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Biochemical characterization, Oxidative Stress and Antifungal Susceptibility in *Acremonium sclerotigenum*

Ayesha Gulzar[1*], Rana Mohammad Zulqarnain[2], Nisha Fatima[3], Fatima Ishaq[4], Mariam Kanwal[5], Kanwal Shahzadi[6], Ammara Aftab[7], Awais Ibrahim[8], Kiran Fatima[9]

[1]Department of Biochemistry, Islamia University Bahawalpur Pakistan
[2]Quaid e Azam Medical College Bahawalpur, Pakistan
[3]College of Animal Science and Technology, Southwest University, Chongqing China
[4]Department of Zoology, Lahore College for Women University, Punjab Pakistan
[5]Department of Allied Health Professionals, Government College University Faisalabad, Punjab Pakistan
[6]Department of Medical Laboratory Technology, Government College University Faisalabad, Punjab Pakistan
[7]Department of Zoology Government College University, Lahore, Pakistan
[8]Institute of Zoology, University of the Punjab New Campus, Lahore Pakistan
[9]Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad, Punjab Pakistan

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***Corresponding author:** Ayesha Gulzar
Department of Biochemistry, Islamia University Bahawalpur Pakistan

Abstract | **Original Research Article**

Acremonium is a polyphyletic genus known as *cephalosporium* formerly widely spread in environment commonly found in soil, decaying vegetation, plant debris and decaying food. *Acremonium sclerotigenum* isolated from clinical samples of tinea infected patients from Punjab Pakistan. Goal of the study is to achieve biochemical characterization of the *A.sclerotigenum* and to analyze the effect of physiological stress and terbinafine susceptibility in test strain. *A.sclerotigenum* was analyzed for oxidative, osmotic stress adaptability by culture characteristics, protease and lipase qualitative and activity assays and antifungal terbinafine susceptibility testing for isolated *Acremonium* strain. Study revealed that *A.sclerotigenum* is not resistant to osmotic and oxidative stress. Level of extracellular proteolytic enzymes is low although lipase enzyme found to be active at a moderate level. *A.sclerotigenum* has very low MIC for terbinafine. A detailed research on the mechanism involving in anti-fungal resistance in *A.sclerotigenum*, is still to be explore.

Keywords: *Acremonium Sclerotigenum*, Qualitative Assay, Activity Assay, Antifungal, Terbinafine.

INTRODUCTION

Species of *Acremonium* are saprophytic moulds that are found in soil and decomposing vegetation and are extensively spread in nature. Intravascular catheters, immunosuppression, and penetrating wounds are risk factors for invasive *Acremonium* infections. In immunocompetent individuals, the fungus can also result in cutaneous infections including mycetomas, which are infections in traumatised or exposed extremities (Hilmioglu *et al*., 2015). A range of hydrolysing enzymes are secreted by filamentous fungus. Keratinases are a special class of proteolytic enzymes that catalyse the hydrolysis of keratins. They are essential enzymes in the fungal invasion of epidermal tissue. About 300 common fungal species were evaluated for extracellular keratinase production in a recent study. On agar plates containing soluble keratin, 54% of the fungi flourished and produce extracellular proteases. *Acremonium* were one of the most active fungal strains (Friedrich *et al*., 1999).

Initiation of infection occur by adherence of fungal infectious agent to host keratinized tissues. After adhesion spore undergo germination and incorporate in stratum corneum tissues to gain nutrients and to develop strong attachment. Dermatophytes then produce pathogenic agents including both enzymatic and non-enzymatic. Dermatophytes penetrate in epidermal layer, rich in keratin such as nails. skin and hair and colonize there (Alcazar-Fuoli *et al.*, 2014). Protease produced by dermatophytes cultured in media supplemented with protein as substrate act as sole and pure source of nitrogen. Phospholipase production by dermatophytes was observed on catalysis of phopholipase substrate in to lipophilic compounds and fatty acids. Major enzymes involved in virulence are proteases, phospholipases and keratinases (Alcazar-Fuoli *et al*., 2014). A research

where enzymatic and antibacterial properties of fungal endophytes were examined, exhibiting a range of enzymatic activity, including lipase, tyrosinase, cellulase, L-glutaminase, and L-asparaginase, were discovered. Study revealed that in a varied endophytic community, mostly of members belong to the genus *Acremonium* (Job, N, *et al*., 2015).

Oxidative stress has important role to protect fungal cells from harmful oxidative agents and reactive oxygen species (ROS) produced normally inside the cell as a result of different metabolic reactions (Angelova *et al.*, 2005). Enhanced in H2O2 level induced the alternative mechanism of respiration in *Acremonium* chryosgenum. Oxidative stress leads to accumulation of modified proteins. Carbonylation damage to fungal cellular proteins act as marker for the accumulation of these oxidative modified proteins (Angelova *et al.*, 2005). Environmental oxidative agents including ionizing, UV radiations and other agents those generate reactive intracellular oxygen species such as redox cycling compounds paraquat and menadione. They can cause oxidative stress which induces oxidation of intracellular proteins. Cellular response to oxidative stress by reactive oxygen species is the immediate induction of mRNAs which encode detoxification of oxidative stress and repair proteins (da Silva Dantas *et al.*, 2015). When a fungal infection occurs, innate immune cells like neutrophils and macrophages assemble and activate the NADPH oxidase complex on cellular membranes, producing a potent oxidative burst and copious amounts of reactive oxygen species (ROS) that kill the fungal infection by inducing DNA breakage, lipid peroxidation, and protein cross-linking and fragmentation (Loh, J. T *et al*., 2023).

Since opportunistic fungal pathogens are linked to higher rates of morbidity and mortality, invasive fungal infections have become a major worldwide health concern. In spite of this, the available medications to treat fungal infections are extremely restricted. Another area of worry is the development of resistance linked to the persistent use of these medications. Since fungal enzymes are necessary for the fungus to proliferate and establish itself in the host, they are among the most significant and promising targets for treatment research (Ramakrishnan *et al*., 2016). The most effective antifungal drugs against yeast were voriconazole, ketoconazole, and itraconazole; in vitro action against dermatophytes was demonstrated by terbinafine, voriconazole, and itraconazole (Silva *et al*., 2014). In initial therapy Amphotericin B is suggested in addition to fluconazole and ketoconazole (Fincher RM *et al*., 1991). Several clinical cases on *acremonium* infection treatment are published. It appears that voriconazole at least based on case series, will be the preferred treatment option for *Acremonium* (Fakharian *et al*., 2015). *Acremonium* is known to produce variety of proteases and keratinases along with novel secondary metabolites and vital bioactive elements (Tian J *et al*., 2017). Study is aimed to analyze for the biochemical, physiological characterization and antifungal susceptibility in *Acremonium sclerotigenum*.

MATERIAL AND METHODS

Sample collection and culturing

From the Civil Hospital in Bahawalpur, samples were taken from the patients suffering with tinea infections. Samples were cultured on SDA media. Spores were extracted from the SDA slant and suspended in autoclaved distilled water.

3.1) Relative Stress Adaptability of *A.sclerotigenum*

3.1.1 Oxidative stress

The investigation was conducted to identify the effect of oxidative stress generated by two different chemical compounds individually. Firstly, two different concentrations i.e 2mM and 6mM of benzoic acid were prepared, added to autoclaved SDA media separately. 15mL of SDA poured into the petri plates. Inoculated with 3µL of spore suspension and incubated for 7 days at 30°C. Following incubation, growth of strain at different concentration of benzoic acid in medium was compared with control. Similarly, hydrogen peroxide in two different concentrations 2mM and 6mM was prepared and added to autoclaved SDA media separately. 15mL of SDA poured into the petri plates. Inoculated with 3µL of spore suspension and incubated for 7 days at 30°C. Following incubation, growth of strain at different concentration of H_2O_2 in media was compared with control. Experiment was performed in duplicates (Ismail F *et al*., 2021).

3.1.2 Salt Stress

Salt stress by NaCl was performed by preparing 2mM and 6mM NaCl in autoclaved SDA medium. Inoculated with *A.sclerotigenum* and incubated for 7 days at 30°C. After the incubation period completed growth was compared with control. Experiment was performed in duplicates (Ismail F *et al*., 2021).

3.2) Protease Qualitative Assay

Protease medium supplemented with casein 1%, glucose 0.1%, yeast extract 1%, K_2HPO_4 1%, KH_2PO_4 0.05%, $MgSO_4$ 0.01% and agar 2%. Inoculated with *A.sclerotigenum* and incubated at 30°C for seven days. A clear halo zone formation around the colony indicates the protease secretion by *A.sclerotignum* (Suryawanshi *et al*., 2017).

3.3) Protease Activity Assay

Protease Activity was determined using Folin's reagent, Sodium carbonate solution, Trichloroacetic acid solution (TCA), Sodium Hydroxide solution, Hydrochloric acid solution, Phosphate buffer solution (pH7), Lactate buffer solution (pH3, pH5), Borate buffer solution (pH9), Casein solution, Tyrosine working solution. Standard solutions for standard curve calibration using Tyrosine (Cupp-Enyard *et al*., 2008).

Preparation of Test Samples

1-2 g protease sample dissolved with the buffer solution. The solution further diluted to 10-15 U/mL with the buffer solution. The casein solution was incubated in a water bath at 40 ± 0.2 °C for 5 min.

Activity Test

For Blank:

1mLof test sample incubated at 40°C for 2min.Trichloroacetic acid solution (2mL) added, mixed and incubated at 40°C for 10 min. Casein solution (1mL) was added and mixed well. Leaved at rest for 10 min. Filtered the solution through filter paper and 5mL sodium carbonate solution was added. 1mL Folin's reagent working solution added, incubated at 40°C for 20min. Absorbance at 680 nm was measured.

For Test Samples:

1mL of test sample incubated at 40°C for 2min. 1mL casein solution added and mixed well. Leaved at R.T for 10 min. TCA (2mL) added, mixed and incubated at 40°C for 10 min. Filtered the solution through filter paper and 5mL sodium carbonate solution was added. 1mL Folin's reagent working solution added, incubated at 40°C for 20min. Absorbance at 680 nm was measured. Protease activity calculated using $\boldsymbol{X = A \times V1 \times V2 \times n/m \times t}$.

where, A is the reading from the standard curve at the given Abs680;

V1 is the total volume of the diluted sample solution (D.2) in mL;

V2 is the total volume of the reaction mixture in ml. V2 equals to 4 (Cupp-Enyard *et al*., 2008).

3.4) Lipase Qualitative Assay:

The lipase medium prepared with peptone 1%, sodium chloride 5%, calcium chloride 0.01% and agar 2%. The medium was autoclaved and 1% tween 80 was added to the molten medium at approximately 50°C. The enriched medium was mixed thoroughly and poured in sterile petridish. The spore suspension was inoculated and incubated at 37°C for 7 days. A clear halo zone of precipitation around the colony indicated lipase production (Elavarashi *et al*., 2017).

3.5) Lipase Activity Assay:

The isolates were cultured on solid state fermentation (SSF) containing 10mL of mineral media and 5g of wheat bran. The crude enzyme was recovered from the moldy wheat bran. Vegetable oil (8.9 mL) was treated with 1.0 mL of crude and 0.1 mL of Tween 80 was used as emulsifying agent. The reaction mixture was mixed with vortex mixer for 1 min. It was then incubated in orbital incubator at 45 °C for 30 min at 180rpm. The reaction was stopped by adding 25mL warm ethanol to 1mL of the hydrolysate. It was further agitated manually for 1min and then titrated (Osho *et al*., 2021).

N. (Vs - Vb). MM/10.m

N = NaOH normality

Vs = volume of base used in sample titration (mL)

Vb = volume of base used in blank titration (mL)

MM = Molecular Mass of the predominant fatty acid (g)

m = sample mass (g) (de Sousa *et al*., 2010).

3.6) Antifungal susceptibility Testing

Terbinafine was used to study the antifungal susceptibility of *A.sclerotigenum*. SDA medium was prepared with terbinafine concentration elected according to their MIC (Minimum Inhibitory Concentration) value. Strain for terbinafine drug sensitivity was tested at MIC value such as 0.08µg/mL in accordance with recently approved EUCAST method (Zalacain *et al.*, 2011). Stock solution (1µg/mL) was prepared in DMSO (Dimethyl sulfoxide). Drug sensitivity was tested by inoculation of 3µl of conidial suspension 2×10^2mL. Culture plates were incubated at 30°C for 7 days then compared with control (Motedayen *et al.*, 2018).

Table 3.1: Illustrates *A.sclerotigenum* MIC values against terbinafine

MIC50	MIC90
0.125µg/mL	0.500µg/mL

RESULTS

4.1) Relative Stress Adaptability of *A.Sclerotignum*

4.1.1 Oxidative Stress

The investigation was conducted to identify the effect of oxidative stress generated by two different chemical compounds individually. Oxidative stress by benzoic acid and hydrogen peroxide was tested at two different concentrations (2mM and 6mM) and compared with control. Benzoic acid produced oxidative stress to the growth of *Acremonium sclerotigenum.* At 2mM benzoic acid affect the growth of *acremonium* to a limited extent but at 6mM Benzoic acid totally diminished the growth of *A.sclerotigenum* shown in figure 4.1. Hydrogen peroxide produced oxidative stress and affect the growth of *A.sclerotigenum* at all tested concentrations. Growth was inhibited by H_2O_2 at 6mM as shown in figure 4.2 and table 4.1.

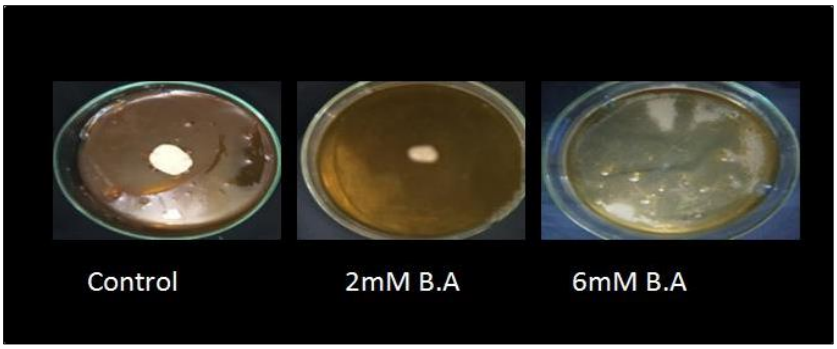

Figure 4.1: Exhibits phenotypic results of Oxidative stress by Benzoic acid on the growth of *A.sclerotigenum*

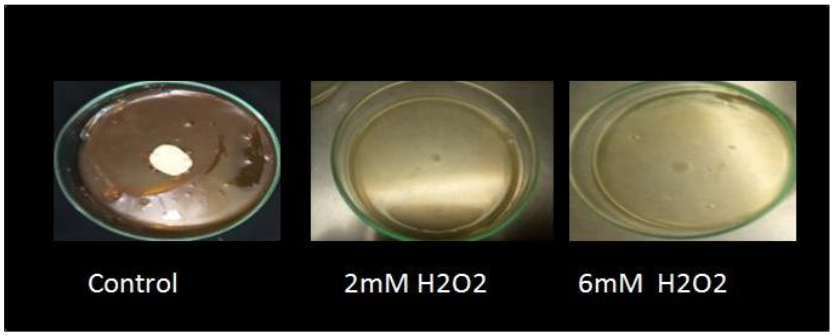

Figure 4.2: Phenotypic results of oxidative stress by H2O2 on the growth of *A.sclerotigenum.*

Concentration (mM)	**Oxidative stress** (Benzoic Acid)	**Oxidative stress** (Hydrogen peroxide)
2mM	+	+
6mM	+	+

4.1.2 Salt Stress

Salt stress by NaCl was performed by preparing 2mM and 6mM NaCl in SDA media. After incubation growth was compared with control. NaCl disturbed homeostasis thus induced osmotic stress in *A.sclerotigenum*. Growth was diminished to a significant level at both 2mM and 6mM NaCl concentrations as indicated in figure 4.3 and table 4.2.

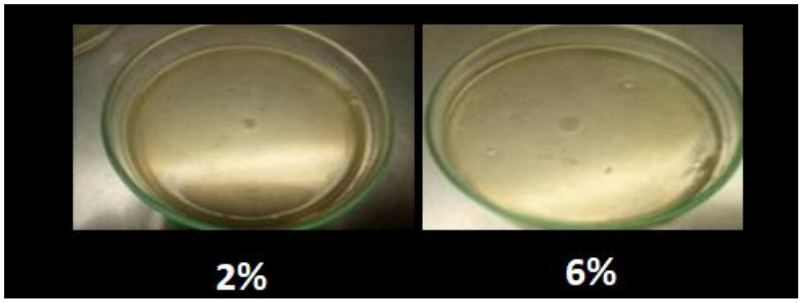

Figure 4.3: Phenotypic results of osmotic stress on the growth of *A.sclerotigenum*

Table 4.2: Osmotic stress by NaCl at two different concentrations was analyzed, +ve sign shows effect of stress on *A.sclerotigenum* growth

Concentration (%)	**NaCl**
2%	+
6%	+

4.2) Protease Qualitative Assay

Protease mineral media prepared where casein was added as a substrate. After incubation of 7 days at 30°C. Colony appeared but there was no clear zone formation round the colony which indicated that protease secretion was absent in *A.sclerotigenum* as showen in figure 4.4.

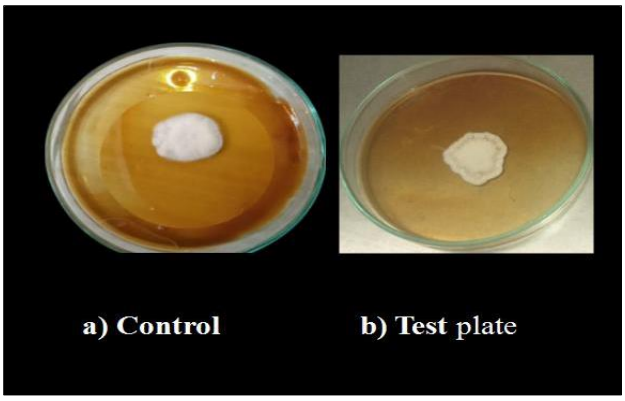

Figure 4.4: showing a) control and b) protease plate. Comparison between control and test plate clearly indicates no halo zone around the colony appeared.

4.3) Protease Activity Assay

In order to investigate the proteolytic activity of *Acremonium Sclerotigenum*. Proteolytic activity assay was performed to study how much protease enzyme is active in *A.sclerotigenum* Test sample was incubated with casein solution (prepared in buffer with specific pH) then TCA was added to stop the enzyme substrate reaction. Folin's reagent acts as derivating agent gave blue color which indicated the release of amino acid. Absorbance measured at 680nm. Absorbance value indicated a very small amount of activity of protease at specific pH which was calculated by using formula. There was no significant enzyme activity found in tested strain. *A.sclerotigenum* showed no enzyme activity at strong acidic pH as well as at strong basic pH. A very small level of protease activity was observed from pH 6-8. Optimum protease activity 169.52U/ml was measured at neutral pH in *A.sclerotigenum* as showen in figure 4.5.

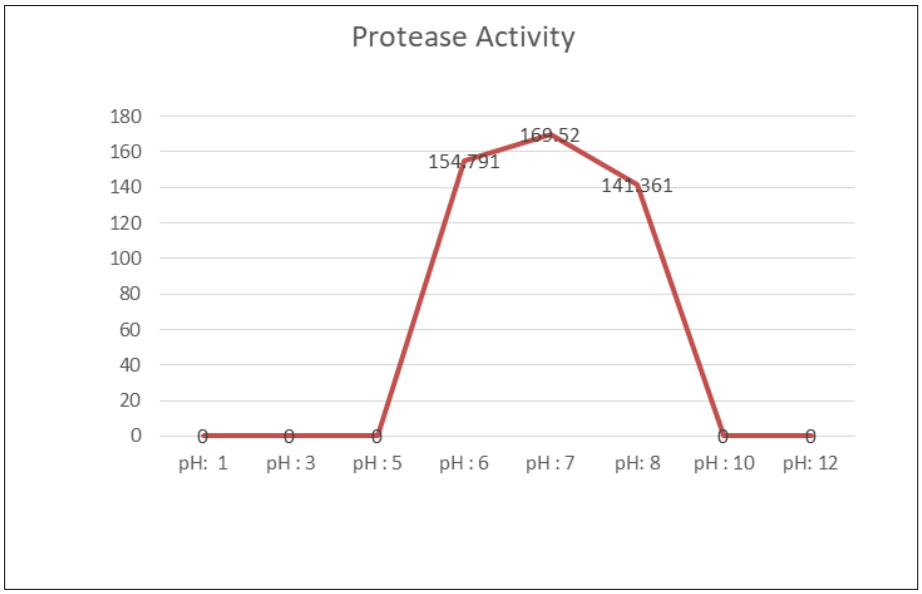

Figure 4.5: pH range on X- axis plotted against protease activity (µ/ml) at Y- axis; results indicate that *Acremonium sclerotigenum* showed a very low level of protease activity from pH 6-8 whereas optimum pH for protease activity is neutral pH (pH7)

4.4) Lipase Qualititative Assay

Lipase medium was prepared where tween 80 was added as a substrate. After incubation at 37˚C for 7 days colony appears, there was a clear zone formation around the colony which indicated the lipase secretion from *A.sclerotigenum* as showen in figure 4.6.

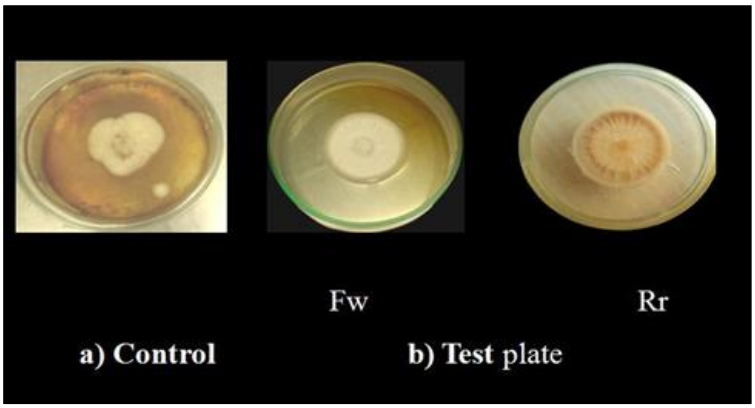

Figure 4.6: represents a) plate as control and b) plate as a test plate with both forward and reverse view having substrate for lipase, indicating a halo zone formation around the colony in test plate which declared the secretion of lipase enzyme in the tested strain.

4.5) Lipase Activity Assay

Lipase activity in *Acremonium sclerotigenum* was determined by modified method of Lin *et al*., 2008. First strain was cultured on solid state fermentation containing mineral media and wheat bran. Tween 80 and wheat bran act as a substrate for lipase activation. After the fermentation crude enzyme extract was extracted which was then titrated against NaOH. Enzyme activity obtained after the titration was 32U/g, which represented a moderate lipase activity in *A.sclerotigenum.*

4.6) Terbinafine Antifungal Testing

SDA medium was prepared with elected terbinafine concentration set according to MICs range of the strain. Terbinafine stock solution 0.1µg/ mL was prepared. Strain for terbinafine drug sensitivity was tested. MICs value such as 0.08µg/mL in accordance with recently approved EUCAST method. Results showed that *A.sclerotigenum* has very low MICs for terbinafine. *A.sclerotigenum* found sensitive against terbinafine as supported by phenotypic results shown in figure 4.7.

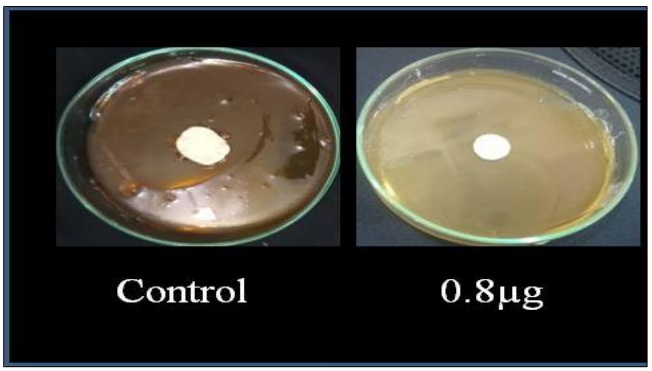

Figure 4.7: Illustrates the antifungal susceptibility of *A.sclerotigenum* against terbinafine.

DISCUSSION

Acremonium is a major polyphyletic genus that includes about 200 species. *Acremonium* are mostly soil saprobes and considered as opportunist pathogens to human and animals (Perdomo *et al.*, 2011). *A.sclerotigenum* is a type of filamentous fungi those are associated with spoilage of food. It has association with many animals and plant sources, shows capability to grow at high temperature relatively with some general compatability i.e morphological. Contamination of the foods those are heat disinfected is facilitated by its heat tolerance ability (Summerbell *et al.*, 2018). *A.sclerotigenum* also known to be called by its valid earliest name *A. egyptiacum*. *A.sclerotigenum* acts as human opportunistic pathogen, food contaminant, probable dermatological agent and heat-tolerant spoilage organism. Industrially, it is already in exploratory use as a producer of the antibiotic ascofuranone (Summerbell *et al.*, 2018). According to an estimate 20% of human population is affected by dermatophytes worldwide (Al Hasan *et al*., 2004). In United States tinea pedis is known to be second common disease of skin and it is estimated that 15% of population is affected by it (Al Hasan *et al.*, 2004).

In order to examine the adaptability of the oxidative stress Hydrogen peroxide was used at 2 different concentrations (i.e 2mM, 6mM). The results showed that *A.sclerotigenum* was inhibited by hydrogen peroxide stress at both tested concentrations. Mechanism of hydrogen peroxide stress involved in the cytotoxicity of H_2O_2 with release of toxic oxygen species like $OH^{\cdot}$ (free hydroxyl radicals). Reactive oxygen species act as powerful oxidative agents and produce damage to DNA,

RNA (nucleic acid), cellular proteins and lipids (Ismail F *et al*., 2021).

Benzoic acid induced oxidative stress on *A.sclerotigenum* growth. There was a gradual decrease in growth with increase in benzoic acid concentration. At 2mM oxidative stress by benzoic acid affect the growth to almost 50%. At 6mM, oxidative stress by benzoic acid inhibited the *A.sclerotigenum* growth. Mechanism of oxidative stress by benzoic acid involves the deposition of benzoic acid in fungal cells. This accumulation reduces fungal cellular pH (cytoplasmic pH) and leads to cell damage (Warth ADJA *et al*., 1991). Hence it is concluded from study that *A.sclerotigenum* is sensitive to oxidative stress produced by benzoic acid as well as hydrogen peroxide. NaCl produced osmotic stress that affects the growth of *A.sclerotigenum.* NaCl imbalances the Na^+/k^+ ion gradient of cellular membrane and decreases intracellular K^+ ions concentration that leads to metabolic deregulation of Na^+ ions and affect the growth of fungal cell (Kane J *et al*., 1975).

Protease production assay revealed that there was no protease secretion observed in *A.sclerotigenum* supported by phenotypic results as there was no formation of clear holo zone around the colony. To further investigate the protease activity in *A.sclerotigenum* protease activity assay was performed. Results obtained from the experimentation showed there was no significant protease activity in *A.sclerotigenum.* A little protease activity was found from pH6-pH8. Optimum protease activity 169.52U/ml was measured at neutral pH which may induce pathogenicity with degradation of keratin on skin surface layer of host stratum corneum and colonizes (Elavarashi E *et al*., 2017).

Phenotypic results showed that lipase excretion in *A.sclerotigenum* as there was a clear holo zone formation around the colony. *A.sclerotigenum* was found to excrete lipase enzyme as supported by phenotypic results. Enzyme activity obtained was 32U/g obtained from calculations after titration. There was a moderate level of lipase activity observed in *Acremonium sclerotigenum.* By using the optimized conditions the MICs of terbinafine for *A.sclerotignum* isolates was determined. Our data show that terbinafine was the most active antifungal agent tested against dermatophytes.

A.sclerotigenum found sensitive against terbinafine as supported by phenotypic results. Terbinafine has been effective to treat *Acremonium* species (Garcia-Effron *et al.*, 2004). Antifungal susceptibility test has demonstrated that *Acremonium* has very low MICs for terbinafine as comparison to other antifungal agents (Perdomo *et al.*, 2011).

CONCLUSION

A saprophytic hyaline fungal mould with pathogenic potential, *Acremonium* can infect people, particularly those with weakened immune systems. Morphological characteristics of *A.sclerotigenum* revealed that *A.sclerotigenum* was sensitive to oxidative stress induced by benzoic acid, as it disturb the cellular pH which leads to cell damage. *A.sclerotigenum* is not adaptable to in vitro stress such as oxidative and osmotic stress. Osmotic stress affects the growth of *A.sclerotigenum* produced by NaCl. There is no significant protease activity in *A.sclerotigenum. A.sclerotigenum* was found to excrete lipase enzyme as supported by phenotypic results. *A.sclerotigenum* is liable to lipase production that may have potential role in virulence. There was a moderate level of lipase activity observed in *Acremonium sclerotigenum.* Moreover, study predicted that *A.sclerotigenum* may induce pathogenic infection with lipase excretion in hot and humid weather. Terbinafine acts as potential antifungal agent against *A.sclerotigenum. A.sclerotigenum* has very low MICs against terbinafine and it acts as potent antifungal agent against *A.sclerotigenum* and may use to treat dermatophytosis. Due to rise in acremonium pathogenicity and reoccurrence of infections. It is required to conduct a thorough investigation into the mechanism underlying *A. sclerotigenum*'s antifungal resistance.

REFERENCES

- Al Hasan, M., Fitzgerald, S. M., Saoudian, M., Krishnaswamy, G. J. C., & Allergy, M. (2004). Dermatology for the practicing allergist: *Tinea pedis and its complications*, *2*(1), 1-11.
- Alcazar-Fuoli, L., & Mellado, E. J. B. J. O. H. (2014). *Current status of antifungal resistance and its impact on clinical practice*, *166*(4), 471-484.
- Angelova, M. B., Pashova, S. B., Spasova, B. K., Vassilev, S. V., & Slokoska, L. S. J. M. R. (2005) *Oxidative stress response of filamentous fungi induced by hydrogen peroxide and paraquat*, *109*(2), 150-158.
- Cupp-Enyard, C. (2008). Sigma's non-specific protease activity assay-casein as a substrate. *JoVE (Journal of Visualized Experiments)*, (19), e899.
- da Silva Dantas, A., Day, A., Ikeh, M., Kos, I., Achan, B., & Quinn, J. J. B. (2015). Oxidative stress responses in the human fungal pathogen, *Candida albicans*, *5*(1), 142-165.
- de Sousa, J. S. (2010). "Application of lipase from the physic nut (Jatropha curcas L.) to a new hybrid (enzyme/chemical) *hydroesterification process for biodiesel production." 65*(1-4), 133-137.
- Elavarashi, E. (2017). "*Enzymatic and non-enzymatic virulence activities of dermatophytes on solid media." 11*(2), DC23
- Fakharian, A., Dorudinia, A., Darazam, I. A., Mansouri, D., & Masjedi, M. R. (2015). Acremonium pneumonia: Case report and literature review. *Tanaffos*, *14*(2), 156.
- Fincher, R. M., Fisher, J. F., Lovell, R. D., Newman, C. L., Espinel-Ingroff, A., & Shadomy, H. J. (1991).

Infection due to the fungus Acremonium (cephalosporium). *Medicine*, *70*(6), 398-409.

- Friedrich, J., Gradišar, H., Mandin, D., & Chaumont, J. P. (1999). Screening fungi for synthesis of keratinolytic enzymes. *Letters in Applied Microbiology*, *28*(2), 127-130.
- Garcia-Effron, G., Gomez-Lopez, A., Mellado, E., Monzon, A., Rodriguez-Tudela, J. L., & Cuenca-Estrella, M. (2004). In vitro activity of terbinafine against medically important non-dermatophyte species of filamentous fungi. *Journal of Antimicrobial Chemotherapy*, *53*(6), 1086-1089.
- Hilmioglu, S., Metin, D. Y., Tasbakan, M., Pullukcu, H., Akalin, T., & Tumbay, E. (2015). Skin infection on both legs caused by Acremonium strictum (case report). *Annals of Saudi Medicine*, *35*(5), 406-408.
- Ismail, F., & Ghani, A. (2021). Akbar SJRJoBR, Sciences A. Emergence of Antifungal Azole Resistance in the Fungal Strains of Tinea corporis, Tinea capitis, Tinea cruris and Tinea pedis from the Locality of Southern Punjab, *Pakistan*, *12*(1), 24-38.
- Job, N., Manomi, S., & Philip, R. (2015). Isolation and characterisation of endophytic fungi from Avicennia officinalis. *Int J Res Biomed Biotechnol*, *5*(1), 4-8.
- Kane, J., & Fischer, J. (1975). The effect of sodium chloride on the growth and morphology of dermatophytes and some other keratolytic fungi. *Canad J Microb*, *21*(6), 742-9
- Loh, J. T., & Lam, K. P. (2023). Fungal infections: immune defense, immunotherapies and vaccines. *Advanced Drug Delivery Reviews*, *196*, 114775.
- Motedayen, N., Hashemi, S. J., Rezaei, S., & Bayat, M. J. J. J. O. M. (2018) In-vitro evaluation of antifungal activity of terbinafine and terbinafine nano-drug against clinical isolates of dermatophytes, 11(5).
- Osho, M. B. (2021). "Screening, optimization and characterization of extracellular lipase of Aspergillus niger ATCC 1015." 40-44.
- Perdomo, H., Sutton, D. A., García, D., Fothergill, A. W., Cano, J., Gené, J., Summerbell, R. C., Rinaldi, M. G., & Guarro, J. (2011). Spectrum of clinically relevant Acremonium species in the United States. *Journal of Clinical Microbiology*, *49*(1), 243-56.
- Ramakrishnan, J., Rathore, S. S., & Raman, T. (2016). Review on fungal enzyme inhibitors–potential drug targets to manage human fungal infections. *RSC advances*, *6*(48), 42387-42401.
- Silva, L. B., De Oliveira, D. B. C., Da Silva, B. V., De Souza, R. A., Da Silva, P. R., Ferreira-Paim, K., ... & Andrade, A. A. (2014). Identification and antifungal susceptibility of fungi isolated from dermatomycoses. *Journal of the European Academy of Dermatology and Venereology*, *28*(5), 633-640.
- Summerbell, R. C., Gueidan, C., Guarro, J., Eskalen, A., Crous, P. W., Gupta, A. K., Gené, J., Cano-Lira, J. F., Van Iperen, A.and Starink, M. J. M. (2018) The Protean Acremonium. A. sclerotigenum/egyptiacum: Revision, *food contaminant, and human disease*, *6*(3), 88.
- Suryawanshi, H. (2017). "Screening, identification of alkaline proteases producing fungi from soil of different habitats of Amalner Tahsil [Maharashtra] and their applications." 5(3), 397-402.
- Tian, J., Lai, D., & Zhou, LJMRiMC. (2017). Secondary metabolites from Acremonium fungi: *Diverse structures and bioactivities*, *17*(7), 603-32.
- Warth, A. D. J. A. (1991). Microbiology e. Mechanism of action of benzoic acid on Zygosaccharomyces bailii: effects on glycolytic metabolite levels, energy production, and intracellular Ph, 57(12), 3410-4.