Research Article

Effect of Growth Conditions on Antibacterial Activity of *Trichoderma harzianum* against Selected Pathogenic Bacteria

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Abstract | Bacterial infections are prevalent in crops and humans, therefore, the need for novel and effective antibacterial compounds to treat, prevent and resist is highly demanded. Luckily fungi are potential producer of antibacterial natural products. The present study was initiated to investigate the antibacterial activity of *Trichoderma harzianum* against phyto and human pathogenic bacterial strains. *T. harzianum* was grown under different conditions and their extracts were evaluated, using disc diffusion method against tomato wilt causing bacteria (*Xanthomonas campestris* and *Clavibacter michiganensis*). Extracts that resulted in strong activity were fractionated into acetonitrile and ethyl acetate followed by testing against human pathogenic bacterial strains (*Eschericia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*) using 96 well microtitre plate method. The Ethyl acetate fraction showed maximum zone of inhibition (24.25±1.06 mm) against *X. campestris* and (23.15±2.12 mm) against *C. michiganensis* on growth nutrient broth (GNB) medium as compared to other culture media (Potato dextrose broth and yeast extract broth). Similarly maximum zone of inhibition was observed in optimized parameters like shaking flask culture, 25 °C temperature, media of pH 5 and light fermentation. The EtOAc extract exhibited the minimal value of MIC (10.41±4.50 μg/mL) against gram negative *E. coli*, while its acetonitrile fraction yielded (6.50±2.25 μg/mL) of MIC against *S. aureus*. Hence, it is revealed from the results that *T. harzianum*is a potential source of antibiotic drugs as it showed a wide range of bioactivity against both phyto and human pathogenic bacteria.

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Introduction

Food production needs to be increased to meet the need of rapidly expanding world population. Unfortunately, our plants suffer from diseases caused by both agents of abiotic and biotic nature; among biotic factors, diseases caused by bacteria are more potent (Sabat et al., 2009). Also, bacterial resistance to the available pesticides/bactericides due to extensive use and misuse of antibiotics has been observed in the last decades (Nascimento et al., 2000), which means that the current available antimicrobial agents are insufficient to control microbial infections (Cowan, 1999; Bax and Mullan, 2000). Development of new drugs, especially in the area of infectious diseases is one of the most important challenges in today's world. Fungi as a source of natural products have attracted great attention of the scientists due to their diversity. The multiplicity in fungal metabolites keeps them interesting candidates to be used as novel yet potent drugs in future. Besides, fungus is known to acquire a significant role in biotechnological applications because of diverse secondary metabolites and their products are recently exploited as potent biopesticides (Prami-

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la and Dubey, 2004).

In past, plants were considered to be the only source of treatment for various diseases but the production of diverse bioactive metabolites from fungi has changed the trend (Strobel, 2003). Some fungi have the potential to produce toxins (antibiotics) that can destroy other microbes even at low concentration (Islam et al., 2005). The diversity of these toxins has shown various activities against both prokaryotes and eukaryotes (Faull, 1988). Fungi can produce a variety of metabolites belong to flavonoids, alkaloids and terpenoids that has antibacterial, antiviral, anti-inflammatory, antitumor, and antifungal properties (Guo et al., 2007; Yu et al., 2010; Aly et al., 2011).

Trichoderma harzianum belongs to ascomycete group (Order Hypocreales), which is extracted mainly from soil and decomposed organic material. The species are distributed globally and their isolates are characterized for producing abundant spores (conidia) in culture media with green shade having fast growth rate. They exist as saprophytes that require minimum nutrition and are efficient against variety of microbes (Grondona et al., 1997). *Trichoderma sp*. has also been reported to produce excessive metabolites with antimicrobial activities (Vinale et al., 2008). This study was investigated to observe the antibacterial potential of *T. harzianum*, isolated from soil of Khyber Pakhtunkhwa, Pakistan, against both phytopathogenic and human pathogenic bacteria. *T. harzianum* was grown in laboratory under different growth conditions to study the effect of antibacterial compounds on selected bacteria. Extracts that showed strong activities were fractionated into ethyl acetate and acetonitrile followed by testing for minimal inhibitory concentration (MIC).

Materials and Methods

Isolation of *T. harzianum*

Rhizosphere soil was collected upto a depth of 6 cm from different areas of Peshawar Khyber Pakhtunkhwa, including Malakandher farm as well. Soil samples were put in zipped polyethylene plastic bags and brought to Research Laboratory of Natural Product, Department of Agricultural Chemistry, University of Agriculture Peshawar. The protocol of Gaddeyya et al. (2012), was employed in the isolation of fungi from soil using serial dilution method. Isolate was purified using potato dextrose agar (PDA) medium by incubation at 27 °C for 7 days and stored at 4 °C for further work.

Morphological characterization

Fungus was identified based on morphological characterization using the method of Domsch et al. (1980). Identification was done through light microscope by studying macroscopic features, i.e. shape, growth and color of the cultured colonies as well as microscopic features, like structure of conidia, hyphae and spore size.

Effect of nutrient media

Three different types of cultural broth media: Potato dextrose broth (PDB), Yeast extract broth (YEB) and Growth nutrient broth (GNB) were prepared for fermentation of *T. harzianum* (Jain and Pundir, 2011) with the aim to check the effect of nutrient media on the antibacterial activity and to select the suitable medium for further studies.

Effect of static and shaking flask culture

T. harzianum was grown under static and shaking conditions to check the effect of aeration on the release of bioactive compounds and its activity. The spores were inoculated in (1 L) conical flask containing 200 mL selected (GNB) medium and kept at room temperature on shaking incubator with 150 rpm for 12 days (Enman et al., 2008). For comparison, the spores were inoculated in (1 L) conical flask containing 200 mL selected media and kept in the same incubator with no shaking for 12 days. Extracts obtained were tested for antibacterial activity.

Effect of light

The spores of fungus were inoculated in (GNB) growth medium and fermented for 12 days at day light. During fermentation fungal growth was observed (Fanelli et al., 2012). To compare the efficiency of antibacterial compounds produced, same conditions were used for fermentation with the exception of wrapping aluminium foil around cultural flasks and kept them in dark. The effect of light and dark conditions on the production of bioactive compounds was confirmed by the antibacterial bioassay.

Effect of pH

Prepared media (1 L) was distributed into five different conical flasks. The pH of the media was adjusted as pH 5,6,7,8 and 9 by adding drop wise, either 1 N HCl or NaOH. After pH adjustment, the media in

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each flask was sterilized at 121 °C for 20 min, followed by the inoculation of spore suspension using sterile wire loop in clean laminar flow hood. Fermentation was carried out in an incubator (27 °C) in order to test the effect of pH on antibacterial activity of the mycelia (Jain and Pundir, 2011).

Effect of temperature

The spores of *T. harzianum* was inoculated in sterilized (GNB) (200 mL) media at 25 °C , 30 °C and 35 °C to check the effect of temperature on the production and effectiveness of the bioactive compounds. The extracts obtained after fermentation were tested for antibacterial activity (Mogensen et al., 2009; Sood, 2011).

Extraction of secondary metabolites

After cultivation of fungus under different fermentation conditions, the mycelia were separated from broth through vacuum filtration. After drying, the mycelia were homogenized in ethyl acetate for 24 hours in order to extract secondary metabolites. Ethyl acetate was then removed with the help of rotary evaporator at 46 °C. The collected organic crude extracts were finally tested against phytopathogenic bacteria using disc diffusion method (Zain et al., 2008).

Disc diffusion susceptibility test

According to Tong et al. (2011), the colonies of phytopathogenic bacteria (*Xanthomonas campestris* and *Clavibacter michiganensis*) were adjusted to approximately 1×10^8 cfu/ mL in sterile nutrient broth medium. Approximately, 50 µl of bacterial suspension was streaked on nutrient agar plate with sterile cotton swab followed by incubation at 37 °C for 12 hrs. On the next day, 20 µl of test sample (1mg crude extract/ 1mL 10% dimethyl sulfoxide or DMSO was added to 6mm sterile whatman filter paper disc, positioned on the surface of nutrient agar medium plate containing the test bacteria. Negative control used in this test was 10% DMSO, whereas for positive control Streptomycin was used. Petri plates were incubated at 37 °C for 24 hrs and inhibition zones were measured in millimeters.

Determination of minimal inhibitory concentration (MIC)

Ethyl acetate extract and acetonitrile fraction were tested for MIC against human pathogenic bacterial strain [two gram negative bacteria (*Escherichia coli* and *Pseudomonas aeuroginosa*) and one gram positive bacteria (*Staphylococcus aureus*)] following the procedure described by National Committee for Clinical Laborotory Standards (2002). Suspension of bacterial strains were made in 3% Tryptic soy broth (TSB) medium at 37 °C for 12 hours followed by two times washing with 10mM Tris buffer (pH 7.4) and quantified at OD_{600}. In this set of experiments, 96-well plates (U-shaped, untreated polystyrene) were used. Samples were prepared through serial dilution with tris buffer and bacterial cells were added to a final volume of 100 µL. Plates were incubated in aerobic incubator at 37 °C , for 6, 8 and 10 hours according to the growth period of particular bacteria.

Statistical analysis

The experiments were carried out in three replicates and the results of the data are presented as mean ± SD (standard deviation) using statistical software Statistix 8.1 (Steel and Torrie, 1997).

Results

Morphological characterization

Trichoderma harzianum was isolated from soil of Peshawar, Khyber pakhtunkhwa Pakistan using serial dilution method that was purified on potato dextrose agar medium. The microbial colonies were identified through morphological characteristics. The colour of the *T. harzianum* colonies was observed dark green. *Trichoderma* has two types of conidiophores and phialides arrangements. Conidiophores are mostly smooth and rounded while phialides are crowded having link with conidiophores. The walls of conidiophores arouse were smooth and the heads of conidia were subglobose with recorded size of 3-4 µm (Figure 1).

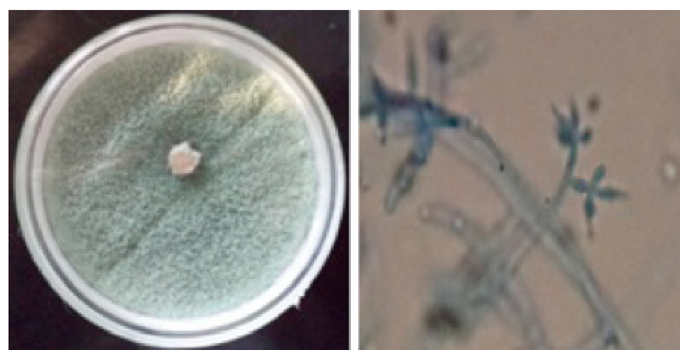

Figure 1: *Pure culture and microscopic image of T. harzianum*

Disc diffusion susceptibility test of different parameters

Effect of growth media

Ethyl acetate extract of *T. harzianum* obtained from

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three different growth media were tested against phytopathogenic bacteria (*X. campestris* and *C. michiganensis*) through disc diffusion assay and zone of inhibitions were recorded (Figure 2). The crude extract obtained on growth nutrient broth (GNB) medium showed maximum zone of inhibition 24.25±1.06 mm against *X. campestris* and 23.15±2.12 mm against *C. michiganensis* followed by the extract obtained from potato dextrose broth (PDB) medium. While the extract obtained from Yeast extract broth (GYEB) medium showed no activity against both the phytopathogenic bacterial species. The inhibition zone in positive control (Streptomycin) regarding *X. campestris* and *C. michiganensis* was found to be 28±1.41 mm and 31±1.41 mm, respectively (Table 1).

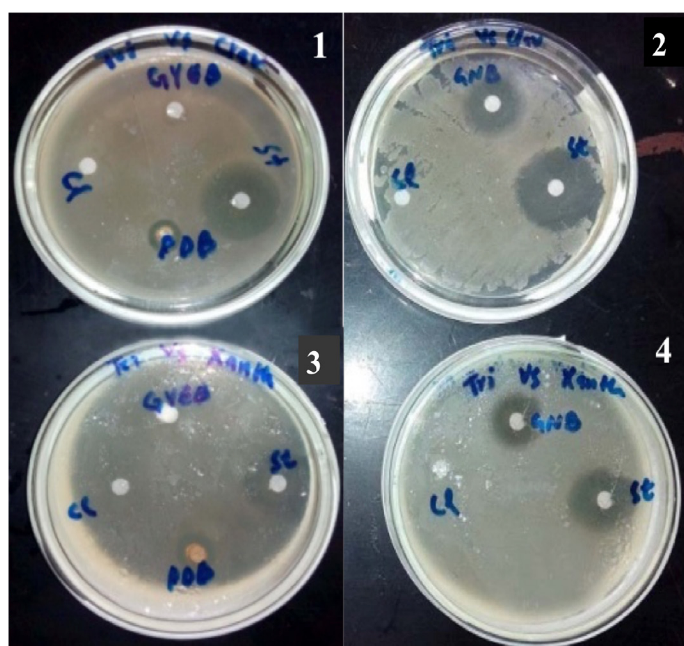

Figure 2: *Antibacterial activity of crude extract of Trichoderma harzianum grown on different media against Clavibacter michiganensis (1,2) and Xanthomonas campestris (3,4)*

Table 1: *Zone of inhibition of Trichoderma harzianum extract obtained from different growth media against Xanthomonas campestris and Clavibacter michiganensis using disc diffusion method.*

Fungus/ Standard	Culture Media	Zone of Inhibition (mm)±SD	
		Xanthomonas campestris	*Clavibacter michiganensis*
T. harzianum	GNB	24.25±1.06	23.15±2.12
	PDB	15.75±1.06	13.25±0.35
	GYEB	0±0.00	0±0.00
Streptomycin		28±1.41	31±1.41

Effect of static and shaking flask culture

Ethyl acetate extract obtained from growth nutrient broth (GNB) medium showed maximum zone of inhibition against wilt causing bacteria and therefore, selected for further optimization. Extract of *T. harzianum* obtained from both shaking and static flask culture of GNB medium were tested against phytopathogenic bacteria (*X. campestris* and *C. michiganensis*) through disc diffusion assay and zone of inhibitions were recorded (Figure 3). The crude extract of *T. harzianum* obtained from shaking flask culture inhibited the growth of *X. campestris* by 25.25±0.35 mm and *C. michiganensis* by 23.75±1.06 mm, while it was 19±0.70 mm and 16.25±0.35 mm, respectively in static flask culture extract. Positive control (Streptomycin) showed 29.5±0.70 mm zone of inhibition against *X. campestris* and 31.25±0.35 mm against *C. michiganensis* (Table 2).

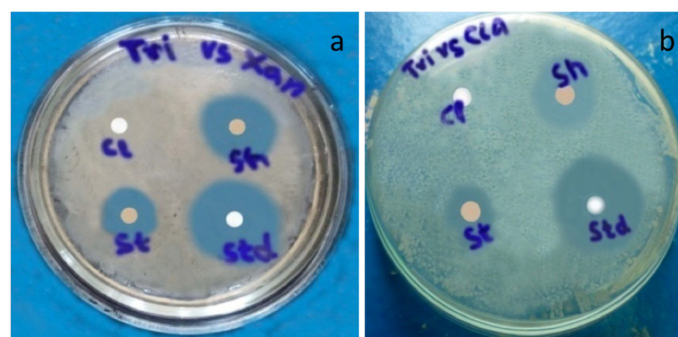

Figure 3: *Antibacterial activity of crude extract of Trichoderma harzianum from (Shaking (Sh) & static (St) flask culture against Xanthomonas campestris 3(a)and Clavibacter michiganensis 3(b)*

Table 2: *Zone of inhibition of Trichoderma harzianum extract obtained from shaking and static flask cultures against Xanthomonas campestris and Clavibacter michiganensis*

Fungus/Std	Flask Culture	Zone of inhibition (mm) ±SD	
		X. campestris	*C. michiganensis*
T. harzianum	Shaking	25.25±0.35	23.75±1.06
	Static	19±0.70	16.25±0.35
Streptomycin		29.5±0.70	31.25±0.35

Effect of light

T. harzianum was cultured in growth nutrient broth (GNB) medium in shaker at 150 rpm at both light and dark conditions. The ethyl acetate extracts obtained were tested against phytopathogenic bacteria (*X. campestris* and *C. michiganensis*) through disc diffusion method and zone of inhibitions were meas-

ured (Figure 4). The crude extract of *T. harzianum* obtained in light fermentation inhibited the growth of *X. campestris* by 25.25±1.06 mm and *C. michiganensis* by 22.5±0.55 mm. The recorded zone of inhibition in dark against *X. campestris* was 24±0.70 mm and *C. michiganensis*was 20.75±0.35 mm. Positive control (Streptomycin) showed 29.25±1.06 mm zone of inhibition against *X. campestris* and 31.75±2.47 mm against *C.michiganensis* (Table 3).

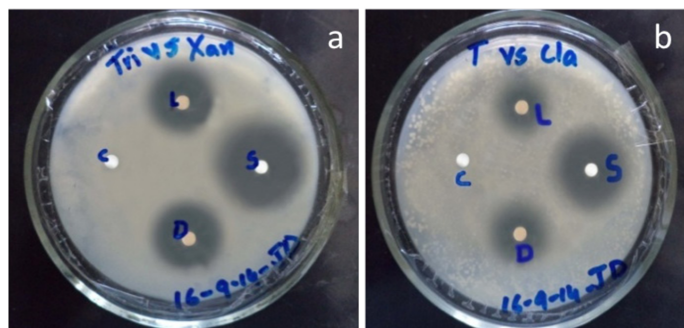

Figure 4: *Antibacterial activity of crude extract of Trichoderma harzianum from (Light (L) & Dark (D) fermentation against Xanthomonas campestris 4(a) and Clavibacter michiganensis 4(b)*

Table 3: *Zone of inhibition of Trichoderma harzianum extract obtained from light and dark fermentation against Xanthomonas campestris and Clavibacter michiganensis.*

Fungus/Std	Fermentation	Zone of inhibition (mm) ±SD	
		X. campestris	*C. michiganensis*
T. harzianum	**Light**	25.25±1.06	22.5±0.55
	Dark	24±0.70	20.75±0.35
Streptomycin		29.25±1.06	31.75±2.47

Effect of pH

Ethyl acetate extract of *T. harzianum* obtained from GNB medium in shaking flask culture at light condition from different pH values were tested against phytopathogenic bacteria (*X. campestris* and *C. michiganensis*) (Figure 5). *T. harzianum* showed fast growth at pH 5 with maximum activity, i.e. 26.25±0.35 mm zone of inhibition against *X. campestris* and 23.5±0.45.70 mm against *C. michiganensis*. The antibacterial activity was decreased as the pH was increased from 5. At pH 6 it showed 19.5±0.65 mm zone of inhibition against *X. campestris* and 18.75±0.35 mm against *C. michiganensis*. At neutral pH, it yield 17.5±0.70 mm zone of inhibition against *X. campestris* and 16.5±0.75 mm against *C. michiganensis*. At alkaline pH 8 and 9, slow growth was observed with less activity against *X. campestris* 15±0.707 mm and 12.75±0.3535 mm and *C. michiganensis* 13.5±0.55 mm and 11.5±0.15 mm) (Table 4). Positive control (Streptomycin) showed 29.75±0.35 mm zone of inhibition against *X. campestris* and 31.5±0.25 mm against *C. michiganensis*.

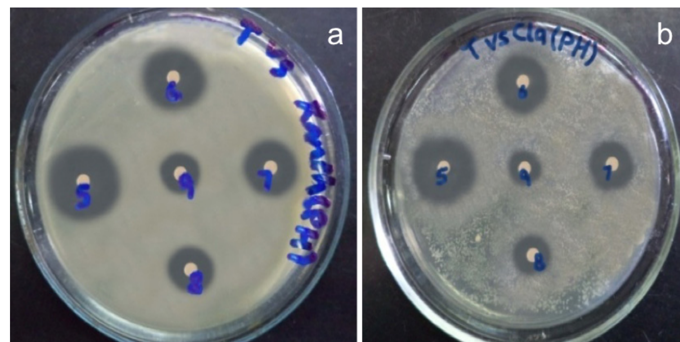

Figure 5: *Antibacterial activity of crude extract of Trichoderma harzianum of (pH 5,6,7,8,9) against Xanthomonas campestris 5(a) and Clavibacter michiganensis 5(b)*

Table 4: *Zone of inhibition of Trichoderma harzianum extract obtained from different pH against Xanthomonas campestris and Clavibacter michiganensis.*

Fungus/Std	pH	Zone of inhibition (mm) ±SD	
		X. campestris	*C. michiganensis*
T. harzianum	**5**	26.25±0.35	23.5±0.45.70
	6	19.5±0.65	18.75±0.35
	7	17.5±0.70	16.5±0.75
	8	15±0.70	13.5±0.55
	9	12.75±0.35	11.5±0.15
Streptomycin		29.75±0.35	31.5±0.25

Table 5: *Zone of inhibition of Trichoderma harzianum extract obtained at different temperature against Xanthomonas campestris and Clavibacter michiganensis.*

Fungus/Std	Temperature	Zone of inhibition (mm) ±SD	
		X. campestris	*C. michiganensis*
T. harzianum	25 °C	23.5±0.45	24.25±0.35
	30 °C	18.25±0.35	18.75±1.06
	35 °C	14±0.70	15.75±0.35
Streptomycin		28.25±0.35	29.5±0.70

Effect of temperature

After the optimization of growth conditions for *T. harzianum*, the ethyl acetate extracts were studied for antibacterial activity against phytopathogenic bacteria (*X. campestris* and *C. michiganensis*) (Figure 6). *T. harzianum* showed 23.5±0.45 mm zone of inhibition against *X. campestris* and 24.25±0.35 mm against *C. michiganensis* at 25 °C . At 30 °C it yielded

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18.25±0.35 mm zone of inhibition against *X. campestris* and 18.75±1.06 mm against *C. michiganensis*. On the contrary, at 35 °C both yield and bioactivity was reduced to 14±0.70 mm inhibition zone against *X. campestris* and 15.75±0.35 mm inhibition zone against *C. michiganensis*. Positive control (Streptomycin) showed 28.25±0.35 mm zone of inhibition against *X. campestris* and 29.5±0.70 mm against *C. michiganensis* (Table 5).

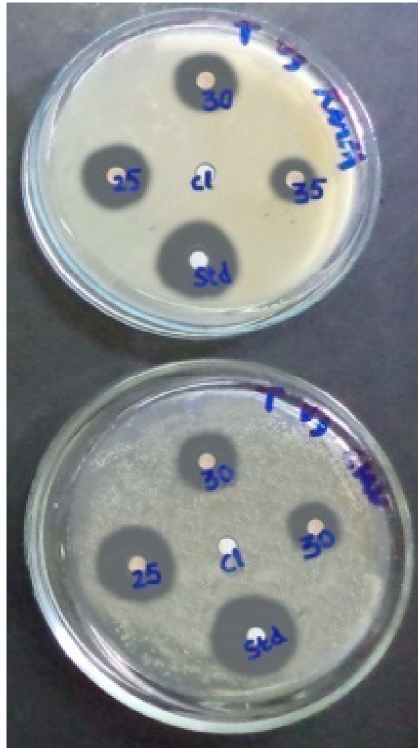

Figure 6: *Antibacterial activity of crude extract of Trichoderma harzianum (at 25,30 & 35 °C) against Xanthomonas campestris (above) and Clavibacter michiganensis (below)*

Microdilution assay

Acetonitrile fraction and ethyl acetate fraction of *T. harzianum* were screened for minimal inhibitory concentration (MIC) against human pathogenic bacterial strains (*Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*) and results were recorded in µg/ml (Figure 7). MIC values of organic crude extract and acetonitrile fraction of *Trichoderna harzianum* are presented in Table 6. The MIC test indicated that organic crude extract exhibited the minimal value of MIC against gram negative *Escherichia coli* 10.41±4.50 µg/mL. While, MIC value of 41.66±18.04 µg/mLwas recored for *Pseudomonas aeruginosa* and 83.33±36.08 µg/mL for *Staphylococcus aureus*. Similarly, the MIC of acetoniltrile fraction against *Escherichia coli* was 83.33±36.08 µg/mL, followed by 72.91±47.73 µg/mL against *Pseudomonas aeruginosa,* and 6.50±2.25 µg/mL for *Staphylococcus aureus*. The MIC value recorded for positive control (Streptomycin) against *Escherichia coli* was 5.63±8.9 µg/mL and for *Pseudomonas aeruginosa* was 7.84±9.45 µg/mL, while it exhibited the minimal value 5.09±11.4 µg/mL for the gram positive *Staphylococcus aureus*.

Discussion

Fungi are rich source of bioactive compounds (drugs etc.) that have drawn the attention of natural product chemists for combating the current bacterial resistance, which resulted in the discovery of novel antibiotics with unique scaffold (Luzhetskyy et al., 2007). Naturally occurring antibiotics are reported mostly from soil borne fungi that are less explored organisms

Figure 7: *Minimum inhibitory concentration of ethyl acetate extract of T. harzianum against P. aeruginosa*

Table 6: *Minimal Inhibitory Concentration of ethyl acetate and acetonitrile fraction of Trichoderma harzianum against human pathogenic bacterial strains using dilution method ELISA plate method.*

Fungus/Std	Fractions	MIC (µg/mL) ±SD		
		Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Trichoderm harzianum	Ethyl acetate	10.41±4.50	41.66±18.04	83.33±36.08
	Acetonitrile	83.33±36.08	72.91±47.73	6.50±2.25
Streptomycin		5.63±8.9	7.84±9.45	5.09±11.4

(Hara-Kishore et al., 2007) and therefore many investigations are carried out to isolate compounds from fungi to fight pathogenic microbes. The first step in these investigations is the isolation of fungi from its natural habitat (Hunter-Cevera et al., 1999). *Trichoderma harzianum* was isolated from soil, purified and identified using light microscope and studied upto species/generic level (Figure 1). Macroscopic and microscopic features were in correspondence to the findings of (Sutton, 1980; Alexopoulos et al., 1996; Kirk et al., 2001).

Suitable medium for the production of bioactive compounds is very important, that satisfy the requirements for the metabolite production (Stanbury et al., 1997). El-Tayeb et al. (2004) and Rizk et al. (2007) optimized various cultural conditions for the production of fungal metabolites. Fungal extracts obtained from different growth media showed different zone of inhibitions against phytopathogenic bacteria in disc diffusion method. The medium in which fungus produced potent bioactive compounds and showed maximum inhibition zone was selected. Here maximum zone of inhibition was recorded from crude extract of growth nutrient broth medium (GNB), in comparison to the crude extracts of other two broth media; yeast extract broth (GYEB) and potato dextrose broth (PDB) media indicating that this fungus can produce bioactive compounds in the medium having nutrient composition of [10 grams glucose (Carbon source), 5 grams peptone (Nitrogen source) and 3 grams yeast extract (mixture of amino acids, peptides, water soluble vitamins, minerals etc] (Figure 2). Slininger and Shea-Wilbur (1995) also studied the effect of growth media on antibacterial activity, and suggested that secondary metabolites are highly effected by the nutrient composition of growth media. Ramos and Said (2011) described that nutrient composition of broth media can increase/improve antibacterial activity. Vijaykumar et al. (2013) suggested that specific growth media can result in production of high concentration of bioactive compound that can increase the activity. Our results are in agreement with findings of above cited mycologists who demonstrated growth nutrient broth as an important factor for fungal bioactive compounds production.

It is evident from literature that shaking flask culture can produce more bioactive metabolites as compared to static flask culture (Helmholz et al., 1999). Bhattacharyya and Jha (2011) optimized various cultural conditions and concluded that shaking flask culture has significant impact on bioactive compounds production during fermentation. Our results also confirmed that shaking flask culture may produce a bit more bioactive secondary metabolites as compared to static flask culture (Figure 3).

Fang et al. (2010) optimized various growth conditions of fungus for production of bioactive antimicrobial compounds and did not observe any drastic change in the compounds effected by light and dark. Our results also showed similarity in antibacterial activity obtained from light and dark fermentation (Figure 4). Jain and Pundir (2011) also observed a slight change in activities from fungal extracts grown under light and dark conditions.

Secondary metabolite production is markedly effected by pH value of the medium. Cell biomass is directly affected by the hydrogen or hydroxyl ion or indirectly by dissociating degrees of substances in medium. Change of pH is necessary for enzymatic activity, and intermediate products of microbes (Rizk et al., 2007). pH 5 was found optimum in our findings that showed maximum antibacterial activity by *T. harzianum* against phytopathogenic bacteria (Figure 5). Similar results are also described by Nishihara et al. (2001). Jain and Pundir (2011) also suggested that slight acidic medium can result in maximum and potent bioactive compounds.

Optimization of physical factor (temperature) can significantly affect the yield of bioactive compounds (Llorens et al., 2004). Our results were according to the findings of (Suzuki et al., 1997 and Fang et al., 2007) who demonstrated 25-27 °C temperature as optimum/ideal for production of antimicrobial compounds (Figure 6). Jain and Pundir (2011) optimized cultural conditions for soil fungal metabolite production and reported maximum antibacterial activity of the extract obtained at temperature 25 °C . Miao et al. (2006), described that growth of fungus increases with increase in temperature from 15 to 30 °C but it slows down when temperature exceeds 30 °C , similarly antibacterial activity of the fungus was observed maximum at temperature ranging from 15 to 25 °C , whereas it was decreased at 30 °C and completely stopped at 35 °C .

Many useful bioactive metabolites have been produced by fungi that are consumed in drug discovery,

this field of pharmacology is fascinating and therefore production of safe compounds for treating different diseases is a hot research spot nowadays (Ramesh et al., 2014). Leelavathi et al. (2014) suggested that *T. harzianum* possess antimicrobial properties against microbes and inhibited the growth of clinical isolates *Staphylococcus aureus*, *E. coli* and *Klebsiella*. MIC recorded against bacteria was (50-100 µg/ml). *T. harzianum* showed good activity in 96 well microtitre plate dilution method against human pathogenic bacterial strains, hence possessing some medical importance as well (Figure 7). Its acetonitrile fraction and ethyl acetate extract inhibited both gram positive and gram negative bacteria and the minimum inhibitory concentration values observed fall within the cut off value of (Kuete, 2010) which are [significant = (MIC ≤ 100 µg/ ml), moderate = (100 - 625 µg/ ml) and weak = (MIC ≥ 625 µg/ ml).Our findings fall in significant zone of MIC. The MIC values of *T. harzianum* were quite promising when compared with the MIC values of Xiao-Yan et al. (2006).

Conclusions

Trichoderma harzianum possess antibiotic properties, as it inhibited both bacteria (Phyto and human pathogenic). This fungus is a rich source of antibacterial compounds which can be promising agent against agricultural and medical pathogenic bacteria. Different activities recorded from different media and parameters suggested that this fungus can produce different metabolites of interest under different environmental conditions, hence improved and novel bioactive compounds can be isolated that might be used in the development of efficient agricultural and pharmaceutical antibacterial agents. This fungus should be explored further to combat the developing resistance and upcoming challenges in disease management both in plants and human beings. Fungal antibiotics are of natural origin that are environment friendly and less toxic, therefore exploitation of this fungus could be an efficient source of biopesticide. Further research is needed to determine structures of these antibacterial compounds for commercialization proposes.

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Author's Contribution

Jawad Anwar carried out research and wrote the manuscript. Dr. Zafar Iqbal supervised him.

References

Alexopoulos, C.J., C.W. Mims and M. Blackwell. 1996. Introductory Mycology. 4th edn. John Wiley, US.

Aly, A.H., A. Debbab and P. Proksch. 2011. Fungal endophytes, unique plant inhabitants with great promises. Appl. Microbiol. Biotechnol. 90: 1829-1845 https://doi.org/10.1007/s00253-011-3270-y

Bax, R., and V. Mullan. 2000. The millennium bugs-the need for and development of new antibacterials. Int. J. Antimicrob. Agent. 16: 51-59. https://doi.org/10.1016/S0924-8579(00)00189-8

Bhattacharyya, P.N., and D.K. Jha. 2011. Optimization of cultural conditions affecting growth and improved bioactive metabolite production by a subsurface Aspergillus strain TSF 146. Int. J. Appl. Biol. Pharmac. Technol. 2(4):133-143

Cowan, M.M. 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev.12(4): 564-582.

Domsch, K.H., W. Gamas and T.H. Anderson. 1980. Compendium of Soil Fungi. P. 168-169 and 559-560. In Academic press, New York, USA.

El-Tayeb, O.M., M.M.N. Hussein, A.A. Salama, H.F. El-Sedawy. 2004. Optimization of industrial production of rifamycin B by Amycolatopsis mediterranei. II. The role of gene amplification and physiological factors in productivity in shake flasks. Afric. J. Biotechnol. 3: 273-280. https://doi.org/10.5897/AJB2004.000-2075

Enman, J., D. Hoge, K.A. Berglund and U. Rova. 2008. Production of the bioactive compound eritadenine by submerged cultivation of Shiitake (Lentinusedodes) Mycelia.*J. Agric. Food Chem.* 56(8): 2609-2612. https://doi.org/10.1021/jf800091a

org/10.1128/AEM.71.7.3786-3796.2005

Fanelli, F., M.S. Heydt, M. Haidukowski, A. Susca, R. Geisen, A. Logrieco and G. Mule. 2012. Influence of light on growth conidiation and fumonisin production by *Fusarium verticillioides*. Fungal boil. 116(2): 246- 248.

Fang, F., H. Vi, K. Shiomi, R. Masuma, Y. Yamaguchi, C.G. Zhang, X.W. Zhang and S.Omura. 1997. Two new components of the aspochalasins produced by Aspergillus sp. J. Antibiot. 50: 919-925 https://doi.org/10.7164/antibiotics.50.919

Faull, J.L. 1988. Competitive antagonism of soilborne plant pathogens: Fungi in Biological control systems. P. 125-140. Manchester University Press, Manchester, UK.

Gaddeyya, G., P.S. Niharika, P. Bharathi and P.K.R. Kumar. 2012. Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. Adv. Appl. Sci. Res. 3(4): 2020-2026

Grondona, I., R. Hermosa, M. Tejada, M.D. Gomis, P.F. Mateos, P.D. Bridge, E. Monte and I. Garcia-Acha. 1997. Physiological and biochemical characterisation of Trichoderma harzianum, abiological control agent against soilborne fungal plant pathogens. Appl. Environ. Microbiol. 63: 3189-3198

Guo, B., Y. Wang, X. Sun and K. Tang. 2007. Bioactive Natural Products from Endophytes. A Review. Biochem. Microbiol.44: 136-142. https://doi.org/10.1134/S0003683808020026

Hara-Kishore, K., S. Misra, D.R. Chandra, K.V.V.R. Prakash and U.S. Murty. 2007. Antimicrobial efficacy of secondary metabolites from *Glomerella cingulata*. Braz. J. Microbiol. 38: 150-155. https://doi.org/10.1590/S1517-83822007000100031

Helmholz, H., P. Etoundi and U. Lindequist. 1999. Cultivation of the marine Basidiomycete Nia vibrissa. J. Biotech. 70: 203-206. https://doi.org/10.1016/S0168-1656(99)00073-5

Hunter-Cevera, J., and A. Belt. 1999. Isolation of cultures. In: Demain and Davies (Eds.). Manual of Industrial Microbiology and Biotechnology. ASM Press. Washington, DC.

Islam, M.T., H. Yasuyuki, D. Abhinandan, I. Toshiaki and T. Satoshi. 2005. Suppression of damping-off-disease in host plants by the rhizoplane bacterium Lysobacter sp. strain SB-K88 is linked to plant colonization and antibiosis against soilborne peronosporomycetes. Appl. Environ. Microbiol. 71: 3786-3796 https://doi.

Jain, P., and R.K. Pundir. 2011. Effect of fermentation medium, pH and temperature variations on antibacterial soil fungal metabolite production. J. Agric. Tech. 7(2): 247-269

Kirk, P.M., P.F. Cannon, J.C. David and J.A. Stalpers. 2001. Ainsworth and Bisby: Dictionary of Fungi. 9th ed. CAB International, UK.

Kuete, V. 2010. Potential of Cameroonian plants and derived products against microbial infections. A review. Plant medica. 76(14): 1479-1491. https://doi.org/10.1055/s-0030-1250027

Leelavathi, M.S., L. Vani and P. Reena. 2014. Antimicrobial activity of Trichoderma harzianumagainst bacteria and fungi. Int. J. Curr. Microbiol. App. Sci. 3(1): 96-103.

Llorens, A., R. Matco, M.J. Hinojo, A. Logrieco and M. Jimenez. 2004. Influence of the interactions amongecologicalvariablesinthecharacterization of Zearalenone producing isolates of Fusarium spp. Syst. Appl. Microbiol. 27:253–260. https://doi.org/10.1078/072320204322881871

Luzhetskyy, A., S. Pelzer and A. Bechthold. 2007. The future of natural products as a source of new antibiotics. Curr. Opin. Investig. Drugs. 8: 608-613.

Miao, L., T.F.N. Kwong and P.Y. Qian. 2006. Effect of culture conditions on mycelial growth, antibacterial activity, and metabolite profiles of the marine-derived fungus Arthrinium c.f. saccharicola. Appl. Microbiol. Biotechnol. 72: 1063–1073. https://doi.org/10.1007/s00253-006-0376-8

Mogensen, M.J., F.N. Kristian, A.S. Robert, C.F. Jens and T. Ulf. 2009. Effect of temperature and water activity on the production of fumonisins by Aspergillus niger and different Fusarium species. BMC, Microbiol. 9: 281. https://doi.org/10.1186/1471-2180-9-281

Nascimento, G.F., L. Juliana, C.F. Paulo and L.S. Giuliana. 2000. Antibacterial activity of plant extracts and Phytochemcicals on antibiotic resistant bacteria. Braz. J. Microbial. 31: 247-256.

National Committee for Clinical Laborotory Standards. 2002. Performance standards for antimicrobial susceptibility testing, twelfth information supplement. M100-S12. NCCLS. Wayne. https://doi.org/10.1590/S1517-83822000000400003

Nishihara, Y., E. Tsujii, Y. Yamagishi, K. Sakamoto,

Y. Tsurumi, S. Furukawa, R. Ohtsu, T. Kino, M. Hino, M. Yamashita and S. Hashimoto. 2001. FR 198248 a new antiinfluenza agent isolated from Aspergillus terreusNo. 13830. J. Antibiot. 54: 136-143. https://doi.org/10.7164/antibiotics.54.136

Pramila, T., and N.K. Dubey. 2004. Exploitation of natural products as an alternative strategy to control post harvest fungal rotting of fruit and vegetables. Posthar. Biol. Technol. 32:235-245. https://doi.org/10.1016/j.postharvbio.2003.11.005

Ramesh, V., C. Karunakaran and A. Rajendran. 2014. Optimization of submerged culture conditions for mycelial biomass production with enhanced antibacterial activity of the medicinal macro fungus Xylariasp. Strain R006 against drug resistant bacterial pathogens. Curr. Res. Environ. Appl. Mycol. 4(1): 88-98.

Ramos, H.P., and S. Said. 2011. Modulation of biological activities produced by an endophytic fungus under different culture conditions. Adv. Biosci. and Biotech. 2: 443-449. https://doi.org/10.4236/abb.2011.26065

Rizk, M., T. Abdel-Rahman and H. Metwally. 2007. Factors affecting growth and antifungal activity of some Streptomycesspecies against Candida albicans. J. Food Agric. Environ.5: 446-449.

Sabat, J., and N. Gupta. 2009. Development of modified medium for the enhancement in antifungal activity of P. steckii (MF1 Mangrove Fungi) against Verticillium Wilt pathogenic fungi of rose. Braz. Arch. Boil. Technol. 52(4): 809-818. https://doi.org/10.1590/S1516-89132009000400003

Slininger, P.J., and M.A. Shea-Wilbur. 1995. Liquid culture pH, temperature carbon and nitrogen source regulate phenazine productivity of the take all biocontrol agent pseudomonas fluorescence. Appl. Microbial. Biotechnol. 37: 388-392. https://doi.org/10.1007/BF00210998

Sood, M. 2011. Effect of temperature of incubation on the growth, sporulation and secondary metabolites production of *Aspergillus umbrosus*. J. Phytol. 3(4): 35-37.

Stanbury, P.F., A. Whitaker and S.J. Hall. 1997. Principles of fermentation technology. Elsevier. London.

Steel, R., and J.H. Torrie. 1997. Principles and procedures of statistic, 3rd Edn, MC Graw Hill book co, Inc., Singapore. Pp. 172-177.

Strobel, G.A. 2003. Endophytes as sources of bioactive products. Microb. Infect. 5: 535-544. https://doi.org/10.1016/S1286-4579(03)00073-X

Sutton, B.C. 1980. The Coelomycetes: Fungi Imperfecti with Pycnidia, Acervuli and Stromata. Common wealth Mycological Institute, Kew, England.

Suzuki, K., A. Kuwahara, H. Yushida, S. Fujita, T. Nishikiori and T. Nakagava. 1997. NF00659AJ, A2, A3, BI and B2, novel antitumor antibiotics produced by Aspergillussp. NF00659. J. Antibiot. 50: 344-347. https://doi.org/10.7164/antibiotics.50.318

Tong, W.Y., I. Darah and Z. Latifah. 2011. Antimicrobial activities of endophytic fungal isolates from medicinal herb Orthosiphonstamineus Benth. J. Med. Plant Res. 5(5): 831-836.

Vijaykumar, S.J., N.K. Sasidharannair, B. Nambision and C. Mohandas. 2013. Optimization of media and temperature for enhanced antimicrobial production by bacteria associated with Rhabitis sp. Iran. J. Microbial. 5(2): 136-141.

Vinale, F., K. Sivasithamparam, E.L. Ghisalberti, R. Marra, S.L. Woo and M. Lorito. 2008. Trichoderma plant pathogen interactions. Soil Biol. Biochem. 40: 1-10. https://doi.org/10.1016/j.soilbio.2007.07.002

Xiao-Yan, S., S. Qing-Tao, X. Shu-Tao, C. Xiu-Lan, S. Cai-Yun and Z. Yu-Zhong. 2006. Broad-spectrum antimicrobial activity and high stability of Trichokonins from Trichoderma koningii SMF2 against plant pathogens. FEMS Microbiol. Lett. 260: 119-125. https://doi.org/10.1111/j.1574-6968.2006.00316.x

Yu, H., L. Zhang, L.Z. Li, C. Guo, L. Li, W. Sun and L. Qin. 2010. Recent developments and future prospects of antimicrobial metabolites produced by endophytes. Microbiol. Res.165: 437-449. https://doi.org/10.1016/j.micres.2009.11.009

Zain, M.E., A.S. Awaad, N.A. Al-Jaber and D.J. Maitland. 2008. New phenolic compounds with antifungal activity from Aspergillus isolated from desert soil. J. Saudi Chem. Soc.12(1): 107-114.