












# The Study of Causative Organisms Affecting Fungal Nail Perforation Test

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## ABSTRACT

**Objective:** To evaluate the capacity of dermatophytes, non-dermatophyte, and yeast to invade nails.

**Materials and Methods:** Nine normal nails were clipped from healthy volunteers and sterilized in an autoclave. Each nail was inoculated with one of nine fungal species, including dermatophytes, non-dermatophyte (*Neoscytalidium dimidiatum*, *N. dimidiatum*), and yeast (*Candida albicans*, *C. albicans*) for two, four, and eight weeks at 26 °C. A positive result was determined by the presence of fungal hyphae or pseudohyphae penetrating the nail plate.

**Results:** Fungal penetration increased with longer durations. At two weeks, *Trichophyton mentagrophytes* (*T. mentagrophytes*) variety *mentagrophytes*, *Nannizzia gypsea*, *Microsporum canis*, and *N. dimidiatum* showed positive nail perforation. At four weeks, *T. mentagrophytes* variety *interdigitale* also tested positive. The remaining fungi (*Trichophyton rubrum*, *Trichophyton tonsurans*, *Epidermophyton floccosum*, and *C. albicans*) demonstrated negative test results at eight weeks.

**Conclusion:** This *in vitro* study confirmed the highly virulent dermatophytes can penetrate the nail plate in a short time. Furthermore, this study highlighted the significant virulence of *N. dimidiatum* in nail invasion.

**Keywords:** dermatophytes, fungal infection, nail perforation test, non-dermatophytes, onychomycosis

## INTRODUCTION

Onychomycosis is a common disorder among the elderly population.<sup>1</sup> However, most patients do not seek diagnosis or treatment due to its asymptomatic nature and minimal impact on quality of life. The prevalence of onychomycosis is estimated to be 4-10% among general population,<sup>2,3</sup> while the prevalence of onychomycosis in Thailand was reported about 1.7% among all the patients visiting the Institute of Dermatology.<sup>4</sup> Several factors increase the risk of

onychomycosis, including diabetes mellitus, peripheral vascular disease, frequent use of slippers, corticosteroid use, and repetitive foot injury.<sup>4-6</sup> Additionally, the causative organism plays a key role in determining prognosis and achieving a cure.

Onychomycosis can be caused by dermatophytes, non-dermatophyte molds and yeast.<sup>6-8</sup> While dermatophyte infections occur worldwide, non-dermatophyte infections are more common in tropical countries like Thailand and Brazil.<sup>5,9</sup> Pathogenic non-dermatophytes play an

important role in nail infection and vary by region.<sup>7,10-12</sup> In Thailand, *Neoscytalidium dimidiatum* (*N. dimidiatum*) and *Fusarium* spp. are common non-dermatophyte molds.<sup>5,6,13</sup> The explanation for non-dermatophytes like *N. dimidiatum* to be reported more prevalent in tropical countries is that they are frequently found in the soil of tropical and subtropical zones.<sup>14</sup> *Candida* spp. is the leading yeast pathogen in fingernail onychomycosis in women, particularly women engaged in wet work.<sup>8</sup>

*N. dimidiatum* commonly causes both nail and superficial skin infections, with its prevalence rising over time.<sup>5,7,10,12</sup> In France, *N. dimidiatum* accounts for up to 34% of fungal infections, with skin infections accounting for 65.3% of cases and onychomycosis 34.7%.<sup>10</sup> In Thailand, its prevalence in onychomycosis has been reported at 20%-36.4%.<sup>4,6</sup> However, the pathogenesis of *N. dimidiatum* infection remains unclear.

In order to diagnose onychomycosis, each patient should have a history taking and physical examination. To further confirm the diagnosis, diagnostic laboratory tests such as potassium hydroxide preparation for microscopic examination, and fungal culture, should be performed. In several instances, nevertheless, the diagnosis is challenging. The histopathology from nail clipping is required to confirm the diagnosis<sup>3</sup> by presence of hyphae or pseudohyphae in the nail plate.<sup>15</sup> Currently, some studies have proposed the pathogenesis of onychomycosis that the nail keratin was destroyed by extracellular enzymes produced from fungi.<sup>16</sup> Furthermore, the site and pattern of nail invasion influence the type of onychomycosis which varies depending on the virulence of the fungal species. However, the exact mechanisms including the time of penetration after inoculation and the depth of penetration by which different fungal species cause onychomycosis remain poorly understood due to limited research. Thus, the *in vitro* study can help to determine these mechanisms without confounding factors. This study aims to investigate the nail-perforation ability of dermatophytes, non-dermatophyte molds, and yeasts using an *in vitro* model.

## MATERIALS AND METHODS

The protocol for this pilot study (Siriraj Protocol Number: 067/2558) was submitted to the Ethics Committee and was officially approved as exempt. Nine normal fingernails were clipped from a healthy volunteer whose age 29 years and screened using

potassium hydroxide preparation and fungal cultures, both of which revealed negative results for infection. Nine fungal species including dermatophytes (*Trichophyton mentagrophytes* (*T. mentagrophytes*) variety *mentagrophytes*, *T. mentagrophytes* variety *interdigitale*, *Trichophyton rubrum* (*T. rubrum*), *Trichophyton tonsurans* (*T. tonsurans*), *Epidermophyton floccosum* (*E. floccosum*), *Nannizzia gypsea* (*N. gypsea*), *Microsporum canis* (*M. canis*)), non-dermatophyte (*N. dimidiatum*), yeast (*Candida albicans*, *C. albicans*) were chosen from the reported pathogens of onychomycosis. The nails were then sterilized in an autoclave and inoculated with one of nine fungal species in sterile water for two, four, and eight weeks at 26 °C. After incubation, the nails were fixed in 10% formalin, stained with periodic acid-Schiff, and microscopically examined by two independent dermatologists. Disagreements were resolved through consensus-based discussion. A positive result was defined by the presence of fungal penetration.

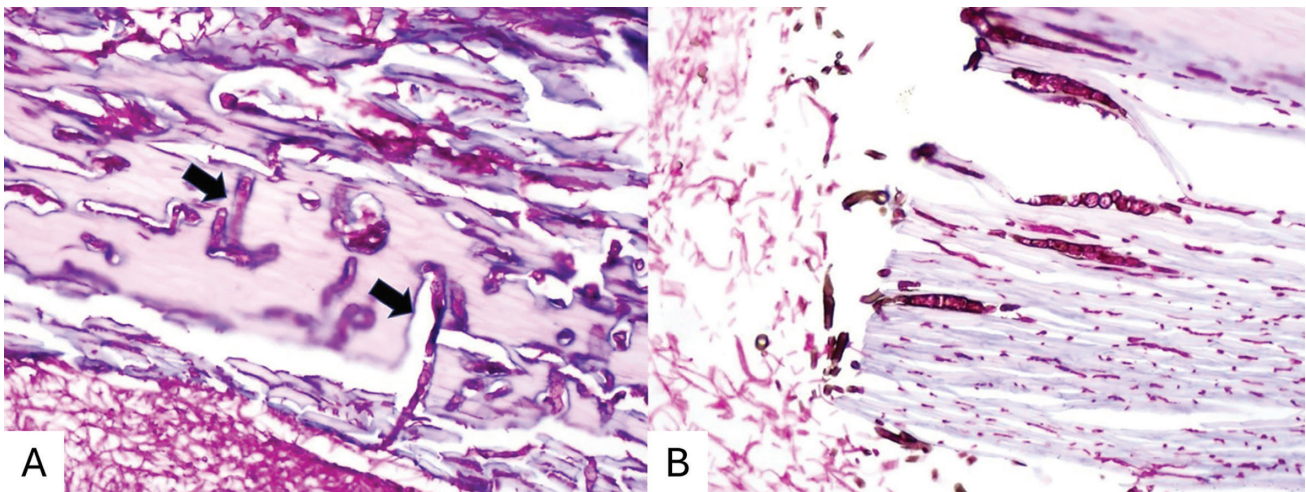
## RESULTS

The nail perforation results are shown in **Figure 1** and **Table 1**. At two weeks, positive penetration was observed for *T. mentagrophytes* variety *mentagrophytes*, *N. gypsea*, *M. canis*, and *N. dimidiatum*. *T. mentagrophytes* variety *interdigitale* exhibited penetration at four weeks. The remaining fungi (*T. rubrum*, *T. tonsurans*, *E. floccosum*, and *C. albicans*) showed no signs of nail penetration.

## DISCUSSION

This study demonstrated the nail-perforation ability of dermatophytes, non-dermatophytes and yeasts. The results of this *in vitro* study may reflect the onset and severity of onychomycosis depending on the fungal virulence. The higher virulence fungi such as *T. mentagrophytes* variety *mentagrophytes*, *N. gypsea*, *M. canis*, and *N. dimidiatum* could produce more severity of onychomycosis and earlier onset than lower virulence fungi. Additionally, it is challenging to get therapeutic improvement when treating onychomycosis from certain fungi, particularly *N. dimidiatum*.<sup>17</sup> This could account for its pathogenicity.

The results support *N. dimidiatum* as a virulent agent in onychomycosis, given its ability to penetrate the nail *in vitro*. *N. dimidiatum* is a common non-dermatophyte with distinct characteristics.<sup>5</sup> It was able to penetrate the nail within two weeks, similar to *T. mentagrophytes*. Following *N. dimidiatum*



**Figure 1** Fungal Penetration of the Nail Plate

(A) *Trichophyton mentagrophytes* variety *mentagrophytes* showed elongated hyphae invading the nail plate perpendicularly (arrow). (periodic acid-Schiff stain, original magnification X400)

(B) *Neoscytalidium dimidiatum* demonstrated brownish septate hyphae with arthroconidia penetrating the nail plate longitudinally. (periodic acid-Schiff stain, original magnification X400)

**Table 1** Results of Nail Invasion for Each Fungus

Fungi	Result	Duration of Nail Invasion (weeks)
<i>Trichophyton mentagrophytes</i> variety <i>mentagrophytes</i>	+	2
<i>Trichophyton mentagrophytes</i> variety <i>interdigitale</i>	+	4
<i>Trichophyton rubrum</i>	-	-
<i>Trichophyton tonsurans</i>	-	-
<i>Nannizzia gypsea</i>	+	2
<i>Microsporum canis</i>	+	2
<i>Epidermophyton floccosum</i>	-	-
<i>Neoscytalidium dimidiatum</i>	+	2
<i>Candida albicans</i>	-	-

penetration, nail damage was seen both clinically and histologically, indicating that its virulence contributes to the quick and severe onset of onychomycosis. Previous reports have linked this mechanism to its ability to produce extracellular enzymes such as amylase, proteases, and lipase, which degrade keratin. Its proteolytic activity facilitates keratin breakdown, enabling penetration and contributing to nail and cutaneous fungal infections.<sup>12</sup>

Although previous research has demonstrated the ability of *T. mentagrophytes* and *M. canis* to perforate nails,<sup>18-21</sup> findings on *T. rubrum* and *T. tonsurans* have been inconsistent.<sup>18,21</sup> This disparity may be due to environmental factors, such as continuous shaking during inoculation<sup>21</sup> and variations in culture media.<sup>18</sup> Interestingly, different fungal subspecies exhibit varying onsets of nail perforation, as observed with *T. mentagrophytes*.

Conversely, *E. floccosum* and *C. albicans*, showed no evidence of nail perforation. This suggests that mammalian keratin penetration by fungi depends on multiple factors, including environmental conditions and host immune response.

This study had some limitations. As a pilot study, it involved a small nail sample size. Additionally, longer culture periods may be necessary to confirm the negative results. Lastly, the results from *in vitro* study may alter from *in vivo* study because there are host factors such as immune status, underlying diseases and environmental factors such as humidity which can affect the ability of fungal penetration into nails. Thus, these factors should be studied in future research.

## CONCLUSION

This study confirmed that the highly virulent dermatophytes can penetrate the nail plate in a short time causing onychomycosis. Furthermore, *N. dimidiatum* can invade the nail plate under *in vitro* conditions, which may explain its virulence in the development of onychomycosis. Additionally, certain dermatophyte species were shown to penetrate the hard keratin of nail plates.

## Conflict of Interest

All authors declare that there are no conflicts of interest related to this study.

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## Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data is not publicly available due to privacy or ethical restrictions.

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