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Full Length Article

Molecular Characterization and Susceptibility Profile of Indigenous *Arthroderma multifidum* to Common Antifungals

Saba Sana[1†*], Shamsa Niaz[2†], Ali Ahmad Sheikh[2], Sikander Ali[1], Asma Abdul Latif[3], Shahgull[4], Huma Mujahid[5], Iqra Liaqat[6] and Iqra Bashir[7]

[1]*Department of Microbiology, Ikram ul Haq Institute of Industrial Biotechnology, Government College University Lahore, Pakistan*
[2]*Institute of Microbiology, University of Veterinary and Animal Sciences Lahore, Pakistan*
[3]*Department of Zoology, Lahore College for Women University Lahore, Pakistan*
[4]*Department of Bioinformatics and Biosciences, Capital University of Science and Technology, Islamabad, Pakistan*
[5]*Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences Lahore, Pakistan*
[6]*Department of Pharmacology & Toxicology, University of Veterinary and Animal Sciences Lahore, Pakistan*
[7]*Department of Botany, University of Veterinary and Animal Sciences Lahore, Pakistan*
[*]For correspondence: saba.sana@gcu.edu.pk; saba.sana@uvas.edu.pk
[†]Contributed equally to this work and are co-first authors
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Abstract

Dermatophytes are group of fungi have inherent potential of skin, hair and nail infection. *Arthroderma multifidum* is a diverse fungus of zoonotic nature among dermatophytes. The main objective of this study was isolation, characterization, and antifungal susceptibility profile of *A. multifidum.* Clinical samples of skin scrapping (n =13) from humans and animals were collected and initially screened by KOH (10%) preparations. Samples (n = 09) were positive for presence of fungal fragments. The positive samples were cultured on Dermatophytes Test Medium (DTM) and isolates were identified by conventional method of fungal identification. Out of 09 total selected samples, 03 animal samples (out of 07 KOH positive animal samples) were positive for *A. multifidum* by culture. The identified isolates were processed for molecular characterization targeting the internal transcribed regions using ITS1 and ITS4 primers by polymerase chain reaction. ITS region of one isolate was sequenced (Sanger sequencing method). The sequence was analyzed using BLAST, submitted to NCBI data base and phylogenetic tree was constructed using MEGA11. Susceptibility of *A. multifidum* to antifungals (azole group, nystatin and amphotericin B), homeopathic drugs (used for local treatment of skin infections) and Thymoquinone was evaluated by disc/well diffusion and minimum inhibitory concentration (MIC) of antifungals was determined by micro-broth dilution method. Variations were observed in susceptibility of three isolated to different antifungals. The highest susceptibility to Fluconazole was observed (ZOIs ranged from 72.00 ± 4.46 to 25.00 ± 2.51 mm). For Thymyquinone, ZOIs ranged from 31.00 ± 3.46 to 24.66 ± 2.51 mm. No inhibitory effect of homeopathic drugs was observed. Micro-broth dilution showed least MIC by azole group (≥ 0.0156 ± 0.00 μg/mL), for Thymoquinone it was ≥6.25±0.00 mg/mL. It is concluded that *A. multifidum* infection must be properly diagnosed and treated with effective antifungals. Homeopathic is not a good option for dermatophytosis caused by *A. multifidum.*

Keywords: Dermatophytes; *Arthroderma multifidum*; Molecular characterization; Antifungal; Susceptibility; Thymoquinone

Introduction

Dermatophytes are keratin degrading fungi infecting the skin, hair nail and feathers collectively known as dermatophytoses. Commonly it is called ringworm and Tinea (Surendran *et al.* 2014). This group includes seven genera of filamentous fungi: *Trichophyton*, *Epidermophyton*, *Microsporum*, *Nannizia*, *Paraphyton*, *Lophophyton* and *Arthroderma* affecting human and animals (Moskaluk and VandeWoude 2022). Among these the most commonly isolated species are *Microsporum canis (M. canis)*, *Trichophyton violaceum (T. violaceum)*, *T. mentagrophytes*, *T. rubrum* and *Epidermophyton floccossum (E. floccossum).* The World Health Organization (WHO) reported that 20–25% of world's population of all age groups is suffering from dermatophytoses. It is prevalent in tropical areas where humidity rate is high. Increase in fungal diseases has been reported since last two years (Pires *et al.* 2014; Colosi *et al.*

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2020). Dermatophytes have three different environment habitats: anthropophiles (living on human), geophiles (living in soil) and zoophiles (living on animals). This grouping influences clinical presentation. However, species in these groups may switch their preferred habitat (Moskaluk and VandeWoude 2022). *T. rubrum* and *E. floccosum* are well adapted to human host, leads to chronic infections. *M. canis*, *T. mentagrophytes* and *Arthroderma benhamiae* (*A. benhamiae)* from animals, *Microsporum gypseum (M. gypseum)* and *Nannizia gypsea* (*N. gypsea)* from soils cause mild infection in patients (Burstein *et al.* 2020).

The Presence of certain factors predisposes a host to development of disease. These factors are high population density and poor hygienic practices, presence of animal reservoirs around human, co-morbidity (diabetes/HIV) along with moisture, broken/injured skin in the form of any micro-injury to skin. This disease changes the lifestyle and quality of life as it takes long time to recover even in case of effective therapy (Al-Khikani 2020; Jaishi *et al*. 2022).

Antifungals are used for the treatment of dermatophytes belong to azoles, allylamines and polyenes. These antifungals are for topical application in the form of ointment as well as for oral use. With the increase in use of antifungals for managing dermatophytoses, the resistance is also on rise (Pashootan *et al.* 2022). The resistance development is associated with target site modification, activation of signaling pathways to stress response, biofilm in dermatophytes and imbalance in drug uptake and drug efflux (Verma *et al.* 2021). The relevancy of any of these mechanisms to antifungal resistance is hard to define because sometime these processes act simultaneously in the host cell (Martinez-Rossi *et al*. 2018). Overexpression of efflux pumps is a major mechanism in acquiring resistance against azole group. Mutation in squalene epoxidase gene is linked with allylamine resistance. However limited studies have been carried out on biofilm formation by dermatophytes (Verma *et al.* 2021).

Understanding of resistance/tolerance mechanism, new target sites and search for new antifungal compounds must focus to curtail dermatophytes infections. Synthetic antifungals targeting the cellular metabolic pathways are under investigation particularly for systemic fungal infections. However, a few drugs are under trial against dermatophytes. Common antifungals are becoming less effective. So, there is dire need to find new approaches to curtail infections (Nouri *et al*. 2023). Plants are among the good sources and provide limitless compounds with antifungal properties. Phytochemical effects on cellular metabolism streamlined the antifungal investigation of these compounds against dermatophytes (Martinez-Rossi *et al.* 2018). One of the popular strategies to control these infections is combinational therapy. The effect may be additive, antagonistic, or synergistic. Sometimes, resistance to one antifungal may be compensated by the second agent leading to resistance modulation (Augostine and Avery 2022).

Thymoquinone is the main bioactive compound of *Nigella sativa* volatile oil. It is known for its therapeutic effects including antioxidant, anticancer, antiviral, antibacterial, anti-inflammatory, anticonvulsant and antifungal activity (Aftab *et al.* 2021; Badary *et al*. 2021). Antifungal activity of Thymoquinone against *Microsporum* sp. was reported by Mahmoudvand *et al*. (2014) against *Candida* sp. by Almshawit and Macreadie (2017) and synergistic effect against oral thrush (candidiasis) with nystatin by Zincir *et al.* (2022).

Arthroderma is the most diverse genus of dermatophytes. *A. multifidium* is an important specie of zoonotic nature. First time it was isolated from rabbit hair in 1963. A sporadic case in human was reported in diabetic patients (Chen *et al*. 2023). Drouot *et al.* (2009) reported the zoonotic species of *Arthroderma* in skin samples collected from pets. *A. multifidum* has inherent potential (keratinolytic) to infect humans along with animals (Al-Bader *et al.* 2023). So the people dealing with animal and veterinary professionals are at higher risk of getting infection. Immunosuppression status and exposure could lead to disease development in humans (Chen *et al.* 2023). In most of the cases fungal infections remains undiagnosed due to lack of diagnostic facilities. So, more relevant studies are needed to find the humans prevalence of *A. multifidu.* Current study focused on *A. multifidum* susceptibility profile to different antifungal options used for treatment of skin infections caused by dermatophytes.

Materials and Methods

Dermatophytosis is a diseased condition caused by dermatophytes. Dermatophytes develop resistance to available antifungals. This leads to treatment failure. In current study, the effect of common antifungals will be evaluated to find out an effective therapeutic agent.

Sample collection

Clinical samples of infected skin were collected for the isolation of dermatophytes. Skin scrapings from human and animals (n = 13) were selected as samples for fungal isolation. Samples were procured from communities of rural areas as well as from pet center University of Veterinary and Animal Sciences (UVAS) Lahore. Samples were placed in sterile plastic boxes and were labelled properly (Shalaby *et al.* 2016). Then samples were transported to the Mycology laboratory at room temperature.

Screening of samples by potassium hydroxide mount

Initial screening was carried out by KOH (potassium hydroxide) mount (Gupta *et al.* 2014). Samples were mixed in 40% KOH followed by observation under compound microscope at 1000X magnification. The samples positive for fungal elements (hyphae and spores) were selected for the isolation of dermatophytes.

Isolation and identification of *A. multifidum*

For isolation of *A. multidium* the samples were inoculated on dermatophytes test medium (DTM). The sample embedded on the DTM plates; plates were incubated in upright position in the incubator until the growth appeared at 25 ± 3ºC. The plates were examined routinely from obverse as well as from reverse sides for growth appearance. The plates having no growth after 28 days were considered negative and discarded ultimately and the positive samples were proceeded accordingly (Poluri *et al*. 2015). For purification, single and multi-spot techniques were used. The purified fungi were identified based upon of macroscopic and microscopic characters. Macroscopic characters (Colony colour, texture, pigment production and diameter) of fungi were observed on obverse and reverse side of growth. Microscopic structures of purified fungal cultures were observed under bright field microscopy by cellophane tape and slide culture method. Different characters were noted including shape and arrangement of spores and type of hyphae (Gnat *et al.* 2021).

Molecular confirmation by Polymerase Chain Reaction

The isolates of fungi were confirmed by Polymerase Chain Reaction (PCR). Internal transcribed region of genome was targeted for amplification. The fungal mycelia were used for the extraction of DNA using genome extraction kit (Thermo Scientific Gene JET Genomic DNA Purification Kit) following the instruction of manufacturer. The extracted DNA was quantified (ng/μL) by Nanodrop. For visualization of DNA, gel electrophoresis was performed with 1% of agarose gel. Polymerase chain reaction (PCR) was carried out in MiniAmp™ Thermal Cycler.

ITS1 and ITS4 regions were targeted using primers (Forward primer: ITS1: 5′-TCCGTAGGTGAACCTGCGG-3′ & ITS4: 5′-TCCTCCGCTTATTGATATGC-3′). Reaction mixture (25 μL) were prepared in PCR tubes using 2X master mix (12.5 μL), Primers (ITS1 & ITS4, 1 μL/each), nuclease free water (6 μL) with template DNA (5 μL). PCR were carried by (Dalis *et al*. 2018). The Initial denaturation was carried out at 94ºC for 3 min, denaturation at 94ºC for 45 s, annealing at 52ºC for 1 min, extension at 72ºC for 1 min and final extension at 72ºC for 7 min and infinite (∞) time at 4ºC to prevent from degradation. Thirty-five cycles were run for amplification. Amplicons were detected by agarose gel electrophoresis on 2% gel. Gel was observed under UV trans-illuminator with DNA ladder (100 base pairs) to detect the amplification of targeted region (Naseif *et al.* 2020). The isolates were confirmed and captured in pictorial form.

Sequence analysis

The PCR products were sent for sequence analysis to Lab Genetics for sanger sequencing. The received sequence was compared in NCBI BLAST website with a reference sequence. Isolates of *A. multidium* were selected for further experiment.

Antifungal susceptibility profile

The standardized inoculum was prepared for the antifungal activity. The Muller Hinton agar (MHA) was used for susceptibility testing. For testing of antifungal activity pour plate method was used. The inoculum (100 μL) was taken in sterilized petri plates followed by pouring of sterile molten agar (45–50ºC). The antibiotic chloramphenicol (0.05 g/L) was added in medium. After solidification, wells were formed with pasture pipette and base of wells were sealed with sterilized molten agar. Then 100 μL of antifungals (Fluconazole, Itraconazole, Amphotericin B, and Nystatin), homeopathic drugs (Sulphur, basilinum, cantharis and hydrocotyl) was added into wells and incubated at 25 ± 3ºC in upright position till appearance of growth. After incubation, zone of inhibitions (ZOIs) were measured in mm and compared (Prajapati *et al.* 2019). Minimum Inhibitory Concentration (MIC) of antifungals was also determined by micro-broth dilution method. So in this experiment we found concentration of antifungals to which *A. multifidum* do not inhibit or kill. MIC was read as minimum concentration of antifungals inhibiting visible growth of fungus (Pfaller *et al*. 2011).

Antifungal activity of thymoquinone

The agar well diffusion test was performed to check antifungal activity of Thymoquinone as mentioned above. For testing, 100 μL of Thymoquinone was poured into wells. Following incubation ZOIs were measured. MIC of Thymoquinone against resistant isolates was determined by micro broth dilution method. MIC was read as minimum concentration of plant extracts inhibiting visible growth of fungus (Pfaller *et al.* 2011).

Data analysis

Data during this study was analyzed by one way ANOVA at 5% level of significance of ($P \geq 0.05$).

Results

In the current study *A. multifidum* species were isolated, characterized by molecular methods and evaluated for antifungal profile to antifungals and Thymoquinone.

Dermatophyte distribution in samples

Out of 13 skin scrapping samples, 09 samples were positive by direct microscopy (KOH mount). Two samples from human and seven from animals were detected positive. Out of 09 total positive samples, 03 samples were positive *A. multifidum* by culture method from animal skin scraps (n = 07) only.

Table 1: Macroscopic and Microscopic characteristics of *A. multifidum*

Macroscopic Characters		Colony Diameter (cm)			Microscopic Characters
Obverse	Reverse	05 day	07 day	14 day	
Pure white, later convert to beige, cottony texture Uniform, round flat growth	Light yellow at older age	3.4	4.5	5.8	Hyaline, septate hyphae, Small ovoid and pyriform shape macroconidia.
		3.7	4.4	5.9	
		3.9	4.4	5.6	Microconidia absent

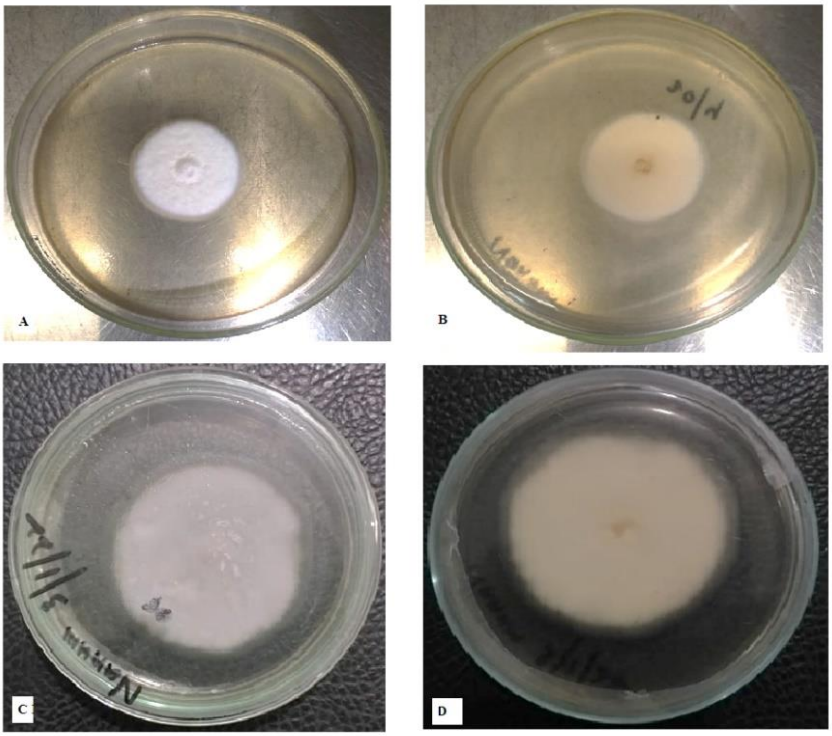

Fig. 1: Identification of *A. multifidum.* **A**: Immature colony (Obverse side view), **B**: Immature colony (Reverse side view), **C**: Colony on maturation (Obverse side), **D**: Colony on maturation (Reverse side)

Identification and characterization of fungi

Fungi identified were belonging to dermatophyte and non-dermatophyte groups. Among dermatophytes species *A. multifidum*, suspected isolates were further selected. The macroscopic and microscopic characters of selected fungi are in (Table 1). In PCR isolates were confirmed by amplifying genus specific ITS1 and ITS4 (650–700 bps) and sequence analysis confirmed the isolates as *A. multifidum* (Fig. 1 and 2). Sequence analysis was obtained and submitted to NCBI (Accession no. OP102681). The phylogenetic tree (bootstrap method) is in (Fig. 3).

Antifungal susceptibility profile

Initially for screening the susceptibility of isolates of *A. multifidum*, against Amphotericin B, Fluconazole, Nystatin, Itraconazole were checked by well/disc diffusion method (Fig. 4 and 5). Highest mean zone of inhibition was produced by Fluconazol (72.00 ± 3.46) against *A. multifidum* 1 and least was 22.00 ± 2.51 against *A. multifidum 2.* However complete resistance (100%) was observed against homeopathic drugs (Sulphur, Bacillinum, Hydrocotyl and Cantharis), represented in Fig. 6. MIC of antifungals was calculated. In this case least MIC was shown by azole group antifungals. Mean MIC of Fluconazole and Itraconazol was observed ≥ 0.0156 ± 0.00 μg/mL against *A. multifidum* isolate 1 and *A. multifidum* 3 respectively (Table 2; Fig. 7–8).

Antimicrobial activity of thymoquinone

Thymoquinone was screened to evaluate antimicrobial effect on isolates of *A. multifidum* by agar well diffusion method in triplets. After that MIC was determined. Thymoquinone was used against fungal isolates. Zones of inhibition (ZOI) were measured in millimeters (mm). The compound depicted the best results and sensitivity was noticed towards all isolates of *A. multifidum*. ZOIs are mentioned in the table. The highest mean ZOI was 31.00 ± 3.46 produced by Thymoquinone against *A. multifidum* 2 (Table 3; Fig. 4). The mean MIC was ≥ 6.25 ± 0.00 (Table 4; Fig. 4).

Table 2: Minimum inhibitory concentration of antifungals *A. multifidum*

Isolates	Antifungals (mcg/mL)	MIC ≥ (mcg/mL)	Mean ± S.D
A. multifidum 1	Nystatin (25)	0.195	0.195 ± 0.00[b]
		0.195	
		0.195	
	Fluconazole (2)	0.0156	0.0156 ± 0.00[a]
		0.0156	
		0.0156	
	Amphotericin B (50)	0.195	0.26 ± 0.11[b]
		0.195	
		0.39	
	Itraconazole (2)	0.0156	0.0208 ± 0.01[a]
		0.0156	
		0.0312	
A. multifidum 2	Nystatin (25)	0.195	0.26 ± 0.11[b]
		0.39	
		0.195	
	Fluconazole (2)	0.0156	0.0208 ± 0.01[a]
		0.0156	
		0.0312	
	Amphotericin B (50)	0.195	0.325 ± 0.11[b]
		0.39	
		0.39	
	Itraconazole (2)	0.0156	0.0156 ± 0.00[a]
		0.0156	
		0.0156	
A. multifidum 3	Nystatin (25)	0.78	0.39 ± 0.33[a]
		0.195	
		0.195	
	Fluconazole (2)	0.0156	0.0208 ± 0.01[a]
		0.0312	
		0.0156	
	Amphotericin B (50)	0.195	0.195 ± 0.00[a]
		0.195	
		0.195	
	Itraconazole (2)	0.0156	0.0208 ± 0.01[a]
		0.0156	
		0.0312	

Different superscripts are significantly different and same superscripts are non-significantly different.

Table 3: Zone of inhibition of Thymoquinone against *A. multifidum*

Isolates	Thymoquinone	Zone of inhibition	Mean ± S.D	*P* value
A. multifidum 1	100 µL	27, 29	24.66 ± 2.51[a, b]	0.029
		25, 35		
		22, 29		
A. multifidum 2		29	31.00 ± 3.46[b]	
		35		
		29		
A. multifidum 3		23	30.00 ± 6.08[b]	
		33		
		34		

*Different superscripts are significantly different and same superscripts are non-significantly different

Discussion

Dermatophyte infections of humans and animals are recognized as a major health problem in several parts of the world and affect approximately 25% of the world's population (Keshwania *et al.* 2023). In North America, East Asia and Europe, different studies have documented a varied prevalence rate of dermatophytosis. The distribution of dermatophytes and etiological agents varies with geographic location.

A study was conducted in Turkey by Metintas *et al.* (2004) reported that *T. rubrum* (43%) was commonly isolate etiologic agent of tinea pedis and tinea corporis, and it was followed by *T. mentagrophytes* (20%) and *M. canis* (13%). Some species of dermatophytes are widely distributed whereas others are geographically restricted. According to Ridzuan *et al.* (2021), *M. canis* was found to be the most frequently isolated species from dogs followed by *T. mentagrophytes* and *Trichophyton* sp. Like other countries on the globe, Pakistan has also reported the presence of dermatophytes. In Khyber Pakhtunkhwa Pakistan, 88.31% of samples were found positive. Tinea corporis (35%) was

Table 4: MIC of Thymoquinone against *A. multifidum*

Isolates	Thymoquinone	MIC (mg/mL)	Mean ± S.D
Arthroderma multifidum 1	100 µL	6.25	6.25 ± 0.00
Arthroderma multifidum 2		6.25	
Arthroderma multifidum 3		6.25	

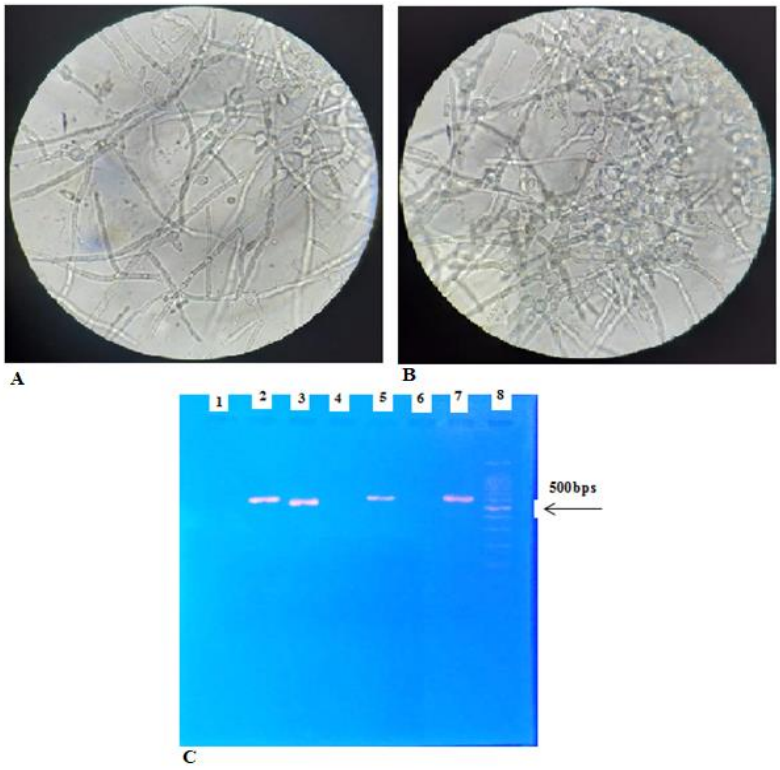

Fig. 2: Identification and Molecular Characterization of *A. multifidum.* **A** & **B**: Microscopic view (400X magnification), **C**: Agarose gel (2%), Lane 8: DNA Ladder (100 bps), Lane 2, 5 and 7 amplicons (669 bps) of *A. multifidum* by ITS1 & ITS4 amplification. Lane 5: Amplicons of other dermatophyte. Lane 1, 4 & 6 negative for amplification

the most predominant type of infection followed by Tinea capitis (22%). The fungi identified were *T. rubrum, T. mentagrophytes, E. floccosum* (Usman *et al.* 2021). A study in UHS Lahore (Shakir *et al.* 2019) has been conducted. *T. mentagrophytes* was prominent fungus (32%) followed by *T. violaceum* (28%) and *T. rubrum* (12%). Most of the studies are focusing on three major genera of dermatophytes in human and animal; *Microsporum*, *Trichophyto* and *Epidermophyton.* In current study, *A. multifidum* was also isolated as a pathogen of dermatophytosis. Several studies described *A. multifidum* isolation as keratinolytic and pathogenic fungus. Isolation was carried out from rook (*Corvus frugilegus)* colony for evaluation as biological agent for disposal of waste feathers (Bohacz *et al*. 2020) and *Arthroderma* sp. from caves in Tatra Mts., Slovakia (Ogorek *et al.* 2022). First isolation of this fungus was from rabbit holes/rabbit hair (Dawson 1963). It was also reported to cause infection in diabetic patient (Fowora *et al*. 2021). However, a zoonotic case of human infection by *A. multifidum* in a case study was reported by Chen *et al*. (2023). In current study, specie was isolated from skin scrapping of animals. So, *A. multifidum* is a diverse fungus of zoonotic importance.

A study conducted in India reported epidemics of dermatophytosis. The conventional antifungals were less responsive to ointments in 3 out of 5 cases which lead the patient to opt for homoeopathy. All 5 cases were recovered with homoeopathic treatment (Roy *et al.* 2021). Another study in India revealed the effectiveness of homoeopathic medicines, herbals, siddha medicines, ayurvedic medicines for treatment of dermatophyte fungal infection as alternative of conventional system (Naik *et al*. 2023). In a previous study homeopathic had shown the antifungal activity against the dermatophytes infection (Uttamchandani and Patil 2019). Above studies revelaed the homeopathic drugs as an effective alternative therapy. In current study carried out in

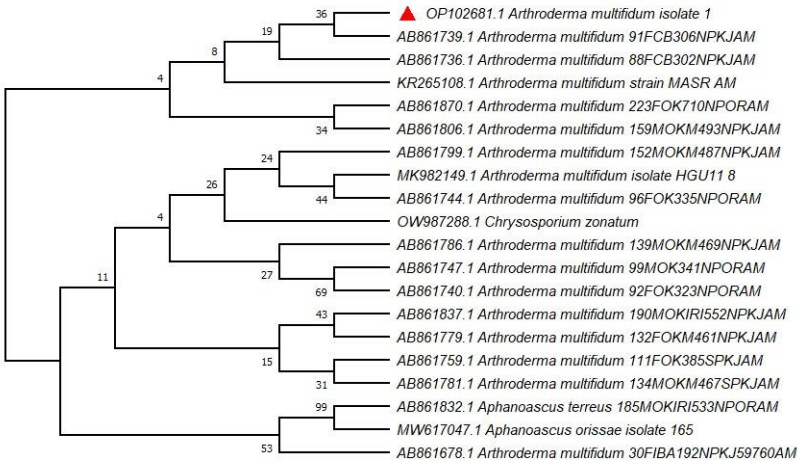

Fig. 3: The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 10 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 695 positions in the final dataset. Evolutionary analyses were conducted in MEGA11

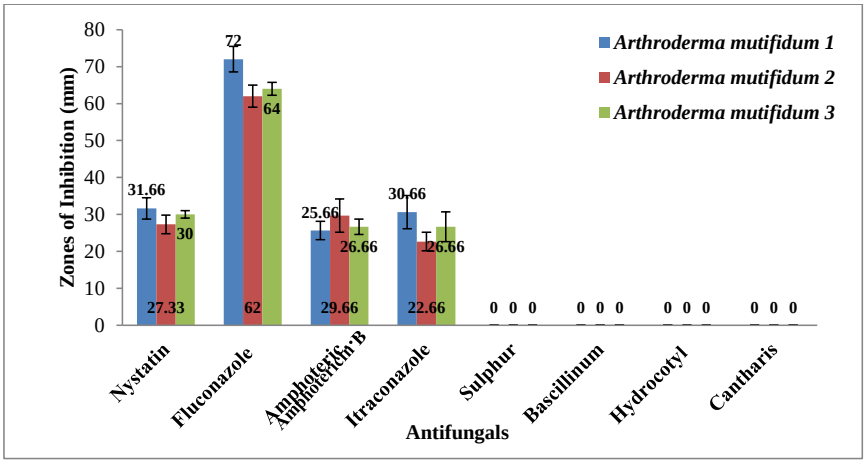

Fig. 4: Antifungal susceptibility of *A. multifidum* to antifungals

Pakistan, *in-vitro* activity showed no antifungal activity. This study contradicts previous studies. This may be the differences in parameters in case study and *in-vitro* activity.

Medicinal plants and their extracts have been in use for hundreds of years for the treatment of many infectious diseases. A study showed that essential oil and various extracts of *Nigella sativa* have antifungal effect on *T. mentagrophytes* (Mahmoudvand *et al.* 2014). Thymoquinone (TQ) lowered the MICs of standard antimicrobials when used in combination with antifungals. TQ also evaluated for antifungal effect against some very important fungal pathogens including *Candida albicans* and certain dermatophytes (Khan *et al.* 2021). A recent study showed complete inhibition of thirty pathogens at concentration of 1mg/mL by TQ. TQ was found to be the best antifungal compound against all of the tested dermatophytes and yeasts, followed by thymol in *N. sativa* extract (Taha *et al.* 2010). As in the previous study the antifungal activity of ether extract of *N. sativa* seed and its active principle TQ was tested against dermatophytes species *T. rubrum* and *T. mentagrophytes* (Mahmoudvand et al. 2014). Antifungal activity of leaf extract of *Acacia nilotica* against *A. multifidun* was evaluated and dose dependent inhibitory response was reported (Fowora *et al.* 2021). *N. sativa* as a source of anti-dermatophyte drugs strengthens its use in medicine for the treatment of fungal skin infections (Aljabre *et al*. 2005). This is the first study reported the effect of homeopathic drugs and TQ on *A.*

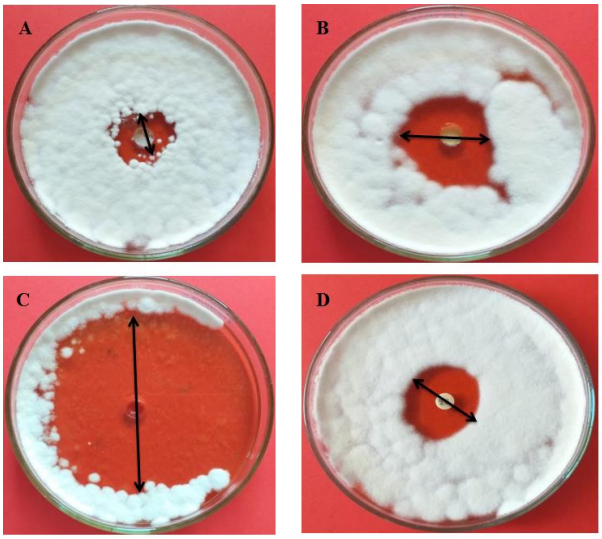

Fig. 5: Antifungal susceptibility testing of *A. multifidum.* Antifungal activity in form of zone of inhibition appeared around the wells/Disc. Zone of inhibitions were measured in millimeter (mm). A**:** Zone of Inhibition of Itraconazole, **B:** Zone of Inhibition of Nystatin C**:** Zone of Inhibition of Fluconazole **D:** Zone of inhibition of Amphotericin B

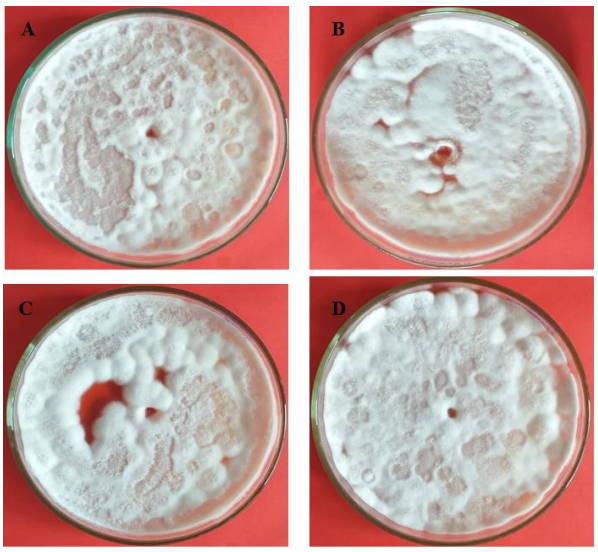

Fig. 6: Susceptibility of *A. multifidum* to homeopathic drugs. No activity (Absence of zone of inhibition around the wells having drugs) was detected in homeopathic drugs against the selected isolates**.** A**:** Sulphur, **B:** Cantharis, **C:** Bacillinum, **D:** Hydrocotyl

multifidum isolated from animal skin scrapings.

Conclusion

A. multifidium is a causative agent of skin infections in animals and is a possible zoonotic pathogen. The fungal diseases are underestimated in whole world, which leads to

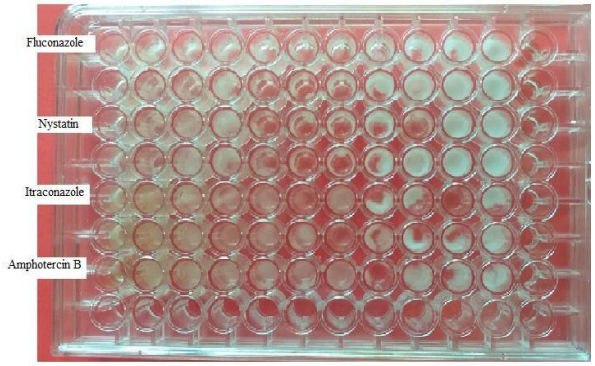

Fig. 7: Minimum inhibitory concentration of *A. multifidum*

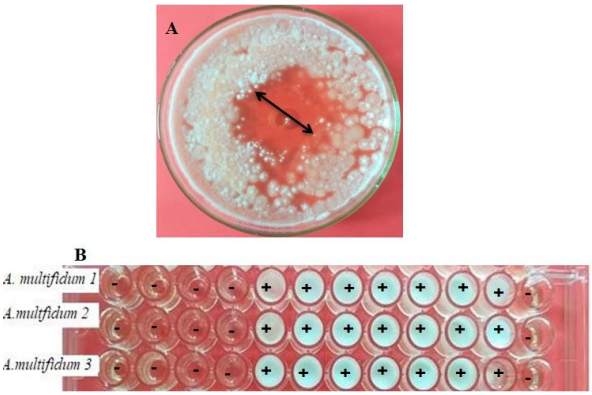

Fig. 8: Antifungal activity of thymoquinone against *A. multifidum*

serious threat to humans and animal species. Different antifungals include allopathic, homeopathic drugs have been used to treat dermatophytosis. But due to high aggression of infection these acquired resistance. Thymoquinone, which is a natural compound of plant; *Nigella sativa* have been evaluated for antimicrobial potential. Current study suggests that the action of homeopathic drugs is generalized for skin infection; skin infection by dermatophytes (*A. multifidium*) must be treated with effective antifungal agent. In current study, homeopathic medicine and antifungals were evaluated against dermatophytes. This study will help the local community to control dermatophytosis by effective therapeutics.

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Author Contributions

SS and AAA planned the experiments, SN and IB conducted the experiments. SA, HM and AAL facilitated in

data analysis, SG and IL helped in article formatting.

Conflicts of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper

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