JBAAR

Journal of Bioscience and Applied Research

https://jbaar.journals.ekb.eg

SPBH

Determination of the inhibitory activity of the fungi extract *Trichoderma koningii* against some multidrug-resistant bacteria species

Shaymaa Naji Dahham [1], Hayfaa Rajab Alwan1 [2], Ahmed Hussein Dhayea [3], Mohammed Nadhir Maaroof [4]

1 Department of Chemistry, College of Education for Pure. sciences/Tikrit university, Tikrit, Iraq.

2 Department of Biology, College of Education for pure sciences/Tikrit university. Tikrit, Iraq.

3 Salah Al-Din Education Directorate, Ministry of Education, Iraq.

4 Department of Biology, College of Sciences /Tikrit University, Tikrit, Iraq.

DOI:10.21608/jbaar.2025.431243

Abstract:

Twenty-two samples were collected from patients attending Tikrit Teaching Hospital, Tikrit, Iraq. The samples were cultured on mannitol salt agar, blood agar, and MacConkey agar. The results showed that 15 samples gave positive growth with 68.18%. Identification results showed 8 gram-positive bacteria isolates (53.3%) and 7 gram-negative bacteria isolates (46.7%). *Staphylococcus aureus* was the most isolated, were 4 isolates (26.66%), followed by *Staph. epidermidis* and *E. coli* were 3 isolates for each species (20%), and two isolates for each *P. aeruginosa* and *K. pneumonia* with 13.33% for each one, in addition, one isolate for each *P. mirabilis* and *P. aeruginosa* with 6.66% for each one.

Sensitivity results showed that all isolates were resistant to most antibiotics used in the current study: Tobramycin, Cloxacillin, Metronidazole, Ceftazidime, and Carbenicillin. while some isolates were sensitive to Amikacin, Levofloxacin. *E. coli* is highly resistant to Meropenem, Ceftazidime, and Co-trimoxazole. Gram-positive isolates are highly resistant to Meropenem and ceftazidime.

Aqueous extract of secondary metabolites of *Trichoderma koningii* showed a high inhibitory effect on Gram-positive bacteria, more than Gram-negative bacteria. *P. aeruginosa* showed high sensitivity with inhibition diameters of 20, 29, and 35 at concentrations of 50, 75, and 100%, respectively.

While the alcoholic extract showed less inhibitory activity than the aqueous extract against isolates. *E. coli* was most sensitive to alcoholic extract, especially at concentrations of 57 and 100 %, with inhibition diameters of 25 and 29 mm, respectively. While *Staph. aureus* was highly sensitive to alcoholic extract with inhibition diameters 18,25,32 mm at concentrations 50,75, and 100%, respectively. *Staph. haemolyticus*.

Keywords: *Trichoderma koningii*, pathogenic bacteria, inhibition activity, Antibiotic resistance.

Introduction:

Nature, with all its terrestrial and marine components, is a treasure and an important source for many living organisms of medical and industrial importance. The most important of these biological elements are fungi and their products of secondary metabolites, which previous studies have proven their importance in the biological control of a large number of pathogens. Many secondary metabolites have been isolated, which are chemical compounds that can kill or inhibit the growth of human pathogens. A general name has been given to these metabolites, which are among the most important

Received: November 15, 2024. Accepted: January 16, 2025. Published: June 1, 2025

biological compounds that represent the largest and most diverse of natural products [1].

Antibiotics have made it possible to treat serious bacterial infections such as meningitis and bacteremia that were previously untreatable and therefore fatal to these pathogens. In recent decades, the overuse and misuse of antibiotics, combined with social habits and economic factors, have accelerated the spread of antibiotic-resistant bacteria, rendering drug therapy against many bacterial strains ineffective. Currently, at least 700,000 people die each year worldwide due to antimicrobial resistance [2,3].

The global distribution of this fungal group and its potential as a source of novel, medicinally useful chemicals (such as alkaloids, polysaccharides, terpenoids, phenols, glycosides, tannins, and flavonoids) have piqued researchers' interests in the past forty years [4,5]. This compound shows great potential in the realms of medicine and pharmacology, particularly in the fight against germs and the treatment of long-term illnesses like diabetes and cancer [4, 6,7].

Trichoderma koningii is one of the most studied fungi after it was described by Oudemans and Koning in 1902 [8], which indicated its major function in the biological control of plant diseases, especially fungal diseases [9,10,11]. Many species differ from each other in phenotypic characteristics, including shape and growth rate [9]. Among these studies, a study proved that this type of fungus produces a compound through secondary metabolism, which is 6-Pentyl alpha pyrone, as an effective inhibitor of spore germination [12,13]. The Trichokonins compound was also distinguished and characterized, which has antibacterial activity, in addition to its ability to control Other fungal diseases that affect plants, also indicated that some compounds resulting from the secondary metabolism of *T. koningii* and *T. harzianum* killed 100% of nematodes that infect root nodules [14].

MATERIAL AND METHODS

1. Isolation and identification of bacteria

Twenty-two samples were collected from different infection sources from patientswho came to Tikrit Teaching Hospital in Tikrit city in Iraq. Samples were cultured on Mannitol salt agar, blood agar, and MacConkey agar and incubated at 37°C for 24 hours. The bacteria were initially identified based on the morphological characteristics of the colonies and examined microscopically after being dyed with Gram stain. Various biochemical tests were carried out to identify the isolated bacteria [15], and the identification was confirmed using a Vitek 2 Compact System.

2. Culture media

2.1. The commercially prepared culture

MacConkey agar, Nutrient Agar, Mannitol Salt Agar, and Brain Heart Infusion broth were used, as the media were prepared and pH values were adjusted and sterilized as per the manufacturer's instructions.

2.2. Culture media prepared in the laboratory

Blood agar medium

It was prepared by adding sterilized sheep blood with a concentration of (5% v/v) to the prepared blood agar base medium according manufacturer's instructions [16].

3. Antibiotic sensitivity test:

All isolates accordance with CLSI 2022 recommendations[17]. An antimicrobial susceptibility test was performed on Mueller-Hinton agar using the Kirby-Bauer disk diffusionmethod. A loopful of bacteria (three to five pure colonies) was emulsified in 5 mL of sterile normal saline and gently stirred to create a homogeneous suspension. The suspension's turbidity was then corrected to meet the McFarland 0.5 requirement for density. To standardize the inoculum size, a sterile cotton swab was dipped into the suspension, and any excess was removed by gently rotatingthe swab against the surface of the tube. The swab was then

used to distribute the bacteria evenly over the entire surface of Mueller-Hinton agar. The inoculation plates were driedfor 3–5 min at room temperature. Then, by using a disk dispenser, selected antimicrobial disks were positioned on the plate and incubated for 16–18 h at 35–37°C. Table 1 lists the antimicrobial agents that were evaluated at the various concentrations. Diameters of thezone of inhibition around the disks were measured using a digital caliper. The result was interpreted as sensitive, intermediate, and resistant based CLSI 2022 guidelines.

4: Isolation of *Trichoderma koningii*:

Samples were collected from different areas in Tikrit city. The upper layer was removed by about 3-5 cm from the soil surface. A sample was taken from it, and after mixing it well, 25 g of it was taken and added to a 500 ml flask containing 225 ml of sterile distilled water. The flasks were shaken well for three minutes. After that, 1 ml of the mixture was taken using a sterile pipette and transferred to a sterile test tube containing 9 ml of sterile distilled water. Then, the necessary series of dilutions was carried out up to 10-5. 0.1 ml of the dilutions 10-4 and 10-5 were taken and spread using a glass spreader in a Petri dish containing sterile Potato dextrose agar medium with two replicates for each sample. Then, the dishes were incubated in an incubator at a temperature of 25 °C for 8-2 days. After that, the growing colonies were examined, and a separate transfer process was carried out for each of them. On Sabouraud dextrose agar and Potato dextrose agar [18], fungal species were identified based on colony shape and colour.

4-1: Preparation of *Trichoderma koningii* extracts:

After the incubation period, the work was carried out in a sterile environment, after which the formed mycelium was separated by filtration through a filter unit (0.45 Mm Nalgene), then the filtrate was centrifuged for 15 minutes at 1000 rpm, then the filtrate was collected and filtered again using a second Nalgene filter unit (0.45 Mm Nalgene). After obtaining the fungal extract, it was placed in glass tubes, then the tubes were covered with adhesive wax tape (Parafilm) [19]. After obtaining the fungal extract, freeze-drying was carried out using a Lypholizer device for a certain period of time until a dry powder was obtained.[20].

4-2: Preparation of the aqueous extract of *Trichoderma koningii*

Utilizing the methodology of Hu et al. [21], 50, 75, and 100 grams of Trichoderma koningii extract powder were introduced into a sterile glass flask containing 100 milliliters of distilled water, subjected to heating for 2 hours at 80 °C, and thereafter incubated in a vibrating incubator for 24 hours at 37 °C To obtain the dry extract powder, the mixture was filtered through filter paper in glass tubes, and then it was lyophilized for twenty-four hours. Additionally, the powder was then sealed and frozen until it was required. The method was performed multiple times to obtain sufficient extract. Dissolve it in 20% DMSO, transfer it to polypropylene tubes, and store until needed.

4-3: Preparation of the alcoholic extract of *Trichoderma koningii*:

The study [20] was adjusted to utilize 50, 75, and 100 g of Trichoderma koningii extract powder per 100 ml of 70% ethyl alcohol in a 1-liter glass beaker with a rubber cap to avoid evaporation. The combination was shaken in a 37 0 C incubator for 24 hours. The method was repeated to extract enough. Store frozen until use. The precipitate was dissolved in 20% DMSO and refrigerated in polypropylene tubes until use.

4-4: Preparation of bacterial suspension

The bacteria were cultured in the broth and incubated for 24 hours at 37°C. The bacterial suspension was then prepared for use in the test of inhibitory activity of the *Trichoderma koningii* extract at 0.5 McFarland standard, which is equal to 1.5×10^{18} CFU/ml.

4-5: Determination of the inhibitory activity of

Trichoderma koningii extract:

Agar well diffusion assay on Mueller Hinton Agar was used to test the inhibitory activity of *Trichoderma koningii* extract against 7 species of pathogenic bacteria that are multi-antibiotic resistant. These are *Staphylococcus aureus*, *Staph. epidermidis*, *Staph. haemolyticus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Klebsiella pneumonia*. The bacteria were spread on the surface of the medium, and then 0.2 ml of each concentration of *Trichoderma koningii* extract (100 mg/ml) was mixed with DMSO dissolved medium at 45°C. were transferred to 6 mm wells and incubated at 37°C for 24 hours, and the diameters of the inhibition zones were measured and recorded [22].

5: RESULTS AND DISCUSSION:

5-1: Isolation and identification

A total of 22 samples were collected from different infection sources. The results showed that 15 samples gave positive bacterial growth with a percentage of 68.18%. The isolates were identified based on the microscopically morphological characteristics and by using various biochemical tests for both Gram-positive and negative bacteria. The identification was confirmed using the Vitek2 compact system. The identification showed that the number of gram-positive bacteria was 8 isolates with an isolation percentage of 53.3%, while the gram-negative bacteria were 7 isolates with a percentage of 46.7%, as shown in Table 1.

Isolates identified as E. coli and K. pneumoniae were recognized by their growth on MacConkey agar, exhibiting a distinctive odor and rose colonies resulting from lactose fermentation in the medium. The isolates identified as P. mirabilis manifested as small, pale colonies that were slightly convex, round, and possessed smooth edges on MacConkey agar plates, exhibiting non-fermentative behavior towards lactose. Furthermore, *P. mirabilis* isolates exhibited swarming movement on blood agar. P. aeruginosa was recognized through its proliferation on cetrimide medium. P. aeruginosa displays a blue-green hue owing to the production of pyocyanin. *Staphylococcus aureus* is distinguished from other staphylococci by its capacity to ferment mannitol; during incubation, the medium's color shifts from pink to yellow due to a pH alteration, indicating mannitol fermentation. The coagulase test confirms the identification of *Staphylococcus aureus* and assists in distinguishing it from other Staphylococcus species. Only *Staphylococcus aureus* produces the coagulase enzyme. *Staphylococcus epidermidis* and *Staphylococcus lentus* exhibit a negative response to Gram staining, do not ferment mannitol, and do not generate coagulase. Additionally, *Staphylococcus lentus* ferments mannose. Therefore, it may be distinguished from other coagulase-negative staphylococci. All isolates were confirmed using the Vitek 2 compact system, as shown in the identified form in Figure 1.

The predominant isolated bacterium was *Staphylococcus aureus*, with four isolates constituting 26.66%. This was succeeded by *Staphylococcus epidermidis* and *Escherichia coli*, each with three isolates, representing 20%. Additionally, there were two isolates of *Klebsiella pneumoniae*, accounting for 13.33%, alongside two isolates of *Pseudomonas aeruginosa*. Furthermore, one isolate each of *Proteus mirabilis* and *Pseudomonas aeruginosa* was identified, each comprising 6.66%.

5-2: Antibiotic susceptibility of bacterial isolates

The susceptibility of bacterial isolates to antibiotics has been determined following the Clinical and Laboratory Standards Institute (CLSI, 2022). Three gram-positive isolates and four gram-negative isolates, obtained from various sources of infection, were evaluated against ten different antibiotics. The results in Table 2 indicated that all isolates exhibited

resistance to the majority of antibiotics employed in the current study, including Tobramycin, Metronidazole, Cloxacillin, Ceftazidime, and Carbenicillin. However, some isolates demonstrated sensitivity to Levofloxacin, Amikacin, and Aztreonam, while they remained resistant to Norfloxacin. Escherichia coli, the most frequently isolated bacterium, showed significant resistance to Meropenem, Ceftazidime, and Co-trimoxazole. The gram-positive isolates exhibit significant resistance to Meropenem and ceftazidime.

Bacterial isolates' susceptibility to antibiotics is not continuous and changes over time and in different environments. This necessitates the frequent testing of prevalent bacterial pathogens' antibiotic susceptibility profiles in various communities [23]. Furthermore, he discovered that fluoroquinolone antibiotic treatment resulted in significant elevations in the MIC, potentially linked to treatment failure. Antibiotic resistance in Enterobacteriaceae and certain gram-positive cocci exhibits significant alterations in characteristics due to the prevalent presence of resistance transfer factors (RTF). β-Lactamase is expressed by the majority of resistance genes, yet some bacteria may exhibit resistance from other classes, as they pick upresistance genes from their surroundings or other bacteria. [24] Some of β-lactamases have been imaged from the chromosomally encoded genes that occur spontaneously in some species to conjugative plasmids, increasing their spreading ability among other species. RTF may be transmitted to drug-sensitive strains by conjugation, exhibiting kinetics analogous to F transfer in *E. coli* [25].

Table 1: Biochemical and morphological properties of isolated bacteria.

Tests \ Bacteria species		*E. coli*	*P. mirabilis*	*K. pneumonia*	*P. aeruginosa*	*Staph. Aureus*	*Staph . epidermidis*	*Staph. haemolyticus*
Gram stain		G^- ve	G^- ve	G^- ve	G^- ve	G^+ ve	G^+ ve	G^+ ve
Catalase		+	+	+	+	+	+	+
Oxidase		-	-	-	+	-	-	-
mannitol fermentation		-	-	-	-	+	-	-
IMVIC	IND	+	-	-	-	ND	ND	ND
	MR	+	-	-	-	ND	ND	ND
	VP	-	+	+	-	ND	ND	ND
	C	-	+	+	+	ND	ND	ND
lactose fermentation		+	-	+	-	-	-	-
coagulase production		ND	ND	ND	-	+	-	-

G^- ve: Gram negative bacteria, G^+ ve: Gram positive bacteria, IND: indole, MR: methyl red, VP: Voges proskauer, C: Citrate utilization, ND: not done

bioMérieux

Laboratory Report

Customer: System #:

Printed by: Labadmin

Patient Name: dr.Shaymaa

Card Type: GP Bar Code: 2421836403190610 Testing Instrument: 0000148FFC51 (9517)

Card Type: AST-P592 Bar Code: 3721634403306644 Testing Instrument: 0000148FFC51 (9517)

Setup Technologist: Laboratory Administrator(Labadmin)

Organism **Selected Organism: Staphylococcus aureus**

Comments:	

Identification Information	**Card:** GP	**Lot Number:** 2421836403	**Expires:** Apr 6, 2024 12:00 CST
	Status: Final	**Analysis Time:** 7.78 hours	**Completed:** Oct 25, 2023 17:50 CDT
Organism Origin	VITEK 2		
Selected Organism	**Staphylococcus aureus** Bionumber: 030002167763231 Confidence: Low discrimination		
Analysis Organisms and Tests to Separate: Low Discrimination Organism Staphylococcus aureus Pyrro.Ary.(1),VP(99), Staphylococcus pseudintermedius Pyrro.Ary.(99),VP(10),			
Analysis Messages: The following antibiotic(s) are not claimed: Ampicillin, Gentamicin High Level (synergy), Streptomycin High Level (synergy),			
Contraindicating Typical Biopattern(s) Staphylococcus aureus AlaA(1),AGLU(79),PHO S(99), Staphylococcus pseudintermedius LeuA(88),PHOS(99),dMAN(1),			

Susceptibility Information	**Card:** AST-P592	**Lot Number:** 3721634403	**Expires:** Aug 18, 2024 13:00 CDT
	Status: Final	**Analysis Time:** 11.92 hours	**Completed:** Oct 25, 2022 21:58 CDT

8

Figure 1: All identified isolates were confirmed using the Vitek 2 compact system

Table 2: Antimicrobial sensitivity of bacterial isolates against antibiotics

antibiotics / bacteria	MEM	SXT	NOR	MET	LEV	CX	CAZ	PY	ATM	AK
E.coli	R	R	R	S	S	R	R	R	S	R
K.pneumoniae	R	R	R	R	S	R	R	R	R	S
P.mirabilis	R	S	S	R	R	R	R	R	S	S
Pseudomonas aeruginosa	R	R	R	R	S	R	R	R	R	S
Staph.aureus	R	R	S	R	S	R	R	R	S	S
Staph. epidermidis	R	R	S	R	R	R	R	R	R	S
Staph. haemolyticus	R	R	S	R	S	R	R	R	S	R

MEM: Meropenem, PY: Carbencillin, CAZ: Ceftazidime, CX: Cloxacillin, ATM: Aztreonam, LEV: Levofloxacin, MET: Metronidazole, NOR: Norfloxacin, SXT: Co-trimoxazole, AK: Amikacin

5-3: Inhibitory efficacy of *Trichoderma koningii* extracts against several antibiotic-resistant bacterial strains:

The inhibitory activity results against seven antibiotic-resistant bacterial species demonstrated significant variation in the efficacy of the secondary metabolite extract from the fungus *Trichoderma koningii*, influenced by the extract concentration (50%, 75%, and 100%) and the solvent type, either water or alcohol. The findings indicated that the inhibitory activity of the secondary metabolite extract from the fungus *Trichoderma koningii* escalated with higher concentrations and was also contingent upon the bacterial species employed in the assay. The results of inhibition showed that the *Trichoderma koningii* aqueous extracts have inhibitory activity against some of the bacterial species that are multi-resistant to antibiotics. As shown in Table 3, their effect on gram-positive bacteria was higher than that on the negative bacteria, except the *Pseudomonas aeruginosa* with diameters 20, 29, and 35, which showed high sensitivity for all concentrations. 50, 75, and 100% respectively). The lowest effect of the fungal extracts was on the *Klebsiella pneumonia* with diameters of 15, 23, and 25, which showed high resistance to their different concentrations. (50, 75, and 100% respectively).

While the alcoholic secondary metabolite extract of the *Trichoderma koningii* fungus showed less inhibitory activity than the aqueous extract against the seven bacterial isolates that showed multiple resistance to antibiotics, as shown in Table 4. The results showed that *E. coli* is the most sensitive Gram-negative isolate to the alcoholic extract of the *Trichoderma koningii*, especially at concentrations of 57 and 100 % and with an inhibition diameter of 25 and 29 ml respectively, While bacteria showed higher sensitivity to 50% concentration of alcoholic extract with inhibition diameter 16 ml, The lowest effect of the fungal extracts was on the *Proteus mirabilis* with diameters 13, 19, 21 ml which showed high resistance to their different concentrations(50, 75, and 100% respectively). While *Staph. aureus* was a highly sensitive Gram-positive bacterial isolate to the activity of the alcoholic extract of the *Trichoderma koningii* fungus and with inhibition diameter 18,25,32 ml at the concentrations 50,75 and 100%, respectively, while *Staph. haemolyticus* showed less sensitivity to the activity of the alcoholic extract at concentrations of 75 and 100% and with an inhibition diameter of 20, 27 ml, respectively.

Table 3: Inhibition activity of aqueous extract of *Trichoderma koningii* measured by millimeters.

Bacterial species	Concentration (mg/ml)		
	50	**75**	**100**
E. coli	18	28	32
Klebsiella pneumonia	15	23	25
Proteus mirabilis	16	25	26
Pseudomonas aeruginosa	20	29	35
Staphylococcus aureus	19	28	34
Staph. epidermidis	21	25	33
Staph. haemolyticus	17	24	30

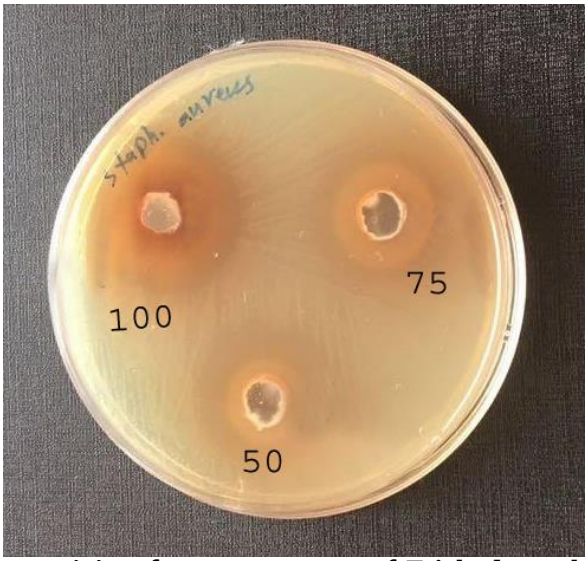

Fig. 2 Inhibitory activity of aqueous extract of *Trichoderma koningii against Staph.aureus*

Table 4: Inhibition activity of alcoholic extract of *Trichoderma koningii* measured by millimeters.

Bacterial species	Concentration (mg/ml)		
	50	**75**	**100**
E. coli	18	28	32
Klebsiella pneumonia	15	23	25
Proteus mirabilis	16	25	26
Pseudomonas aeruginosa	20	29	35
Staphylococcus aureus	19	28	34
Staph. epidermidis	21	25	33
Staph. haemolyticus	17	24	30

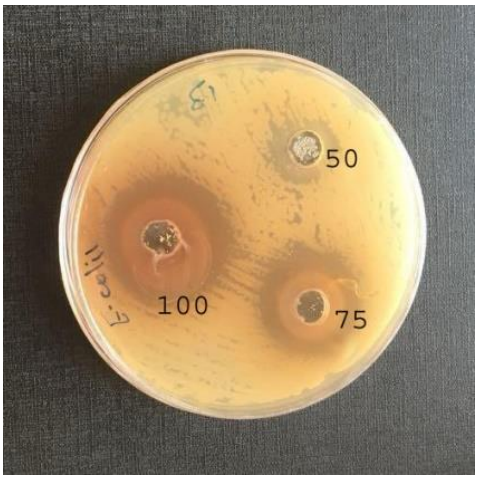

Fig. 2 Inhibitory activity of the alcohol extract of *Trichoderma koningii against E. coli*

It is known that different fungi of the genus Trichoderma can produce degradative enzymes that are used in the biological control of many diseases, perhaps due to their prevalence and ease of growth in various culture media [26,27]. The inhibitory effect of the fungal extract was highly inhibitory in the isolated bacterial species, This may be attributed to the biological activity of the ability of these secondary metabolites to break down peptide bonds in the prokaryotic ribosomes that manufacture proteins [28]. The results of the current study showed that it had an inhibitory effect on bacteria of both species, *Staph. aureus* and *E. coli* were similar to a number of previous studies [29]. which confirmed that *T. koningii* metabolites have biological activity in inhibiting a number of Gram-positive and Gram-negative bacterial species in addition to molds and yeasts. This activity is attributed to the production of enzymes that degrade chitinases, β-glucanases, proteins, and cell wall-degrading enzymes [30]. It was also indicated that it can secrete degrading enzymes and produce antibiotics and enzymes that break down the cell wall, such as chitinases and glucanases, which make up the fungal cell walls [31,32].

T. koningii can produce the compounds Trichokonins and Trichodermine, which are characterized by their inhibitory activity against bacteria and fungi, as shown by the biological activity of each of them against the isolated microbial species [33]. It has been shown that Trichokonins are one of the Peptaibols compounds, which are a large group of secondary metabolites of soil fungi, including the genus Trichoderma, from which it has been indicated that more than 190 compounds can be extracted from their growth extracts [34]. Peptabiols are characterized by the presence of an unnatural amino acid, a-aminoisobutyric acid (Aib), which has been shown to have the ability to form ion channels in the two lipid layers of the cell membrane, allowing the destruction of the membrane by changing the permeability of the membrane [35]. There is a study indicating its role in preventing the production of the energy-synthesizing enzyme adenosine triphosphate (ATPase) in the energy houses (mitochondrion), which hinders the growth and reproduction of other bacteria. In addition, these compounds can change the shape of the outer membrane of bacterial cells, thus hindering their reproduction or causing their death [36].

Conclusion:

The *T. koningii* isolated from the soil were able to produce secondary metabolic compounds in their extracts after growing them on the production

medium for 7 days at 30 ° C as Trichokonins and Trichodermine. These metabolic compounds had different inhibitory activity against some species of bacteria that are multi-resistant to antibiotics, and their inhibitory ability increased with increasing the concentration used, in addition to the fact that the aqueous extract of the fungus was more effective than the alcoholic extract in inhibiting multi-resistant bacteria and all the extracts in this study exhibited potent antioxidant activity.

Conflict of interest: NIL

Funding: NIL

References:

[1] Qin S, Krohn K, Hussain H, Schulz B, Draeger S. Pestalotheols EH. (2011) Antimicrobial metabolites from endophytic fungus Ascocarpe sp. Eur J Org Chem; 5163-5166.

[2] Munita, J. M. and Arias, C. A. (2016). Mechanisms of antibiotic resistance. Microbiology spectrum, 4(2).

[3] Blair, J. M., Webber, M. A., Baylay, A. J., Ogbolu, D. O., Piddock, L. J. (2015). Molecular mechanisms of antibiotic resistance. Nature Reviews Microbiology, 13(1), 42.

[4] Deka, A. C., Sarma, I., Dey, S., & Sarma, T. C. (2017). Antimicrobial properties and phytochemical screening of some wild macrofungi of Rani-Garbhanga reserve forest area of Assam, India. *Advances in Applied Science Research*, *8*(3), 17-22.

[5] Culliao, A. G. L., Lumang-ay, R. S., Kingat, G. M. T., Colallad, T. P., & Canaria, M. V. P. (2020). Preliminary Bioactivity Screening of Crude Extracts of Six Wild Macrofungi from Pine Forests in Benguet and Mt. Province, Philippines. *Manila Journal of Science*, *13*, 74-88.

[6] Badalyan, S. M., & Rapior, S. (2020). Perspectives of biomedical application of macrofungi. *Current Trends Biomedical Engineering & Biosciences*, *19*(5), 556024.

[7] Bi-allelic ACBD6 variants lead to a neurodevelopmental syndrome with progressive and complex movement disordersKaiyrzhanov, R., Rad, A., Lin, S.-J., ... Houlden, H., Maroofian, R., Brain, 20 Conference Paper.

[8] Tran N. Ha (2010) USING TRICHODERMA SPECIES FOR BIOLOGICAL CONTROL OF PLANT PATHOGENS IN VIET NAM J. ISSAAS Vol. 16, No. 1:17-21.

[9] Metcalf DA &Wilson CR (2001) The process of antagonism of Sclerotium cepivorum in white rot affected onion roots by Trichoderma koningii. Plant Pathol 51: 249–257.

[10] Escande AR, Laich FS & Pedraza MV (2002) Field testing of honeybee-dispersed Trichoderma spp. to manage sunflower head rot (Sclerotinia sclerotiorum). Plant Pathol 51: 346–351.

[11] Perell'o ´o A, M onaco C, Sim´on MR, Sisterna M & Bello GD (2003) Biocontrol efficacy of Trichoderma isolates for tan spot of wheat in Argentina. Crop Prot 22: 1099–1106.

[12] Worasatit N, Sivasithamparam K, Ghisalberti EL, Rowland C (1994). Variation in pyrone production, lytic enzymes, and control of Rhizoctonia root rot of wheat among single-spore isolates of Trichoderma koningii. Mycological Research 98 1357–1363.

[13] Gary J. Samuels, Sarah L. Dodd, Bing-Sheng Lu, Orlando Petrini, Hans-Josef Schroers, and Irina S. Druzhinina (2006). The Trichoderma koningii aggregate species. Stud Mycol. 56: 67–133

[14] Sankaranarayanan C, Hussaini SS, Kumar PS, Prasad RD (1997). Nematicidal effect of fungal filtrates against root-knot nematodes. Journal of Biological Control 11: 37–41.

[15] Leber A L (2016) *Clinical Microbiology.* Procedures Handbook, 4th ed, vol 2. Washington, DC: ASM Press.

[16] Atlas, R. M. (2010). Handbook of microbiological media. 4th. Ed. Taylor and Francis Group, New York, USA.

[17] CLSI.(2022). Clinical and Laboratory Standards Institute**.** Performance Standards for Antimicrobial Susceptibility Testing. 32nd ed. Wayne, PA, USA: CLSI supplement M100; 2022.

[18]Benson (2001). Microbiological Applications Lab Manual. 8th ed., The McGraw-Hill companies, USA.

[19] Chakraborty, R., M.S.Srinivasan and S.K.Raghanan.Proteases by a new *Aspergillus niger* during solid substrate fermentation. *Journal.* Microbiology and Biotechnology.. 1995;10:17-30.

[20] Rashan, I.J.; Abed, A.A. and Aziz, A.A. (1992). Further observation on the pharmacological activities of the aqueous extract of Aristolochia bottae stems. Fitoloerapial XIII; 4:350-352.

[21] Hu, H., Zhang, Z., Lei, Z., Yang, Y., Sugiura, N., (2009). Comparative studies of activity and antiproliferative effect of hot water and ethanol extracts from the mushroom Inonotus obliquus. Journal of Bioscience and Bioengineering 107 (1), 42–48.

[22] CLSI. (2015). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. Approved standard M100-S25. CLSI, Wayne, PA

[23] Tula MY, Filgona J, Kyauta SE, Elisha R. Screening for some virulent factors among bacterial isolates from surfaces of hospital fomites and hands of healthcare workers. Cell Mol Biomed Rep 2022;3:9-16.

[24] Joseph WG, Oti BV, Tsaku AP, Ajegena SA, Ajegena BK. Molecular detection of beta-lactam resistance genes in *Staphylococcus aureus* isolated from women in Nasarawa State, Nigeria. Int J Healthc Med Sci 2018;4:60-

[25]. Alvarez M, Tran JH, Chow N, Jacoby GA. Epidemiology of conjugative plasmid-mediated AmpC β-lactamases in the United States. Antimicrob Agents Chemother 2004;48:533-7.

[26] Whipps JM, Lumsden RD (2001) Commercial Use of Fungi as Plant Disease Biological Control Agents – Status and Prospects: Fungi as Biocontrol Agents – Progress, Problems and Potential, CABI Publishing, Wallingford, pp 9-22.

[27]New virulence factor of normal Flora *E. coli*, Issa, A.H., Almayah, A.A., Ibrahim, H.K.Systematic Reviews in Pharmacy, 2020, 11(2), pp. 71–76.

[28]CARRASCOL, M. BARBACID and D. VAZQUEZ (1973) THE TRICHODERMIN GROUP OF ANTIBIOTICS, INHIBITORS OF PEPTIDE BOND FORMATION BY EUKARYOTIC RIBOSOMES. *Biochimica et Biophysica Acta*, 312 (1973) 368-376.

[29] Song Xiao-Yan, Shen Qing-Tao, Xie Shu-Tao, Chen Xiu-Lan, Sun Cai-Yun & Zhang 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

[30] Vinale F.;R.Marre; F.Scala; E.L.Ghisalbert; M/Lorito; K. Sivasthamparam.(2006) Major secondary metabolites produced by two

commercial Trichoderma strains active against different phytopathogens. Letters in Applied Microbiology 43 (2) 143-148.

[31] Schirmbock M, Lorito M, Wang YL, Hayes CK, Arisan-Atac I, Scala F, Harman GE, Kubicek CP (1994) Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics, molecular mechanisms involved in the antagonistic action of Trichoderma harzianum against phytopathogenic fungi. Applied and Environmental Microbiology 60, 4364-4370

[32] Elad Y (2000) Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Protection 19, 709-714

[33] Su H-N, Chen Z-H, Song X-Y, Chen X-L, Shi M, et al. (2012) Antimicrobial Peptide Trichokonin VI-Induced Alterations in the Morphological and Nanomechanical Properties of Bacillus subtilis. PLoS ONE 7(9) Volume 7 | Issue 9 | e45818 doi:10.1371/journal.pone.0045818

[34] Whitmore, L. & Wallace, B. A. (2004). The Peptaibol Database: a database for sequences and structures of naturally occurring peptaibols. Nucleic Acids Res 32 (Database issue), D593–D594

[35] Chugh, J. K. & Wallace, B. A. (2001). Peptaibols: models for ion channels. Biochem Soc Trans 29, 565–570.

[36] Crawford J.M.; Townsend, C.A. New insights into the formation of fungal aromatic polyketides. Nat. Rev. Microbiol. 2010, 8, 879-889.