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Killer Activity of *Hanseniaspora uvarum* Isolated from Dates Vinegar: Partially Purification and Characterization of Killer Toxin

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Abstract:

This study was conducted to isolate and identify killer yeast *Hanseniaspora uvarum* from dates vinegar and measurement the ability of this yeast to produce killer toxin. The antimicrobial activity of the concentrated supernatant containing partially purified concentrated killer toxin was also detected against several pathogenic bacteria and yeast species, which includes two types of yeast *Rhodotorula mucilaginosa* and *Candida tropicalis* and four human pathogenic bacteria *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. In addition, the antagonistic activity of examined yeast have been studied toward four types of fungi, where two are pathogenic for human *Trichophyton mentagrophytes* and *Trichophyton rubrum* and two are plant pathogens *Fusarium solani* and *Sclerotinia sclerotiorum*. The results of killer toxin production experiments revealed the ability of yeast to produce killer toxin with molecular weight at 18 kDa by 12 % SDS electrophoresis. The optimal conditions for killer toxin production were studied, and their antimicrobial activity was determined. The results revealed that killer toxin production was increased at 4 % NaCl, the highest inhibition zone was 20 mm for *S. aureus*, while the lowest inhibition zone was 7 mm for *E. coli*. Killer activity was increased at pH 4 and the best inhibition zone obtained was about 16 mm for *K. pneumoniae*, while 8 mm for *E. coli* and *C. tropicalis*. The temperature was also affect the production of killer toxin, where 25 °C is the best temperature for toxin production of examined yeast, The best killer activity was 21 mm for *C. tropicalis*. The antagonistic activity of killer yeast *H. uvarum* toward pathogenic fungal growth was determined and showed killer activity about 61.11, 44.44, 33.33 and 24.44 % against *T. mentagrophytes*, *T. rubrum*, *F. solani* and *S. sclerotiorum* in comparison to the control.

Key words: Antimicrobial activity, *Hanseniaspora uvarum*, Killer toxin, Yeast.

Introduction:

Since the killer phenomenon in the yeast of *Saccharomyces cerevisiae* was firstly discovered by Makower and Bevan (1), Killer activity has been found to be widespread among different types of yeasts (2, 3). Killer yeasts secrete an extracellular proteinaceous toxin considered to be lethal to other strains of yeasts name as sensitive yeasts, which belong to the same or congeneric species, filamentous fungi and bacteria (3). So far, it has been generally regard that the killer toxins their sensitive cells through various mechanisms, such as inhibition of DNA replication, induction of membrane permeability changes, arrest of the cell cycles in G1 phase and others. Moreover, in some cases, a killer toxin can interfere with cell wall synthesis of sensitive cells by inhibiting β -1, 3-glucan synthase or by hydrolysis of the major cell

wall component β -1, 3- glucans, mannans, chitin, etc. by interacting with receptors in the cell wall and cytoplasmic membrane. Finally, ion leakage occurs due to the formation of channels in the cytoplasmic membrane and blocking of calcium uptake (4, 5, 6). The capability of killer toxins production can confer an advantage over more sensitive competitive strains growing in a fermentative process. More than 11 different killer toxins have been studied and identified, many types of yeasts defined as killer toxins such genera as *Saccharomyces*, *Williopsis*, *Debryomyces*, *Pichia*, *Torulasporea*, *Ustilago*, *Hanseniaspora*, etc. (4, 7-10). *H. uvarum*, the sporogenous form of *Kloeckera apiculata* so-called "apiculatus Yeasts" was reported to produce killer toxins (11). *H. auvarum* is frequently found on ripe fruits (12), especially on grape and fermentation microbiome. The abundance of this yeast also associated with grapes and alcoholic fermentation (13, 14). More studies reported that the *H. uvarum* isolated from fermented beverage such as cider

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(15). *H. uvarum* is considered as a biocontrol agent against some phytopathogenic molds such as *Botrytis cinerea* on grapes and strawberries (16, 17). *Penicillium* spp. (Fruit rot) on citrus (17). Studies and reports revealed that the phenomenon of production of killer toxins by the yeasts associated and widespread in alcohol fermentation for beverage production such as in breweries (18), wine (19), plants and sugarcane producing plants (20). Killer toxins produced by *H. uvarum* have shown that the production of this protein is dependent on specific environmental factors, especially pH and temperature that significantly affected the killing activity against other sensitive microorganisms, the optimum activity of the killer toxin was detected at pH 4.2-4.6 and at temperature of 25-35°C (11). The aim of the present study is to isolate and identify local yeast isolate have the ability to produce killer toxins and investigate the lethal activity of these toxins against several types of microbial pathogens.

Materials and Methods:

Isolation of Killer Yeast:

Naturally Dates vinegar was collected from markets in Iraq/Baghdada, The samples were collected in sterile containers and transported to the laboratory and preserved at 4°C before analysis. Samples were enriched in Yeast Extract peptone dextrose broth medium (YEPD), approximately 10 ml of vinegar inoculated in 250 ml Erlenmeyer flasks with 100 ml of YEPD consisting of 1% Yeast extract, 2% Peptone, 2% Dextrose, pH adjusted at 4.5, broth medium containing 5% (v/v) glycerol buffered with citrate phosphate. The flakes were incubated on rotary shaker incubator at 30 °C and 120 rpm for 48 h. The culture was streaked on YEPD agar supplemented with 100 mg/L chloramphenicol for reduction the growth of bacteria, after that, the growing colonies were isolated and purified to obtained pure colonies. Yeast isolated with highest killing activity was identified by molecular method.

Identification of killer Yeast by molecular method:

DNA of the yeast isolate was extracted using Wizard Genomic DNA Extraction Kit according to the manufacturer's directions (Promega, A1125, and Technical Manual, USA), Yeast isolate identification was performed after Polymerase Chain Reaction (PCR) amplification of the Internal Transcribed Spacer (ITS1-5.8S rDNA-ITS2) regions. The PCR reaction was achieved using 0.5 µM of universal primers ITS1 (5'TCCGTAGGTGAACCTGCGG3') and ITS4 (5'TCCTCCGCTTATTAGTATGC-3') under the

following conditions: Initial denaturation at 95 °C for 5 minutes, followed by 30 cycles of denaturing at 95 °C for 30 Sec, annealing at 55 °C for 30 sec, extension at 72 °C for 1 min and final extension cycle at 72 °C for 10 min, then the program was held at 4 °C. In this case, the amplified DNA was detected by using electrophoresis by resolving the products on 1% agarose gel in 1X TBE buffer. The gel was stained with ethidium bromide and visualized the ampilified DNA under UV illuminator and photographed. The ITS region obtained was purified and sequenced by Medigene company using AB13730 XL automatic DNA sequencing by using forward and reverse primers. Identification of yeast isolate was confirmed by subjecting the obtained sequences to a basic local alignment search tool (BLAST) on the NCBI database.

Sensitive Yeasts and pathogenic Bacterial Isolates:

The sensitive Yeasts *R. mucilaginosa* and *C. tropicalis*, In addition to bacterial pathogens included *S. aureus*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* were spurced from the laboratories of microbiology / University of Baghdad, College of Science (for women) / Department of Biology, These isolates were isolated from clinical samples and identified and characterized according to morphological, biochemical and physiological tests. All pathogenic isolates were subcultured and maintained on selective media for further use.

Production of Killer Toxin:

To assess the production of killer toxin by *H. uvarum*, a loopful of inoculum from a 48 h culture was transferred to a 250 mL Erlenmeyer flask containing 100 mL of YEPD broth medium, followed by incubation at 130 rpm for 72 h in the dark. Using a method described by (21), the yeast cells were separated from the supernatant by centrifugation at 5000 xg for 10 min at 4 C, The supernatant was adjusted to a final glycerol concentration of 15% and concentrated to a volume of 10 ml by ultrafiltration with appropriate device size and membrane (Vivaspin®6- 5-kDa-cutoff membrane PES, Sartorius, Germany). This partially purified concentrated supernatant was used as a concentrated killer toxin after sterilization through a 0.45 µm pore-size membrane Millipore filters (21).

One Dimensional SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

In order to identify the production of yeast killer toxins, SDS-PAGE was conducted. A resolving gel (12 %) and (4 %) stacking gel were used. The killer toxin was prepared by mixing with 6x SDS loading dye and electrophoresed at a

constant 100 v until the dye reached to the bottom. Gels were stained with Coomassie blue and destained in de-staining buffer until the proteins became visible.

Screening of Killer Activity.

We assayed killer toxin activity of the concentrated supernatant containing partially purified killer toxin with a diffusion test according to (22). YEPD agar medium containing (0.003% (w/v)) methylene blue, this medium was buffered to pH 4.5 with 0.5 M sodium citrate/phosphate. The medium was seeded with sensitive yeast *R. mucilaginosa* and *C. tropicalis* at a final density of 1×10^5 cells mL/mL before being poured. The petri dishes were incubated for 1-2 h until the medium be solidified. Wells were cut in the medium using a sterile cork borer (5 mm diameter) and agar pieces removed with a sterile scalpel. Finally, 100 μ L of the concentrated killer toxin extract was added to each well and incubated at 25 °C for 72 h and checked daily. The existence of an inhibition zone around the wells was recorded and measured, 100 μ L of uninoculated YEPD medium was added to well and used as a control (C). The assay was performed in triplicate (10, 21). For the antibacterial activity of partially purified concentrated killer toxin, cells of pathogenic bacteria were grown in nutrient broth (Difco) for 24 h with gentle shaking 120 rpm at 37 °C, and a 100 μ L of 1×10^5 cells was spread out on Mueller Hinton Agar (Difco). Wells in the size of 5 mm were cut by using sterile cork borer, and up to 100 μ L of the concentrated killer toxin extract was added to each well. Then, the plates were incubated at 37 °C for 24-48 h and the diameter of clear zone around the wells was measured (23), 100 μ L of uninoculated MHA medium was added to well and used as a control (C). The assay was prepared three times from three independent biological replicates.

Protein estimation

Protein estimation in concentrated supernatant was executed by Lowry method (24) using bovine serum albumin as a standard.

Optimal Growth Condition for Production of Killer Toxin

The optimal growth conditions for the killer toxin production by *H. uvarum* were investigated. The production medium YEPD broth was prepared as above and killer yeast cells were inoculated from stock culture and grown in 250 mL Erlenmeyer flasks containing 100 mL, this medium was supplemented once with different NaCl concentrations (2, 4, 6, 8 and 10%), or either prepared under different pH values (4, 4.5, 5, 6 and

7), and incubation time was carried out at various temperatures (15, 20, 25, 30 and 35 °C), Killer yeast was incubated at different conditions for 72 h with gentle shaking. The killer activity of concentrated supernatant containing partially purified killer toxin was evaluated towards pathogenic yeasts and bacterial isolates using YEPD methylene blue and Muller Hinton Agar, The killer activity was measured as the diameter of the inhibition zone. Killer yeast growth under different conditions was monitored by measurement of OD₆₀₀ nm of each condition in comparison to the prevalent media as a control (blank). The growth was observed every 2 h starting from OD₆₀₀ \approx 0.1 to approximately OD₆₀₀ \approx 2.5.

Screening of the Antagonistic Activity.

Killer yeast was screened *in vitro* as a biocontrol agent against four strains of fungal pathogens using the dual culture technique. Mycelial disks of 5 mm diameter from the actively growing culture were cut from all tested fungal pathogens, all disks were taken and plated on one side of a petri dish with potato dextrose agar (PDA) medium and incubated at 25 °C for 24 h. On the other side of the petri dish a loopful of the killer yeast was streaked orthogonally. The negative control consisted in PDA plates inoculated only with 5 mm disks of mycelial growth of fungal pathogens without yeast, all plates were incubated for 7 days at 28 °C. Three biological replicates from three independent technical replicates were conducted, The fungal colony diameter was periodically measured, and the inhibition of radial growth reduction was measured and calculated according to the formula of (25).

Inhibition of radial growth (RI) % = $[(C-T)] \times 100$

Where, C (control) is the average diameter of fungal colonies in the absence of *H. uvarum* and T (treatment) is the average diameter of fungal colonies in culture petri dishes.

Statistical Analysis

All experiments were carried out in triplicate repeated at least three times. The data obtained from investigation the effect of optimal growth factors on the killer activity of killer toxin produced by *H. uvarum* against microbial pathogens were analyzed statistically using One-way ANOVA by the SPSS statistical package version 19.0. The means were compared by Duncan's multiple range test at P<0.05.

Results and Discussion

Identification of Isolated Yeast

The killer yeast isolated from Dates vinegar, collected from Baghdad city, Iraq, was

identified according to sequencing of ITS regions using specific primers. The forward (ITS1) and reverse (ITS4) primers were used to amplify the region of the 5.8S rDNA giving 678 pb of PCR product. The amplicon was sequenced and analyzed, and then aligned using BLAST at NCBI. A total similarity (99%) was found between the sequence obtained from isolated killer yeast and

closely related taxa retrieved from (BLASTN) GenBank database, this yeast was identified as *H. uvarum* according to the results of alignment (Figure 1). The sequence of 5.8S ITS rDNA region of yeast isolate was deposited in the GeneBank NCBI database with the accession number is MH333237.

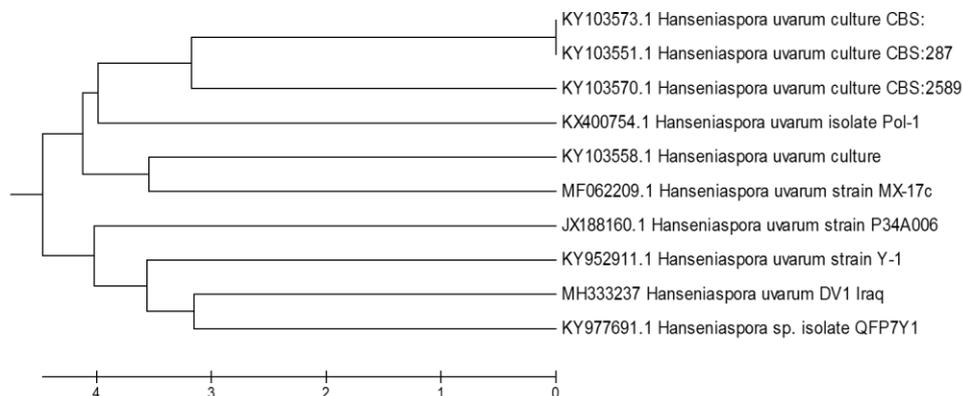


Figure 1. Phylogenetic tree of *H. uvarum* and closely related taxa retrieved from BLASTN (NCBI) drawn based on the nucleotide sequences of ITS-rDNA region, the UPGMA method analysis was used to reconstruct a phylogenetic tree with the MEGA6 program.

To determine the molecular weight of killer toxin production from *H. uvarum*, one dimensional SDS-PAGE (12%) was used. The partially purified concentrated killer toxin was detected with the molecular weight about 18 kDa compared with the corresponding standard ladder (Figure 2).

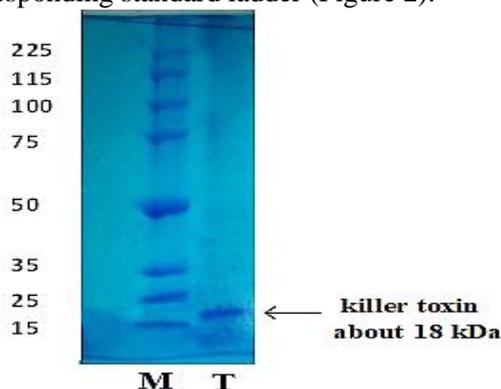


Figure 2. One-dimensional SDS-PAGE (12%) with Coomassie brilliant blue R (Sigma-Aldrich) staining. Lane T, Partially purified killer toxin produced by *H. uvarum*. Lane M, the PageRuler™ plus Unstained Rec. Protein ladder (Promega).

Protein estimation

Protein content in concentrated supernatant of *H. uvarum* culture for all optimal growth

conditions was measured by using Lowry method, the results were shown in the Table 1. Obtained data showed the highest production of protein were 139.6, 106.4 and 194.21 mg/ml at 2% NaCl, pH 4.5 and 25 °C respectively, these samples showed the highest inhibition zone when investigated against microbial pathogens.

Table 1. Protein estimation using the Lowry method in the concentrated supernatant of *H. uvarum*

Growth factors	Protein Concentration mg/ml	Protein Concentration mg/100µl
NaCl con. %	2	102.3
	4	139.6
	6	88.6
	8	34.1
	10	33.78
pH values	4	114.3
	4.5	109.4
	5	102.0
	6	101.4
Temperatures °C	7	57.87
	15	45.8
	20	160.51
	25	194.21
	30	121.03
	35	67.72

Screening of Killer Activity

In this study, effects of initial NaCl concentration, pH and initial temperature on growth and killer toxin production of *H. uvarum* were

Investigated in the shake flask batch experiment, the inhibition activity of partially purified concentrated killer toxin produced by *H. uvarum* under these conditions was tested using

agar diffusion well method against four pathogenic bacteria and two yeast species. The isolated killer yeast was grown in culture medium supplemented with various NaCl concentrations 2, 4, 6, 8, and 10%. The results of the effect of salt concentration on the production of killer toxin were summarized in (Figure 3). The optimum inhibitory effect against all pathogens was obtained when the growth medium supplemented with 4% NaCl compared with control. The means diameters of the inhibition zone around wells filled with partially purified

killer toxin produced under different concentrations of NaCl were varied, and the largest inhibition zone was appeared when the killer yeast grown at 4% of NaCl, as shown in (Figure 3), the inhibition diameters were 20, 13, 7, 9, 12, 11 mm for *S. aureus*, *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *R. mucilaginosa* and *C. tropicalis* respectively. A slight inhibition was obtained at 2% of NaCl, whereas the other concentrations of NaCl don't have any effect on tested pathogens compared with treatment of control.

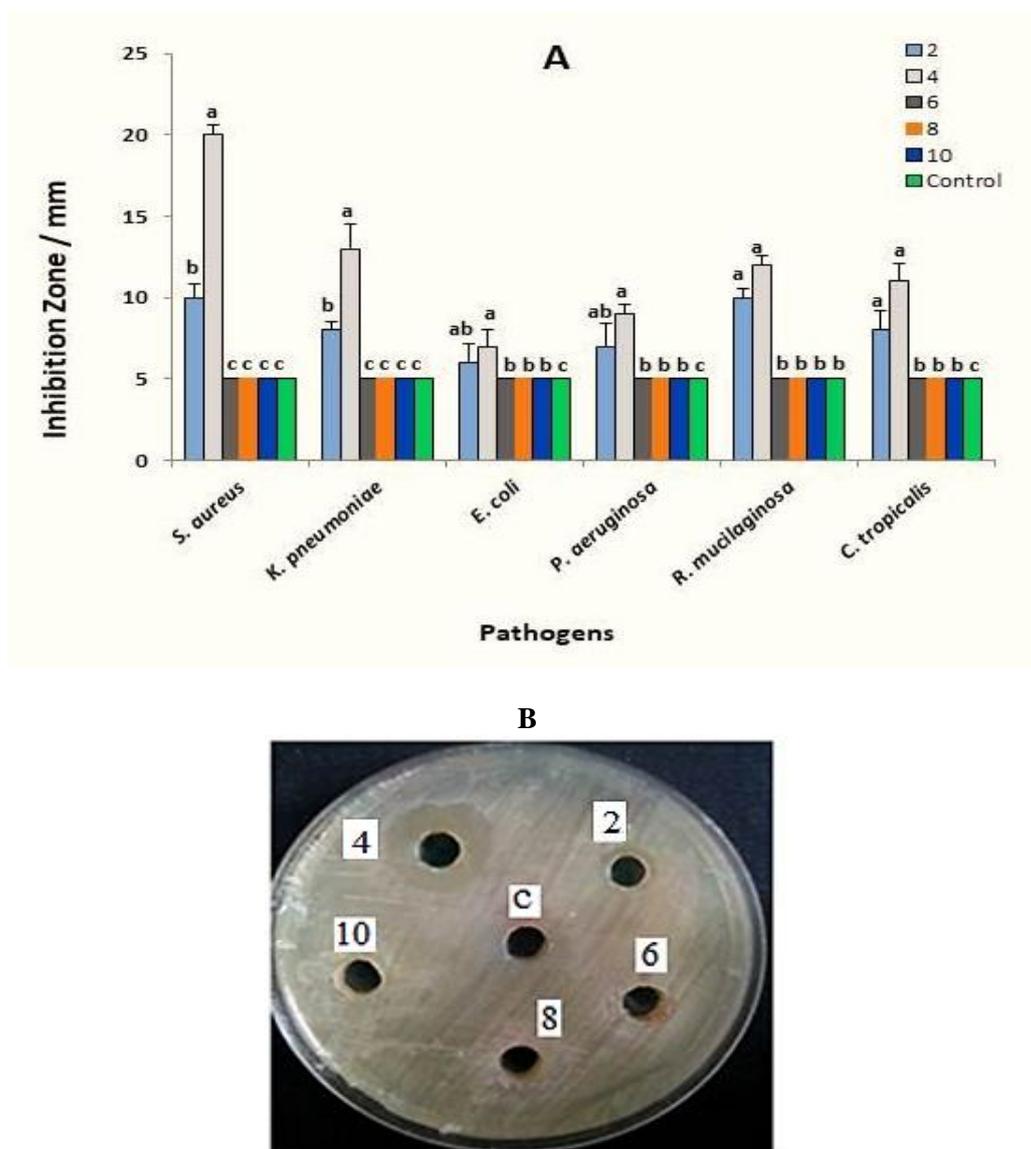


Figure 3. A- Effect of different NaCl concentrations (2,4,6,8 and 10%) on the killer activity of killer toxin produced by *H. uvarum* against pathogenic bacteria and yeasts, the killer activity was estimated by measuring the inhibition zone formed around the wells filled with concentrated supernatant. **B-** Inhibition zone caused by *H. uvarum* concentrated supernatant in Mueller Hinton Agar seeded with *S. aureus* at 37°C for 24-48 h. Vertical bars indicate standard deviations, Different letters above bars indicate statistical significance at $P<0.05$.

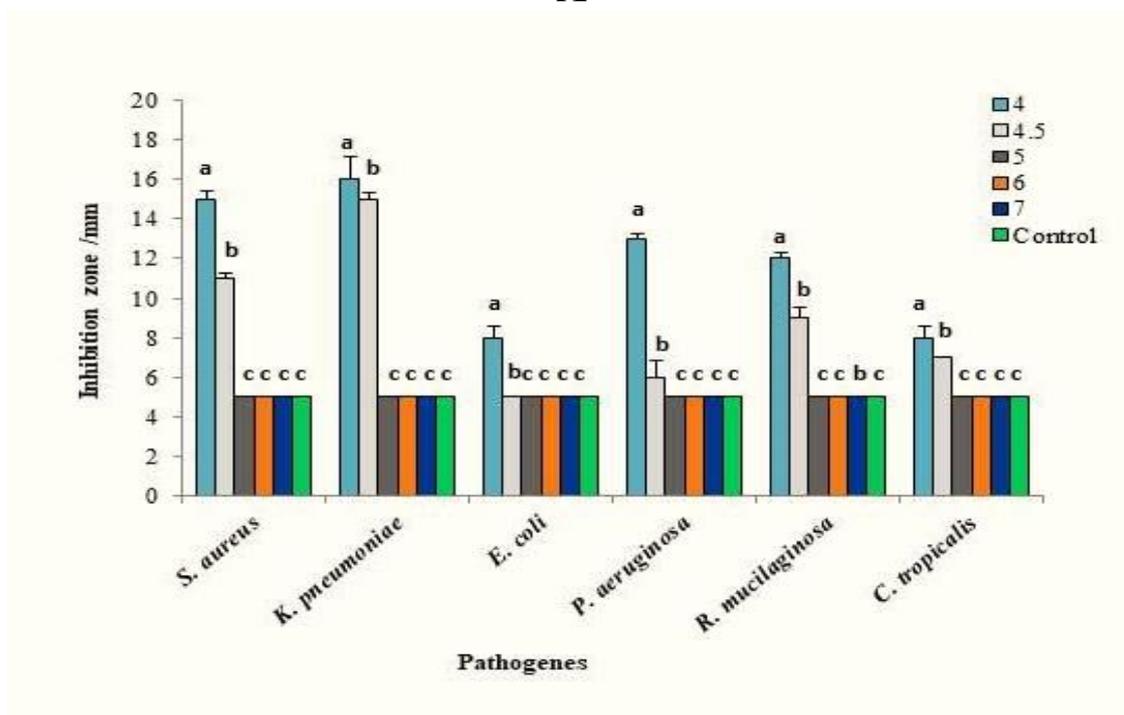
The effects of the initial pH value on the production of killer toxin and killer activity of *H. uvarum*, this was achieved by preparing the YEPD medium with different pH values separately 4, 4.5,

5, 6 and 7 to investigate the optimum pH value for killer toxin production. The data showed that the optimum pH was 4.0 and the highest inhibition zone was obtained at this pH value. The means of

diameters were 15, 16, 8, 13, 12 and 8 for *S. aureus*, *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *R. mucilaginosa* and *C. tropicalis* respectively, From the results, we observed a moderate killer activity

under pH 4.5 was observed and the means of inhibition zone are shown in (Figure 4), whereas no effect was observed at pH 5, 6 and 7.

A



B

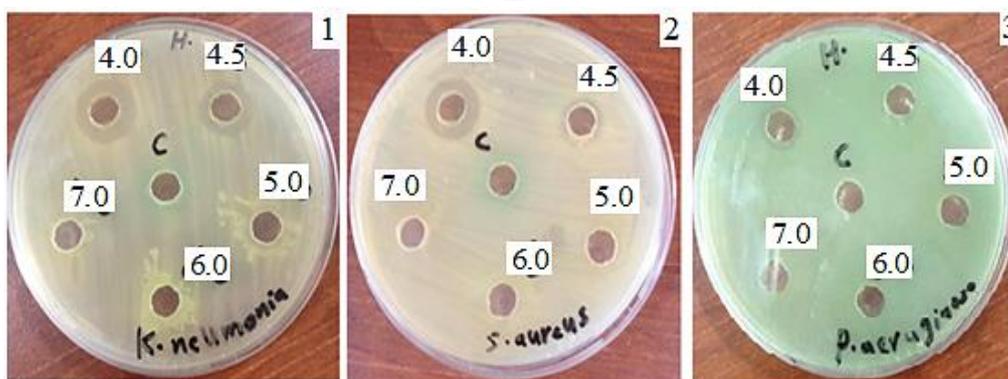


Figure 4. A- Effect of pH on the killer activity of killer toxin produced by *H. uvarum* against pathogenic bacteria and yeasts, the killer activity was estimated by measuring the inhibition zone formed around the wells filled with concentrated supernatant. B- inhibition zone caused by *H. uvarum* concentrated supernatant in Mueller Hinton Agar seeded with 1-*K. pneumoniae* 2- *S. aureus*, 3- *P. aeruginosa* at 37°C for 24-48 h. Vertical bars indicate standard deviations, Different letters above bars indicate statistical significance at $P < 0.05$.

In order to determine the effect of initial temperature on production of killer toxin and its antimicrobial activity against tested pathogens, the YEPD medium inoculated with tested yeast was incubated at various temperatures separately 15, 20, 25, 30 and 35°C. From the results shown in (Figure 5), the optimal incubation temperature for killer toxin production and significantly killer activity was below 25 and 30°C. The antimicrobial activity of concentrated supernatant containing partially

purified killer toxin at these temperatures was measured as an inhibition zone formed around the wells filled with this concentrated supernatant, from the results it can be noticed that after 48 h incubation at 25 and 30°C the means of diameters were 13, 14, 13, 13, 20, 21 and 11, 12, 12, 9, 10 and 10mm for *S. aureus*, *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *R. mucilaginosa* and *C. tropicalis* respectively, while other temperature degrees showed low or no effect on the tested pathogens.

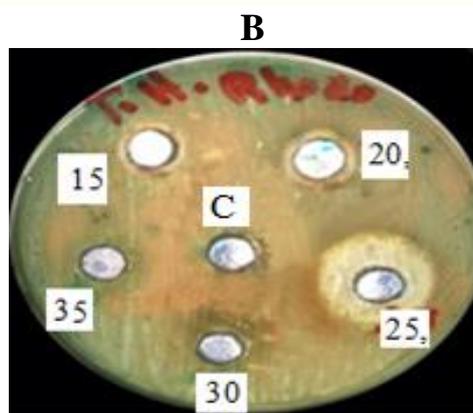
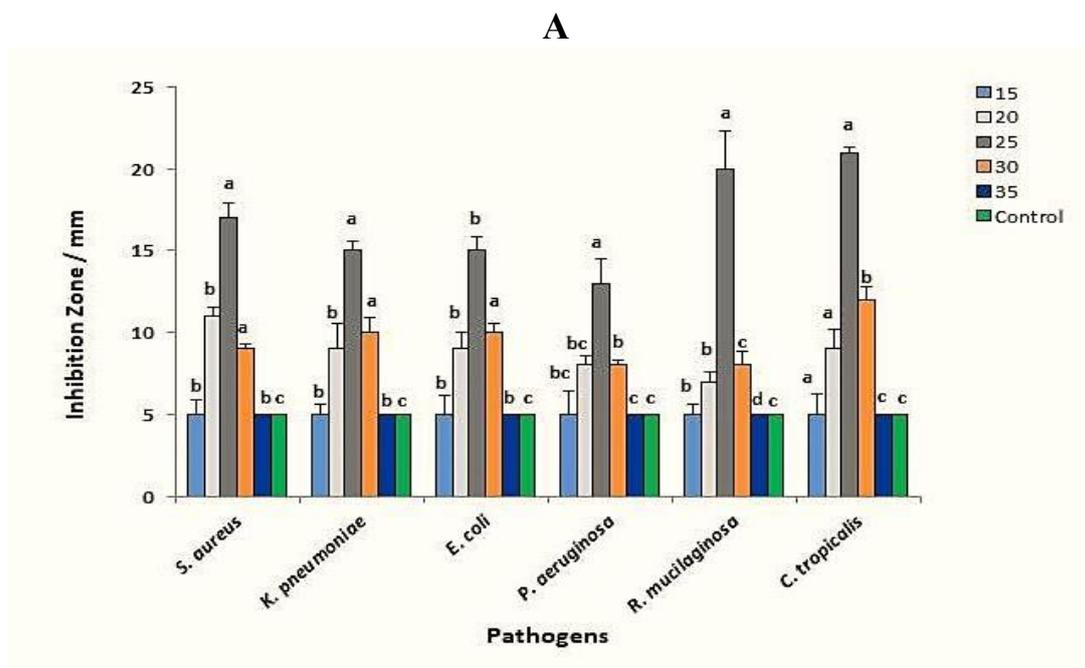


Figure 5. A- Effect of temperature on the killer activity of killer toxin produced by *H. uvarum* against pathogenic bacteria and yeasts, the killer activity was estimated by measuring the inhibition zone formed around the wells filled with concentrated supernatant. **B-** Inhibition zone caused by *H. uvarum* killer toxin in YEPD agar medium containing (0.003% (w/v)) methylene blue seeded with *R. mucilaginosa* at 25°C for 72 h. Vertical bars indicate standard deviations, Different letters above bars indicate statistical significance at $P < 0.05$.

Antagonistic Activity of *H. uvarum* Against Pathogenic Fungi *In vitro*

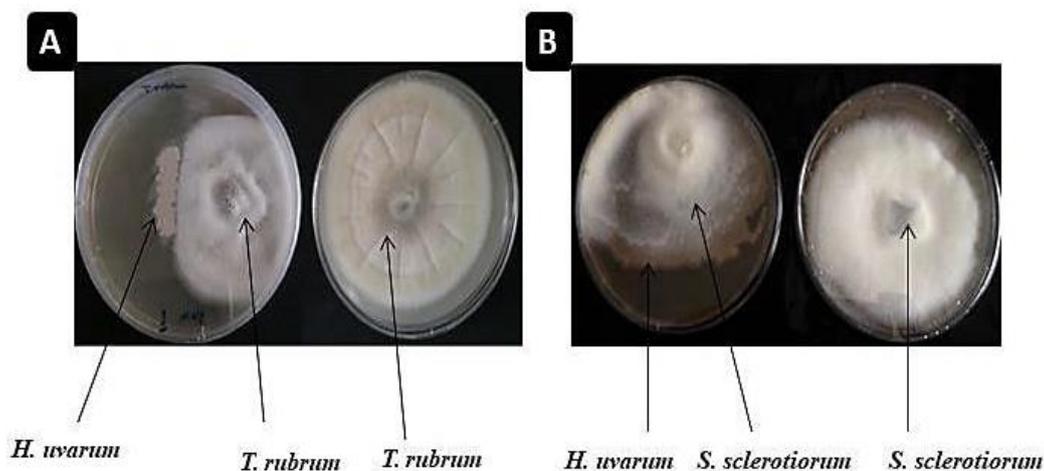
To investigate the antagonistic activity of killer yeast *H. uvarum* against some pathogenic fungi *in vitro*, four strains of fungal pathogens were selected, two for human via *T. mentagrophytes* and *T. rubrum* and two for plants via *F. solani* and *S. sclerotiorum*. This experiment was accomplished by measuring the radial growth inhibition of tested fungal pathogens by dual plate assay in PDA medium. As shown in table 2 and (Figure 6), it is revealed that *H. uvarum* possess a significant

antagonistic effect against these four fungal pathogens, the percentage of inhibition ranged from 61.11 to 24.44%, from the results illustrated in Table 1, the radial growth of fungal mycelia of the human pathogens *T. mentagrophytes* and *T. rubrum* was significantly inhibited and the percentage of radial growth inhibition was 61.11 and 44.44% respectively, while the antagonistic effect of yeast against plant pathogens is not high, efficiency is moderate and the percentage of radial mycelial growth inhibition was 33.33% and 24.44% for *F. solani* and *S. sclerotiorum* respectively.

Table 2. The antagonistic effect of *H. uvarum* against fungal pathogens, as determined by percentage of mycelial radial growth inhibition after 10-14 days of incubation at 25±2 °C

Fungal pathogens	Diameter of colony in control/mm	Diameter of colony in treatment/mm	Percentage of inhibition (%)
<i>T. mentagrophytes</i>	70	15	61.11a
<i>T. rubrum</i>	90	50	44.44ab
<i>F. solani</i>	90	60	33.33a
<i>S. sclerotiorum</i>	90	68	24.44b

Values in the same column followed by the same letter are not statistically different by Duncan's multiple range test at ($p < 0.05$)

**Figure 6. In Vitro antagonistic activity of *H. uvarum* A- towards *T. rubrum*, and B- *S. sclerotiorum* on PDA medium after 10-14 days incubation at 25±2 °C compared with treatment of control plates.**

H. uvarum was isolated in large number from different natural sources, this yeast is widely distributed and abundant in fruits, especially on grapes and fermentation microbiome, also associated with alcoholic fermentation particularly in the initial stages (14). This can be attributed to the high content of sugars and nutrients in fruits that are very important for growth of many types of yeasts (26). Ten yeasts were isolated from the rotten and spoilage date fruits in Saudi Arabia, one isolated yeast was yielded large amount of bio-ethanol and identified as *H. uvarum* (27). This yeast is isolated from naturally fermented date molasses and is used to produce high amount of bio-ethanol from non-expensive source called date palm molasses as a fermentation substrate (28). Several workers in the field of killer yeasts and production of killer toxins reported that the presence of interactions between *H. uvarum* and *S. cerevisiae* during alcoholic fermentation (21) is associated with the phenomenon of the production of killer toxin (11, 29). The killer toxin produced by *H. uvarum* was estimated as 18 kDa (30).

In most researches, it has been indicated that the culture conditions potentially affect the production of killer toxin and the optimal conditions should be found empirically (31). In general, Yeasts exhibit maximum killer activity under acidic conditions and pH ranging between 3-4.5, also most

of yeasts have optimum temperature at 25-30°C (32). In a previous study (22) it has been reported that the addition of salts (NaCl) affect the expression of killer trait, and showed that the concentration up to 6% from NaCl is important to increase the killer activity of some killer yeasts and this concentration is similar to the concentration of salts in the fermenting food like in the fermenting olive brine. The data of our study is in agreement with the data obtained by (33) who revealed that the yeasts grow in high concentrations of salts were able to produce large amount of killer toxin and killed a wide range of sensitive yeast strains in culture media supplemented with 4% NaCl.

Biocontrol is the application of selected specific microbes with antagonistic potential towards other pathogen and used as alternative solution to reduce the effect of synthetic pesticides on human health and the ecosystem. Many studies recorded the detection of new microbial strains with antimicrobial phenomenon and used it against several types of microbial pathogens particularly mold (34). Recently, the use of microbes as biocontrol agents has become an effective method for managing and controlling the fungal pathogens, particularly in the fruit and vegetables of plants (35). Widely range of yeasts naturally present in the environments, especially on fruit surfaces, phyllosphere, roots, soil, can be found in insect,

animal tracts, marine water (31). Yeasts were used as a biocontrol agents because it has several mechanisms of action to reduce diseases and control of pathogens, These mechanisms are found in the wide range taxa of yeasts, that include the ability of these yeasts to multiply rapidly and to compete the pathogens for nutrients and space, play an important role in the inhibition of mycelial growth or conidia germination in molds by secreting of many types of antimicrobial substances and antibiotics, induction of resistance in the host tissues, production of lytic enzymes attack and degrade the cell wall (36, 34). The current study shows that *H. uvarum* isolated from dates vinegar has a significant control on the effectiveness against four potentially pathogenic fungi, this is the first work to investigate the antagonistic activity of this yeast isolated from dates vinegar against *T. rubrum*, *T. mentgrophytes*, *F. solani* and *S. sclerotiorum*. The potential action of *H. uvarum* as a bio-control agent has been previously studied and reported and investigated. This yeast shows inhibitory effect on spore germination and reduce the growth of mycelia of *Bacillus cinerea*. In this field, it was found that using of *H. uvarum* alone and in combination with chemicals include salicylic acid or sodium bicarbonate for controlling the most destructive postharvest disease of grapes caused by *B. cinere* and called Gray mold (37).

Conclusion:

In this study, killer yeast *H. uvarum* isolated from industrial sources is almost new. We investigated the antimicrobial activity of concentrated supernatant containing partially purified killer toxin against some microbial pathogens. *H. uvarum* can produce toxic proteins or glycoproteins causing inhibition of sensitive yeast isolates and some pathogenic bacteria, Many yeast strain isolated from industrial sources produce killer toxins which inhibit the growth of other microorganisms espceailly yeast strains. There are many parameters play an important role in the increasing of killer activity and the amount of killer toxin found in the concentrated supernatant such as NaCl, pH, Temperature. In the present work we determined the antagonistic activity of killer yeast *H. uvarum* against four pathogenic fungi and the results showed a good inhibition of radial growth of these pathogenic fungi.

Conflicts of Interest: None.

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الفعالية القاتلة لخميرة *Hanseniaspora uvarum* المعزولة من خل التمر: تنقية وتعريف جزئي للذيفان القاتل

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الخلاصة:

هدفت الدراسة الى عزل وتعريف الخميرة القاتلة *Hanseniaspora uvarum* من خل التمر وتحديد قابليتها على انتاج الذيفانات القاتلة، كذلك اختبار القابلية القاتلة لهذه الذيفانات تجاه مجموعة من المايكروبات الممرضة متمثلة بنوعين من الخمائر هي *Rhodotorula mucilaginosa* و *Candida tropicalis* واربعة انواع من البكتريا الممرضة هي *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* بالاضافة الى انه تم اختبار الفعالية التضادية *H. uvarum* تجاه اربعة انواع من الفطريات اثنين منها ممرضة للإنسان تضمنت *Trichophyton mentagrophytes* and *Trichophyton rubrum* واثنين ممرضة للنبات تضمنت *Fusarium solani* and *Sclerotinia sclerotiorum*، تجربة اختبار قابلية الخميرة على انتاج الذيفانات القاتلة اشارت الى قابلية الخميرة على انتاج ذيفان قاتل وزنه الجزيئي بحدود 18 كيلو دالتون باستخدام تقنية الترحيل الكهربائي 12% SDS. الظروف المثلى لأنتاج الذيفان القاتل تم دراستها والفعالية التضادية قد حددت، النتائج اوضحت ان انتاج الذيفان القاتل يزداد بوجود 4% ن ملح الطعام NaCl و اعلى منطقة تثبيط كان مقدارها 20 ملمتر تجاه بكتريا *S. aureus* في حين كانت اقل منطقة تثبيط تم الحصول عليها كانت 7 ملمتر تجاه بكتريا *E. coli*، الفعالية القاتلة كانت قد زادت عند رقم هيدروجيني 4 pH وكان مقدار افضل منطقة تثبيط هي 16 ملمتر تجاه بكتريا *K. pneumoniae* و اقل منطقة تثبيط كانت 8 ملمتر تجاه بكتريا *E. coli* و خميرة *C. tropicalis*، درجة الحرارة ايضاً كان لها تأثير في كمية انتاج الذيفانات القاتلة وكانت درجة الحرارة 25 مئوي هي الافضل للأنتاج وبلغت افضل مناطق التثبيط 21 ملمتر تجاه خميرة *C. tropicalis*. الفعالية التضادية لخميرة *H. uvarum* تجاه نمو الفطريات الممرضة كانت قد حددت وبأظهرت فعالية قاتلة بلغت 61.11، 44.44، 33.33 و 24.44% تجاه *T. mentagrophytes*, *T. rubrum*, *F. solani* and *S. sclerotiorum* مقارنة بمعاملة السيطرة.

الكلمات المفتاحية: الذيفانات القاتلة، *Hanseniaspora uvarum*، خميرة، الفعالية المضادة للمايكروبات .