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A BIOCHEMICAL ANALYSIS ON SOME FUNGAL ISOLATES AGAINST THEIR GROWTH AND SPORULATION

SHAMSHAD UL HAQ*, MEENAKSHI SHARMA, SUBHASH GORA, SUNITA MAHARIYA, NEETU JAIN AND SONIYA BAIRWA

Medical Mycology and Microbiology Laboratory,
Department of Botany, University of Rajasthan, Jaipur, Rajasthan-302004, India

ABSTRACT

Several biochemical parameters represent distinguishable vital consequences on fungal physiology and morphological changes. The effect of various culture conditions supplemented with different biochemicals, was examined to identify optimal and tolerated conditions for fungal growth and sporulation. In the present study, the effect of Sodium chloride (NaCl), Indole-Acetic acid (IAA) and Indole-Buteric acid (IBA) were analyzed on five different fungal isolates namely, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum gypseum*, *Microsporum canis* and *Chrysosporium tropicum.* Analysis revealed that the remarkable growth and sporulation were identified at between 0.5 % to 3% NaCl concentrations within *T. mentagrophytes*, *T. rubrum*, *M. gypseum* and *C. tropicum* but retarded response was seen over exceeding 3% NaCl concentration. Moreover, *T. mentagrophytes* revealed remarkable response against 0.5 to 10 ppm IAA and 1 to 5 ppm (IBA) concentration. Similarly, *T. rubrum* reflected inducible changes at between 0.5 to 3 ppm concentration of both IAA and IBA. Thus, each fungus revealed a characteristic differentiation in their morphological features against the biochemical tolerance and offered a possibility for recognition and categorization of these fungi.

KEYWORDS: ***Fungal Isolates, Sodium chloride, Indole-Acetic acid (IAA) and Indole-Buteric acid (IBA)***

SHAMSHAD UL HAQ

Medical Mycology and Microbiology Laboratory, Department of Botany, University of Rajasthan, Jaipur, Rajasthan-302004, India

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INTRODUCTION

Varieties of biochemical and physiological factors have a significant impact on the growth and development in microorganisms and manage their metabolism towards increase or decrease growth with certain morphological measurable changes. Accordingly, the fungal growth and their sporulation also influenced by various biochemical and physiological factors like, nutrients, ionic strength, pH, temperature, acid and alkaline conditions, light, aeration and water activity. Additionally, the elevated concentrations of salts in the growth medium have a variable impact on the several parameters of fungal growth and development, such as cell growth and multiplication, utilization of the primary carbon and energy source is reduced and change in concentration of metabolic products[1]. In accordance, some dermatophytes and related arthrodermataceous anamorphs showed species-specific growth responses on raising the level of Sodium chloride in the culture medium[2]. Afterward, few dermatophytes revealed a notably response on their morphology against low level of salts addition and revealed differntiating effect on their growth and development[2, 3]. Some of the salts like sodium metabisulfite and propyl-paraben showed limited effect on mycelial growth and spore germination of several fungal isolates[4]. Moreover, Sodium chloride concentrations found to be more useful than calcium chloride, ammonium chloride and manganese chloride in controlling some *Fusarium* spp[5]. However, some inhibitory effect of salts such as, ammonium bicarbonate, potassium benzoate, potassium sorbate and sodium benzoate, has been seen on mycelial growth *of F. oxysporum f. sp. melonis*[6]. On the other hand, the effect of various culture parameters, such as carbon sources, nitrogen sources, pH and temperature, has been also examined in *Aspergillus terreus* against solubilization of phosphorus from the insoluble form and differential biochemical changes were identified during growth and development in *Aspergillus terreu*[7].Moreover, several types of growth hormones are also important indispensable to regulate microbial growth and represents a differentiating impact on microbial physiology and phenotypical appearances[8]. Therefore, the study on fungal cultivations have been documented to identify various concentration of different hormones against their growth and development[9]. singnificantly, the various concentration of cytokinin have been evaluated against growth and development of *Aspergillus oryzae*, *A. terreus*, *A. niger* and *Alternaria alternate*[10]. Besides, the combination of plant growth hormones with ribitol revealed synergistically increased growth of *Ramalina farinacea and Ramalina fastigiata*[11]. Considerably, the derivative of auxins like Indole Acitic Acid (IAA) and Indole Butyraic Acid (IBA) have an impact on fungal physiology and their reflection can differ from one species to species against different doses of hormones. While, several studies have shown that auxins (IAA and IBA) trigger cellular elongation, mycelial growth and sporulation in several fungal isolates[12]. Furthermore, the addition of each hormone like, Indole-3-acetic acid (IAA) and kinetin (KIN) in the culture media, significantly enhanced the biomass of *Mucor indicus* and chitosan production but this inverse response was seen at higher concentrations of growth hormones[12]. In the present study, the effect of various concentration of Sodium chloride (NaCl) and plant growth hormones such as Indole-Acetic acid (IAA) and Indole-2-Butyric acid were evaluated on some fungal isolates against their morphological and physiological changes.

MATERIALS AND METHODS

Fungal isolates

The pure strain of five different fungus was used for the analysis against biochemical tolerance and these strain were maintained in (6.5%) sabouraud's dextrose agar (SDA) medium supplemented with chloramphenicol (16 µg/ml) and cycloheximide (0.5 µg/ml), in our laboratory, Medical Mycology and Microbiology Laboratory, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India. The fungal isolates namely, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum gypseum*, *Microsporum canis* and *Chrysosporium tropicum* were used for the effect of Sodium chloride (NaCl) and hormones on test fungal isolates against their growth and development. The effect of various culture conditions of test biochemical supplemented in the media was analysed on selected fungi. Growth and sporulation studies were compared with control (absent of test chemical) and all the experiments were conducted in triplicates. The degree of sporulation of the test fungal isolates was determined using standard method which is described by Tuite[13] & Wilson and Knight[14] . The obtained data from the experiment was used for graphical representation using Microsoft Office Excel 2007.

Effect of Sodium chloride (NaCl) concentration

The effect of various NaCl concentrations was studied on the growth and sporulation of selected fungal isolates and one series of Sabouraud dextrose agar (SDA) slants supplemented with 0.5, 1, 3, 5 and 10% (w/v) NaCl concentrations along with NaCl-free controls were prepared for the analysis[2]. Each fungal isolates was inoculated onto SDA slant encompassing the entire range of salt concentrations for each type of basal medium. After inoculation, growth from a point inoculum source was recorded after two weeks at 25°C to 28°C temperature. An individual species was considered to be strongly inhibited at a given salt concentration if its colony diameter was less than 2mm after two weeks. The micromorphology of all cultures were studied against each NaCl concentration.

Effect of synthetic hormones

The role of synthetic plant growth hormones was analyzed on the growth and sporulation of test fungi and two series of SDA slants were prepared. One series consisted of SDA slants supplemented with 0.5, 1, 3, 5 and 10 ppm concentrations of Indole-Acetic acid (IAA) along with control (hormones absent). The second series consisted of SDA slants supplemented with 0.5, 1, 3, 5 and 10 ppm concentrations of Indole-2-Butyric acid (IBA) and control was made without IBA. These different concentrations of growth hormones were used to evaluate the comparative effect of hormones against growth and sporulation in the selected fungi. .

RESULTS

The effect of various concentrations of both Sodium chloride (NaCl) and plant growth hormones namely; Indole-Acetic acid (IAA) and Indole-2-Butyric acid (IBA), were examined on the selected fungal isolates against growth and sporulation.

Effect of Sodium chloride (NaCl) on fungal isolates

The effect of different concentrations (0.5, 1, 3, 5 and 10%) of NaCl was examined on five fungal isolates and all the select fungi showed significant response against various concentration of Sodium chloride (NaCl) supplemented in SDA slants which are shown in figure 1. While, *T. mentagrophytes* revealed measurable growth response between 0.5 to 3% NaCl concentrations but excellent result was seen at 0.5% NaCl concentrations and further growth was restricted onward 5% concentrations of NaCl (Fig. 2). At this concentration, colonies appeared as white, cottony and velvety growth was seen after 14 days of incubation at 25^{o}C. Similarly, *T. mentagrophytes* showed remarkable sporulation at 3% NaCl concentration but poor sporulation was observed at 0.5% and 1 % concentration with some spiral hyphae (Table 1). The micromorphology of microconidia was reported as subspherical in shape at 3% concentration. The notably growth response was seen in *T. rubrum* at 1% and 3% NaCl concentration but best growth was observed at 1% concentration and further, growth was suppressed on raising concentration (Fig. 2). At between 0.5 to 3.0% NaCl concentrations, the colonies were seen as granular, wrinkled, white to yellow and reverse yellowish white. The maximum sporulation was found at 0.5% NaCl concentration with microconidia having pyriform along the sides of the hyphae and macroconidia found to be pencil in shape (Table 1). On increasing NaCl concentration, the poor sporulation was observed at 1% and 3.0% concentration with only racquet hyphae. Therefore, both growth and sporulation were seen as continuously retarded fashion at higher (5% and 10%) NaCl concentrations.Additionally, *M. gypseum* showed excellent growth response at 0.5% NaCl concentrations but onward this concentration, growth was inhibited and colonies found to be spreading, granular and buff to cinnamon coloured growth (Fig 2). Excellent sporulation was observed at 1 % and 3 % concentration and macroconidia was slightly rounded with truncate bases and microconidia found to be clavate shaped (Table 1). At 0.5% concentration, growth was good but sporulation was observed poor. Only hyphae were seen at 0.5% NaC1 concentration and no sporulation takes place.Similarly, substantially growth of *M. canis* was obtained at lower NaCl concentrations with colonies having white to dense surface and deep yellow colour but tolerance was seen onward high NaCl concentration (Fig. 2). While, considerable sporulation was identified at between 0.5% to 5% NaCl concentrations and most excellent response was found at 3% and 5% NaCl concentration (Table 1). At lowest concentration, only few macroconidia with spindle shaped were found but only hyphae was observed at 10% concentration with no sporulation. It was found that *M. canis* grew well between 0.5% to 5% but excellent sporulation was reported at 3% and 5% NaCl concentrations.The fifth fungal isolate was *C. tropicum* which represented better growth response at between 0.5% to 3% NaCl concentrations with white and velvety growth as well as excellent growth was reported at 3% (Fig. 2). Similarly, remarkable sporulation was seemed at between 0.5% to 3% NaCl concentrations but 3% concentration showed best sporulation response with pyriform microconidia and truncate bases were observed (Table 1). Moreover, spiral hyphae was seen at 5% NaC1 concentration but poor sporulation was observed. Finally, *C. tropicum* represented NaCl tolerance over their 3% concentration.

Response of hormones on growth and sporulation on fungal isolates

Two fungus namely; *T. mentagrophytes* and *T. rubrum* showed a remarkable growth and sporulation against exogenous addition of synthetic hormone (IAA and IBA) compare to other and best result are shown in figure 3. The inducible response of IAA was observed from 0.5 to 10 ppm concentration on growth and sporulation of *T. mentagrophytes* as compared to control (Fig.4). The considerable growth was seen at 10 ppm IAA and significantly sporulation was figured throughout the concentrations. Therefore, the constructive responses of IAA were reported on growth and sporulation in *T. mentagrophytes* at all concentrations (Table 2). Similarly, the response of various ppm concentration of IBA was studied in *T. mentagrophytes* regarding their growth and sporulation. The considerable growth was reported at between 1 to 5ppm concentration and best result was seen at 1 ppm IBA concentration (Fig. 4). Furthermore, measurable sporulation was found between 0.5 to 5 ppm concentration but excellent response reported at 3 ppm and 5ppm concentration (Table 2). Thus, *T. mentagrophytes* revealed inhibitory effect for both growth and sporulation at high ppm concentration of IBA.Similarly, both IAA and IBA response were studied on *T. rubrum* against their growth and sporulation. The appreciable growth response of IAA was seen at 0.5 ppm and 1 ppm concentrations and best growth was observed at 0.5 ppm concentration while excellent sporulation was observed at 3 ppm IAA concentration (Fig. 5 and Table 2). Furthermore, the increasing ppm concentration of IAA showed a depressing effect and no growth was seen at 10% ppm IAA concentration. Moreover, *T. rubrum* revealed considerable growth response at between 0.5 to 3% ppm IBA concentrations and continuously reducing response was seen till 10 ppm IBA concentration (Fig. 5). Furthermore, the upright sporulation was seen between 0.5% to 3% ppm concentration and inhibition was displayed on increasing concentrations (Table 2). Thus, *T. rubrum* displayed suppression on their growth and sporulation at higher concentration.

Table 1

Effect of various concentration of Sodium chloride (NaCl) on sporulation of selected fungal isolates

Serial no.	Fungus	Sodium chloride (NaCl) Treatments					
		0.50%	1%	3%	5%	10%	Control
1	*Trichophyton mentagrophytes*	+	+	++++	0	0	+++
2	*Trichophyton rubrum*	++++	+	+	0	0	++
3	*Microsporum gypseum*	+	++++	++++	0	0	+++
4	*Microsporum canis*	+++	+++	++++	++++	+	++
5	*Chrysosporium tropicum*	++	+++	++++	+	0	+++

Sporulation was scored in the following manner:
++++= Excellent sporulation; +++ = Good sporulation; ++ = Fair sporulation; + = Poor sporulation; 0 = No sporulation

Table 2

Effect of various concentration of growth hormones Indole-2-Acetic acid (IAA) and Indole-2-Butaric acid (IBA) on test fungi against sporulation

Serial no.	Fungus	Treatment concentration (ppm)	Indole-2-Acetic acid (IAA)	Indole-2-Butaric acid (IBA)
1	*Trichophyton mentagrophytes*	0.5	+++	++
		1	++	++
		3	++++	++++
		5	++++	+++
		10	+	+
		control	+++	+++
2	*Trichophyton rubrum*	0.5	++	+++
		1	+++	+++
		3	++++	++++
		5	0	+
		10	0	0
		control	++++	+++

Sporulation was scored in the following manner:
++++= Excellent sporulation; +++ = Good sporulation; ++ = Fair sporulation; + = Poor sporulation; 0 = No sporulation

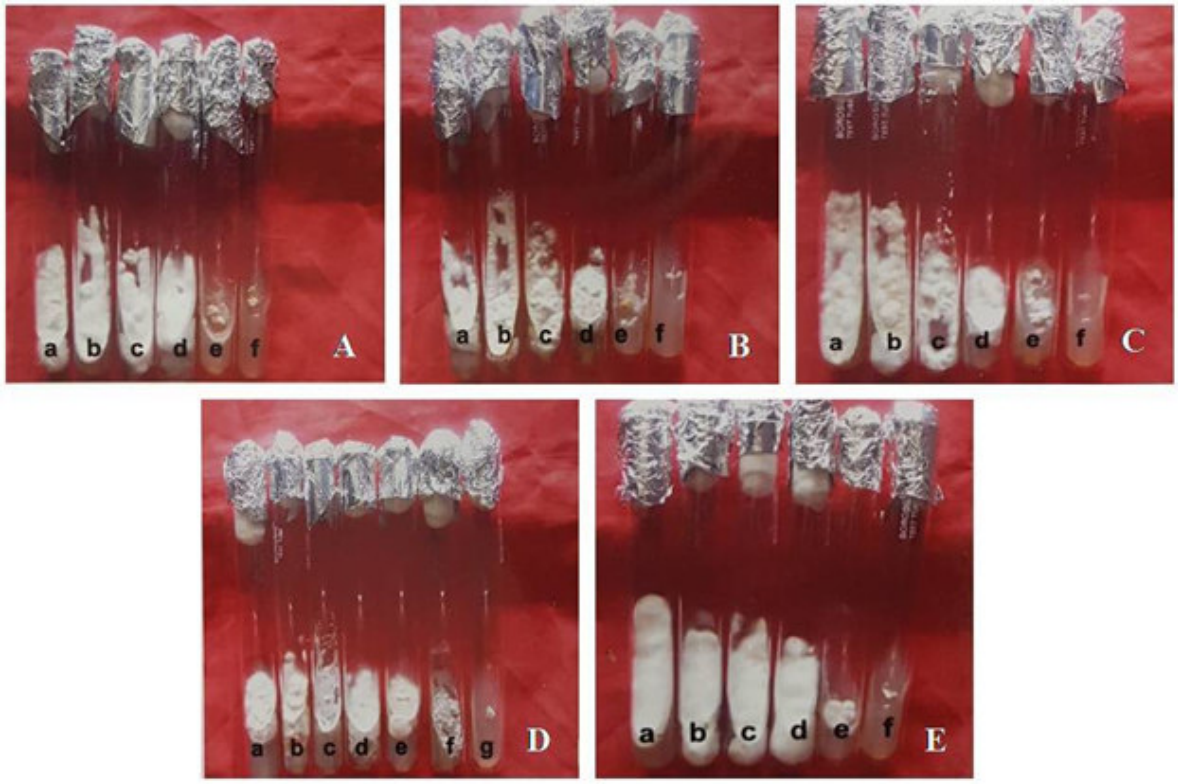

Figure 1

Photographs represent the effect of different concentrations of Sodium chloride (NaCl) on five fungal isolates which are as follows: A. Trichophyton mentagrophytes, B. Trichophyton rubrum, C. Microsporum gypseum, D. Microsporum canis and E. Chrysosporium tropicum. This image represents sabouraud dextrose agar (SDA) slants supplemented with various NaCl concentrations which are as follows: (a) Control, (b) 0.5%, (c) 1.0%, (d) 3.0%, (e) 5.0%, (f) 10.0% but M. canis also examined at 15% (g) NaCl concentration.

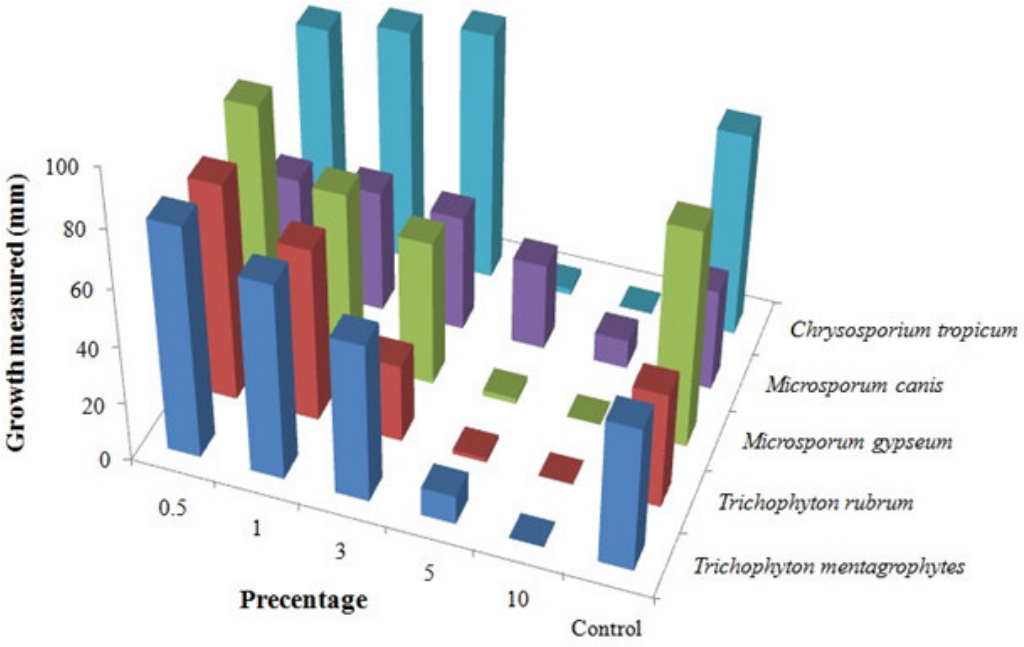

Figure 2
Effect of different concentration of sodium chloride (NaCl) concentration against growth of selected fungal isolates.

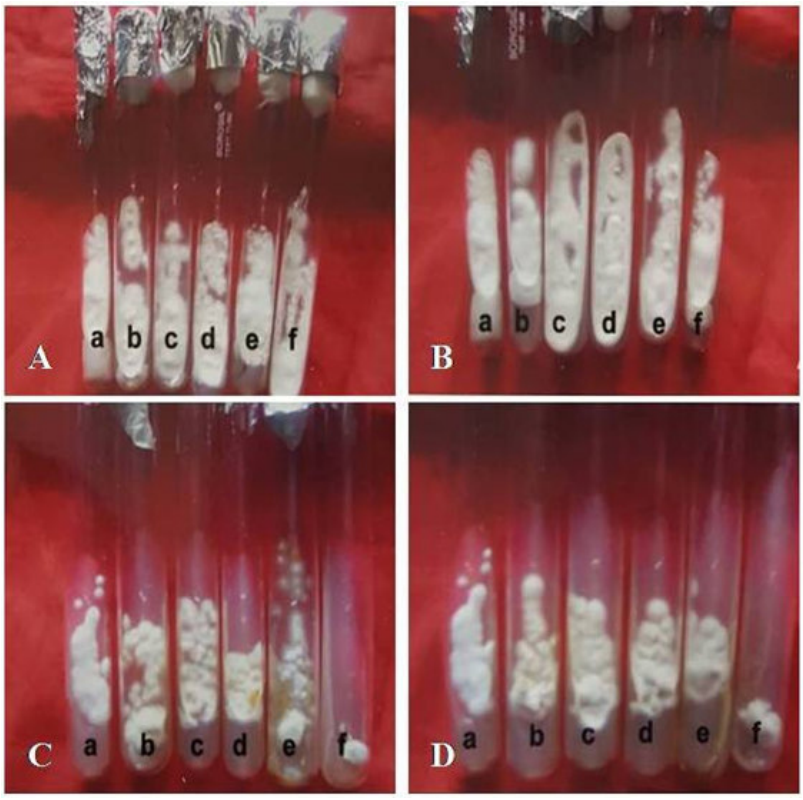

Figure 3
Photograph represents effect of Indole-Acetic (IAA) acid and Indole-2-Butyric acid (IBA) on Trichophyton mentagrophytes (A and B) and Trichophyton rubrum (C and D). In this figures, various concentrations of IBA and IAA which are as follow; (a) control (b 0.5 ppm (c) 1.0 ppm (d) 3.0 ppm (e) 5.0 ppm and (f) 10.0 ppm.

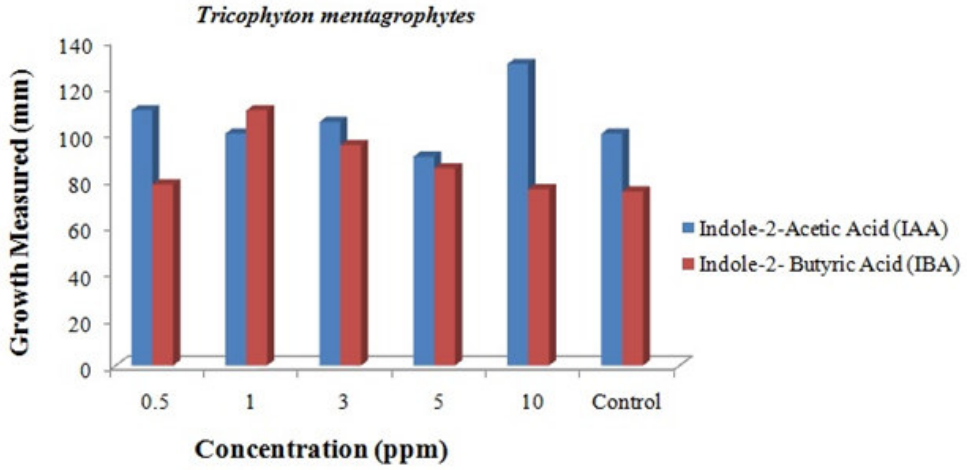

Figure 4
Effect of Indole-2-Acetic acid (IAA) and IBA (Indole-2-Butaric acid) on Tricophyton Mentagrophytes against their growth and development

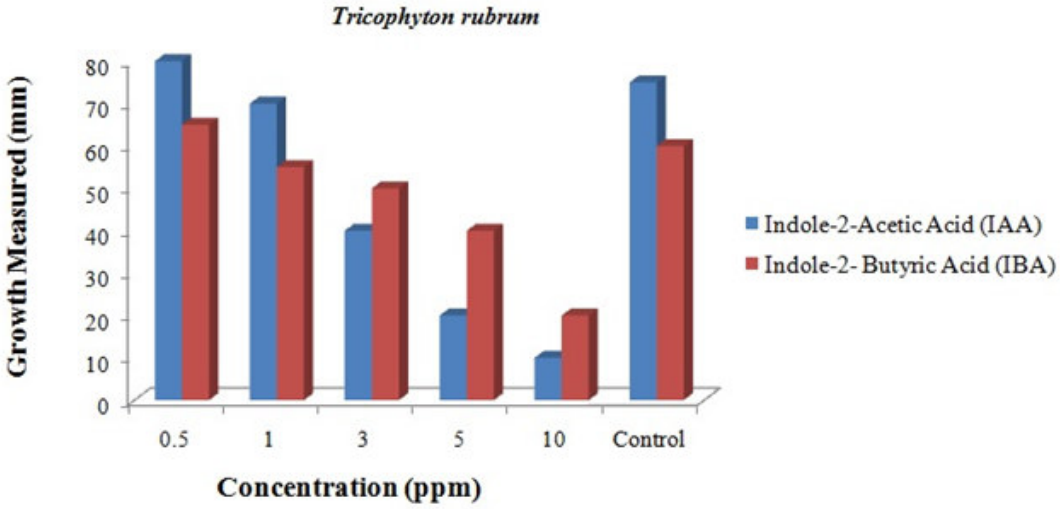

Figure 5
Effect of Indole-2-Acetic acid (IAA) and IBA (Indole-2-Butaric acid) on Tricophyton rubrum against their growth and development

DISCUSSION

The present study was intended to decipher the morphological and taxonomical changes amongst five different fungal isolates against some biochemical tests (Sodium chloride and hormones). Earlier, several studies have been documented on various fungi and compared effect on their growth and development against several biochemical tests[15-18]. Here, the fungal isolates namely *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum gypseum*, *Microsporum canis* and *Chrysosporium tropicum* were tested on various concentration of Sodium chloride (NaCl) supplemented in SDA medium. The remarkable growth and sporulation were identified at between 0.5 % to 3% NaCl concentrations within *T. mentagrophytes*, *T. rubrum*, *M. gypseum* and *C. tropicum* but the retarded response was seen over exceeding 3% NaCl concentration. This result is in compliance with earlier report which explained the most satisfactory response was seen at 3% NaCl concentration and the growth was further decreased at above 3% concentration but macroconidia formation was seen at 3% and 5% concentration[19]. However, *M. canis* showed a tolerance towards high (>10%) NaCl concentration and this high salt tolerance is in agreement with previous report on some fungal isolates which revealed tolerance towards high NaCl concentration at ranged between 6% to 11% against 196 strains of genus *Aspergillus* (21 different species) and in 87 strains of dermatophytes (4 different species)[20].Additionally, the growth suppressing response against higher NaCl concentrations (onward 12 %) was also reported on the growth and morphology of some dermatophytes and keratinolytic fungi[2]. Moreover, Kane and summerbell[21] who found Sodium chloride as aid in identification of *Phaeoannellomyces werneckii* and other medically important dermatiaceous fungi and reported *P. werneckii* found to be distinguished by its tolerance against high NaCl concentration (15%). Likewise, the Sodium chloride tolerance was evaluated in strains of *Epidermophyton floccosum* and *E. stockdale* at different concentration ranging from 0 to 10% and found separation towards their diameter of colonies development[22]. Similarly, the

different level of NaCl tolerance was reported on various fungi isolated from Salt pan which were as follow; *T. mentagrophytes* (0.8%), *Malbranchea* spp. (0.8%), *Uncinocarpus reesii* (1.6%), *C. fluviale* (1.6%), *Malbranchea aurantiaca* (2.4%), *Trichophyton terrestre* (3.2%), *Ctenomyces serratus* (4.0%), *C. tropicum* (5.6%), *Microsporum gypseum* complex (7.2%) and *Chrysosporiumindicum* (12.0%)[23].Significantly, only two fungi i.e. *T. mentagrophytes* and *T. rubrum* revealed excellent growth and sporulation against two synthetic hormones (IAA and IBA). It was found that plant growth hormones influenced the growth of microorganisms and their cell composition beside of their effect on cell elongation, tissue swelling, cell division, and formation of adventitious roots[12]. In the present study, the remarkable response was reported at lower hormones concentration, ranged between 0.5% to 3% ppm concentration and inhibition was displayed on increasing concentrations. This observation is in compliance with earlier studies which reflected a significant response on various fungi at lower hormone concentrations[24-27]. Similarly, Reddy and Strzelczyk[28] reported that the growth response on *Rhizoctonia solani* was markedly influenced by various concentrations of gibberellins (GA3) and kinetin but IAA revealed depressing growth response. Furthermore, the plant growth hormone abscisic acid (ABA) showed inhibitory effect on *Colletotrichum acutatum* but mycelial growth of *C. acutatum* found to be upright on Potato Dextrose Agar (PDA) inoculated with 2 mM of ABA[29]. Additionally, the effect of some growth regulators such as progesterone, testosterone and estradiol were used which revealed retarded fungal growth of anthropophilic species (*T. rubrum*, *T. tonsurans* and *E. floccosum*), zoophilic strains (*T. mentagrophytes*, *M canis*)[30]. Accordingly, *T. rubrum* and *E. floccosum* showed the highest sensitivity to androgenic hormones amongst derrnatophytes[31]. Furthermore, Wang et al.[32] reported increase dry weight of the lichen-forming fungus *Nephromopsis ornate* using 2,3,5-triiodobenzoic acid (TIBA) and indole-3-butyric acid (IBA). Several functional activities as well as retarded effects of plant growth hormones have been observed on microbial growth and development[8, 33-36]. Furthermore, the several earlier studies have been concentrated on several fungi for the enhancement of their biomass production and enrichment of metabolic products by using regulatory role of different plant growth hormones[12, 34 , 37].

CONCLUSION

This study was intended to examine the response of some biochemical like Sodium chloride test and hormones on five different fungal isolates (*Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum gypseum*, *Microsporum canis* and *Chrysosporium tropicum*). The distinguished tolerance revealed by each fungus against biochemical effect and different morphological variations were considered in the form of changing in their colony pattern, growth rate and sporulation. Thus, we can say that the biochemical analysis is an important criteria to identify specific fungal isolate by using chemical tolerance in relation to their microscopic morphologies, colony features and growth requirements.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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Reviewers of this article

Dr.Premlata Singariya

UGC - Post Doctoral Fellow ,
Dept. of Botany ,
University of Rajasthan ,
Jaipur 302004.

Prof.Dr.Prapurna Chandra Rao

Assistant Proffessor, KLE University,
Belgaum, Karnataka

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal
of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International
Journal of Pharma and Bio sciences.

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