophyton rubrum*, *Epidermophyton floccosum* and others (Yue et al., 2015). Based on Table 5.5, the non-dermatophyte fungus that infects the toenails of construction workers is the fungus *Rhizopus* sp. A total of 4 adult respondents and 7 elderly respondents showed positive infection with the fungus *Rhizopus* sp.

Based on Tables 1 and Table 2, hygienic aspects of construction workers in Lamongan Regency, it can be seen that the majority of respondents pay less attention to hygienic aspects, 55% didn't cut their nails once a week, 70% do not wash their hands with soap, 70% do not wash their feet after work. 45%, 45% don't use footwear when working, 0% don't use gloves when working, 100% work in the rice fields for a full day, 0% work in the fields for a full month, 0% don't shower after working, no Using a towel after washing hands and feet 85% of the time, and not using antiseptics after work 100%. Hygienic aspects greatly influence the emergence of dermatophyte and non-dermatophyte fungal infections on nails.

Personal protective equipment that should be used as protective equipment when working, so that microorganisms do not enter the nails, was not noticed by a group of construction workers (Widiawati et al., 2023). Soil contaminated by fungus will easily get into the nails of construction workers when they work without wearing gloves and footwear (Zebua, 2021). Non-dermatophyte nail fungus, namely *Rhizopus* sp. These can get into the feet of farmers who don't cut their toenails. These factors can trigger non-dermatophyte fungal infections in the toenails of construction workers in Lamongan Regency.

Long and unkempt nails will become a place for various dirt containing bacteria, fungi, viruses and germs to stick. Transmission of dermatophyte and non-dermatophyte fungi includes dirty hands and feet. The habit of construction workers who work without using PPE (Personal Protective Equipment) will make it easier for their toenails and hands to become infected with nail fungus because construction workers' work is in direct contact with soil and a dirty environment, especially in terms of workers' behavior as workers who tend not to pay attention to personal hygiene. such as hoeing sand, lifting bricks, mixing sand and cement on dirty ground without wearing personal protective equipment, not wearing footwear or gloves when working (Gupta et al., 2022).

Another factor that can influence the onset of fungal infections is age. Based on Table 5.3, it shows that the majority of respondents aged 46-65 were positively infected with non-dermatophyte fungi. The age group that suffers most from onychomycosis is an age group that has many predisposing factors, for example working in areas prone to wetness, trauma and lots of sweating, so the risk of suffering from onychomycosis is greater compared to other age groups (Hazarika et al, 2019). The prevalence of non-dermatophyte fungal infections increases with age, the prevalence increases due to an increase in a person's immunosuppression, and the habit of not using footwear (Ameen, 2010)

Nail fungus infection is a factor that greatly influences the quality of human resources, because it can interfere with work productivity. In general, factors that influence nail fungus infections include poor environmental sanitation and personal hygiene conditions (Ezomike et al., 2021). To reduce the prevalence of nail fungus infection by breaking the chain, namely improving environmental sanitation and personal hygiene, is the most important effort to break the chain of transmission of nail fungus disease. Availability of adequate facilities and infrastructure, washing hands with soap after finishing work, always using personal protective equipment when working, and keeping nails clean (Paul et al., 2017).

The relationship between construction workers and onychomycosis fungal infections is their daily habits. Many construction workers have a habit of not maintaining cleanliness, such as rarely washing their feet and hands after work. Apart from that, there are also many construction workers who don't wear footwear when working, and even cut their toenails. This triggers the growth of fungus on the toenails of construction workers. Another factor that can influence the onset of fungal infections is age. The age group that suffers most from mycosis is the age group that has predisposing factors such as direct contact with the soil. The prevalence of dermatophyte fungal infections will increase with age and a person's habits (Jha et al., 2021).

Management of onychomycosis had been reported. Topical antifungals are advised for mild to moderate disease and have better safety profiles, whereas oral antifungals are typically advised for moderate to severe onychomycosis and have higher cure rates (Falotico et al., 2022). Previous study shows that several important risk factors for onychomycosis, including age, obesity, tinea pedis, PVD, venous insufficiency, diabetes mellitus, and HIV. The

prevention of primary infections and recurrences of onychomycosis necessitates the identification and management of these risk factors (Albucker et al., 2023). Common household surfaces can act as breeding grounds for dermatophytes and other pathogens. Previous study confirmed that shared home items like bed linens, nail trimmers, and shoes can spread dermatophytes. Pets, bedding, and household cleaning supplies can all act as long-term reservoirs of infection due to the dermatophytes' persistence (Jazdarehee et al., 2022).

5. Conclusion

There is growth of the fungus *Rhizopus* sp. Non-dermatophyte groups cause onychomycosis in SDA media both macroscopically and microscopically. There is growth of the fungus *Rhizopus* sp. Non-dermatophyte groups cause onychomycosis in PDA media both macroscopically and microscopically. There is no difference in the growth of the fungus *Rhizopus* sp. on PDA media and SDA media both macroscopically and microscopically. There are differences in the growth of the fungus *Rhizopus* sp. Non-dermatophyte groups causing onychomycosis based on age.

Compliance with ethical standards

Disclosure of conflict of interest

The author declares no conflict of interest.

Statement of ethical approval

This study design had been approved by ethical committee of Delima Husada Gresik Health Analyst Academy with registered number 031/IV.LPPM.EC/2023

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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